

PLANT PIGMENTS (ANTIOXIDANTS) OF MEDICINAL PLANTS *MALVA SILVESTRIS* L. AND *MALVA MOSCHATA* L. (MALVACEAE)

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ABSTRACT

Qualitative-quantitative structure of plant pigments in wild plants *Malva silvestris* L. and *Malva moschata* L. (*Malvaceae*), which were collected in 20 locations in Sarajevo area and surroundings, was tested during spring and summer in 2003. Acetone extracts of both categories were made and rising paper-chromatography done for the purpose of qualitative analysis. Quantitative analysis was done by spectrophotometry. Chlorophyll a, chlorophyll b and xanthophylls presence was confirmed by separation of pigments from acetone extract of these plant species. Spectrophotometric analysis of acetone extracts showed these results (given in mg/L): chlorophyll a 2,386, chlorophyll b 0,332 and carotenoides 1,037. Data given in mg/g dry substance are: chlorophyll a $1,193 \times 10^{-2}$, chlorophyll b $1,66 \times 10^{-3}$, and carotenoides $5,185 \times 10^{-3}$. Pigments structure (in mg/L) in species *Malva moschata* is 1,6 for chlorophyll; 1,419 for chlorophyll b; and 0,364 for carotenoides. Data given in mg/g are: chlorophyll a 8×10^{-3} , chlorophyll b $7,09 \times 10^{-3}$, and carotenoides $1,82 \times 10^{-3}$. Considering that species *Malva moschata* L. grows on ecologically clear soils as opposed to well-known medicinal species *Malva silvestris* L., and considering the production of phytomass, phytochemical structure and pharmacological influence it can be considered very medical and be given advantage over this wider spread category.

KEY WORDS: chlorophyll, carotene, plant pigments, paper-chromatography, spectrophotometry, medical plant, edible wild plant, Bosnia and Herzegovina.

INTRODUCTION

Plant pigments have a very important role in the protection of photosynthetic system, as well as protection of plant organism itself from the negative influences of sun radiation. Basic pigments chlorophylls have a special defense role, especially chlorophyll a and b. Accessory pigments carotenoides are very important in that process (2). Nowadays, chlorophylls and carotenoides have a very important role in prevention and therapy of different diseases of human beings, including immune system, different forms of skin disease, and characteristic antioxidative influence is attributed to them. Chlorophylls show antimicrobial and deodorant activity, and they support vitamin B synthesis in organism. (3) Carotenoides are protectors of chlorophyll. They have an important role in strengthening of immune system, cardiovascular system, and act preventively against cancer (4). Otherwise, plant pigments are water insoluble. Extremely lipophilic chlorophyll appears in most of these plants in two chemicaly related forms as *bluish green chlorophyll a* and yellow green *chlorophyll b*. Their ratio is most frequently 3:1. Chlorophylls fluoride lively red in short wave ultraviolet light (2). Besides green chlorophylls, in less quantity, liposoluble orange red and yellow carotenoides, are regular constituents of plastids in these plants. Those are unsaturated hydrocarbons, and in their chemical structure they are identical to tetraterpents. Because of numerous conjugated double bonds, they are orange or red colored lipophilic stains (lipochromes). β -carotene is the most frequent among them. Its chain ends form beta-ion rings, like in many others carotenoides (α -karoten). Xanthophylls are derivatives of carotene that contain oxygen. Lutein, which corresponds to beta-carotene, has one OH group on every ring. It does not act as vitamin, although it oxidizes even in darkness. Protective function is attributed to carotenoides of photosynthetic apparatus in regard to chlorophyll (2). For α and β - carotene, besides pro-vitamin activity, positive effects on immune system, cardiovascular disease was proven, and they reduce a risk from some forms of cancer, too (5). Types of plant species *Malva L.* (family *Malvaceae*) have been used as origins of medical substances from ancient times. But, most of them, like *M. sylvestris* and *M. moschata*, are widely used as healthy wild vegetable in preparation of wide dietetics spectrum. This activity, in great part, can be attributed to participation of basic and accessory pigments, which appear in genus *Malva* species in larger quantity (6). Both, flower and leaf of *M. sylvestris* and *M. neglecta*

species are in official usage, and they prescribe by numerous pharmacopoeia. Those are: German Pharmacopoeia (DAB 6 and DAB 7), then Pharmacopoeia Helvetica edition quinta and edition sexta (Bern 1933 and Bern 1971) and Ph Jug I and II. They are used like infusions for respiratory organs treatment - Species pectorals and Species althaeae, and for skin disease treatment – Species emollients. Concentration of anthocyanins is sufficient for achieving therapeutic effect in making infusion for cough and tonsillitis. Apart from that, French pharmacopoeia (Pharmacopoeia Française IX edition, Paris, 1974) lists *Malva alcea*, *Malva rotundifolia* and *Malva sylvestris* as officinal. It is important to mention that *Malvae sylvestris floss* is the official drug in European pharmacopoeia (European Pharmacopoeia, fourth edition, Strasbourg, 2002), Czechoslovakian Pharmacopoeia Ph. Bs II (Pharmacopoeia Bohemoslovenica, edition secunda, Praha, 1964) and in Polish Pharmacopoeia III (Ph.Pol.) II – Pharmacopoeia Polska III, PZWL Warszawa, 1954). Basic metabolites in genus *Malva* specis are: mucus, pectin, starch, sucrose, irridoide glycosides, vitamins, some tannin, mineral substances, fats, anthocyanine, ferments and others (6, 7, 8). Since *Malva sylvestris*, in the biggest part of its distribution, mainly grows in nitrified soils, which are frequently loaded with heavy metals, pesticides and other residues, its usage is limited in humane medicine. Therefore, it is important to find and test species with similar pharmacological-physiological effects, and which grow on ecologically favorable soils. One of those species is *Malva moschata L.* It finds its optimum in clear soils of mountain meadows, and it reaches high biomass production for the balanced collection and application in healthy phytopharmaceuticals production. The basic goal of this work is to test qualitative and quantitative contents of plant pigments in *Malva sylvestris* species which grows on ecologically more burdened soils and *M. moschata* which grows on ecology more favorable soils, and after that estimate their potentials in modern dietetic and phytotherapy.

MATERIAL AND METHODS

MATERIAL

Testing was done on plant material of wild plants *Malva sylvestris* and *Malva moschata*. These plants belong to a wider spread family Malvaceae, order Malvales, subclass Dillenidae, class Magnoliopsida and phylum Magnoliophytina (9).

METHODS

Methods of field work

Plant material was collected by hand in dry weather. Healthy specimens were collected in ecologically favorable soils. Surface parts of plant *Malva sylvestris* were collected in twenty locations in Sarajevo region. *Malva moschata* samples were collected from twenty locations on Igman mountain at altitude of 1000-1300 m in the summer and they were regularly conserved. This region of sampling is marked by coordinates: 18° and 18°:30' of east longitude and 43° 30' and 44° of north latitude.

Methods of laboratory work

Determination of qualitative-quantitative pigments characteristics was done, after extraction, by one-dimensional rising paper-chromatography and by spectrophotometry (10, 11, 12).

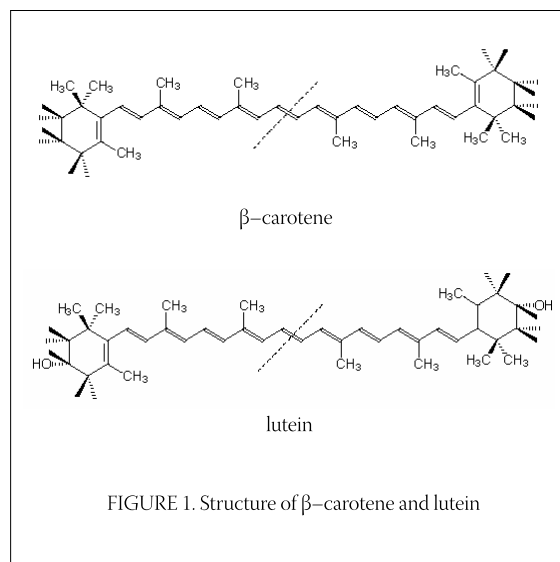
Chloroplast pigments separating by method of chromatography

One-dimensional rising paper-chromatography was used for determination of pigments. Chromatographic paper Whatman N°1 was used, and standard technique and reagents. Acetone extract of pigment was used as a sample. Eluent (medicinal gas:petroleum-ether:acetone=100:25:20) was used for separation. Time of separation was from 20 to 30 minutes. The distance from the start line corresponds molecular mass values in reverse order. Thus, the closest to the start line will be:

- Chlorophyll b Mr = 907 (green-yellow fraction)
- Chlorophyll a Mr = 893 (light green fraction)
- Then above chlorophyll a in wider area xanthophylls place and their molecule masses range from Mr=552 for cryptoxanthene to Mr=600 for violaxanthene. Colors of this fraction are different nuances of yellow.
- Carotene fraction Mr=536 yellow orange color is the closest to the front line (2).

Spectrophotometric analysis of chlorophyll and carotenoides from acetone solution

Quantitative analysis of chlorophyll a and b and carotenoides is based on their extraction with acetone, followed by spectrophotometric analysis. Measure-



ments are done on wavelengths from 662 nm, because it is absorbance maximum for chlorophyll a, and 644 nm, because it is absorbance maximum for carotenoides. Measurements were done on spectrophotometer (Bansch & Lomb, General vertretong und Service – Stelle, Digitana AG 8003, Zurich). Reading of absorbance is on wave length (λ) 662, 644 and 440 nm, and after that calculations are done:

- chlorophyll a = $9,784 \times A_{662} - 0,990 \times A_{644}$
- chlorophyll b = $21,426 \times A_{644} - 4,650 \times A_{662}$
- carotene = $4,695 \times A_{440} - 0,268 \times (a+b)$

where 9,784; 0,990; 21,426; 4,650; 0,268 are molar absorbance coefficients according to Wetstein for absolute acetone and thickness of quvete of 1 cm, and A-light absorption. Calculated values are in mg/L, and then we calculate raw substance in mg/g according to the following formula:

$$C = \frac{C_1 \times V \times R}{G \times 1000}$$

where:

C = content of certain fraction in mg/g of raw substance

C₁ = concentration of pigments calculated according to formula (mg/L)

V = extract volume

R = extract dilution (if it exists)

G = sample mass (dry or raw material)

1000 = factor of transforming of g into mg (12).

RESULTS

QUALITATIVE ANALYSIS

Chloroplasts pigments separation by one-dimensional paper-chromatography

Paper chromatograms were obtained for every tested species. Identification of pigments was done based on characteristic color: chlorophyll b is light green fraction, chlorophyll a is dark green, and xanthophylls – yellow; and also on the bases of their position on chromatogram. Presence of chlorophyll a; chlorophyll b and xanthophylls was proved in analyzed species of Malva genus. Chromatogram of cultivated carrot – *Daucus carota* L. var *sativa* was used as reference (Figures 2. and 3.).

QUANTITATIVE ANALYSIS

Spectrophotometric pigments analysis

Spectrophotometric analysis was done for quantitative analysis of pigments. Water was used as reference. Following results were obtained on the basis of measurements: (Table 1.). By entering these results in formula for pigments quantity calculation we got the values given in Table 2. Calculated values are in mg/L of acetone extract, and after that values are calculated according to given formula and they are in mg/g of dry mass for both species (Table 2.)

PIGMENT	PLANT SPECIES			
	MALVA SILVESTRIS		MALVA MOSCHATA	
	mg/L	Mg/g dry mass	Mg/L	Mg/g dry mass
CHLOROPHYLL A	2,3867	1,193x10 ⁻²	1,600	8x10 ⁻³
CHLOROPHYLL B	0,3320	1,66x10 ⁻³	1,419	7,09x10 ⁻³
CAROTENOIDES	1,037	5,185x10 ⁻³	0,364	1,82x10 ⁻³

TABLE 2. Comparative spectrophotometrical analysis of plant pigments based on the mean values gotten from 20 samples



FIGURE 2. Rising one-dimensional chromatography of plant *Malva sylvestris* (left) and cultivated carrot *Daucus carota* var. *sativa* (right)

DISCUSSION

QUALITATIVE-QUANTITATIVE ANALYSIS OF PIGMENTS

Quantitative ratios between the pigments in both analyzed species show certain deviations from usual standards. For most of C₃ plants the ratio of chlorophylls a and b is 2,5 to 3:1, while in C₄ plants that ratio is bigger and usually is 4-5:1. Chlorophyll a and b ratio is very high and amounts to 7,2:1 in *Malva sylvestris*, and that can be attributed to the specific structure of chloroplasts and to the fact that analyzed chlorophyll originates from leaf mesophyll. Chlorophyll a and b ratio in mountain plant *Malva moschata* is 1,2:1 and which is a result of high intensity of sun lights. It is a known fact that this ratio is lower in plants that grow in high intensity of light and they contain less chlorophyll a. Analysis of

Reported for treatment	48 patients with dg: M75.5
Reported for treatment	20 patients with dg: M75.4
Reported for treatment	30 patients with dg: M75.3
Reported for treatment	659 patients with dg: M75.2
Reported for treatment	229 patients with dg: M75.1
Reported for treatment	129 patients with dg: M75.0
TOTAL M75.	1.115 patients

TABLE 1. Diagnosis structure in shoulder periartthritis



FIGURE 3. Chromatogram of plant *Malva moschata* (left) and cultivated carrot *Daucus carota* var. *sativa* (right)

PLANT SPECIES	CHLOROPHYLL A AND B RATIO	TOTAL CHLOROPHYLL AND CAROTENOIDES RATIO
<i>MALVA SILVESTRIS</i>	7,2: 1	2,6:1
<i>MALVA MOSCHATA</i>	1,2: 1	8,3

TABLE 3. Quantitative proportions between some pigments

total chlorophyll and carotenoides ratio is significantly different. In normal circumstances it is usually 5:1. But, that proportion in plant *Malva silvestris* is 2,6:1, and in plant *Malva moschata* is extremely high 8,3:1 (2). Chlorophyll b, as dark green fraction, was separated nearest to the start, and chlorophyll a as light green fraction, and we compared it with the same fraction separated in chromatogram of cultivated carrot as a reference. Chlorophyll presence was confirmed in both categories.

POTENTIAL FOR USAGE IN MODERN PHYTOTHERAPY

Common mallow *Malva silvestris* is wider spread plant. It has been favorite food and medical plant in the Balkans peninsula from ancient times. It is particularly appreciated as vegetable. Fresh leaves contain about 120 mg% vitamin C and as much as 18mg% carotene. Leaves contain considerable quantity of iron and calcium. Therefore, they are considered the healthiest vegetable in this area (12). Leaves and flowers of *Malva silvestris* were used for the treatment of difficult diseases of respiratory tract, including malignant diseases of this system.

Decoct of this plant is effective agent against middle ear inflammation (13, 14, 15). This species has proven to be specially healthy food in soldiers feeding in nature, and in the cases of mass catastrophe as well (16, 17, 18). In *Malva moschata* species, till now, no systematic studies of nutritive and pharmacological active substances was done. But, it is supposed that this species contains similar substances like the wider spread species and that it can be used in the same purpose. *Malva silvestris* usually grows on nitrified ground, by roads, near traffic arteries, on soils, which can be loaded with heavy metals, pesticides and other residuum with unfavorable influence on human health. Because of that this species from natural soils has or may have limited usage. Plant species *Malva moschata*, grows far from human settlements, in mountain or sub-mountain zone, by edges of meadows in the zone of coniferous forests and mixed deciduous and conifer forests, on the grounds which are natural and unburden with organic and inorganic pollutants. Therefore, this species has excellent possibilities in preparation of different nutritive and phytopharmacs in relation to majority of species of *Malva genus* and it has big comparative advantage in this respect.

CONCLUSION

Wild medicinal plant species *Malva silvestris* and *Malva moschata* contain different plant pigments or antioxidants. Chlorophyll a, chlorophyll b and xanthophylls presence was verified by separation of pigments from acetone extract of these plant species. Spectrophotometric analysis of acetone extract showed these results (given in mg/L): chlorophyll a 2,386, chlorophyll b 0,332 and carotenoides 1,037. Data given in mg/g dry substance are: chlorophyll a $1,193 \times 10^{-2}$, chlorophyll b $1,66 \times 10^{-3}$, and carotenoides $5,185 \times 10^{-3}$. Pigments content (in mg/L) in category *Malva moschata* is 1,6 for chlorophyll; 1,419 for chlorophyll b; and 0,364 for carotenoides. Data given in mg/g are: chlorophyll a 8×10^{-3} , chlorophyll b $7,09 \times 10^{-3}$, and carotenoides $1,82 \times 10^{-3}$. There is high potential for application of plant pigments in the prevention and modern therapy therefore main and accessory plant pigments play important role as antioxidants and as anticancer means. Species *Malva moschata* has more advantages as an effective antioxidant means than other plant species *Malva silvestris*.

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