Leaves, Flowers, Immature fruits and Leafy flowered stems of *Malva sylvestris*:

A comparative study of the nutraceutical potential and composition

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Abstract

Malva sylvestris is widely used in Mediterranean and European traditional medicine and ethnoveterinary for the treatment of external and internal inflammation, as well as injuries. Moreover, its use is not only limited to therapeutic purposes; the species is also locally regarded as a food wild herb. Considering that antioxidants and free radical scavengers can exert also an anti-inflammatory effect, the extracts of different parts of the medicinal/edible plant *M. sylvestris* (leaves, flowers, immature fruits and leafy flowered stems) were compared for their nutraceutical potential (antioxidant properties) and chemical composition. Particularly, mallow leaves revealed very strong antioxidant properties including radical scavenging activity (EC$_{50}$ = 0.43 mg/mL), reducing power (0.07 mg/mL) and lipid peroxidation inhibition in liposomes (0.04 mg/mL) and brain cells homogenates (0.09 mg/mL). This part of the plant is also the richest in nutraceuticals such as powerful antioxidants (phenols, flavonoids, carotenoids, and tocopherols), unsaturated fatty acids (eg. α-linolenic acid), and minerals measured in ash content.

Keywords: *Malva sylvestris*; Portuguese Ethnobotany; Leaves; Flowers and stems; Fruits; Nutraceuticals
1. Introduction

Antioxidant defences are essential for survival. They include antioxidant enzymes (superoxide dismutases, catalase), sulphydryl groups as in glutathione, and glutathione-associated enzymes such as peroxidises and transferases. These defences maintain the balance between essential oxidative processes and reactive oxygen-mediated cell regulation, and an excessive production of free radicals that constitutes oxidative stress. So do we need antioxidants from the diet as well? The evidence from large-scale intervention studies is that pure antioxidants may do more harm than good. Epidemiological evidence still indicates that plant-derived foods can protect against cancer and cardiovascular disease (Collins, 2009).

Malva sylvestris L. (Malvaceae), usually known as common mallow, is native to Europe, North Africa and Asia, and its traditional use has been documented since a long-time ago, although little clinical evidence is available. The Greeks and Romans claimed for its emollient and laxative properties and several ethnobotanical surveys conducted in Europe (Table 1) highlight the potential of such neglected local resource, which use is today at the brink of disappearance. Roots, shoots, leaves, flowers, fruits, and seeds are applied in infusions, decoctions, poultices, liniments, lotions, baths and gargles (Camejo-Rodrigues et al., 2003; Novais et al., 2004; Pardo de Santayana, 2004; Carvalho, 2005; Ferreira et al., 2006; Natali and Pollio, 2007; Guerrera and Leporatti, 2007; Quave et al., 2008; Leporatti et al., 2009; Neves et al., 2009). Traditionally, the medicinal applications of the common mallow treat specified disorders of several systems of the body, such as the digestive system, the respiratory, the genitourinary, the muscular and skeletal system, as well as skin disorders and injuries. Besides the most widely recognised anti-inflammatory properties, some other pharmacological and clinical effects are frequently mentioned. The common
mallow is considered to have diuretic, laxative, spasmolytic, lenitive and choleretic effects. It is also used as bronchodilator, expectorant, antitussive, anti-diarrheal and highly recommended for acne and skin care, and as antiseptic, emollient and demulcent (Carvalho, 2005; Quave et al., 2008; DellaGreca et al., 2009; Leporatti et al., 2009; Neves et al., 2009). Edible uses are concerned with folk gastronomy and with those uses generally included in so-called minor nourishment (Guarrera, 2003; Carvalho 2005). Young leaves are eaten raw in salads, leaves and shoots are consumed in soups and as boiled vegetables. Immature fruits are sucked or chewed by children, shepherds and hunters (Pardo de Santayana, 2004; Carvalho, 2005; Neves et al., 2009). It seems that most of the times, leaves are perfectly wholesome and no adverse effects are recorded concerning human consumption, although some authors have reported harmful effects in livestock because when grown on nitrogen rich soils, the plant tends to concentrate high levels of nitrates in its leaves (Cooper and Johnson, 1984; Rivera and Obón de Castro, 1991).

The biological activity of this plant may be attributed to antioxidants, such as polyphenols, vitamin C, vitamin E, β-carotene, and other important pythochemicals. Polyphenols are secondary plant metabolites, widely distributed in plants and foods of plant origin. Their health benefits (vasodilatatory, anti-inflammatory, anticancerogenic, antiviral and antibacterial effects) arise from the antioxidative effects of these phytochemicals, which are based on their ability to scavenge different free radicals leading to the protection of biological molecules against oxidation (Rackova et al., 2009). Among all the antioxidants present in plant cells, vitamin C (ascorbic acid) plays a relevant role in controlling ROS (Reactive Oxygen Species) homeostasis through enzymatic and non enzymatic reactions, acting in different cell compartments (Locato et al., 2009). Tocopherols, collectively known as vitamin E, are lipophytic antioxidants, essential dietary components for mammals and
exclusively synthesised by photosynthetic organisms. Of the four forms (α, β, γ and δ), α-
tocopherol is the major vitamin E form present in green plant tissues, and has the highest
vitamin E activity, possibly due to a preferential absorption and distribution of this
molecule in the human body (Caretto et al., 2009). Unsaturated fatty acids, linoleic and
linolenic acids, are not synthesised by mammals and therefore important dietary
requirements. Like vitamins, they are required for growth and good health, and hence are
called essential fatty acids. Plants are able to synthesise linoleic and linolenic acids and are
the source of these fatty acids in our diet (Zubay, 2006).

Herein, we intend to present a comparative study of the composition in nutraceuticals
(phenolics, flavonoids, carotenoids, ascorbic acid, tocopherols, sugars, fatty acids) and
antioxidant properties of different parts of *Malva sylvestris* (leaves, flowers, immature
fruits and leafy flowered stems), in order to valorise all the plant as functional food or even
pharmafood.

2. Materials and methods

2.1. Samples

Aerial parts of *Malva sylvestris* were gathered in July 2009, in the Natural Park of
Montesinho territory, Trás-os-Montes, North-eastern Portugal, according to local
consumers’ recommendations, particularly those concerning fruit ripening and convenient
gathering period and practices. Taking in account the Portuguese folk pharmacopoeia and
the local edible uses (*Table 1*), four different samples – young leaves, flowers, leafy
flowering steams and immature fruits – were prepared for posterior analysis (*Figure 1*).

Morphological key characters from the Flora Iberica (Castroviejo, 2005) were used for
plant identification. Voucher specimens are deposited in the Herbário da Escola Superior Agrária de Bragança (BRESA). Each sample was lyophilized (Ly-8-FM-ULE, Snijders, HOLLAND) and kept in the best conditions for subsequent use.

2.2. Standards and Reagents

Acetonitrile 99.9%, n-hexane 95% and ethyl acetate 99.8% were of HPLC grade from Lab-Scan (Lisbon, Portugal). All the other solvents were of analytical grade purity: methanol and diethyl ether were supplied by Lab-Scan (Lisbon, Portugal), while toluene and sulphuric acid were supplied by Sigma Chemical Co. (St. Louis, MO, USA). The fatty acids methyl ester (FAME) reference standard mixture 37 (fatty acids C4 to C24; (standard 47885-U) was purchased from Sigma (St. Louis, MO, USA), as also other individual fatty acid isomers, ascorbic acid, tocopherol standards (α, β, γ and δ), sugar standards (D(-)-fructose, D(+)-glucose anhydrous, D(+)-melezitose, D(+-) raffinose pentahydrate, D(+-)-sucrose) and D(+-)-trehalose, and the standards used in the antioxidant activity assays: trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), gallic acid and (+)-catechin. Racemic Tocol, 50 mg/mL, was purchased from Matreya (PA, USA). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA). All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, USA).

2.3. Evaluation of the nutraceutical potential

A fine dried powder (20 mesh; ~1 g) was extracted by stirring with 30 mL of methanol at 25 °C at 150 rpm for 1 h and filtered through Whatman No. 4 paper. The residue was then extracted with one additional 30 mL portion of methanol. The combined methanolic
extracts were evaporated at 35 °C under reduced pressure (rotary evaporator Büchi R-210), re-dissolved in methanol at a concentration of 10 mg/mL, and stored at 4 °C for further use.

2.3.1. DPPH radical-scavenging activity

This methodology was performed using an ELX800 Microplate Reader (Bio-Tek Instruments, Inc), according to Barros et al. (2009). The reaction mixture in each one of the 96-wells consisted of one of the different concentrations of the extracts (30 μL) and aqueous methanolic solution (80:20 v/v, 270 μL) containing DPPH radicals (6×10⁻⁵ mol/L). The mixture was left to stand for 60 min in the dark. The reduction of the DPPH radical was determined by measuring the absorption at 515 nm. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discolouration using the equation: % RSA = [(A_{DPPH} - A_{S})/A_{DPPH}] × 100, where A_{S} is the absorbance of the solution when the sample extract has been added at a particular level, and A_{DPPH} is the absorbance of the DPPH solution. The extract concentration providing 50% of radicals scavenging activity (EC₅₀) was calculated from the graph of RSA percentage against extract concentration. Trolox was used as standard.

2.3.2. Reducing power

The different concentrations of the extracts (0.5 mL) were mixed with sodium phosphate buffer (200 mmol/L, pH 6.6, 0.5 mL) and potassium ferricyanide (1% w/v, 0.5 mL). The mixture was incubated at 50 °C for 20 min, and trichloroacetic acid (10% w/v, 0.5 mL) was added. The mixture (0.8 mL) was poured in the 48-wells, as also deionised water (0.8 mL) and ferric chloride (0.1% w/v, 0.16 mL), and the absorbance was measured at 690 nm in the
Microplate Reader described above (Barros et al., 2009). The extract concentration providing 0.5 of absorbance (EC$_{50}$) was calculated from the graph of absorbance at 690 nm against extract concentration. Trolox was used as standard.

2.3.3. Inhibition of β-carotene bleaching

A solution of β-carotene was prepared by dissolving β-carotene (2 mg) in chloroform (10 mL). Two millilitres of this solution were pipetted into a round-bottom flask. After the chloroform was removed at 40°C under vacuum, linoleic acid (40 mg), Tween 80 emulsifier (400 mg), and distilled water (100 mL) were added to the flask with vigorous shaking. Aliquots (4.8 mL) of this emulsion were transferred into different test tubes containing different concentrations of the extracts (0.2 mL). The tubes were shaken and incubated at 50°C in a water bath (Barros et al., 2009). As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm using a spectrophotometer (Analytikjena 200-2004). A blank, devoid of β-carotene, was prepared for background subtraction. β-Carotene bleaching inhibition was calculated using the following equation:

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\text{(β-carotene content after 2h of assay/initial β-carotene content)} \times 100.
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The extract concentration providing 50% antioxidant activity (EC$_{50}$) was calculated by interpolation from the graph of β-carotene bleaching inhibition percentage against extract concentration. Trolox was used as standard.

2.3.4. Inhibition of lipid peroxidation using thiobarbituric acid reactive substances (TBARS)
Brains were obtained from pig (*Sus scrofa*) of body weight ~150 Kg, dissected and homogenized with a Polytron in ice-cold Tris–HCl buffer (20 mM, pH 7.4) to produce a 1:2 (w/v) brain tissue homogenate which was centrifuged at 3000 g for 10 min. An aliquot (0.1 mL) of the supernatant was incubated with the different concentrations of the extracts (0.2 mL) in the presence of FeSO₄ (10 μM; 0.1 mL) and ascorbic acid (0.1 mM; 0.1 mL) at 37°C for 1 h. The reaction was stopped by the addition of trichloroacetic acid (28% w/v, 0.5 mL), followed by thiobarbituric acid (TBA, 2%, w/v, 0.38 mL), and the mixture was then heated at 80 °C for 20 min. After centrifugation at 3000 g for 10 min to remove the precipitated protein, the colour intensity of the malondialdehyde (MDA)-TBA complex in the supernatant was measured by its absorbance at 532 nm (Barros et al., 2009). The inhibition ratio (%) was calculated using the following formula: Inhibition ratio (%) = [(A – B)/A] x 100%, where A and B were the absorbance of the control and the extract solution, respectively. The extract concentration providing 50% lipid peroxidation inhibition (EC₅₀) was calculated from the graph of TBARS inhibition percentage against extract concentration. Trolox was used as standard.

2.4. Composition in nutraceuticals

2.4.1. Phenolics

Total phenolics were estimated based on procedures described by Wolfe et al. (2003) with some modifications. An aliquot of the extract solution (1 mL) was mixed with Folin-Ciocalteu reagent (5 mL, previously diluted with water 1:10 v/v) and sodium carbonate (75 g/L, 4 mL). The tubes were vortexed for 15 s and allowed to stand for 30 min at 40 °C for colour development. Absorbance was then measured at 765 nm. Gallic acid was used to
calculate the standard curve (0.05-0.8 mM; $y = 1.9799x + 0.0299; R^2 = 0.9997$), and the results were expressed as mg of gallic acid equivalents (GAEs) per g of extract.

Flavonoids content was determined using the method of Jia et al. (1999), with some modifications. An aliquot (0.5 mL) of the extract solution was mixed with distilled water (2 mL) and subsequently with NaNO₂ solution (5%, 0.15 mL). After 6 min, AlCl₃ solution (10%, 0.15 mL) was added and allowed to stand further 6 min, thereafter, NaOH solution (4%, 2 mL) was added to the mixture. Immediately, distilled water was added to bring the final volume to 5 mL. Then the mixture was properly mixed and allowed to stand for 15 min. The intensity of pink colour was measured at 510 nm. (+)-Catechin was used to calculate the standard curve (0.0156-1.0 mM; $y = 0.9186x - 0.0003; R^2 = 0.9999$) and the results were expressed as mg of (+)-catequin equivalents (CEs) per g of extract.

2.4.2. Ascorbic acid

Ascorbic acid was determined according to the method of Klein and Perry (1982). The extract (150 mg) was re-extracted with metaphosphoric acid (1%, 10 mL) for 45 min at room temperature and filtered through Whatman Nº 4 filter paper. The filtrate (1 mL) was mixed with 2,6-dichloroindophenol (9 mL) and the absorbance was measured within 30 min at 515 nm against a blank. Content of ascorbic acid was calculated on the basis of the calibration curve of authentic L-ascorbic acid (0.006-0.1 mg/mL; $y = 3.0062x + 0.007; R^2 = 0.9999$), and the results were expressed as mg of ascorbic acid per g of extract.

2.4.3. Carotenoids
β-Carotene and lycopene were determined according to the method of Nagata and Yamashita (1992). The extract (150 mg) was vigorously shaken with 10 mL of acetone–hexane mixture (4:6) for 1 min and filtered through Whatman No. 4 filter paper. The absorbance of the filtrate was measured at 453, 505, 645 and 663 nm. Contents of β-carotene and lycopene were calculated according to the following equations: lycopene (mg/100 mL) = \(-0.0458 \times A_{663} + 0.204 \times A_{645} + 0.372 \times A_{505} - 0.0806 \times A_{453}\); β-carotene (mg/100 mL) = \(0.216 \times A_{663} – 1.220 \times A_{645} - 0.304 \times A_{505} + 0.452 \times A_{453}\). The results were expressed as mg of carotenoid per g of extract.

2.4.4. Tocopherols

Tocopherols content was determined following a procedure previously optimized and described by Barros et al. (2009). BHT solution in hexane (10 mg/mL; 100 μL) and IS solution in hexane (tocol; 50 μg/mL; 400 μL) were added to the sample prior to the extraction procedure. The samples (~500 mg) were homogenized with methanol (4 mL) by vortex mixing (1 min). Subsequently, hexane (4 mL) was added and again vortex mixed for 1 min. After that, saturated NaCl aqueous solution (2 mL) was added, the mixture was homogenized (1 min), centrifuged (5 min, 4000 g) and the clear upper layer was carefully transferred to a vial. The sample was re-extracted twice with hexane. The combined extracts were taken to dryness under a nitrogen stream, redissolved in 2 mL of n-hexane, dehydrated with anhydrous sodium sulphate, filtered through a 0.22 μm disposable LC filter disk, transferred into a dark injection vial and analysed by HPLC. The HPLC equipment consisted of an integrated system with a Smartline pump 1000 (Knauer, Germany), a degasser system Smartline manager 5000, an AS-2057 auto-sampler and a
2500 UV detector at 295 nm (Knauer, Germany) connected in series with a FP-2020 fluorescence detector (Jasco, Japan) programmed for excitation at 290 nm and emission at 330 nm. Data were analysed using Clarity 2.4 Software (DataApex). The chromatographic separation was achieved with a Polyamide II (250 x 4.6 mm) normal-phase column from YMC Waters (Japan) operating at 30°C (7971 R Grace oven). The mobile phase used was a mixture of n-hexane and ethyl acetate (70:30, v/v) at a flow rate of 1 mL/min, and the injection volume was 20 µL. The compounds were identified by chromatographic comparisons with authentic standards. Quantification was based on the fluorescence signal response, using the internal standard method. Tocopherol contents in the samples are expressed in mg per 100 g of dry sample.

2.4.5. Fatty Acids

Fatty acids were determined by gas-liquid chromatography with flame ionization detection (GC-FID)/capillary column as described previously by the authors (Heleno et al., 2009), and after the following trans-esterification procedure: fatty acids (obtained after Soxhlet extraction) were methylated with 5 mL of methanol:sulphuric acid:toluene 2:1:1 (v:v), during at least 12 h in a bath at 50 °C and 160 rpm; then 3 mL of deionised water were added, to obtain phase separation; the FAME were recovered with 3 mL of diethyl ether by shaking in vortex, and the upper phase was passed through a micro-column of sodium sulphate anhydrous, in order to eliminate the water; the sample was recovered in a vial with Teflon, and before injection the sample was filtered with 0.2 µm nylon filter from Millipore. The fatty acid profile was analyzed with a DANI model GC 1000 instrument equipped with a split/splitless injector, a flame ionization detector (FID) and a Macherey-Nagel column (30 m x 0.32 mm ID x 0.25 µm d). The oven temperature program was as follows: the
initial temperature of the column was 50 ºC, held for 2 min, then a 10 ºC/min ramp to 240
ºC and held for 11 min. The carrier gas (hydrogen) flow-rate was 4.0 mL/min (0.61 bar),
measured at 50 ºC. Split injection (1:40) was carried out at 250 ºC. For each analysis 1 µL
of the sample was injected in GC. Fatty acid identification was made by comparing the
relative retention times of FAME peaks from samples with standards. The results were
recorded and processed using CSW 1.7 software (DataApex 1.7) and expressed in relative
percentage of each fatty acid.

2.4.6. Sugars

Free sugars were determined by high performance liquid chromatography coupled to a
refraction index detector (HPLC-RI) as described by Heleno et al. (2008). Dried sample
powder (1.0 g) was spiked with the melezitose as internal standard (IS, 5 mg/mL), and was
extracted with 40 mL of 80% aqueous ethanol at 80 ºC for 30 min. The resulting
suspension was centrifuged (Centorion K24OR- 2003 refrigerated centrifuge) at 15,000 g
for 10 min. The supernatant was concentrated at 60 ºC under reduced pressure and defatted
three times with 10 mL of ethyl ether, successively. After concentration at 40 ºC, the solid
residues were dissolved in water to a final volume of 5 mL. Soluble sugars were determined
by using HPLC (Knauer, Smartline system) at 35 ºC. The HPLC system was equipped with
a Knauer Smartline 2300 RI detector and with a Eurospher 100-5 NH2 column (4.6 x 250
mm, 5 mm, Knauer). The mobile phase was acetonitrile/deionized water, 7:3 (v/v) at a flow
rate of 1 mL/min. The results are expressed in g/100 g of dry weight, calculated by internal
normalization of the chromatographic peak area. Sugar identification was made by
comparing the relative retention times of sample peaks with standards.
2.4.7. Macronutrients

The samples were analysed for chemical composition (moisture, protein, fat, carbohydrates and ash) using the AOAC procedures (AOAC, 1995). The crude protein content ($N \times 6.25$) of the samples was estimated by the macro-Kjeldahl method; the crude fat was determined by extracting a known weight of powdered sample with petroleum ether, using a Soxhlet apparatus; the ash content was determined by incineration at 600±15 °C; reducing sugars were determined by DNS (dinitrosalicylic acid) method. Total carbohydrates were calculated by difference: Total carbohydrates = 100 – (g protein + g fat + g ash). Total energy was calculated according to the following equations: Energy (kcal) = $4 \times (\text{g protein} + \text{g carbohydrate}) + 9 \times (\text{g lipid})$.

2.5. Statistical analysis

For each one of the species three samples were analysed and also all the assays were carried out in triplicate. The results are expressed as mean values and standard deviation (SD) or standard errors (SE). The results were analyzed using one-way analysis of variance (ANOVA) followed by Tukey’s HSD Test with $\alpha = 0.05$. This treatment was carried out using SPSS v. 16.0 program.

3. Results and discussion

3.1. Evaluation of the nutraceutical potential

*Malva sylvestris* is widely used in local traditional medicine mainly for the treatment of external and internal inflammation and inflammation-related diseases such as rheumatism (Conforti et al., 2008). Considering that antioxidants and free radical scavengers can exert
also an anti-inflammatory effect (Geronikaki and Gavalas, 2006; Conforti et al., 2008), the
effects of different components of M. sylvestris were studied for their nutraceutical
potential, particularly antioxidant potential. The scavenger capacity was evaluated
measuring the decrease in DPPH radical absorption and measuring the neutralization of
linoleate-free radical and other free radicals formed in the system which attack the highly
unsaturated β-carotene models. The reducing power was measured by the conversion of a
Fe³⁺/ferricyanide complex to the ferrous form. Furthermore, the inhibition of lipid
peroxidation in brain tissue was measured by the colour intensity of MDA-TBA complex.
All the samples proved to have antioxidant activity (Table 2) being more significant for
leaves (lowest EC₅₀ values), with the exception of TBARS assay in which leafy flowered
stems presented lowest values. The leaves sample revealed better DPPH radical scavenging
activity (0.43 mg/mL) and lipid peroxidation inhibition (0.09 mg/mL) than leaves from
Italy (0.61 mg/mL and > 1 mg/mL, respectively; Conforti et al., 2008). Nevertheless, the
Italian leaves gave a slight lower EC₅₀ value for β-carotene bleaching inhibition (0.03
mg/mL) than the leaves analysed in the present report (0.04 mg/mL). Other Italian authors
(DellaGreca et al., 2009) reported, in a study with aerial parts of Malva sylvestris, 24% of
DPPH scavenging activity at 20 µg/mL. Ethanolic extracts of Portuguese samples, analysed
by Ferreira et al. (2006), were not active against DPPH radicals. Therefore, the methanolic
extracts used by us proved to be more effective. Another report on mallows from Turkey
(El and Karakaya, 2004) revealed scavenging effects on hydrogen peroxide of 46.19% at
0.05 g/mL, which is significantly worst than our results. Methanolic extracts of mallow
seeds from Scotland (Kumarasamy et al., 2007) presented 0.97 mg/mL as EC₅₀ value for
DPPH radical scavenging activity which proves that, leaves, flowers and leafy flowered
stems have a higher antioxidant potential than mallow seeds. Otherwise, fruits seem to have
the lowest antioxidant capacity (highest EC$_{50}$ values) when compared with the other parts.

3.2. Nutraceuticals composition

The yields of the methanolic extraction and the nutraceuticals composition of the different
parts of *Malva sylvestris* are given in Table 3. It was not observed any correlation between
the extraction yield and the nutraceuticals contents. Phenolics were the major antioxidant
components; leaves revealed the highest content in phenolics (386.45 mg/g of extract),
flavonoids (210.81 mg/g) and carotenoids (0.19 mg/g). The amounts found in our sample of
leaves were higher than the ones found in Italian leaves (28 and 4.77 mg/g, respectively for
phenolics and flavonoids) (Conforti et al., 2008). The highest amount of ascorbic acid was
found in flowers (1.11 mg/g of extract), while leaves presented the lowest levels (0.17
mg/g). In Trás-os-Montes, Portugal, the decoction of the flowers is mainly used for proper
washing and skin care as well as for topical treatments for acne (Carvalho, 2005). These
uses are according to the high vitamin C (powerful antioxidant) content found in mallow
flowers.

Fruits revealed the lowest levels of nutraceuticals including phenols, flavonoids and
carotenoids (Table 3) which is in agreement with its lowest antioxidant activity, measured
by the four assays (Table 2).

Tocopherols content in the different parts of *Malva sylvestris* was also determined and the
results are given in Table 4. The values point to the existence of differences in what
concerns tocopherols composition. $\alpha$-Tocopherol was the major compound in all the parts,
and $\delta$-tocopherol was not detected in mallow fruits. Leaves presented the highest content of
tocopherols (106.51 mg/100g of dry weight; Table 4) while immature fruits revealed the
lowest content (2.61 mg/100g). As far as we know, nothing is described in literature about vitamin E in mallow, an important lipophilic antioxidant, which has been proved to be important in reducing the risk of cardiovascular diseases, enhancing immune status and modulating important degenerative conditions associated with aging. Tocopherols act as a free radical scavengers (i.e., chain-breaking antioxidants) when the phenoxylic head group encounters a free radical (Halliwell and Gutteridge, 1989). α-Tocopherol is an endogenous major lipid-soluble antioxidant that protects cells from the diverse actions of Reactive Oxygen Species (ROS) by donating its hydrogen atom (Burton and Ingold, 1988).

The results for fatty acid composition, total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) of the studied parts of *Malva sylvestris* are shown in Table 5. The major fatty acids found in all the samples were α-linolenic (C18:3), linoleic (C18:2) and palmitic acid (C16:0).

Linoleic acid is a member of the group of essential fatty acids called omega-6 fatty acids, so called because they are an essential dietary requirement for all mammals. The other group of essential fatty acids is the omega-3 fatty acids, for example α-linolenic acid. Linoleic acid has showed positive roles in many diseases like diabetes and cancer prevention (Ip et al., 1991; Horrobin, 1993). Dietary α-linolenic acid has been assessed for its role in cardiovascular health including primary and secondary prevention of coronary heart disease (Connor, 2000; Mozaffarian, 2005). Palmitic acid is one of the most common saturated fatty acids found in animals and plants. The World Health Organization claims there is convincing evidence that dietary intake of palmitic acid increases risk of developing cardiovascular diseases. However, possibly less-disinterested studies have shown no ill effect, or even a favorable effect, of dietary consumption of palmitic acid on...
blood lipids and cardiovascular disease, so that the WHO finding may be deemed controversial (WHO, 2003). Another study showed that palmitic acid has no hypercholesterolaemic effect if intake of linoleic acid is greater than 4.5% of energy (French et al., 2002).

In all the samples, PUFA predominated over MUFA due to the significant contribution of α-linolenic and linoleic acids; leaves presented the highest levels of UFA (~84%) while fruits and flowers revealed the highest contents of SFA (~37%). In all the cases UFA predominate over SFA, ranging from 63 to 84%, being palmitic acid the main SFA found, followed by tricosanoic acid (C23:0). Twenty two fatty acids were identified and quantified. As far as we know, nothing has been reported on fatty acid composition of mallow.

In what concerns sugar composition, Malva sylvestris presented fructose, glucose, sucrose, trehalose and raffinose as main sugars (Table 6). The present study describes for the first time the sugars composition in all the parts of mallow. For leaves sucrose was the most abundant sugar (3.97 g/100g of dry weight), while fructose predominated in flowers (8.72 g/100g) and glucose predominated in immature fruits (1.52 g/100 g) and in leafy flowered stems (4.74 g/100g). Flowers revealed the highest total sugars content, and highest levels of fructose and glucose, while immature fruits showed the lowest levels in total sugars (2.30 g/100 g).

As mentioned before, the leaves, shoots and fruits of Malva sylvestris are edible, even if food uses are not as expanded as the medicinal ones. Mallow greens have similar organoleptic characteristics to other wild vegetables also consumed such as borages (Borago officinalis) and sorrels (Rumex spp.); however, in Portugal, common mallow
greens are less used and recommended than others. Mallow fruits, a nutlet strongly reticulate, are less considered than wild berries and rose hips, but there is evidence that they are moderately consumed (Carvalho, 2005). Therefore, the analysis of nutritional composition of all that parts is very important. The results of the nutrients composition and estimated energetic value (expressed on dry weight basis) are shown in Table 7. Leafy flowered stems revealed the highest moisture content (77.26 g/100 g), while immature fruits showed the lowest moisture content (45.60 g/100 g). Carbohydrates, calculated by difference, were the most abundant macronutrients and were higher than 71%. Protein was found in low levels and varied between 3.26 g/100 g in immature fruits and 14.26 g/100 g in leafy flowered stems. Fat was the less abundant macronutrient being lower than 9%. Total sugars determined by HPLC-RI were higher than reducing sugars obtained by DNS method due to the contribution of non-reducing sugars such sucrose and trehalose. The highest energetic value is guaranteed by immature fruits (393.45 Kcal/100 g of dry weight) mainly due to the contribution of fat, while leaves gave the lowest energetic contribution (Table 7). Ash content was higher in leaves (13.53 g/100 g) and lower in flowers (10.76 g/100 g). The results show that the consumption of mallow greens can be interesting for their nutraceuticals such as toopherols composition, as pointed out by 20% of the informants interviewed in Trás-os-Montes. The traditional custom of chewing fruits or eaten them as snacks seems appropriate for the particular purpose of satisfying hunger in view of their carbohydrates content and energetic value (Carvalho, 2005).

It is known that many plants that have medicinal value are used as condiment or aromatic (Hardy, 2000; Ferreira et al., 2006). Malva sylvestris is one of this kind of plants, and the present study may stimulate its use as functional food or even pharmafood. Particularly,
mallow leaves revealed very strong antioxidant properties including radical scavenging activity, reducing power and lipid peroxidation inhibition in liposomes and brain cells homogenates. This part of the plant is also the richest in nutraceuticals such as powerful antioxidants: phenols, flavonoids, carotenoids and tocopherols, unsaturated fatty acids: particularly α-linolenic acid, and minerals measured in ash content. The low levels of total fat found in leaves are responsible for its low energetic value. Mallow flowers revealed the highest contents in ascorbic acid, carbohydrates and, particularly, sugars such as fructose and glucose. The immature fruits revealed the lowest nutraceutical potential (lowest concentrations of phenols, flavonoids, carotenoids, tocopherols, sugars, UFA and proteins, and highest antioxidant activity EC₅₀ values) with the highest levels of total fat, saturated fatty acids (eg. palmitic acid) and, therefore, caloric value.

Acknowledgements

The authors are grateful to the Foundation for Science and Technology (Portugal) for financial support to the research centre CIMO and L. Barros grant (SFRH/BPD/4609/2008).

References


<table>
<thead>
<tr>
<th>European country</th>
<th>Plant part used</th>
<th>Medicinal application</th>
<th>Medicinal use</th>
<th>Edible use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Portugal, Spain, France</td>
<td>roots</td>
<td>chewed, decoction</td>
<td>toothache, genital tract, dermatitis</td>
<td>none</td>
</tr>
<tr>
<td>Portugal, Spain, Italy, France</td>
<td>young leaves</td>
<td>decoctions, infusions, cataplasm</td>
<td>skin, injuries, burns, stomach, diarrhea pectoral, rheumatism</td>
<td>tea, salads, soups</td>
</tr>
<tr>
<td>Portugal, Italy, France</td>
<td>shoots</td>
<td>decoctions, infusions, vapour baths</td>
<td>toothache, genital tract, haemorrhoids, constipation</td>
<td>salads, soups</td>
</tr>
<tr>
<td>Portugal</td>
<td>leafy-flowered stems</td>
<td>ointments, poultices, baths, decoctions, infusions, liniments</td>
<td>cold, cough, throat pain, tonsils, bladder, rheumatism</td>
<td>none</td>
</tr>
<tr>
<td>Portugal, Spain, Italy, France</td>
<td>flowers</td>
<td>decoctions, baths, gargles, lotions, vapour baths, syrups</td>
<td>acne, skin condition, eyes, throat pain, cough</td>
<td>none</td>
</tr>
<tr>
<td>Portugal, Spain, Italy, France</td>
<td>immature fruits</td>
<td>unknown</td>
<td>unknown</td>
<td>snacks, salads</td>
</tr>
<tr>
<td>Spain</td>
<td>seeds/mericarps</td>
<td>maceration</td>
<td>inflamed or injured skin</td>
<td>flavour</td>
</tr>
</tbody>
</table>

Sources: Lieutaghi, 1996; Camejo-Rodrigues et al., 2003; Novais et al., 2004; Pardo de Santayana, 2004; Carvalho, 2005; Ferreira et al., 2006; Natali and Pollio, 2007; Guarrera, 2003; Guarrera and Leporatti, 2007; Natali and Pollio, 2007; Quave et al., 2008; Leporatti et al., 2009; Neves et al., 2009.
Table 2. Nutraceutical potential (measured as antioxidant activity EC\textsubscript{50} values, mg/mL) of different extracts of *Malva sylvestris* (mean ± SD; n=3). In each row different letters mean significant differences (*p*<0.05).

<table>
<thead>
<tr>
<th></th>
<th>Leaves</th>
<th>Flowers</th>
<th>Immature fruits</th>
<th>Leafy flowered stems</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPPH scavenging activity</td>
<td>0.43 ± 0.05 b</td>
<td>0.55 ± 0.07 b</td>
<td>4.47 ± 0.32 a</td>
<td>0.59 ± 0.08 b</td>
</tr>
<tr>
<td>Reducing power</td>
<td>0.07 ± 0.00 c</td>
<td>0.17 ± 0.01 b</td>
<td>1.00 ± 0.05 a</td>
<td>0.10 ± 0.00 c</td>
</tr>
<tr>
<td>β-carotene bleaching inhibition</td>
<td>0.04 ± 0.00 c</td>
<td>0.11 ± 0.00 b</td>
<td>0.68 ± 0.01 a</td>
<td>0.10 ± 0.00 b</td>
</tr>
<tr>
<td>TBARS inhibition</td>
<td>0.09 ± 0.00 c</td>
<td>0.12 ± 0.00 b</td>
<td>0.85 ± 0.04 a</td>
<td>0.05 ± 0.00 d</td>
</tr>
</tbody>
</table>
Table 3. Extraction yields (%) and nutraceuticals composition (mg/g extract) of different extracts of *Malva sylvestris* (mean ± SD; n=3). In each row different letters mean significant differences (*p*<0.05).

<table>
<thead>
<tr>
<th></th>
<th>Leaves</th>
<th>Flowers</th>
<th>Immature fruits</th>
<th>Leafy flowered stems</th>
</tr>
</thead>
<tbody>
<tr>
<td>η</td>
<td>18.49 ± 0.22 b</td>
<td>35.30 ± 0.33 a</td>
<td>10.07 ± 0.09 c</td>
<td>19.41 ± 0.15 b</td>
</tr>
<tr>
<td>Phenolics</td>
<td>386.45 ± 8.54 a</td>
<td>258.65 ± 26.04 c</td>
<td>56.76 ± 2.01 d</td>
<td>317.93 ± 2.61 b</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>210.81 ± 7.99 a</td>
<td>46.55 ± 5.26 c</td>
<td>25.35 ± 2.72 d</td>
<td>143.40 ± 7.86 b</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>0.17 ± 0.05 c</td>
<td>1.11 ± 0.07 a</td>
<td>0.27 ± 0.00 b</td>
<td>0.20 ± 0.04 c</td>
</tr>
<tr>
<td>Carotenoids</td>
<td>0.19 ± 0.00 a</td>
<td>0.03 ± 0.00 c</td>
<td>0.01 ± 0.00 c</td>
<td>0.11 ± 0.00 b</td>
</tr>
</tbody>
</table>
Table 4. Tocopherols composition (mg/100 g of dry weight) of different *Malva sylvestris* components (mean ± SD; n=3). In each row different letters mean significant differences (*p*<0.05).

<table>
<thead>
<tr>
<th>Component</th>
<th>Leaves</th>
<th>Flowers</th>
<th>Immature fruits</th>
<th>Leafy flowered stems</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-tocopherol</td>
<td>83.70 ± 1.99 a</td>
<td>14.03 ± 0.72 c</td>
<td>2.07 ± 0.01 d</td>
<td>28.40 ± 0.26 b</td>
</tr>
<tr>
<td>β-tocopherol</td>
<td>1.48 ± 0.06 a</td>
<td>0.57 ± 0.08 b</td>
<td>0.26 ± 0.01 c</td>
<td>0.57 ± 0.08 b</td>
</tr>
<tr>
<td>γ-tocopherol</td>
<td>20.05 ± 1.05 a</td>
<td>2.53 ± 0.20 c</td>
<td>0.28 ± 0.00 d</td>
<td>5.93 ± 0.13 b</td>
</tr>
<tr>
<td>δ-tocopherol</td>
<td>1.29 ± 0.04 a</td>
<td>0.24 ± 0.05 b</td>
<td><em>nd</em></td>
<td>0.02 ± 0.00 c</td>
</tr>
<tr>
<td>Total tocopherols</td>
<td>106.51 ± 3.07 a</td>
<td>17.37 ± 1.04 c</td>
<td>2.61 ± 0.00 d</td>
<td>34.92 ± 0.06 b</td>
</tr>
</tbody>
</table>

*nd*- not detected
Table 5. Fatty acids composition of different *Malva sylvestris* components (mean ± SD; n=3). In each row different letters mean significant differences (p<0.05).

<table>
<thead>
<tr>
<th></th>
<th>Leaves</th>
<th>Flowers</th>
<th>Immature fruits</th>
<th>Leafy flowered stems</th>
</tr>
</thead>
<tbody>
<tr>
<td>C6:0</td>
<td>0.01 ± 0.00</td>
<td>0.63 ± 0.00</td>
<td>0.10 ± 0.00</td>
<td>0.08 ± 0.01</td>
</tr>
<tr>
<td>C8:0</td>
<td>0.01 ± 0.00</td>
<td>0.03 ± 0.00</td>
<td>0.02 ± 0.00</td>
<td>0.03 ± 0.00</td>
</tr>
<tr>
<td>C10:0</td>
<td>0.02 ± 0.00</td>
<td>0.02 ± 0.12</td>
<td>0.05 ± 0.00</td>
<td>0.07 ± 0.00</td>
</tr>
<tr>
<td>C12:0</td>
<td>0.09 ± 0.01</td>
<td>0.12 ± 0.01</td>
<td>0.10 ± 0.00</td>
<td>0.10 ± 0.00</td>
</tr>
<tr>
<td>C14:0</td>
<td>0.48 ± 0.04</td>
<td>0.90 ± 0.10</td>
<td>0.71 ± 0.04</td>
<td>0.60 ± 0.01</td>
</tr>
<tr>
<td>C14:1</td>
<td>0.22 ± 0.01</td>
<td>0.19 ± 0.01</td>
<td>0.06 ± 0.00</td>
<td>0.16 ± 0.00</td>
</tr>
<tr>
<td>C15:0</td>
<td>0.06 ± 0.00</td>
<td>0.07 ± 0.00</td>
<td>0.19 ± 0.01</td>
<td>0.04 ± 0.00</td>
</tr>
<tr>
<td>C16:0</td>
<td>9.79 ± 1.07</td>
<td>17.17 ± 0.04</td>
<td>19.76 ± 0.46</td>
<td>12.88 ± 0.16</td>
</tr>
<tr>
<td>C16:1</td>
<td>0.08 ± 0.00</td>
<td>0.62 ± 0.04</td>
<td>0.13 ± 0.00</td>
<td>0.10 ± 0.00</td>
</tr>
<tr>
<td>C17:0</td>
<td>0.16 ± 0.01</td>
<td>0.30 ± 0.03</td>
<td>0.47 ± 0.01</td>
<td>0.23 ± 0.00</td>
</tr>
<tr>
<td>C18:0</td>
<td>1.22 ± 0.07</td>
<td>2.36 ± 0.00</td>
<td>2.96 ± 0.06</td>
<td>1.47 ± 0.02</td>
</tr>
<tr>
<td>C18:1n9</td>
<td>3.31 ± 0.42</td>
<td>6.10 ± 0.00</td>
<td>6.15 ± 0.06</td>
<td>3.15 ± 0.02</td>
</tr>
<tr>
<td>C18:2n6</td>
<td>11.96 ± 0.42</td>
<td>23.54 ± 0.16</td>
<td>46.02 ± 0.16</td>
<td>15.70 ± 0.67</td>
</tr>
<tr>
<td>C18:3n6</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>2.23 ± 0.00</td>
</tr>
<tr>
<td>C18:3n3</td>
<td>67.79 ± 0.96</td>
<td>33.50 ± 0.13</td>
<td>10.33 ± 0.08</td>
<td>53.09 ± 0.55</td>
</tr>
<tr>
<td>C20:0</td>
<td>0.52 ± 0.02</td>
<td>1.63 ± 0.03</td>
<td>0.62 ± 0.00</td>
<td>0.97 ± 0.01</td>
</tr>
<tr>
<td>C20:1</td>
<td>0.04 ± 0.00</td>
<td>0.07 ± 0.01</td>
<td>0.18 ± 0.00</td>
<td>0.08 ± 0.00</td>
</tr>
<tr>
<td>C20:2</td>
<td>0.13 ± 0.02</td>
<td>0.10 ± 0.01</td>
<td>0.16 ± 0.01</td>
<td>0.74 ± 0.06</td>
</tr>
<tr>
<td>C20:3n3+C21:0</td>
<td>0.15 ± 0.00</td>
<td>0.20 ± 0.01</td>
<td>0.18 ± 0.01</td>
<td>0.13 ± 0.00</td>
</tr>
<tr>
<td>C22:0</td>
<td>0.74 ± 0.09</td>
<td>1.49 ± 0.01</td>
<td>2.82 ± 0.05</td>
<td>1.39 ± 0.04</td>
</tr>
<tr>
<td>C23:0</td>
<td>2.48 ± 0.26</td>
<td>9.99 ± 0.02</td>
<td>7.77 ± 0.35</td>
<td>5.82 ± 0.07</td>
</tr>
<tr>
<td>C24:0</td>
<td>0.75 ± 0.01</td>
<td>0.98 ± 0.05</td>
<td>1.24 ± 0.08</td>
<td>0.95 ± 0.01</td>
</tr>
<tr>
<td>SFA</td>
<td>16.32 ± 0.90</td>
<td>35.83 ± 0.25</td>
<td>36.79 ± 0.01</td>
<td>24.62 ± 0.15</td>
</tr>
<tr>
<td>MUFA</td>
<td>3.65 ± 0.39</td>
<td>6.84 ± 0.24</td>
<td>6.52 ± 0.06</td>
<td>3.48 ± 0.03</td>
</tr>
<tr>
<td>PUFA</td>
<td>80.03 ± 0.51</td>
<td>57.33 ± 0.01</td>
<td>56.69 ± 0.08</td>
<td>71.90 ± 0.18</td>
</tr>
</tbody>
</table>


*nd* - not detected. Caproic acid (C6:0); Caprylic acid (C8:0); Capric acid (C10:0); Lauric acid (C12:0); Myristic acid (C14:0); Myristoleic acid (C14:1); Pentadecanoic acid (C15:0); Palmitic acid (C16:0); Palmitoleic acid (C16:1); Heptadecanoic acid (C17:0); Stearic acid (C18:0); Oleic acid (C18:1n9c); Linoleic acid (C18:2n6c); γ-Linolenic acid (C18:3n6); α-Linolenic acid (C18:3n3); Arachidic acid (C20:0); Eicosenoic acid (C20:1c); cis-11,14-Eicosadienoic acid (C20:2c); cis-11, 14, 17-Eicosatrienoic acid and Heneicosanoic acid (C20:3n3 + C21:0); Behenic acid (C22:0); Tricosanoic acid (C23:0); Lignoceric acid (C24:0).
Table 6. Sugars composition (g/100 g of dry weight) of different *Malva sylvestris* components (mean ± SD; n=3). In each row, different letters mean significant differences (p<0.05).

<table>
<thead>
<tr>
<th></th>
<th>Leaves</th>
<th>Flowers</th>
<th>Immature fruits</th>
<th>Leafy flowered stems</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fructose</strong></td>
<td>1.82 ± 0.23 c</td>
<td>8.72 ± 0.14 a</td>
<td>0.40 ± 0.03 d</td>
<td>3.53 ± 0.18 b</td>
</tr>
<tr>
<td><strong>Glucose</strong></td>
<td>3.15 ± 0.43 c</td>
<td>7.36 ± 0.13 a</td>
<td>1.52 ± 0.07 d</td>
<td>4.74 ± 0.18 b</td>
</tr>
<tr>
<td><strong>Sucrose</strong></td>
<td>3.97 ± 0.03 b</td>
<td>2.47 ± 0.05 c</td>
<td>0.11 ± 0.03 d</td>
<td>3.30 ± 0.10 a</td>
</tr>
<tr>
<td><strong>Trehalose</strong></td>
<td>2.67 ± 0.11 b</td>
<td>1.47 ± 0.06 c</td>
<td>nd</td>
<td>3.09 ± 0.03 a</td>
</tr>
<tr>
<td><strong>Raffinose</strong></td>
<td>nd</td>
<td>nd</td>
<td>0.26 ± 0.03 a</td>
<td>nd</td>
</tr>
<tr>
<td><strong>Total sugars</strong></td>
<td>11.61 ± 0.51 c</td>
<td>20.02 ± 0.26 a</td>
<td>2.30 ± 0.10 d</td>
<td>14.67 ± 0.49 b</td>
</tr>
</tbody>
</table>
Table 7. Moisture (g/100 g of fresh weight), macronutrients composition (g/100 g of dry weight) and energetic value (Kcal/100 g of dry weight) of different *Malva sylvestris* components (mean ± SD; n=3). In each row, different letters mean significant differences (*p* < 0.05).

<table>
<thead>
<tr>
<th></th>
<th>Leaves</th>
<th>Flowers</th>
<th>Immature fruits</th>
<th>Leafy flowered stems</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>76.30 ± 0.54 b</td>
<td>72.49 ± 1.89 c</td>
<td>45.60 ± 0.97 d</td>
<td>77.26 ± 1.34 a</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>71.46 ± 0.81 c</td>
<td>78.12 ± 0.44 a</td>
<td>74.96 ± 1.10 b</td>
<td>71.89 ±0.35 c</td>
</tr>
<tr>
<td>Proteins</td>
<td>12.25 ± 1.01 b</td>
<td>8.50 ± 0.51 c</td>
<td>3.26 ± 0.25 d</td>
<td>14.26 ± 0.44 a</td>
</tr>
<tr>
<td>Fat</td>
<td>2.76 ± 0.40 b</td>
<td>2.84 ± 0.37 b</td>
<td>8.96 ± 0.22 a</td>
<td>3.09 ± 0.27 b</td>
</tr>
<tr>
<td>Ash</td>
<td>13.53 ± 0.11 a</td>
<td>10.54 ± 0.30 b</td>
<td>12.83 ± 0.78 a</td>
<td>10.76 ± 0.04 b</td>
</tr>
<tr>
<td>Reducing sugars</td>
<td>6.22 ± 0.49 c</td>
<td>13.95 ± 0.16 a</td>
<td>2.09 ± 0.12 d</td>
<td>10.46 ± 0.70 b</td>
</tr>
<tr>
<td>Energy</td>
<td>359.72 ± 1.10 c</td>
<td>372.02 ± 2.13 b</td>
<td>393.45 ± 4.41 a</td>
<td>372.43 ± 1.08 b</td>
</tr>
</tbody>
</table>
Figure 1. Common mallow (Malva sylvestris L.) gathered in Trás-os-Montes, Portugal.

A - Leafy flowered stems; B – Young leaves; C- Immature fruits.