Leaves, Flowers, Immature fruits and Leafy flowered stems of *Malva sylvestris*: A comparative study of the nutraceutical potential and composition

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13 **Abstract**

14 *Malva sylvestris* is widely used in Mediterranean and European traditional medicine and
15 ethnoveterinary for the treatment of external and internal inflammation, as well as injuries.
16 Moreover, its use is not only limited to therapeutic purposes; the species is also locally
17 regarded as a food wild herb. Considering that antioxidants and free radical scavengers can
18 exert also an anti-inflammatory effect, the extracts of different parts of the medicinal/edible
19 plant *M. sylvestris* (leaves, flowers, immature fruits and leafy flowered stems) were
20 compared for their nutraceutical potential (antioxidant properties) and chemical
21 composition. Particularly, mallow leaves revealed very strong antioxidant properties
22 including radical scavenging activity (EC_{50} = 0.43 mg/mL), reducing power (0.07 mg/mL)
23 and lipid peroxidation inhibition in lipossomes (0.04 mg/mL) and brain cells homogenates
24 (0.09 mg/mL). This part of the plant is also the richest in nutraceuticals such as powerful
25 antioxidants (phenols, flavonoids, carotenoids, and tocopherols), unsaturated fatty acids
26 (eg. α-linolenic acid), and minerals measured in ash content.

27

28 *Keywords: Malva sylvestris*; Portuguese Ethnobotany; Leaves; Flowers and stems; Fruits;
29 Nutraceuticals

30

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; Guarrera 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 bronchondilater, 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 lipophylic 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 ºC 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 ºC under reduced pressure (rotary evaporator Büchi R-210), re-dissolved in methanol at a concentration of 10 mg/mL, and stored at 4 ºC 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 ($6x10^{-5}$ 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 = $[(A_{DPPH}-A_S)/A_{DPPH}] \times 100$, 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 (EC_{50}) 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 ºC 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 40ºC 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 50ºC 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 (Analytikijena 200-2004). A blank, devoid of β-carotene, was prepared for background subtraction. β-Carotene bleaching inhibition was calculated using the following equation: (β-carotene content after 2h of assay/initial β-carotene content) × 100. 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)

173 Brains were obtained from pig (*Sus scrofa*) of body weight ~150 Kg, dissected and
174 homogenized with a Polytron in ice-cold Tris–HCl buffer (20 mM, pH 7.4) to produce a 1:2
175 (w/v) brain tissue homogenate which was centrifuged at 3000 *g* for 10 min. An aliquot (0.1
176 mL) of the supernatant was incubated with the different concentrations of the extracts (0.2
177 mL) in the presence of $FeSO_4$ (10 μM; 0.1 mL) and ascorbic acid (0.1 mM; 0.1 mL) at
178 37ºC for 1 h. The reaction was stopped by the addition of trichloroacetic acid (28% w/v, 0.5
179 mL), followed by thiobarbituric acid (TBA, 2%, w/v, 0.38 mL), and the mixture was then
180 heated at 80 ºC for 20 min. After centrifugation at 3000 *g* for 10 min to remove the
181 precipitated protein, the colour intensity of the malondialdehyde (MDA)-TBA complex in
182 the supernatant was measured by its absorbance at 532 nm (Barros et al., 2009). The
183 inhibition ratio (%) was calculated using the following formula: Inhibition ratio (%) = [(A
184 – B)/A] x 100%, where A and B were the absorbance of the control and the extract
185 solution, respectively. The extract concentration providing 50% lipid peroxidation
186 inhibition (EC_{50}) was calculated from the graph of TBARS inhibition percentage against
187 extract concentration. Trolox was used as standard.
188
189 *2.4. Composition in nutraceuticals*
190 *2.4.1. Phenolics*
191 Total phenolics were estimated based on procedures described by Wolfe et al. (2003) with
192 some modifications. An aliquot of the extract solution (1 mL) was mixed with *Folin-*
193 *Ciocalteu* reagent (5 mL, previously diluted with water 1:10 v/v) and sodium carbonate
194 (75 g/L, 4 mL). The tubes were vortexed for 15 s and allowed to stand for 30 min at 40 °C
195 for colour development. Absorbance was then measured at 765 nm. Gallic acid was used to

196 calculate the standard curve (0.05-0.8 mM; $y = 1.9799x + 0.0299$; $R^2 = 0.9997$), and the
197 results were expressed as mg of gallic acid equivalents (GAEs) per g of extract.
198 Flavonoids content was determined using the method of Jia et al. (1999), with some
199 modifications. An aliquot (0.5 mL) of the extract solution was mixed with distilled water (2
200 mL) and subsequently with $NaNO_2$ solution (5%, 0.15 mL). After 6 min, $AlCl_3$ solution
201 (10%, 0.15 mL) was added and allowed to stand further 6 min, thereafter, NaOH solution
202 (4%, 2 mL) was added to the mixture. Immediately, distilled water was added to bring the
203 final volume to 5 mL. Then the mixture was properly mixed and allowed to stand for 15
204 min. The intensity of pink colour was measured at 510 nm. (+)-Catechin was used to
205 calculate the standard curve (0.0156-1.0 mM; $y = 0.9186x - 0.0003$; $R^2 = 0.9999$) and the
206 results were expressed as mg of (+)-chatequin equivalents (CEs) per g of extract.
207
208 *2.4.2. Ascorbic acid*
209 Ascorbic acid was determined according to the method of Klein and Perry (1982). The
210 extract (150 mg) was re-extracted with metaphosphoric acid (1%, 10 mL) for 45 min at
211 room temperature and filtered through Whatman Nº 4 filter paper. The filtrate (1 mL) was
212 mixed with 2,6-dichloroindophenol (9 mL) and the absorbance was measured within 30
213 min at 515 nm against a blank. Content of ascorbic acid was calculated on the basis of the
214 calibration curve of authentic L-ascorbic acid (0.006-0.1 mg/mL; $y = 3.0062x + 0.007$; R^2
215 = 0.9999), and the results were expressed as mg of ascorbic acid per g of extract.
216
217 *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 × A_{663} + 0.204 × A_{645} + 0.372 × A_{505} - 0.0806 × A_{453}; β-carotene (mg/100 mL) = 0.216 × A_{663} – 1.220 × A_{645} - 0.304 × A_{505} + 0.452 × 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

241 2500 UV detector at 295 nm (Knauer, Germany) connected in series with a FP-2020
242 fluorescence detector (Jasco, Japan) programmed for excitation at 290 nm and emission at
243 330 nm. Data were analysed using Clarity 2.4 Software (DataApex). The chromatographic
244 separation was achieved with a Polyamide II (250 x 4.6 mm) normal-phase column from
245 YMC Waters (Japan) operating at 30ºC (7971 R Grace oven). The mobile phase used was a
246 mixture of n-hexane and ethyl acetate (70:30, v/v) at a flow rate of 1 mL/min, and the
247 injection volume was 20 µL. The compounds were identified by chromatographic
248 comparisons with authentic standards. Quantification was based on the fluorescence signal
249 response, using the internal standard method. Tocopherol contents in the samples are
250 expressed in mg per 100 g of dry sample.
251
252 *2.4.5. Fatty Acids*
253 Fatty acids were determined by gas-liquid chromatography with flame ionization detection
254 (GC-FID)/capillary column as described previously by the authors (Heleno et al., 2009),
255 and after the following trans-esterification procedure: fatty acids (obtained after Soxhlet
256 extraction) were methylated with 5 mL of methanol:sulphuric acid:toluene 2:1:1 (v:v),
257 during at least 12 h in a bath at 50 ºC and 160 rpm; then 3 mL of deionised water were
258 added, to obtain phase separation; the FAME were recovered with 3 mL of diethyl ether by
259 shaking in vortex , and the upper phase was passed through a micro-column of sodium
260 sulphate anhydrous, in order to eliminate the water; the sample was recovered in a vial with
261 Teflon, and before injection the sample was filtered with 0.2 µm nylon filter from Milipore.
262 The fatty acid profile was analyzed with a DANI model GC 1000 instrument equipped with
263 a split/splitless injector, a flame ionization detector (FID) and a Macherey-Nagel column
264 (30 m x 0.32 mm ID x 0.25 µm d_f). 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 NH_2 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.

289 *2.4.7. Macronutrients*

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

299

300 *2.5. Statistical analysis*

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

306

307 **3. Results and discussion**

308 *3.1. Evaluation of the nutraceutical potential*

309 *Malva sylvestris* is widely used in local traditional medicine mainly for the treatment of
310 external and internal inflammation and inflammation-related diseases such as rheumatism
311 (Conforti et al., 2008). Considering that antioxidants and free radical scavengers can exert

312 also an anti-inflammatory effect (Geronikaki and Gavalas, 2006; Conforti et al., 2008), the
313 extracts of different components of *M. sylvestris* were studied for their nutraceutical
314 potential, particularly antioxidant potential. The scavenger capacity was evaluated
315 measuring the decrease in DPPH radical absorption and measuring the neutralization of
316 linoleate-free radical and other free radicals formed in the system which attack the highly
317 unsaturated β-carotene models. The reducing power was measured by the conversion of a
318 Fe^{3+}/ferricyanide complex to the ferrous form. Furthermore, the inhibition of lipid
319 peroxidation in brain tissue was measured by the colour intensity of MDA-TBA complex.
320 All the samples proved to have antioxidant activity (**Table 2**) being more significant for
321 leaves (lowest EC_{50} values), with the exception of TBARS assay in which leafy flowered
322 stems presented lowest values. The leaves sample revealed better DPPH radical scavenging
323 activity (0.43 mg/mL) and lipid peroxidation inhibition (0.09 mg/mL) than leaves from
324 Italy (0.61 mg/mL and > 1 mg/mL, respectively; Conforti et al., 2008). Nevertheless, the
325 Italian leaves gave a slight lower EC_{50} value for β-carotene bleaching inhibition (0.03
326 mg/mL) than the leaves analysed in the present report (0.04 mg/mL). Other Italian authors
327 (DellaGreca et al., 2009) reported, in a study with aerial parts of *Malva sylvestris*, 24% of
328 DPPH scavenging activity at 20 µg/mL. Ethanolic extracts of Portuguese samples, analysed
329 by Ferreira et al. (2006), were not active against DPPH radicals. Therefore, the methanolic
330 extracts used by us proved to be more effective. Another report on mallows from Turkey
331 (El and Karakaya, 2004) revealed scavenging effects on hydrogen peroxide of 46.19% at
332 0.05 g/mL, which is significantly worst than our results. Methanolic extracts of mallow
333 seeds from Scotland (Kumarasamy et al., 2007) presented 0.97 mg/mL as EC_{50} value for
334 DPPH radical scavenging activity which proves that, leaves, flowers and leafy flowered

335 stems have a higher antioxidant potential than mallow seeds. Otherwise, fruits seem to have
336 the lowest antioxidant capacity (highest EC_{50} values) when compared with the other parts.
337
338 *3.2. Nutraceuticals composition*
339 The yields of the methanolic extraction and the nutraceuticals composition of the different
340 parts of *Malva sylvestris* are given in **Table 3**. It was not observed any correlation between
341 the extraction yield and the nutraceuticals contents. Phenolics were the major antioxidant
342 components; leaves revealed the highest content in phenolics (386.45 mg/g of extract),
343 flavonoids (210.81 mg/g) and carotenoids (0.19 mg/g). The amounts found in our sample of
344 leaves were higher than the ones found in Italian leaves (28 and 4.77 mg/g, respectively for
345 phenolics and flavonoids) (Conforti et al., 2008). The highest amount of ascorbic acid was
346 found in flowers (1.11 mg/g of extract), while leaves presented the lowest levels (0.17
347 mg/g). In Trás-os-Montes, Portugal, the decoction of the flowers is mainly used for proper
348 washing and skin care as well as for topical treatments for acne (Carvalho, 2005). These
349 uses are according to the high vitamin C (powerful antioxidant) content found in mallow
350 flowers.
351 Fruits revealed the lowest levels of nutraceuticals including phenols, flavonoids and
352 carotenoids (**Table 3**) which is in agreement with its lowest antioxidant activity, measured
353 by the four assays (**Table 2**).
354 Tocopherols content in the different parts of *Malva sylvestris* was also determined and the
355 results are given in **Table 4**. The values point to the existence of differences in what
356 concerns tocopherols composition. α-Tocopherol was the major compound in all the parts,
357 and δ-tocopherol was not detected in mallow fruits. Leaves presented the highest content of
358 tocopherols (106.51 mg/100g of dry weight; **Table 4**) while immature fruits revealed the

359 lowest content (2.61 mg/100g). As far as we know, nothing is described in literature about
360 vitamin E in mallow, an important lipophilic antioxidant, which has been proved to be
361 important in reducing the risk of cardiovascular diseases, enhancing immune status and
362 modulating important degenerative conditions associated with aging. Tocopherols act as a
363 free radical scavengers (i.e., chain-breaking antioxidants) when the phenoxylic head group
364 encounters a free radical (Halliwell and Gutteridge, 1989). α-Tocopherol is an endogenous
365 major lipid-soluble antioxidant that protects cells from the diverse actions of Reactive
366 Oxygen Species (ROS) by donating its hydrogen atom (Burton and Ingold, 1988).
367
368 The results for fatty acid composition, total saturated fatty acids (SFA), monounsaturated
369 fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) of the studied parts of *Malva*
370 *sylvestris* are shown in **Table 5**. The major fatty acids found in all the samples were α-
371 linolenic (C18:3), linoleic (C18:2) and palmitic acid (C16:0).
372 Linoleic acid is a member of the group of essential fatty acids called omega-6 fatty acids,
373 so called because they are an essential dietary requirement for all mammals. The other
374 group of essential fatty acids is the omega-3 fatty acids, for example α-linolenic acid.
375 Linoleic acid has showed positive roles in many diseases like diabetes and cancer
376 prevention (Ip et al., 1991; Horrobin, 1993). Dietary α-linolenic acid has been assessed for
377 its role in cardiovascular health including primary and secondary prevention of coronary
378 heart disease (Connor, 2000; Mozaffarian, 2005). Palmitic acid is one of the most common
379 saturated fatty acids found in animals and plants. The World Health Organization claims
380 there is convincing evidence that dietary intake of palmitic acid increases risk of
381 developing cardiovascular diseases. However, possibly less-disinterested studies have
382 shown no ill effect, or even a favorable effect, of dietary consumption of palmitic acid on

383 blood lipids and cardiovascular disease, so that the WHO finding may be deemed
384 controversial (WHO, 2003). Another study showed that palmitic acid has no
385 hypercholesterolaemic effect if intake of linoleic acid is greater than 4.5% of energy
386 (French et al., 2002).
387 In all the samples, PUFA predominated over MUFA due to the significant contribution of
388 α-linolenic and linoleic acids; leaves presented the highest levels of UFA (~84%) while
389 fruits and flowers revealed the highest contents of SFA (~37%). In all the cases UFA
390 predominate over SFA, ranging from 63 to 84%, being palmitic acid the main SFA found,
391 followed by tricosanoic acid (C23:0). Twenty two fatty acids were identified and
392 quantified. As far as we know, nothing has been reported on fatty acid composition of
393 mallow.
394
395 In what concerns sugar composition, *Malva sylvestris* presented fructose, glucose, sucrose,
396 trehalose and raffinose as main sugars (**Table 6**). The present study describes for the first
397 time the sugars composition in all the parts of mallow. For leaves sucrose was the most
398 abundant sugar (3.97 g/100g of dry weight), while fructose predominated in flowers (8.72
399 g/100g) and glucose predominated in immature fruits (1.52 g/100 g) and in leafy flowered
400 stems (4.74 g/100g). Flowers revealed the highest total sugars content, and highest levels of
401 fructose and glucose, while immature fruits showed the lowest levels in total sugars (2.30
402 g/100 g).
403 As mentioned before, the leaves, shoots and fruits of *Malva sylvestris* are edible, even if
404 food uses are not as expanded as the medicinal ones. Mallow greens have similar
405 organoleptic characteristics to other wild vegetables also consumed such as borages
406 (*Borago officinalis)* and sorrels (*Rumex* spp.); however, in Portugal, common mallow

407 greens are less used and recommended than others. Mallow fruits, a nutlet strongly
408 reticulate, are less considered than wild berries and rose hips, but there is evidence that they
409 are moderately consumed (Carvalho, 2005). Therefore, the analysis of nutritional
410 composition of all that parts is very important. The results of the nutrients composition and
411 estimated energetic value (expressed on dry weight basis) are shown in **Table 7**. Leafy
412 flowered stems revealed the highest moisture content (77.26 g/100 g), while immature
413 fruits showed the lowest moisture content (45.60 g/100 g). Carbohydrates, calculated by
414 difference, were the most abundant macronutrients and were higher than 71%. Protein was
415 found in low levels and varied between 3.26 g/100 g in immature fruits and 14.26 g/100 g
416 in leafy flowered stems. Fat was the less abundant macronutrient being lower than 9%.
417 Total sugars determined by HPLC-RI were higher than reducing sugars obtained by DNS
418 method due to the contribution of non-reducing sugars such sucrose and trehalose.
419 The highest energetic value is guaranteed by immature fruits (393.45 Kcal/100 g of dry
420 weight) mainly due to the contribution of fat, while leaves gave the lowest energetic
421 contribution (**Table 7**). Ash content was higher in leaves (13.53 g/100 g) and lower in
422 flowers (10.76 g/100 g). The results show that the consumption of mallow greens can be
423 interesting for their nutraceuticals such as tocopherols composition, as pointed out by 20%
424 of the informants interviewed in Trás-os-Montes. The traditional custom of chewing fruits
425 or eaten them as snacks seems appropriate for the particular purpose of satisfying hunger in
426 view of their carbohydrates content and energetic value (Carvalho, 2005).
427
428 It is known that many plants that have medicinal value are used as condiment or aromatic
429 (Hardy, 2000; Ferreira et al., 2006). *Malva sylvestris* is one of this kind of plants, and the
430 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 lipossomes 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 EC_{50} values) with the highest levels of total fat, saturated fatty acids (eg. palmitic acid) and, therefore, caloric value.

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549 **Table 1.** Medicinal and edible uses of common mallow (*Malva sylvestris* L.) reported in
550 several european ethnobotanical surveys.

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

551
552 Sources: Lieutaghi, 1996; Camejo-Rodrigues et al., 2003; Novais et al., 2004; Pardo de
553 Santayana, 2004; Carvalho, 2005; Ferreira et al., 2006; Natali and Pollio, 2007; Guarrera,
554 2003; Guarrera and Leporatti, 2007; Natali and Pollio, 2007; Quave et al., 2008; Leporatti
555 et al., 2009; Neves et al., 2009.

556 **Table 2.** Nutraceutical potential (measured as antioxidant activity EC_{50} values, mg/mL) of
557 different extracts of *Malva sylvestris* (mean ± SD; n=3). In each row different letters mean
558 significant differences ($p<0.05$).

	Leaves	Flowers	Immature fruits	Leafy flowered stems
DPPH scavenging activity	0.43 ± 0.05 b	0.55 ± 0.07 b	4.47 ± 0.32 a	0.59 ± 0.08 b
Reducing power	0.07 ± 0.00 c	0.17 ± 0.01 b	1.00 ± 0.05 a	0.10 ± 0.00 c
β-carotene bleaching inhibition	0.04 ± 0.00 c	0.11 ± 0.00 b	0.68 ± 0.01 a	0.10 ± 0.00 b
TBARS inhibition	0.09 ± 0.00 c	0.12 ± 0.00 b	0.85 ± 0.04 a	0.05 ± 0.00 d

559 **Table 3**. Extraction yields (%) and nutraceuticals composition (mg/g extract) of different
560 extracts of *Malva sylvestris* (mean ± SD; n=3). In each row different letters mean
561 significant differences ($p<0.05$).

	Leaves	Flowers	Immature fruits	Leafy flowered stems
η	18.49 ± 0.22 b	35.30 ± 0.33 a	10.07 ± 0.09 c	19.41 ± 0.15 b
Phenolics	386.45 ± 8.54 a	258.65 ± 26.04 c	56.76 ± 2.01 d	317.93 ± 2.61 b
Flavonoids	210.81 ± 7.99 a	46.55 ± 5.26 c	25.35 ± 2.72 d	143.40 ± 7.86 b
Ascorbic acid	0.17 ± 0.05 c	1.11 ± 0.07 a	0.27 ± 0.00 b	0.20 ± 0.04 c
Carotenoids	0.19 ± 0.00 a	0.03 ± 0.00 c	0.01 ± 0.00 c	0.11 ± 0.00 b

562 **Table 4.** Tocopherols composition (mg/100 g of dry weight) of different *Malva*
563 *sylvestris* components (mean ± SD; n=3). In each row different letters mean significant
564 differences (p<0.05).
565

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

566 nd- not detected

567 **Table 5.** Fatty acids composition of different *Malva sylvestris* components (mean ± SD;

568 n=3). In each row different letters mean significant differences ($p<0.05$).

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

569 *nd*- not detected. Caproic acid (C6:0); Caprylic acid (C8:0); Capric acid (C10:0); Lauric
570 acid (C12:0); Myristic acid (C14:0); Myristoleic acid (C14:1); Pentadecanoic acid
571 (C15:0); Palmitic acid (C16:0); Palmitoleic acid (C16:1); Heptadecanoic acid (C17:0);
572 Stearic acid (C18:0); Oleic acid (C18:1n9c); Linoleic acid (C18:2n6c); γ-Linolenic acid
573 (C18:3n6); α-Linolenic acid (C18:3n3); Arachidic acid (C20:0); Eicosenoic acid
574 (C20:1c); *cis*-11,14-Eicosadienoic acid (C20:2c); *cis*-11, 14, 17-Eicosatrienoic acid and
575 Heneicosanoic acid (C20:3n3 + C21:0); Behenic acid (C22:0); Tricosanoic acid
576 (C23:0); Lignoceric acid (C24:0).
577

578 **Table 6.** Sugars composition (g/100 g of dry weight) of different *Malva sylvestris*
579 components (mean ± SD; n=3). In each row, different letters mean significant
580 differences (p<0.05).

	Leaves	Flowers	Immature fruits	Leafy flowered stems
Fructose	1.82 ± 0.23 c	8.72 ± 0.14 a	0.40 ± 0.03 d	3.53 ± 0.18 b
Glucose	3.15 ± 0.43 c	7.36 ± 0.13 a	1.52 ± 0.07 d	4.74 ± 0.18 b
Sucrose	3.97 ± 0.03 b	2.47 ± 0.05 c	0.11 ± 0.03 d	3.30 ± 0.10 a
Trehalose	2.67 ± 0.11 b	1.47 ± 0.06 c	*nd*	3.09 ± 0.03 a
Raffinose	*nd*	*nd*	0.26 ± 0.03 a	*nd*
Total sugars	11.61 ± 0.51 c	20.02 ± 0.26 a	2.30 ± 0.10 d	14.67 ± 0.49 b

581

582 **Table 7.** Moisture (g/100 g of fresh weight), macronutrients composition (g/100 g of
583 dry weight) and energetic value (Kcal/100 g of dry weight) of different *Malva sylvestris*
584 components (mean ± SD; n=3). In each row, different letters mean significant
585 differences (p<0.05).

	Leaves	Flowers	Immature fruits	Leafy flowered stems
Moisture	76.30 ± 0.54 b	72.49 ± 1.89 c	45.60 ± 0.97 d	77.26 ± 1.34 a
Carbohydrates	71.46 ± 0.81 c	78.12 ± 0.44 a	74.96 ± 1.10 b	71.89 ±0.35 c
Proteins	12.25 ± 1.01 b	8.50 ± 0.51 c	3.26 ± 0.25 d	14.26 ± 0.44 a
Fat	2.76 ± 0.40 b	2.84 ± 0.37 b	8.96 ± 0.22 a	3.09 ± 0.27 b
Ash	13.53 ± 0.11 a	10.54 ± 0.30 b	12.83 ± 0.78 a	10.76 ± 0.04 b
Reducing sugars	6.22 ± 0.49 c	13.95 ± 0.16 a	2.09 ± 0.12 d	10.46 ± 0.70 b
Energy	359.72 ± 1.10 c	372.02 ± 2.13 b	393.45 ± 4.41 a	372.43 ± 1.08 b

586

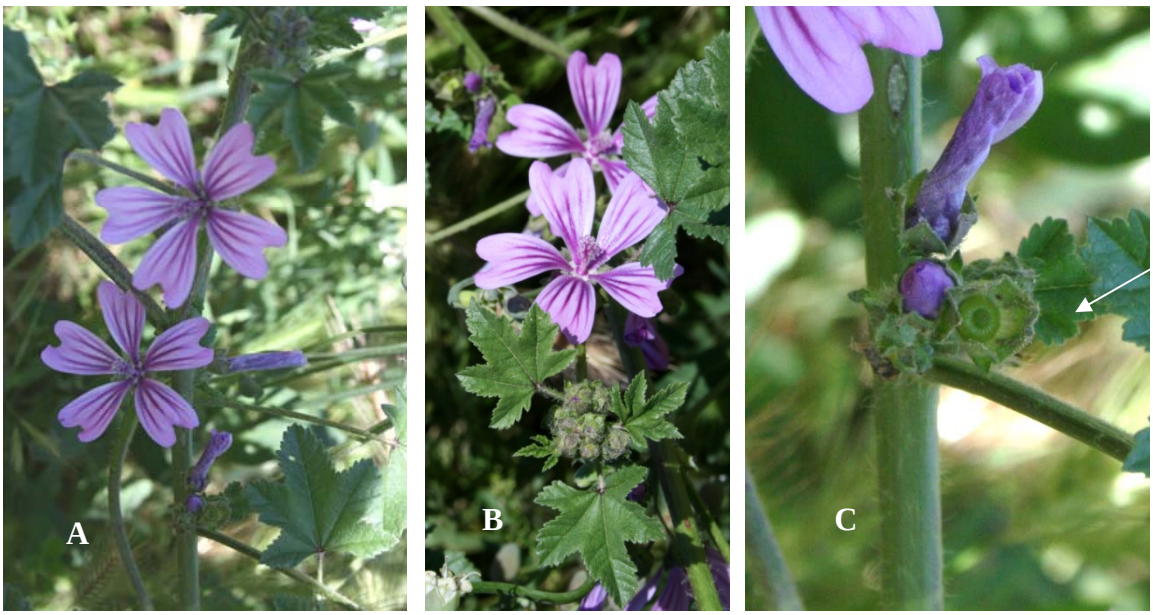

Figure 1. Common mallow (*Malva sylvestris* L.) gathered in Trás-os-Montes, Portugal. A - Leafy flowered stems; B – Young leaves; C- Immature fruits.