UDC 633.882

Anatomical Features of the Marshmallow (*Althaea officinalis* L.) Root

- Original scientific paper -

Dragana RANČIĆ[1], Slobodan DRAŽIĆ[2], Zora DAJIĆ STEVANOVIĆ[1] and Radenko RADOŠEVIĆ[1]
[1]Faculty of Agriculture, Belgrade-Zemun
[2]Institute for Medicinal Plant Research "Dr Josif Pančić", Belgrade

Abstract: Marshmallow (*Althaea officinalis* L.) is a perennial herbaceous plant of the family *Malvaceae*, whose flowers, leaves and, especially roots, are used as drug in both folk and official medicine. Due to a high content of mucilage, the marshmallow root predominately serves for respiratory illnesses remedy. Anatomical features of a marshmallow root structure of different zones (root head, taproot and lateral roots) were observed by the analysis of permanent and temporal microscope slides with the additional application of histochemical methods in order to determine the mucilage localisation in root tissues and cells. Results of anatomical structure of the marshmallow root showed that parenchyma cells and intercellular spaces of parenchyma of the secondary phloem and the secondary xylem represented a spot of the synthesis and the accumulation of the mucilage, whereas the older parts of the root (root head and taproot) could be characterised by dominance of the secondary xylem zone in difference to younger lateral roots with a more expressed zone of the secondary cork, i.e. the secondary phloem. These findings were confirmed by the application of a specific stain for mucilage with alcian blue. The ray cells are considered as the main accumulators of starch, which was confirmed through the application of the iodine reagent. Obtained results might be of a practical importance for the estimation of marshmallow drug quality, since the structure and the size of secretory parenchyma of the root probably corresponds with the content and the chemical composition of its active substances.

Key words: Histochemistry, marshmallow, mucilage, root anatomy.

Introduction

Marshmallow (*Althaea officinalis* L.) is a herbaceous perennial plant of the family *Malvaceae*, which is native to Europe, but now can be found growing in the majority moist spots worldwide.

The species has been known as a medicinal plant since ancient times. The scientific name comes from its ancient uses: *Althos* in Greek means to heal, and the plant was called "the official healer". The fleshly, branchy roots are thick, long and tapering, very tough and pliant, whitish yellow outside, white and fibrous within, and known to contain a relatively high mucilage content making this herb an excellent demulcent, emollient, expectorant, diuretic, anti-inflammatory and expectorant, ***Schmidgall et al***., 2000. The extract of marshmallow exhibited strong antioxidant, bactericidal and anti-inflammatory properties, ***Elmastas et al***., 2004, ***Nosalova et al***., 1993. Besides using it in medicine, both the Romans and the Egyptians ate the root as a vegetable.

The whole plant, particularly the root, abounds with mild mucilage. By "mucilage in plants" is usually thought of substances which are soluble, or at least swell very perceptibly in water, and which upon the addition of alcohol are precipitated in a more or less amorphous or granular mass. Mucilage originates in the plant either as a part of the contents of the cell or as a part of the wall thereof, ***Kraemer*** 1898. In some species of the *Malvaceae* family, mucilage is stored in idioblasts, and in others in the cavities between cells, whereas a number and size of mucilage cells vary among species and genera, ***Pakravan et al***., 2007.

The mucilage is composed of a complex structurally diverse class of neutral and acidic biological macromolecules - polysaccharides with a broad range of physicochemical properties which are responsible for the biological activity of this hydrocolloid. Its framework consists of an acidic polymer rhamnogalacturonan with a high content of acidic sugars, regularly distributed along the chain. A strong pharmacodynamic effect of the polysaccharide fraction from the aqueous extract from *A. officinalis* roots is related to mucilaginous and bioadhesive properties, leading to the formation of polysaccharide layers on inflamed or destructed epithelial mucosa, protecting the irritated cells from local irritation, ***Schmidgall et al***., 2000. The traditional use of aqueous marshmallow extracts is for dry cough due to irritation of the oral, pharyngeal or gastric mucosa. Recent reviews of pharmacological properties indicate that the use of the aqueous marshmallow extract is justified for therapeutic indications for gastric/duodenal ulcers and dry cough, upon effects of its mucilage as an active substance, ***ESCOP***, 2003.

The mucilage content varies considerably with the season. The highest content of the root drug mucilage was determined during the late autumn and winter, with about 11%, the lowest in spring and summer with approximately 5-6%, ***Franz***, 1966. The increase in the mucus quantity was followed by the increase in the dextrose polysaccharides. Simultaneously with the increase of mucilage in autumn its content of glucose-containing polysaccharides increases. This is the reason why the root is usually harvested in late autumn from plants at least two years old.

The aim of this study was to investigate the structure and the organisation of the marshmallow primary and secondary root, as well as, the root tissue and cell morphology to identify root mucilage cells and possible mode of mucilage secretion upon the application of different microscopic and histochemical methods. Since the structure and size of secretory tissue in *Malvaceae* correspond with the content and the chemical composition of its active substances, ***Pakravan et al***., 2007, the obtained results might be of a practical importance for the further estimation of marshmallow drug quality.

Material and Methods

The anatomical investigation of different root zones (root head, taproot and lateral roots) was performed on fresh and fixed samples. The roots were collected in autumn 2008 from plants grown in experimental fields of the Institute for Medicinal Plant research "Dr Josif Pančić" (locations Petrovac and Nova Pazova). Hand-sections of fresh roots were subject to histochemical research using the IKI solution, ***Jensen***, 1962, and the 0.05% aqueous solution of toluidin blue, ***O'Brien et al***., 1964, for the starch and pectin identification, respectively. Samples fixed in FAA (4% v/v formaldehyde, 15% v/v acetic acid, and 50% v/v ethanol) were dehydrated through a gradual series of ethanol (80%, 96% and absolute ethyl alcohol) and xylene, slowly infiltrated with the paraffin embedding medium (Histowax, 56-58°C) and sectioned on 10-20 μm thick sections by sliding microtome. Paraffin was removed from the sections passing slides through series of ethyl alcohol solutions. The sections were stained with hematoxylin and safranin and mounted in Canada balsam. Histochemical investigations of mucopolysaccharides were prepared according to ***Ruzin***, 1999. Deparaffinised and re-hydrated paraffin sections were immersed for 10 minutes in a freshly prepared solution of 1% alcian blue in 3% acetic acid (pH 2.5), rinsed in distilled water 2-3 times per 10 minutes, and immersed in 1% alkaline alcohol (pH $\geq$ 8) for two hours till the appearance of the insoluble blue form (monstral fast blue). After staining, the tissue was dehydrated rapidly through absolute alcohol and xylol, and finally, slides were mounted in Canada balsam. Sections were observed on DMLS LEICA microscope and documented with the digital camera LEICA DC 300.

Results and Discussion

The root of *Althaea officinalis* builds up as a taproot, where the primary root develops into a large taproot producing smaller, lateral roots. The root surface is light brown in colour, often spirally twisted, and roots are internally yellowish white. The root tissues can be categorised into the three tissue systems: dermal, ground and vascular. In roots at the primary growth stage the dermal tissue system consists of only one layer of cells. Some of the epidermal cells are elongated into long

unicellular root hairs. Epidermal cells are typically thin-walled since they are involved in the water absorption. The ground tissue system is composed of the cortex, including the endodermis. The cortex is a major component of the ground root tissue at this developmental stage. It is represented by several layers of loosely arranged parenchyma cells with prominent intercellular spaces. In the central part of the root vascular tissue, xylem and phloem are arranged in a radial tetrarch or a pentarch vascular bundle. During the secondary growth, the root undergoes visible morphological and anatomical changes. The cambium arising in the vascular bundle forms a continuous ring and its cell divisions result in the secondary root growth and root thickening. The cambium layer (2 to 3 celled) forms new cells on the inside and outside of the cambium cylinder forming the secondary xylem and the secondary phloem cells, respectively. At this point, the cork cambium begins to form the periderm, a multi-layered tissue consisting of protective cork cells which contains the suberin. In older roots (head of the root and main root) a large proportion of the root is constituted of the secondary xylem, but in younger ones (e.g. lateral roots) the secondary phloem zone is more prominent (Figure 1). The transverse section of the secondary root shows a somewhat thick whitish bark (cortex) with a brownish periderm, separated from the white xylem (wood) by the well-marked cambium line (Figure 1). The xylem is diffuse porous, made up mainly of lignified elements: vessels, tracheids, fibres and tracheidal fibres. Vessels are very small (25-50 μm and 113 to 262 μm long), mostly solitary or in groups of 2-4, ***Metcalfe*** and ***Chalk***, 1974. Wood fibres exhibit thick walls of the width ranging from 9 to 19 μm. Medullary rays are 3 to 5 cells deep. The cross sectional view of the secondary phloem (Figure 2) shows that it is well developed and consists of sieve tubes, companion cells and phloem parenchyma. The most prominent cells in the zone of the secondary phloem are the phloem fibres. The cells surrounding the patches of fibres are sieve tube members and companion cells. A phloem ray, made of parenchyma cells were identified as well. Rays are made up of parenchyma cells and are useful in lateral transport

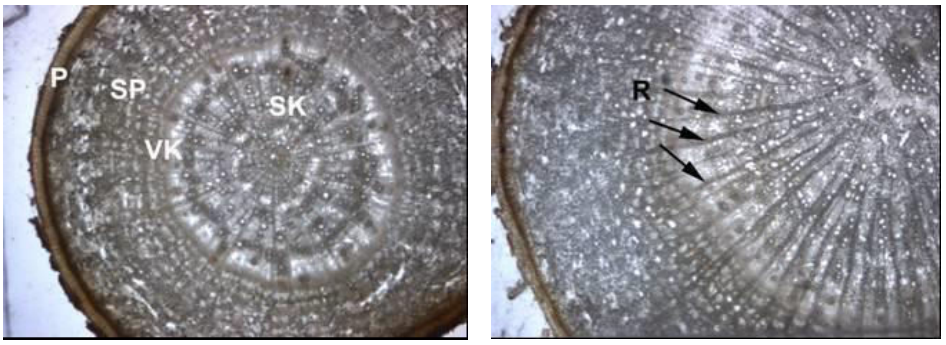

Figure 1. A transverse section (unstained) passing through the root reveals the following details: p - periderm, sp - secondary phloem, vk - vascular cambium, sk - secondary xylem, ve - vessel elements, R - rays (left: taproot; right: the root head (x25))
Poprečni presek (neobojen) sa detaljima: p - periderm, sp - sekundarni floem, vk - kambijum, sk - sekundarni ksilem, ve - traheje, R - zraci (levo: glavni koren; desno - glava korena (x25))

within the root. Rays are usually four cells wide. Some of the parenchymatous cells in the cortical, phloem and xylem region contain large cluster crystals of calcium oxalate mostly of 25 μm to 30 μm in diameter.

Most of parenchymatous cells contain starch grains (Figure 2). After reaction with the IKI solution starch appear blue to black. The starch granules are abundant and mostly simple but a few are compound with two to four components. Individual granules are small, 5 μm to 20 μm in length, whereas most grains were less than 12 μm in diameter, spherical to ovoid or subreniform and rather irregular. They usually have a well marked circular or slit-shaped hilum, ***Metcalfe*** and ***Chalk***, 1974, ***Jackson*** and ***Snowdon***, 1990. Some parenchyma cells are developed as mucilage cells whish are more rounded in outline than the surrounding cells.

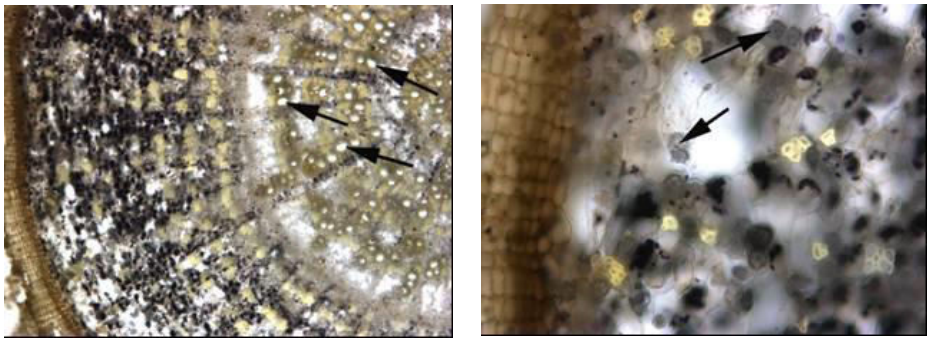

Figure 2. Iodine staining of taproot (transverse section)
Left: Black starch granules in secondary phloem and rays. Note wide vessels (arrowed) in secondary xylem. Many of the smaller cells in this section are xylem vessel members too (x50).
Right: Peridermis and detail of cortex parenchyma cells rich in starch. Note the pattern of alternating small patches of lignified fibres and conductive phloem cells. Cluster crystals of calcium oxalate in some of the parenchyma cells (arrowed) (x200).
Glavni koren obojen Lugolovim rastvorom (poprečan presek)
Levo:Tamne granule skroba u sekundarnom floemu i zraci. U sekundarnom ksilemu se uočavaju široke traheje (označeno strelicama) (x50).
Desno: Periderm i detalj parenhimskih ćelija korteksa koje su bogate skrobom. U sekundarnoj kori uočavaju se grupe lignifikovanih vlakana i ćelija floema (x200).

Mucilage is placed in large mucilage cells and also in large numerous schizogenous and lysigenous mucilage canals presented in the cortex and the pith (Figure 2, Figure 3, Figure 4). The marshmallow root has a large number of mucilage-containing cells in both, the central part of the root and in the root cortex. In very young roots, the mucilage cells are found to be much larger than in the surrounding parenchyma, but there is no indication of any of the cell containing mucilage. However, during the root development, a mucilage layer is formed on the inside of the cell walls. Cells containing mucilage were stained red using safranine (Figure 3) or deep blue u sing alcian blue (Figure 4), but remained unstained by the

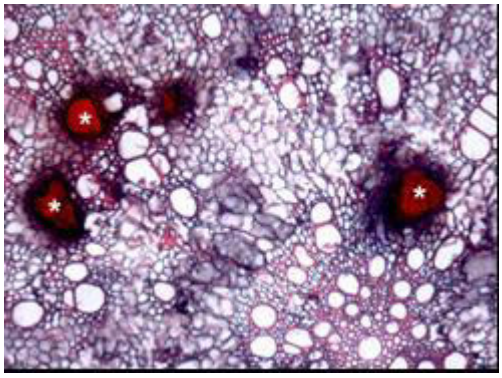

Figure 3. Secondary xylem in taproot stained with safranin and hematoxylin. Asterisks indicate mucilage deposition (x200)
Sekundarni ksilem u glavnom korenu obojen safraninom i hematoksilinom. Zvezdicama su označena mesta akumulacije sluznih materija (x200)

iodine application (Figure 2). According to ***Kraemer***, 1898, mucilage is frequently produced by a metamorphosis of the cell walls. There are various transformations of essential cellulose into mucilage (e.g. roots of *Symphytum*) and other mucilage may doubtless be indebted to starch for its origin (e.g. mucilage of the orchid bulbs). In a general way it may be said that all mucilage from cellulose and starch gives a blue-violet and sometimes a yellow colour with iodine. The mucilage of the root of *Althaea officinalis* is gummy mucilage that swells, but does not dissolve in water and does not colour blue with iodine. However, mucilage cells in the genus *Althaea* are easily stained with 1% ruthenium red, ***Metcalfe*** and ***Chalk***, 1974.

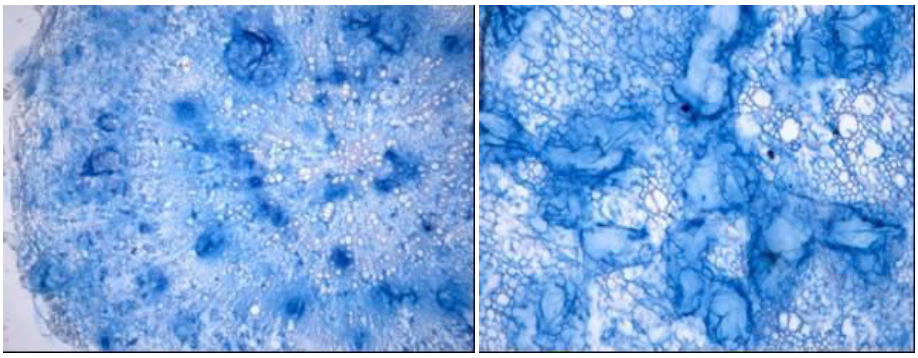

Figure 4. Transverse section through an Althaea root and alcian blue staining showed that the abundant mucilage is situated in mucilage cells and in intercellular. Note deep blue stained mucilage deposits in the cortex and the secondary xylem zone (Left x25, Right x100)
Poprečni presek korena belog sleza i bojenje alcijan plavim pokazuje da se velika količina sluzi nalazi u sluznim ćelijama i međućelisjkim prostorima. Tamnoplavo obojene sluzne materije u korteksu i zoni sekundarnog ksilema (levo x25, desno x100)

The conventional preparative methods that involve aqueous fixatives present the difficulty for searching the mucilage, since most of mucilage is fully hydrated during the fixation, which might cause its leakage from cells and intercellular spaces. The application of toluidine blue has marked the polyphenols, such as lignin and tannins which were stained green to blue-green, while pectin and pectic substances were coloured pink and purple (Figure 5). However, remarkable

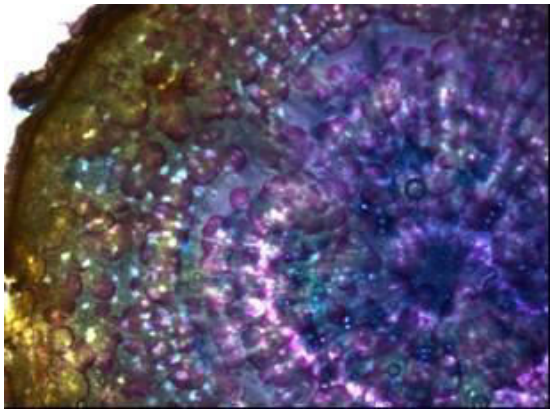

Figure 5. Toluidine blue staining of the taproot. The purple colour indicates the presence of pectin compounds (x25).
Bojenje glavnog korena toluidin plavim. Ljubičasta boja ukazuje na prisustvo pektniskih jedinjena (x25)

staining with toluidine blue was expected, because it is well known that the marshmallow crude drug (*Altaeae radix*) besides mucilage (35%) and starch (37%), is rich in pectin (11%) and other substances, such as sugars (11%), fat (1,25 %) and asparagin (up to 2 %), ***Blumenthal et al***., 2000. Although the root contains 25% to 35% of mucilage, ***Windholz***, 1983, ***Evans***, 1989, the content of the individual, purified mucilaginous polysaccharides is much lower. The mucilage of marshmallow plants is a complex sugar composed of a number of polysaccharides. The structure of the marshmallow polysaccharides is such that they cannot be digested by the human body, and thus, it is believed that they are the major components responsible for therapeutic effects, ***Deters et al***., 2009. The root mucilage contains at least three distinct polysaccharides, namely galakturonorhamnan, glucan and arabinogalaktan (or arabinan and galactan), ***Karawya et al***., 1971, ***Tomoda et al***., 1980, ***Madaus et al***., 1987. According to more recent data, ***Kačurakova et al***., 2000, cell wall polysaccharides of *Althaea officinalis* roots are composed of arabinan, rhamnogalacturonan and glucan. Besides polysaccharides, the root contains pectin, sugars, asparagine, tannins and fatty oil, ***Leung*** and ***Foster*** 1980, ***Windholz***, 1983. Other constituents are flavone glycosides (about 0.2%), phenolic acids, the coumarin scopoletin and starch, ***Deters et al***., 2009.

Conclusion

Althaea officinalis is one of the most appreciable medicinal plants, used as a herbal remedy for therapeutic indications for gastric/duodenal ulcers and dry cough, upon effects of its mucilage as an active substance. The mucilage of marshmallow plants is a complex sugar composed of a number of polysaccharides, pectin, sugars, flavone glycosides, phenolic acids, tannins and starch. The anatomy of the taproot, lateral roots and root head were observed upon the application of standard microscopic and histochemical techniques aiming to identify the mucilage and its localisation within particular root tissues and cells. Results showed that main sites of mucilage are linked to root parenchyma of the secondary phloem and xylem, as well as, large numerous schizogenous and lysigenous mucilage canals presented in the cortex and the pith. Marshmallow root has a large number of mucilage-containing cells in both, the central part of the root and the root cortex. Cells containing mucilage were stained red using safranine or deep blue using alcian blue, but remained unstained by the iodine application. A further research will be focused on relations between the mucilage content and the number and/or the size of the mucilage cells.

Acknowledgements

Authors would like to thank the Ministry of Science of the Republic of Serbia for supporting this study by financing the Project: TR 20113.

References

Blumenthal, M., A. Goldberg and ***J. Brinckmann*** (2000): Herbal Medicine: Expanded Commission E Monographs, Blumenthal, M., A. Goldberg and J. Brinckmann (eds), Austin, TX, American Botanical Council: Integrative Medicine Communications, pp. 244-248.

Deters, A., J. Zippel, N. Hellenbrand, D. Pappai, C. Possemeyer and ***A. Hensel*** (2009): Aqueous extracts and polysaccharides from marshmallow roots (*Althea officinalis* L.). Cellular internalisation and stimulation of cell physiology of human epithelial cells *in vitro*. J. Ethnopharmacol. doi:10.1016/j.jep.2009.09.050.

Elmastas M., L. Ozturk, I. Gokce, R. Erenler, H.Y. Hassan and ***H.Y. Aboul-Enein*** (2004): Determination of antioxidant activity of marshmallow flower (*Althaea officinalis* L.). Anal. lett. **37** (9): 1859-1869.

ESCOP (2003): ESCOP Monograph (European Scientific Co-operative on Phytotherapy) The Scientific Foundation for Herbal Medicinal Products, 2nd ed. Thieme Publishers, New York, pp. 32–37.

Evans, W.C. (1989): Pharmacognosy, 13th ed. Bailliere Tindall, London, U.K.

Franz, G. (1966): Die Schleimpolysaccharide von *Althaea officinalis* und *Malva silvestris*. Planta Med. 14: 90-110.

Jackson, B.P. and ***D.W. Snowdon*** (1990): Atlas of Microscopy of Medicinal Plants, Culinary Herbs and Spices, ed. CBS Publishers Delhi, pp. 257.

Jensen, W.A. (1962): Botanical Histochemistry: Principles and Practice, ed. W.H. Freeman and Co., San Francsco and London, pp. 408.

Kačurakova, M., P. Capek, V. Sasinkova, N. Wellner and ***A. Ebringerova*** (2000): FT-IR study of plant cell wall model compounds: pectic polysaccharides and hemicelluloses Carbohydrate Polymers 43: 195-203.

Karawya, M.S., S.I. Blabaa and ***M.S.A. Afifi*** (1971): Investigation of the carbohydrate contents of certain mucilaginous plants. Planta Medica 20: 14-23.

Kraemer, H. (1898): Origin and detection of mucilage in plants. Amer. J. Pharm. 70: 6.

Leung, A.Y. and ***S. Foster*** (1980): Encyclopedia of Common Natural Ingredients Used in Food, Drugs, and Cosmetics, ed. J. Wiley and Sons New York, NY, USA.

Madaus A., W. Blaschek and ***G. Franz*** (1987): *Althaeae radix* mucilage polysaccharides, isolation, characterization and stability. Pharm. Weekblad Sci. ed. 9: 239.

Metcalfe, C.R. and ***L. Chalk*** (1974): Anatomy of the Dicotyledons, Vol. I and II, ed. Oxford Clarendon Press, London.

Nosalova, G., A. Strapkova, A. Kardosova and ***P. Capek*** (1993): Antitussive activity of a rhamnogalacturonan isolated from the roots of *Althaea officinalis* L., var. *robusta*. J. Carbohydrate Chem. **12** (4/5): 589-596.

O'Brien, T.P., N. Feder and ***M.E. McCully*** (1964): Polychromatic staining of plant cell walls by toluidine blue O. Protoplasma **59** (2): 366-373.

Pakravan, M., H. Abedinzadeh and ***J. Safaeepur*** (2007): Comparative studies of mucilage cells in different organs in some species of *Malva*, *Althaea* and *Alcea*. Pakistan J. Biol. Sc. **10** (15): 2603-2605.

Ruzin. S.E. (1999): Plant Microtechnique and Microscopy, ed. by Oxford University Press, New York, New York, USA.

Schmidgall J., E. Schnetz and ***A. Hensel*** (2000): Evidence for bioadhesive effects of polysaccharides and polysaccharide-containing herbs in an *ex vivo* bioadhesion assay on buccal membranes. Planta Med. **66** (1): 48-53.

Tomoda M., N. Satoh and ***K. Shimada*** (1980): Plant Mucilages. XXIV. The structural features of althaea-mucilage O, a representative mucous polysaccharide from the roots of *Althaea officinalis*. Chem. Pharm. Bull. 28: 824-830.

Windholz M. (1983). The Merck Index, 10th ed. 1983, Merck & Co. Inc., Rahway, NJ, pp. 1301.

Received: 08/10/2009
Accepted: 20/11/2009

UDK 633.882

Anatomske karaktersitike korena belog sleza (*Althea offcinalis* L.)

- Originalni nuačni rad -

Dragana RANČIĆ[1]Slobodan DRAŽIĆ[2], Zora DAJIĆ STEVANOVIĆ[1] i Radenko RADOŠEVIĆ[1]
[1]Poljoprivredni fakultet, Beograd-Zemun
[2]Institut za proučavanje lekovitog bilja "Dr Josif Pančić", Beograd

R e z i m e

Beli slez (*Althaea officinalis* L.) je višegodišnja zeljasta biljka iz familije *Malvaceae*, čiji se cvetovi, listovi i, posebno, koren koriste kao droga, kako u narodnoj, tako i u zvaničnoj medicini. Zbog velikog sadržaja sluzi koren belog sleza se prvenstveno upotrebljava kao sredstvo u lečenju respiratornih obolenja. Anatomske karakteristike građe korena belog sleza iz različitih zona (glava korena, glavni koren i bočni korenovi) proučavane su analizom trajnih i privremenih mikroskopskih preparata, uz primenu različitih histohemijskih metoda, u cilju identifikacije i lokalizacije sluznih materija. Rezultati analize anatomske građe korena belog sleza pokazali su da parenhimske ćelije i intercelularni prostori u parenhimu sekundarnog floema i sekundarnog ksilema predstavljaju mesta sinteze, odnosno akumulacije sluzi, pri čemu kod starijih zona korena (glava korena i glavni koren) se u tom smislu ističe zona sekundarnog ksilema, a u mlađim, bočnim korenovima, zona sekundarne kore, tj. sekundarnog floema, što je potvrđeno i histohemijskim analizama specifičnih bojenja sluznih materija alcijan plavim. Ćelije sržnih zrakova predstavljaju mesta akumulacije skroba, što je pokazano reakcijom sa Lugolovim rastvorom. Prikazani rezultati mogu biti od praktičnog značaja za poznavanje kvaliteta droge belog sleza, utoliko što građa i veličina zona sa sekretornim ćelijama korena ove biljke najverovatnije korespondira sa količinom i hemijskim sastavom aktivne supstance.

Primljeno: 08.10.2009.
Odobreno: 20.11.2009.

Adresa autora:
Dragana RANČIĆ
Poljoprivredni fakultet
Nemanjina 6
11080 Beograd-Zemun
Srbija
E-mail: rancicd@agrif.bg.ac.rs