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Antioxidant properties and some phytochemical components of the edible medicinal *Malva sylvestris* L

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Abstract
This paper presents a phytochemical screening of extracts of *M. sylvestris* leaves and petioles. The antioxidant activity was studied in vitro by determination of DPPH, FRAP and phenolic compounds in the extracts. The soluble carbohydrates were also investigated. Results show that flower extracts have a higher amount of total phenolics with 6.32 ± 0.13 mg GAE/g FW, and total flavonoids with 1.45 ± 0.21mg QE/g FW. In general, more total soluble carbohydrates were found in the flowers 42.9 g/100 g FW, as reducing sugars presented mainly by glucose and fructose were also higher in the flowers – 5.5 g/100 g FW.
The results from current study evaluated mallow flowers as a rich source of bioactive compounds for healthy human nutrition.

Keywords: *Malva sylvestris* L, common mallow, carbohydrates, phenolic content, bioactive compounds

Introduction
Many plants synthesize substances, like phenols, which are useful to human health. Their health benefits (antibacterial, anti-inflammatory, anti-cancerogenic, and antiviral effects) originate from the antioxidative effects of these phytochemicals, which are based on their ability to inhibit various free radicals leading to the preservation of biological molecules against oxidation [1]. *Malva sylvestris* L., known as common mallow and used as food and medicine in Europe since the time of ancient Greece and Rome, is a biennial plant from the Malvaceae family. *Malva sylvestris*, with its 100-120cm height, is traditionally used for the treatment of injuries, inflammation, burn healing, swelling [2], as well as an anti-inflammatory agent for the respiratory and gastrointestinal tracts [3\], skin disorders [4], coughs and mouth irritations (both leaf and flower) [5].
Mallow leaves and flowers feature high amounts of lime[6] and carbohydrates. Carbohydrates give mallow most of its soothing activity, though flavonoids and anthocyanidins may also contribute. Mallow is typically applied as a tea or gargle for these indications. *M. sylvestris* L. has also been used in cancer and ulcer healing, because it is reported to contain flavonoids, tannin, phenolic compounds, ascorbic acid, carotenoids, and tocopherols [4, 7]. It has been claimed that the leaves of this plant have powerful anti-inflammatory, antioxidant and skin tissue integrator properties [7].
The active ingredients are found in the flowers and leaves, which are rich in mucilage, used for their expectorant properties [8]. The plant is largely used to soothe mucous membrane inflammations. *M. sylvestris* is good for skin disorders, as well as demonstrating good antimicrobial and anti-inflammatory activity. Aquatic extract of Malva *sylvestris* L. plant is able to strengthen innate immune system and reduce effect of Candida infection [9].
In culinary technology, young leaves are eaten raw in salads, leaves and shoots are consumed in soups and as boiled vegetables [10, 11]. The biological activity of this plant may be attributed to antioxidants, such as polyphenols, vitamin C, vitamin E, β-carotene, and other important phytochemicals.
In herbal medicine, mallow is classified as a demulcent - a soothing agent that counters irritation and mild inflammation. It has been used for the treatment of colitis and stomatitis, in cases of chronic bronchitis, against furuncle and abscess, contusions and hemorrhoids as well as other dolorous and inflammatory processes [12]. *Malva sylvestris* extracts are also reported for their radical scavenging effect [13].

The main purpose of the present study was to evaluate and identify some of the phytochemical constituents of *M. sylvestris* leaves and flowers. The total phenol content and potential antioxidant activities of the extracts were also evaluated using the spectrophotometric FRAP and DPPH methods.

Materials and Methods

Materials

Aerial parts of *Malva sylvestris* were gathered in March 2018 (Plovdiv, Bulgaria). Two different samples – leaves and flowers – were prepared for analysis. All chemicals used for experiments were at least analytical grade.

Preparation of the extracts

For the extraction of phytochemical compounds (5 g) from *Malva sylvestris* leaves and flowers were extracted with 70% (v.v-1) ethanol in solid to liquid ratio 1:20 (w.v-1). The extraction procedure was performed in an ultrasonic bath (VWR, Malaysia, 45 kHz and 30 W) for 15 min, at 45 °C as previously described by Petkova *et al*. [14]

Determination of Total soluble carbohydrate content

The total soluble carbohydrate content in mallow leaves and flowers extracts were estimated by phenol-sulphuric acid method [15]. Briefly, 0.1 ml of each extract were mixed with 1 ml of 5% phenol, 5 ml of sulphuric acid and placed in a water bath at 30 °C for 20 minutes. The absorbance was measured at 490 nm against blank prepared with d. H_2O. The amount of presented carbohydrates was determined from the calibration curve for glucose used as a standard y = 0.0098x - 0.0465 (R_2=0.998) and the results were calculated as (g/100 g) of fresh weight (FW).

Analysis of reducing sugars content

The reducing sugars were analyzed by PAHBAH method[16]. PAHBAH reagent (0.750 ml) was added to 0.250 ml properly diluted extract. The mixture was boiled for 5 min in a water bath and then was cooled in the ice bath for 5 min. The absorbance was measured at 410 nm against the blank, prepared with d. H_2O. The assay was set up by preparing glucose standard in the concentration range 5–100 µg/ml [17].

Determination of mono-and disaccharides by an HPLC analysis

Chromatographic separations and determination of presented sugars were performed on a HPLC instrument Elite Chrome Hitachi, coupled with refractive index detector (RID) Chromaster 5450. The analysis of mallow leaves and flowers extracts were done on a Shodex® Sugar SP0810 (300 mm × 8.0 mm i.d.) with Pb2+and a guard column Shodex SP - G (5 µm, 6 × 50 mm) operating at 85 °C, mobile phase d. H2O with flow rate 1.0 ml/min, and the injection volume 20 µl [18].

Total phenolic content (TPC)

Total phenolic contents were measured using a Folin-Ciocalteu reagent with some modifications [19]. Briefly, 1 ml Folin-Ciocalteu reagent diluted five times was mixed with 0.2 ml sample and 0.8 ml 7.5% Na_2CO_3. The reaction was performed for 20 min at room temperature in darkness. Then the absorbance was measured at 765 nm against blank. The results were expressed as mg equivalent of gallic acid (GAE) per g fresh weight (FW), according to calibration curve, build in range from 0.02 to 0.10 mg gallic acid used as a standard [20].

Total flavonoid content (TFC)

The total flavonoids content was analyzed by $Al(NO_3)_3$ reagents [21]. In brief, 1 ml properly diluted mallow extract was mixed with 100 µl of 10% Al $(NO_3)_3$, 100 µl 1M potassium acetate and 3.8 ml d. H_2O. The reaction was performed for 40 min at room temperature. The absorbance was measured at 415 nm against blank prepared without addition of 10% Al $(NO_3)_3$. The results were presented as mg equivalents quercetin (QE) per g fresh weight (FW) according to the calibration curve, linear in range of 10-100 µg/mL quercetin as a standard.

DPPH radical-scavenging ability

Each 70% ethanol extract of mallow leaves and flowers (0.15 ml) was mixed with 2.85 ml freshly prepared 0.1mM solution of DPPH in methanol. The sample was incubated for 15 min at 37 °C in darkness. The reduction of absorbance was measured at 517 nm in comparison to the blank containing methanol and% inhibition were calculated. A standard curve was built with 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) in concentration between 0.005 and 1.0 mM. The results were expressed in mM Trolox equivalents (TE) per g fresh weight (FW) [20].

Ferric reducing antioxidant power (FRAP) assay

The assay was performed according to Benzie and Strain [22] with slight modification. The FRAP reagent was freshly by mixing 10 parts 0.3 M acetate buffer (pH 3.6), 1 part 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl and 1 part 20 mM FeCl3.6H2O in d. H2O. The reaction was started by mixing 3.0 ml FRAP reagent with 0.1 ml of investigated extract. The reaction time was 10 min at 37 °C in darkness and the absorbance was measured at 593 nm against blank prepared with methanol. Antioxidant activity was expressed as mM Trolox® equivalents (TE) per g fresh weight (FW) [20].

Statistical analysis

Data were expressed as means ± standard deviations for triplicate determination. Statistical analysis was performed using Microsoft Excel 2016. Differences were considered to be significant at validity of α=0.95.

Results and Discussion

Carbohydrates, essential macromolecules, are widely distributed in plants, glucose is seen as a major metabolic fuel as it is being absorbed into the bloodstream. The results for the carbohydrate content of mallow (*Malva sylvestris*) leaves and flowers were summarized in Table 1. In the investigated flower extracts, only the presence of sucrose, glucose and fructose was found (Fig. 1a). The same sugars were detected in the mallow leaves extracts. In general, more total soluble carbohydrates were found in the flowers 42.9 g/100 g FW.

Table 1: Carbohydrate content in mallow (*Malva sylvestris*) leaves and flowers, g/100 g FW

Sample	Total soluble carbohydrates	Reducing sugars	Sucrose	Glucose	Fructose
Mallow leaves	42.9	2.1	0.46	0.61	0.88
Mallow flowers	47.0	5.5	0.21	0.93	2.03

Flowers showed the highest total sugars content, and the highest levels of fructose and glucose, as fructose predominated with 2.03 g/100g FW. This observation was in accordance with the reports of Barros *et al*. [4] The same observation was found in the leaves. Sucrose was dominant in the mallow leaves 0.46 g/100 FW. Reducing sugars were in a larger quantity in the flowers – 5.5 g/100 g FW. The overconsumption of sucrose is seen as a global problem for people with metabolic problem and *Malva sylvestris* can be recognized as a valuable nutritious source as its carbohydrate content is fairly modest.

Total phenolic content and total flavonoids of *Malva sylvestris* L. samples are presented in Table 2. Phenolic compounds ranged from 6.32 to 1.42 mg GAE/g FW in the leaves and flowers, respectively. These results were higher than the ones reported by Tabaraki *et al*. [23] for pure ethanol extracts from mallow leaves and petioles.

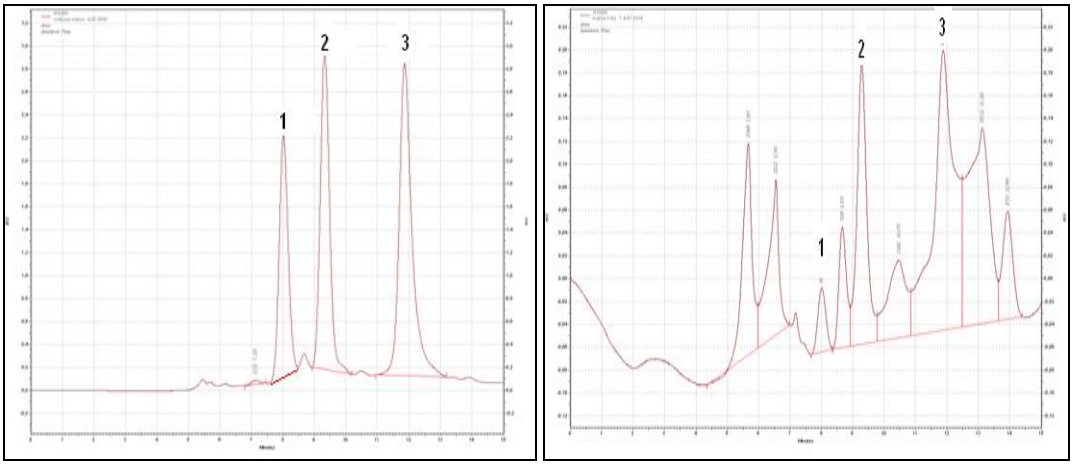

Fig 1a). Fig 1b).

Fig 1: HPLC-RID chromatograms of sugar content in Malva flowers (a) and leaves (b), where 1. sucrose, 2. glucose and 3. fructose

Flavonoids are typical phenolic compounds with multiple biological activities including potent antiallergic and anti-inflammatory and antiviral reactions. The total flavonoids in the mallow leaves and flowers varied from 0.76 to 1.45 mg QE/g FW. Barros *et al*. [4] documented significant quantities of flavonoids: 210.8, 46.6, 25.4 and 143.4 mg/g in the leaves, flowers, immature fruits and flowered stems, respectively. The daily intake of flavonoids with normal food, especially fruit and vegetables, is 1-2 g [24]. The flavonoid intake is being encouraged by physicians, as their intake is proven to simulate some hormones and neurotransmitters, scavenge free radicals, inhibit specific enzymes, and treat numerous diseases [24]. Shelbaya *et al*. [25] reaffirm the presence of tannins, saponins, flavonoids and carbohydrates in extracts of *Malva sylvestris*.

M. sylvestris has previously been reported to possess antioxidant properties. Tabaraki *et al*. [23], Beghdad *et al*. [26] and Barros *et al*. [4] demonstrated in their research the antioxidant potential of extracts obtained from *Malva sylvestris* L. leaves and petioles. Della Greca *et al*. [27] investigated the antioxidant activity of aqueous extract of *M. sylvestris* by its ability to scavenge the 2, 2'-diphenyl-1-picrylhydrazyl (DPPH). In this study, two antioxidant assays were applied – DPPH and FRAP. Flower extracts possessed higher antioxidant potential than leaves which was in contrast to the results presented by Mihaylova *et al.* [13]

The current results could be explained with the higher content of total phenolic compounds accumulated in the flowers (Table 2).

Table 2: Total phenolic content (TPC), total flavonoids content and in vitro antioxidant activity of mallow (*Malva sylvestris*) leaves and flowers

Sample	TPC, mg GAE/g FW	Total flavonoids, mg QE/g FW	FRAP	DPPH
			mM TE/g FW	
Mallow leaves	1.42±0.14	0.76±0.19	4.04±0.85	3.88±0.51
Mallow flowers	6.32±0.13	1.45±0.21	6.01±0.54	5.98±0.43

Contrary to Tabaraki *et al*. [23] the highest antioxidant activity was found in flower extracts of *Malva sylvestris* L. 6.01±0.54 mM TE/g FW (DPPH assay) and 5.98±0.43 mM TE/g FW for FRAP assay, respectively. Other authors also found a higher phenolic and flavonoid contents and antioxidant activity in leaves than in leafy flower stems and flowers, when 95% ethanol as extracting solvent was used. In this case, higher water content (30%) causes extraction of more bioactive compounds, due to their solvation.

Conclusion

The obtained results implied that *Malva sylvestris* leaves and flowers could be a source of phenolic compounds with antioxidant potential. The current study encourages the consumption of edible and medicinal plants, because this plant's presence in human nutrition could assure intake of natural antioxidants containing phenolic acids that are associated with long term health benefits. The work also reveals that *Malva sylvestris* has a potential use in the fields of food preparation and pharmaceuticals.

References

1. Rackova L, Drabikova K, Jancinova V, Perecko T, Smidrka J, Harmatha J. *et al*. Structural aspects of antioxidant action of selected natural polyphenols. Free Rad. Res. 2009; 43:27-97.
2. Pirbalouti AG, Koohpyeh A. Wound Healing Activity of Extracts of *Malva sylvestris* and *Stachys lavandulifolia*. Int. J Biol. 2011; 3(1):174-179.
3. Lust J. The Herb Book, Toronto: Bantam Books, 1974,

262-263.
4. Barros L, Carvalho A, Ferreira I. Leaves, flowers, immature fruits and leafy flowered stems of *Malva sylvestris*: A comparative study of the nutraceutical potential and composition. Food Chem Toxicol. 2010; 48(6):1466-1472.
5. Weiss RF. Herbal Medicine. Gothenburg, Sweden: Ab Arcanum and Beaconsfield: Beaconsfield Publishers Ltd., 1958.
6. Wichtl M. Herbal drugs and phytopharmaceuticals. Boca Raton, FL: CRC Press, 1994, 313-316.
7. Gasparetto J, Martins C, Hayashi S, Otuky M, Pontarolo R. Ethnobotanical and scientific aspects of *Malva sylvestris* L.: a millennial herbal medicine. J Pharm Pharmacol. 2012; 64(2):172-89.
8. Yeole NB, Sandhya P, Chaudhari PS, Bhujbal PS. Evaluation of *Malva sylvestris* and Pedalium murex mucilage as suspending agent. International Journal of Pharm. Tech Research. 2010; 2(1):385-389.
9. Hajyani S, Modaresi M, Madani M. Effect of *Malva sylvestris* L. extract on blood cell parameters in mice with Candida albicans infection. Der Pharma Chemica. 2015; 7(5):302-305.
10. Carvalho A. Etnobotánica del Parque Natural de Montesinho. Plantas, tradición y saber popular en un territorio del nordeste de Portugal. Universidad Autónoma, Madrid, 2005.
11. Neves J, Matosa C, Moutinho C, Queiroz G, Gomes L. Ethnopharmacological notes about ancient uses of medicinal plants in Trás-os-Montes (northern of Portugal). Journal of Ethnopharmacology. 2009; 124:270-283.
12. Esteves P, Sato A, Esquibel M, Buzzi F, Meira A, Filho V. Antinociceptive Activity of *Malva sylvestris* L. Lat. Am. J. Pharm. 2009; 28(3):454-456.
13. Mihaylova D, Popova A, Denkova R, Alexieva I, Krastanov A. In vitro antioxidant and antimicrobial activity of extracts of Bulgarian *Malva silvestris* L. Annuaire de l'Université de Sofia "St. Kliment Ohridski" Faculte de Biologie. 2014; 100(4):41-48.
14. Petkova N, Ivanov I, Denev P, Pavlov A. Bioactive substance and free radical scavenging activities of flour from Jerusalem artichoke (*Helianthus tuberosus* L.) tubers – a comparative study. Turkish Journal of Agricultural and Natural Sciences. 2014; 2:1773-1778.
15. Dubois M, Gilles K, Hamilton J, Rebers P, Smith F. Colorimetric method for determination of sugars and related substances, Anal. Chemistry. 1956; 28(3):350-356.
16. Lever M. A new reaction for colorimetric determination of carbohydrates. Anal. Biochemistry. 1972; 47:273-279.
17. Dimitrova M, Petkova N, Denev P, Aleksieva I. Carbohydrate Composition and Antioxidant Activity of Certain Morus Species, International Journal of Pharmacognosy and Phytochemical Research. 2015; 7(3):621-627.
18. Petkova N, Vrancheva R, Denev P, Ivanov I, Pavlov A. HPLC-RID method for determination of inulin and fructooligosacharides. ASN. 2014; 1:99-107.
19. Stintzing C, Nerbach M, Mosshammer M, Carle R, Yi W, Sellappan S *et al*. Color, betalain pattern, and antioxidant properties of cactus pear (*Opuntia* spp.) clones. J Agric Food Chem. 2005; 53(2):442-451.
20. Ivanov I, Vrancheva R, Marchev A, Petkova N, Aneva I., Denev P. *et al*. Antioxidant activities and phenolic compounds in Bulgarian Fumaria species, International Journal of Current Microbiology and Applied Sciences. 2014; 3(2):296-306.
21. Kivrak I, Duru M, Öztürk M, Mercan N, Harmandar M, Topçu G. Antioxidant, anticholinesterase and antimicrobial constituents from the essential oil and ethanol extract of *Salvia potentillifolia*. Food Chemistry. 2009; 116(2):470-479.
22. Benzie I, Strain J. The Ferric Reducing Ability of Plasma (FRAP) as a measure of antioxidant power: The FRAP Assay. Analytical Biochemistry. 1996; 239(1):70-76.
23. Tabaraki R, Yosefi Z, Ali H, Gharneh A. Chemical Composition and Antioxidant Properties of *Malva sylvestris* L. Journal of Research in Agricultural Science 2012; 8(1):59-68.
24. Havsteen BH. The biochemistry and medical significance of the flavonoids, Pharmacol Ther. 2002; 96(2, 3):67-202.
25. Shelbaya L, Sello A, Kotp M. Antioxidative effect of some Malva sylvestris extracts on oxidation of cotton oil during heating, The 6th Arab and 3rd International Annual Scientific Conference on: Development of Higher Specific Education Programs in Egypt and the Arab World in the Light of Knowledge Era Requirements, 2011, 2164-2179.
26. Beghdad M, Benammar Ch, Bensalah F, Sabri FZ, Belarbi M, Chemat F. Antioxidant activity, phenolic and flavonoid content in leaves, flowers, stems and seeds of mallow (*Malva sylvestris* L.) from North Western of Algeria. African Journal of Biotechnology. 2014; 13(3):486-491.
27. DellaGreca M, Cutillo F, D'Abrosca B, Fiorentino A, Pacifico S, Zarrelli A. Antioxidant and radical scavenging properties of *Malva sylvestris*. Nat Prod Commun. 2009; 4:893-896.