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# LIPID COMPOSITION AND ANTIOXIDANT ACTIVITIES OF THE SEED OIL FROM THREE MALVACEAE SPECIES

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*Abstract* - The oil content and fatty acids, unsaponifiable composition and antioxidant activities of the seed oil from three Malvaceae species (*Malva sylvestris* L., *Malva sylvestris* L. var. *mauritiana* and *Althaea officinalis* L.) from Serbia were determined. The oil yields from the seeds varied from 7.18 to 9.60%. The main fatty acids of the seed oils were linoleic acid (44.14-54.49%), oleic acid (13.00-16.99%) and palmitic acid (11.45-24.29%). A small amount of cyclopropenoid acids, up to 1.85% was also established. The predominant sterol in all seed oils was $\beta$-sitosterol (11.51-17.34 mg/g of oil). The antioxidant potential of all the investigated seed oils was evaluated by radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay.

*Key words*: Malvaceae, seed oils, fatty acids, sterol

## INTRODUCTION

Malvaceae, or mallow family, is a family of flowering plants containing over 200 genera comprising ca. 2,300 species. There are a number of economically important members of this family. Some are best known as fiber plants, such as cotton (*Gossypium* spp.), kenaf (*Hibiscus cannabinus*) and Chinese jute (*Abutilon theophrasti*), whereas others are food plants, such as okra (*Abelmoschus esculentus* or *Hibiscus esculentus*) and several edible *Malva* spp. (*M. nicaensis*, *M. parviflora*, *M. sylvestris* and *M. verticillata* var. *crispa*).

Mallows are perennial plants native to Europe, North Africa, and southwestern Asia. The plants have been naturalized in North America and are cultivated from Western Europe to Russia. They prefer damp areas near the ocean, salt marshes, meadows, sides of ditches and banks of tidal rivers. They grow from 1 to 2 m tall, and the leaves, flowers, and roots have been used for medicinal purposes. The flowers bloom in late spring, and the roots must be at least 2 years old before harvesting (Chevallier 1996, Blumenthal et al., 2000).

*M. sylvestris* is a species of mallow that belongs to the family of Malvaceae known as the common mallow. It is an annual or perennial herb, attaining a height of four feet and is grown widely in Serbia. The high mucilage content of *M. sylvestris* makes it an excellent demulcent that can be used for many applications. In the digestive tract, the fruit mucilage can be used to heal and soothe inflammations such as gastritis, peptic ulcers, enteritis, and colitis (Yeole et al., 2010).

The flowers and leaves of *M. sylvestris* L. var. *mauritiana* have been used in the treatment of catarrhs of the respiratory system and various inflammations of the nasal and oral cavities. Capek et al. (1999) have found that the mucilage isolated from the flowers of this herb, and especially its acidic heteropolysaccharide component, exhibited cough-suppressing activity.

Marshmallow (*A. officinalis*) has demulcent properties. Sometimes it is used to treat inflammations and irritations of the mucous membranes such as the alimentary canal, urinary and respiratory organs. Marshmallow root is traditionally used as a demulcent and emollient for the irritation of oral and pharyngeal mucosa and associated dry cough, mild gastric complaints and for the treatment of minor skin inflammations.

The active components of the Malvaceae species are found in the leaves, flowers, seeds and roots. Several studies document the mucilaginous polysaccharide content in the plant; the primary components are composed of rhamnose, galactose, galacturonic acid, and glucuronic acid (Karawya et al., 1791, Tomoda et al., 1989, Gonda et al., 1990, Classen et al., 2002). Flavonoids, phenolic acids, tannins, and volatile oils have also been studied (Blumenthal et al., 2000, Billeter et al., 1991, Proestos et al., 2005, Cutillo et al., 2006). Malonated anthycyanins have been isolated from the flowers (Takeda et al., 1989, Pourrat et al., 1990, Mas et al. 2000)

The marshmallow leaf contains mucilage polysaccharides (6-9%) composed of arabinogalactans and galacturonorhamnans; flavonoids 8-hydroxyluteolin and 8-$\beta$-gentiobiosid; phenolic acids; tannins; and volatile oil (Newall et al., 1996; Wichtl and Bisset, 1994). The primary chemical constituents of Marshmallow include mucilage, polysaccharides, flavonoids (quercetin, kaempferol), asparagine, tannins, lecithin, and pectin.

Several studies on seeds of the Malvaceae family describe the occurrence of cyclopropenoid fatty acid derivatives. The most commonly found are sterculic (9,10-methylene-9-octadecenoic) and malvalic (8,9-methylene-8-heptadecenoic) acids (Vickery, 1980). These two fatty acids are generally referred to as cyclopropenoid fatty acids (CPEFAs), and have now been found in a large number of seed oils from plant families of the order Malvales (Sterculiaceae, Malvaceae, Bombacaceae and Tiliaceae). Generally, both fatty acids are present together in concentrations that vary up to 60%, depending on the species, and they are usually accompanied by small amounts of the cyclopropenoid analogs, i.e. dihydrosterculic and dihydromalvalic acids. They are also found in leaves, roots and shoots. Sterculic acid is the more active of the two fatty acids whose general action is to inhibit the desaturation of stearic to oleic fatty acid in the animal body with a resultant alteration in membrane permeability or an increase in the melting point of fats.

Compounds containing a cyclopropenoid ring are associated with several biological properties, such as insecticides, antifungal, antibiotic, antiviral, hormonal, carcinogenic or antitumor activities and enzyme inhibitor (Salaun and Baird, 1995; Salaun, 2000). The cyclopropenoid fatty acids manifest a number of unusual properties. The main impetus leading to the discovery of cyclopropenoid fatty acids came from the food and agricultural industries. Cyclopropenoid fatty acids have been investigated extensively owing to their biological effects on animals and their co-carcinogenic properties (Greenberg and Harris, 1982).

No detailed study on the chemical composition of the oil of the seeds of *M. silvestris* var. *mauritanica* has been performed so far. The aim of this study was to determine the oil content as well as the fatty acid and sterol composition of seeds from three Malvaceae species, to evaluate their potential for nutritional and medicinal applications.

## MATERIALS AND METHODS

### *Materials*

Fully mature seeds from three species, *M. silvestris*,

*A. officinalis* and *M. silvestris* var. *mauritanica*, were manually collected in experimental fields at the Institute for Medicinal Plant Research in Pančevo, Serbia (latitude/longitude 44˚ 52` 20`` N, 20˚ 42` 25`` E) in 2010. The seeds were cleaned, placed in glass containers and stored at 20˚C ± 2˚C until used in experiments.

*Preparation of oil extract*

The seeds were milled and the oil was extracted for 6 h with petroleum ether (boiling range 30-60°C) in a Soxhlet apparatus. The extracted oils were dried over anhydrous sodium sulfate and the solvent removed under reduced pressure in a rotary evaporator. The content of fatty acids was expressed as the percentage in the composition of the dry seed powder. The content of sterols was expressed in mg/g of oil.

*Preparation of methyl esters*

Fatty acid methyl esters were prepared following IUPAC methodology without heating. A 100 mg sample of raw seed oil was accurately weighed into a 20 mL centrifuge tube and dissolved in 5 mL *n*-hexane. A methanolic KOH solution (2 mol/L) was added (0.2 mL). The tube was sealed and mixed vigorously for 30 s in a vortex shaker. Saturated NaCl solution (2.0 mL) was added and the organic phase was separated. An aliquot (1.0 µL) of the hexane solution was submitted to GC analysis.

*Gas-liquid chromatography and GC-MS*

The GC and GC-MS analyses were performed on an Agilent 7890A GC system equipped with 5975C inert XL EI/CI MSD and a FID detector connected by capillary flow technology 2-way splitter with make-up gas. A DB23 capillary column (Agilent Technologies, 0.25 mm i.d., 60 m length, 0.25 µm film thicknesses) was used. Samples were injected in split mode (30:1). The injection volume was 1 µl and the injector temperature was 220°C. The carrier gas (He) flow rate was 3.3 ml/min at 60°C (constant pressure mode). The column temperature was 50°C (1 min) then programmed linearly 50-175°C (25°C/min) and 175-235°C (4°C/min) with a final 5-min hold. The transfer line was heated at 235°C. The FID detector temperature was 300°C. EI mass spectra (70 eV) were acquired in *m/z* range of 35-550 atomic mass units (AMU), with 3 min solvent delay. The ion source and quadrupole temperatures were 230°C and 150°C, respectively. Retention times of the fatty acid components of the seeds were compared with the corresponding original fatty acids from GC, and identification of mass spectra of individual components was achieved by comparison with Wiley07 and Nist05 databases.

*Antioxidant activity*

The antioxidant activity of toluene solutions of the seed oils was tested against DPPH radicals using Ramadan and Moersel's method (Ramadan and Moersel, 2006). Briefly, a volume of 1600 µL of 0.1 mM DPPH solution prepared also in toluene was added to 400 µL of the toluene solutions of the oils in concentrations of 0.1, 1.0, 10, and 100 µg/mL, vortexed for 10 s and left in the dark for 1 h at room temperature. Absorbance of the samples at 515 nm was then recorded using a CINTRA 40 GBC spectrophotometer. DPPH scavenging activity was determined from the equation:

$$DPPH (\%) = [1- (A_x/A_0)] * 100$$

The same procedure was carried out in toluene, instead of oil, in order to measure $A_0$. The value $A_x$ was the absorbance of the remaining DPPH after a reaction with the oil solution. Each sample was measured in four different dilutions and every measurement performed in triplicate and subsequently averaged. The $EC_{50}$ value for each oil sample was determined from the graph DPPH (%) = f (c), constructed from four dilutions (concentrations). Butylated hydroxyanisole (BHA), a synthetic antioxidant, was used as reference.

*Analysis of unsaponifiables*

Sterols were determined by GC/MS of the complete unsaponifiable fraction. 3.75 mL of internal

**Table 1.** Lipid percentage and fatty acid composition of three Malvaceae species

| | | *M. silvestris* | *M. silvestris var. mauritana* | *A. officinalis* .L. |
|---|---|---|---|---|
| Fatty acid | % lipid | 9.60 | 7.18 | 9.42 |
| Myristic acid | 14:0 | 0.46 | 0.49 | 0.17 |
| Palmitic acid | 16:0 | 24.29 | 23.22 | 11.45 |
| Margaric acid | 17:0 | 0.19 | 0.18 | 0.18 |
| Stearic acid | 18:0 | 3.68 | 3.06 | 4.07 |
| Behenic acid | 22:0 | 0.44 | 0.23 | 0.21 |
| Lignoceric acid | 24:0 | 0.27 | 0.33 | 0.12 |
| ∑ SAFAs | | 29.33 | 27.51 | 16.20 |
| Palmitoleic acid | 16:1 | 0.33 | 0. 34 | 0.10 |
| Oleic acid | 18:1 | 13.66 | 13.00 | 16.99 |
| ∑ MUFAs | | 13.99 | 13.34 | 17.09 |
| Linoleic acid | 18:2 | 44.16 | 48.30 | 54.49 |
| Linolenic acid | 18:3 | 0.77 | 0.83 | 0.89 |
| ∑ PUFAs | | 45.65 | 49.92 | 55.38 |
| Dihydro malvalic acid | 18:CA | 0.59 | 0.50 | 0.50 |
| Dihydro sterulic acid | 19:CA | 0.82 | 0.92 | 0.92 |
| Malvalic acid | 18:CE | 0.85 | 0.96 | 1.85 |
| Sterulic acid | 19:CE | 0.52 | 0.42 | 0.69 |
| ∑ CPEFAs | | 2.78 | 2.80 | 3.96 |
| 10-ketostearic acid | | 1.11 | 1.62 | 1.25 |
| ∑ | | 91.94 | 95.98 | 95.42 |

SAFAs = saturated fatty acids; MUFAs = monounsaturated fatty acids; PUFAs = polyunsaturated fatty acids; CPEFAs= cyclopropenoid fatty acids. Data are expressed in percentage of relative fatty acids (%).

standard solution (0.1% of cholesterol in methylene chloride) was added to 2.3 g of oil sample and the methylene chloride was evaporated. The prepared sample was saponified with 10 mL of 6 M solution of KOH in water and 15 ml of EtOH (ethanol with up to 5% diethyl ether). The solution was refluxed for 90 min at 70°C (water bath 85-90°C). After saponification, 15 mL of water was added and non-saponifiable compounds were extracted, first with 22.5 mL of petroleum ether and then with 22.5 mL of diethyl ether. The two extracts were combined and washed twice with 20 mL of 0.5 M solution of KOH in water and with 20 ml of 5% NaCl solution in water until the pH of the washing water was neutral. The organic fraction was dried with $Na_2SO_4$ and filtered over folded filter paper into a conical flask. The residue obtained after evaporation was derivatized by adding 1.5 mL of pyridine, 0.2 mL hexamethyldisilazane and 0.1 mL trimethylsilane as silylation agents. The flask was placed in an oven at 70°C for 30 min for completion of the silylation. The derivatized sample was transferred into a vial and was ready for injection in GC. All samples were analyzed within 6 h after derivatization. GC and GC/MS analysis were performed on an Agilent 7890A GC equipped with inert 5975C inert XL EI/CI MSD and FID detector connected by capillary flow technology 2-way splitter with make-up. An HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm) was used. The temperature for the GC oven was programmed from 60°C to 300°C at 3°C/min and held for 10 min. Helium was used as carrier gas at 16.255 psi (constant pressure mode). The sample was analyzed in the splitless mode. The injection volume was 1 μL. The GC detector temperature was 300°C. MS data was acquired in EI mode, with scan range 30-550 *m/z*, source temperature was 230°C and quadrupole temperature was 150°C. Solvent

**Table 2.** Sterol contents of three Malvaceae species (mg/g)

| Compound | *A. officinalis* | *M. sylvestris* | *M. sylvestris var. mauritiana* |
|---|---|---|---|
| Ergosterol | 0.244 | - | - |
| Campesterol | 3.147 | 3.278 | 2.377 |
| Stigmasterol | 2.633 | 3.975 | 2.488 |
| β-sitosterol | 15.66 | 17.34 | 11.51 |
| Fukosterol | 1.309 | 4.021 | 2.504 |
| Gramisterol | 0.063 | 0.307 | 0.239 |
| 3*β*-lanosta-8,24-dien-3-ol | 0.460 | - | - |
| Cycloartenol | 3.717 | 1.107 | 0.933 |
| Avenasterol | 0.173 | 0.587 | 0.238 |
| 3*β*-cholest-5ene-3,25-diol | 0.246 | 0.250 | 0.208 |
| 9,19-ciclolanostan-3-ol | 0.507 | 0.923 | 0.710 |
| n.i. | 0.193 | 0.321 | 0.219 |
| n.i. | 0.189 | 0.121 | 0.064 |

n.i. not identified

delay was 3 min. Identification was confirmed by retention time lock (RTL) method and RTL Adams data base.

## RESULTS AND DISCUSSION

The total oil contents of the seeds are given in Table 1. The oil content of the seeds was 7.18% for *M. silvestris* var. *mauritiana,* 9.42% for *A. officinalis* and 9.60% for *M. silvestris.* Mishina et al. (1975) reported that the seeds of *A. officinalis* contained 15.25% oil. Previously, the total oil content of *M. silvestris* seeds was found to be 16.6% (Mukarram et al., 1984). The difference observed is probably due to the origin of the seed. No detailed study on the chemical composition oil of the seeds of *Malva silvestris* var. *mauritanica* has been performed so far.

The oils were examined by GC-MS with respect to their fatty acid contents. Our data revealed that the most abundant fatty acids in the oils of *M. silvestris*, *M. silvestris* var. *mauritanica* and *A. officinalis* were linoleic (44.16, 48.30 and 54.49%, respectively), palmitic (24.29, 23.22 and 11.45%, respectively), oleic (13.66, 13.00 and 16.99%, respectively), and stearic (3.68, 3.06 and 4.07%, respectively) (Table 1). The *A. officinalis* oil was found to be the richest in linoleic acid quantity (54.49%), followed by *M. silvestris* var. *mauritanica* and *M. silvestris* (48.30% and 44.16%, respectively). The fatty acid profiles of the samples from *M. silvestris* and the sample from *M. silvestris* var. *mauritanica* were similar, while those of the *A. officinalis* sample were quite different, especially for the content of linoleic, palmitic and oleic acids. When comparing these results with the ones previously reported for *A. officinalis* and *M. silvestris* by Mishina et al. (1975) and Mukarram et al. (1984), some differences can be noticed, probably due to the different location of the orchard from where the samples were collected, as well as to the year of harvest.

### *Content of unsaponifiables*

The composition of the sterol fraction, analyzed by

**Table 3.** Antioxidant activity seed oil of three Malvaceae species and BHT

| | $EC_{50}$ (ml/ml)* | $EC_{50}$ (mg/mL) |
|---|---|---|
| *M. sylvestris var. mauritiana* | 0.0318 | 28.97 |
| *Malva sylvestris* | 0.0587 | 52.83 |
| *Althaea officinalis* | 0.0397 | 35.65 |
| BHT | - | 1.10 |

* mL of oil per mL of solution

GLC, is shown in Table 2. The sterol fraction of the *A. officinalis* seed oil consisted mainly of campesterol, stigmasterol, $\beta$-sitosterol, fucosterol and cycloartenol, of which $\beta$-sitosterol was the most predominant. The sterol compositions of the *M. sylvestris* and *M. sylvestris* var. *mauritiana* seed oils were very similar. The dominant sterol was also $\beta$-sitosterol, at concentrations of 17.34 and 11.51%, respectively. *A. officinalis* seed oil contained a higher amount of $\beta$-sitosterol compared to the other two oils. Also, ergosterol and 3$\beta$-lanosta-8,24-dien-3-ol were detected only in *A. officinalis* seed oil.

*DPPH radical scavenging activity*

DPPH is a free radical compound that has been widely used to test the free radical-scavenging ability of various samples due to its stability and characteristic absorption at 515 nm. This test was used to study the radical-scavenging effects of the oils. As antioxidants donate hydrogen radicals to this radical, the absorption decreases.

The antioxidant activity of seed oils from *M. silvestris*, *M. silvestris* var. *mauritiana* and *A. officinalis* was tested against DPPH radical at 0.1, 1.0, 10, and 100 mg/mL concentrations. The highest antioxidant activity was displayed by the oil from *M. silvestris* var. *mauritiana* seeds ($EC_{50}$ = 28.97 mg/mL). The synthetic antioxidant BHT (Butylated hydroxytoluene) had $EC_{50}$ of 1.10 mg/mL under the same conditions. The results showed that all of the oils exerted a similar scavenging effect towards DPPH, exhibiting significant DPPH scavenging activity that was 28-53 times weaker than that of BHT (Table 3). The seed oil of *M. silvestris var. mauritiana* exhibited a 2-fold stronger effect than the seed oil of *M. silvestris*. This activity may have been, in part contributed to by some constituents other than fatty acids, such as tocopherols and phenolic compounds (Tasioula-Margari and Okogeri, 2001; Lee et al., 2002).

## REFERENCES

*Billeter, M. Meier, B.* and *O. Sticher* (1991). 8-hydroxyflavonoid glucuronides from *Malva sylvestris Phytochemistry.* **30**, 987-990.

*Blumenthal, M. Goldberg,* and *A. Brinckmann* (2000). *J.* Herbal Medicine: Expanded Commission E Monographs. Marshmallow leaf. Austin, TX: American Botanical Council; Newton

*Capek, P. Kardočova, A.* and *D. Lath* (1999). A Neutral Heteropolysaccharide from the Flowers of *Malva mauritiana* L. *Chem. Papers.* **53,** 131-136.

*Chevallier, A* (1996). The Encyclopedia of Medicinal Plants, New York

*Classen, B.* and *W. Blaschek* (2002). An arabinogalactan-protein from cell culture of *Malva sylvestris*. *Planta Med.* **68**, 232-236.

*Cutillo, F. D'Abrosca, B. Dellagreca, M. Fiorentino, A.* and *A. Zarrelli* (2006). Terpenoids and phenol derivatives from *Malva silvestris*. *Phytochemistry.* **67**, 481-485.

*Greenberg, A.* and *J. Harris* (1982). Cyclopropenoid fatty acids. *J. Chem. Educ.* **59**, 539–543.

*Gonda, R. Tomoda, M.* and *N. Shimizu* (1990). Structure and anticomplementary activity of an acidic polysaccharide from the leaves of *Malva sylvestris* var. *mauritiana*. *Carbohydr. Res.***198**, 323-329.

IUPAC Standard Methods for Analysis of Oils, Fats and Derivatives, Blackwell Scientific Publications, seventh ed.,

IUPAC Method 2.301; Report of IUPAC Working Group WG 2/87, 1987.

*Karawya, M.S. Balbaa, S.I.* and *M.S. Afifi* (1971). Investigation of the carbohydrate contents of mucilaginous plants. *Planta Med*. **20**, 14-23.

*Mas, T. Susperregui, J. Berke, B. Cheze, C. Moreau, S. Nuhrich, A.* and *J. Vercauteren* (2000). DNA triplex stabilization property of natural anthocyanins. *Phytochemistry.* **53**, 679-687.

*Mishina, A.S. Kornievskia, I.I. Shkurupia, S.M.,* and *V.S. Dolia* (1975) Fatty oil of *Althea officinalis*, stoloniferous valerian and golden wallflower. *Farm Zh*. **5**, 92-93 0

*Mukarram, M. Ishtiaque, A.* and *A. Mashood* (1984). HBr-reactive acids of *Malva sylvestris* seed oil, *Journal of the American Oil Chemists' Society*. **61**, 1060-1067.

*Newall, C.A. Anderson, L.A.* and *J.D. Phillipson* (1996). Herbal Medicines: A Guide for Health-Care Professionals. London, England: Pharmaceutical Press, 188.

*Pourrat, H. Texier, O.* and C. *Barthomeuf* (1990). Identification and assay of anthocyanin pigments in *Malva sylvestris* L. *Pharm. Acta. Helv*. **65**, 93-96.

*Proestos, C. Chorianopoulos, N. Nychas, G.J.,* and *M. Komaitis* (2005). RP-HPLC analysis of the phenolic compounds of plant extracts. Investigation of their antioxidant capacity and antimicrobial activity. *J. Agric. Food Chem*. **53**, 1190-1195.

*Ramadan, M.F.* and *J.T. Moersel* (2006). Screening of antiradical action of vegetable oils. *J. Food Comp. Anal*. **19**, 838-842.

*Salaun, J.* and *M.S. Baird* (1995). Biologically active cyclopropanes and cyclopropenes. *Curr. Med. Chem*. **2**, 511–542.

*Salaun, J.* (2000). Cyclopropane derivatives and their diverse biological activities. *Top. Curr. Chem.* **207**, 1-67.

*Takeda, K. Enoki, S. Harborne, J.B.* and *J. Eagles* (1989). Malonated anthocyanins in Malvaceae: malonylmalvin from *Malva sylvestris*. *Phytochemistry.* **28**, 499-500.

*Tomoda, M. Gonda, R. Shimizu, N.* and *H. Yamada* (1989). Plant mucilages. XLII. An anti-complementary mucilage from the leaves of *Malva sylvestris* var. *mauritiana*. *Chem Pharm Bull*. **37**, 3029-3032.

*Vickery, J.R.* (1980). The fatty acid composition of seed oils from ten plant families with particular reference to cyclopropene and dihydrosterulic acids. *J. Am. Oil Chem. Soc.* **57**, 87-91

*Wichtl, M.* and *N.G. Bisset* (1994) Herbal drugs and phytopharmaceuticals. CRC Press, Stuttgart 65-66

*Yeole,* N.B. *Sandhya*, *P. Chaudhari*, *P.S.* and *P.S. Bhujbal* (2010). Evaluation of *Malva sylvestris* and *Pedalium murex* Mucilage as Suspending Agent. *Int. J. Ph.Tech Res.,* **2**, 385-389.