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# DETERMINATION OF BIOLOGICALLY ACTIVE SUBSTANCES IN TAPROOT OF COMMON CHICORY (*CICHORIUM INTYBUS L.*)

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***Abstract***

*The object of our current study is to determinate the biologically active substance presented in the taproots of Bulgarian medicinal plant common chicory (Cichorium intybus L.). The carbohydrate composition, the amount of total phenols, the total flavanoides content and the antioxidant activity in the obtained sequential ethanol and water extracts has been evaluated. The amount of inulin-type fructans was defined by the resorcinol assay. The sugars, fructooligosacharides and inulin contents of the obtained extracts were analyzed by TLC and HPLC-RID methods. The total phenolic and flavonoid quantities were analysed by using Folin–Ciocalteu's and $Al(NO_3)_3$ reagents, respectively. The antioxidant activity was defined by four method (DPPH, ABTS, FRAP and CUPRAC). The total fructan content in taproots is in range 23 % dw. The presence of monosaccharide glucose, fructose, sucrose and trisaccharides 1-kestose, nystose in the ethanol extracts was observed in the ethanol extracts. The analysis of water extracts revealed the high level of inulin (14 %), total phenols and flavonoids (7 mg/g GAE dw and 2 mg QE/g dw, respectively). The 95 % (v/v) ethanol extracts of roots collected during autumn showed the most well-pronounced antioxidant activity as followed: DPPH - 31 mM TE/g dw, ABTS - 49 mM TE/g dw, FRAP - 28 mM TE/g dw and CUPRAC - 127 mM TE/g dw.*

***Key words****: fructooligosacharides, inulin, common chicory, antioxidant activity*

## INTRODUCTION

*Cichorium intybus* L. known as common chicory or wild succory is a member of Compositae family and has been considered as an important medicinal herb (Kocsis et al., 2001). It is an erect, glandular, biennial plant with a taproot, rosette of 30-70 leaves and stem up to 90 cm height (Ilaiyaraja and Khanum, 2010) (Figure 1). This herb usually grows like a weed or flower near to the roadside and meadows (Ozuturk et al., 2006).

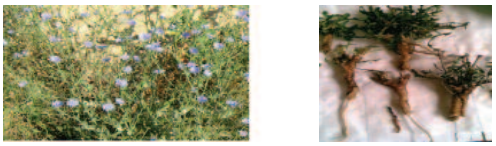

Figure 1. Common chicory (*Cichorium intybus* L.) - aerial parts and roots

The whole plant has numerous applications in food industry and medicine (Ilaiyaraja and Khanum, 2010). Its dried roots were used as a substitute or adulterant in coffee powder (Jung et al., 1996). The young leaves can be added to salads and vegetable dishes, while chicory extracts are used for the production of invigorating beverages. Except because of its nutritive value, wild chicory is used for winter forage for ruminant animal (Ozturk, 2006).

It has been reported that this plant is an important source of fructans and chicoric acid (Milala et al., 2009). It contains also saccharides, organic acids, polyphenols (Jurgonbski et al., 2011) such as chlorogenic acid, caffeic acid derivatives (Kumari et al., 2007). Because of its rich content of biologically active substances such as inulin, coumarins, vitamins, bitter compounds, flavonoids and sesquiterpene lactones, the whole plant extracts is used as anti-diabetic (Pushparaj et al., 2007), antioxidant (Gazzani et al., 2000; Papetti et al., 2002), antibacterial (Petrovic, 2004), immunotoxic (Kim et al., 2003), antihepatotoxic (Zafar and Ali, 1998; Ahmed et al., 2003), antiulcerogenic, anti-inflammatory, appetizer, digestive, stomachic, depurative (Rastogi et al., 1994), for curing

different diseases connected with gastrointestinal system. Chicory roots have been used in folk medicine for livers disorders, gallstone and inflammations of the urinary tract since 17 th century (Kocsis et al., 2001). It has been traditionally used to cure various ailments in Ayurvedic and Unani systems (Ilaiyaraja and Khanum, 2010). Leaves and roots of *Chicorium intybus* L. were also used for purification of blood, for curing arteriosclerosis and they are also considered to possess anti-arthritis, anti-spasmodic, hypotensive and laxative action (Tiwari, 2008).

During the past decade, there is a growing interest in natural plant extracts with potential antioxidant activity, because of their improved healthy effect (Aleksieva et al. 2013; Mihaylova et al. 2013). The expanded application is due to their protective properties against oxidative stress disorders, as well as oxidative damage in food products (Ivanov et al., 2014).

It is well known that polyphenols from plant extracts possessed strong antioxidant activities. Their presences in medicinal plants that are natural source of inulin-type fructans prebiotics additionally increase the biological activity of the obtained extracts (Petkova et al., 2012; Vrancheva et al., 2012). However, there are uncompleted information about presence of inulin, total phenols and flavonoids in root of medicinal plants common chicory (*Cichorium intybus* L.), growth in Bulgaria. Not detailed investigations have been reported regarding evaluation of antioxidant potency of common chicory roots.

Therefore, the aim of the current study was to determinate the content of biologically active substances and to evaluate antioxidant activities of extracts obtained from roots of common chicory (*Cichorium intybus* L.) gathered during the spring and autumn seasons from Bulgaria.

## MATERIALS AND METHODS

The taproots of several random chosen plants of common chicory (*Cichorium intybus* L.) were collected from territory of Bulgaria - Kresna (Blagoevgrad region) and Chehlare village (Sredna Gora Mountain) during May and November 2012. The underground parts were air-dried, finely ground and passed through a 0.5 mm sieve. The prepared samples with approximately 8.7% moisture content were stored in dry containers for further use.

All used reagents and solvents were of analytical grade scale. Carbohydrate standards fructose, sucrose, 1-kestose and nystose have been purchased from Sigma-Aldrich (Steinheim, Germany). Fructooligosacchrides Frutafit®CLR, and inulin Frutafit®TEX were supplied by Sensus (Roosendaal, the Netherlands). Frutafit®CLR contains high level of oligofructoses with the average chain length of 7-9 monomers. Frutafit®TEX is characterized with mean degree of polymerization DP 22.

Ethanol and subsequent water extraction procedure was applied to obtained fructans, total phenols and flavonoids from the taproots of common chicory. The extraction process was carried out as follow: 0.7 g dry chicory roots were extracted three hours with 95 % (v/v) boiling ethanol. Then, the residue was treated three hours with boiling distilled water. The fructan content in the obtained extracts expressed as fructose equivalent were analyzed spectrophotometrically at wavelength 480 nm by resorcinol-thiourea reagent (Petkova and Denev, 2013).

TLC analysis were used to elucidate the presence of mono-, di-, fructooligosaccharides (FOS) and inulin in the ethanol and water extracts from common chicory roots. Five microliters of each sample were performed on silica gel 60 $F_{254}$ plates (Merck, Germany) with mobile phase *n*-BuOH:*i*-Pro:$H_2O$:$CH_3COOH$ (7:5:4:2) (v/v/v/v). The TLC plates were dipped in the detecting reagent diphenylamine-aniline-$H_3PO_4$–acetone (Lingyun et al., 2007), heated and scanned as previously described (Petkova and Denev, 2013).

The sugars, FOSs and inulin content in root extracts were analyzed by HPLC methods. Chromatographic separations were performed on HPLC Shimadzu, coupled with LC-20AD pump, refractive index detector Shimadzu RID-10A. The control of the system, data acquisition, and data analysis were under the control of the software program LC solution version 1.24 SP1 (Shimadzu Corporation, Kyoto, Japan)

The determination of inulin and sugars in water extracts were performed on a Shodex® Sugar SP0810 with $Pb^{2+}$ a guard column (50 × 9.2 mm i.d.) and an analytical column (300 mm × 8.0 mm i.d.) at 85 °C. The mobile phase used for separation was distilled water with flow rate 1.0 ml/min. The injection volume of the samples was 20 µL (Petkova et al., 2013).

Folin–Ciocalteu's method was used for determination of total phenols. The results were expressed as mg gallic acid equivalent (GAE) on dry weight bases (Ivanov et al., 2014).

The total flavonoids content was analysed by $Al(NO_3)_3$ reagent. The absorbance was measured at 415 nm. The results were presented as mg equivalents quercetin (QE) per g dry weight (DW), (Kivrak et al., 2009) according to the calibration curve, linear in range of 10-100 µg/mL quercetin as a standard.

The total antioxidant activity of ethanol and water extracts from taproots of common chicory (*Cichorium intybus* L.) was estimated by forth methods as follows: DPPH (1,1-diphenyl-2-picrylhydrazyl radical), ABTS (2,2`azinobis-(3-ethylbenzthiazoline-6-sulfonic acid)), FRAP (ferric reducing antioxidant power) and CUPRAC (cupric reducing antioxidant capacity).

DPPH assay: 0.15 ml of each extract was mixed with 2.85 ml freshly prepared DPPH solution (0.1 mM in methanol). After incubating for 15 minutes at 37 ºC in darkness, the absorbance at 517 nm was measured with spectrophotometer in comparison to the blank containing methanol and % inhibition were calculated.

For ABTS assay, 0.15 ml extract was mixed with 2.85 ml of the ABTS solution previously diluted with methanol (1:30; v/v). After 15 min at 37 ºC in darkness, the absorbance of formed complex was measured spectrophotometrically at 734 nm (Ivanov et al., 2014).

FRAP assay: 0.1 ml of investigated extracts were added to 3 ml FRAP reagent (0.3 M acetate buffer (pH 3.6): 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ): 20 mM $FeCl_3 \times 6H_2O$ (10:1:1; v/v/v) and allowed to react for 10 min at 37 °C in darkness. The absorbance of the formed coloured product was measured at 593 nm (Benzie and Strain, 1996).

CUPRAC assay: The reaction was started by mixing of 1 ml $CuCl_2 \times 2H_2O$, 1 ml Neocuproine (7.5 ml in methanol), 1ml 0.1 M ammonium acetate buffer; 0.1 ml of analyzed extracts and 1 ml d. $H_2O$. The reaction time was 20 min at 50 °C in darkness. After cooling the absorbance was measured at 450 nm (Marchev et al., 2012). All the results from the determination of antioxidant activity were performed in triplicates and expressed as mM Trolox equivalents (mM TE) by dry weight. All the data were expressed as mean ± standard deviation (SD).

Statistical analysis was performed using MS Excel 2010. The p values less than 0.05 were considered as significantly different.

## RESULTS AND DISCUSSIONS

The results from the TLC analysis of the sequentially obtained ethanol and water of common chicory (*Cichorium intybus* L.) showed that a large number of carbohydrates were successively extracted (Figure 2). The presence of sugars fructose, sucrose, FOSs including 1-kestose, nystose and 7-9 oligomers, equivalent with used standards Frutafit CLR, was established in all investigated 95 % (v/v) ethanol extracts (Figure 2 A).

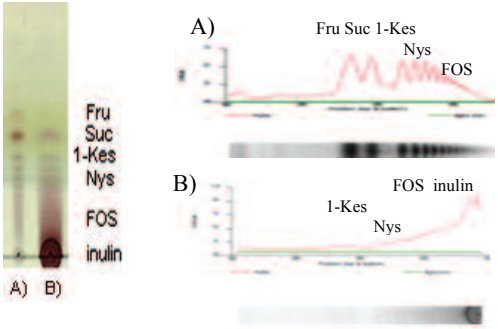


Figure 2. Thin-layer chromatograms of 5 µl A) ethanol and B) water extracts from root of *Cichorium intybus* L., where Fru - fructose, Suc - sucrose, 1-Kes - 1-kestose (GF2), Nys - nystose (GF3), fructooligosaccharides (respectively GF4, GF5, GF6, GF7, GF8) and inulin

In the water extracts, obtained after ethanol pretreatment, except 1-kestose, nystose, FOSs, the presence of high molecular fraction of inulin with DP 22 similar to the used standard was observed (Figure 2 B).

The spectrophotometric analysis showed the low and high molecular fructan fractions expresses as fructose equivalents (Table 1). The

established total carbohydrate content was 23 g/100 g dw. Bagaoutdinova et al. (2001) reported similar results. The detailed analysis of HPLC results of the studied extracts (Table 2) showed that the taproots of common chicory

Table 1. Fructan content expressed as fructose equivalents in the extracts from of common chicory (*Cichorium intybus* L.), g/100 g dw[1] (mean ± SD[2], n=4)

| Location | Harvest time | Low-molecular fraction (Fru[3], Suc[4] and FOSs) | High-molecular fraction (inulin) | Total |
|---|---|---|---|---|
| Kresna | May 2012 | 9.3 ± 0.7 | 14.1 ± 1.2 | 23.4 ± 0.7 |
| Chehlare | November 2012 | 6.8 ± 0.3 | 16.8 ± 0.3 | 23.1 ± 0.6 |

[1]dry weight, [2]SD – standard deviation, [3]Fru – fructose, 4 - sucrose

Table 2. Carbohydrates content in root extracts *Cichorium intybus* L.

| Location | Harvest time | Fructose | Sucrose | 1-Kestose | Nystose | Inulin |
|---|---|---|---|---|---|---|
| Kresna | May 2012 | 1.6 | 2.4 | 1.3 | 0.9 | 12.6 |
| Chehlare | November 2012 | 1.2 | 1.9 | 1.5 | 1.2 | 16.2 |

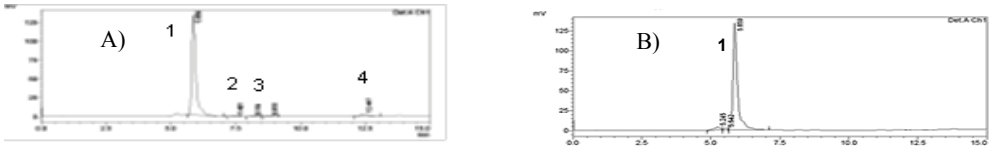


Figure 3. HPLC chromatograms of water extract obtained from taproots of chicory harvest from different locations and season: a) Kresna (May) and b) Chehlare (November), where 1. inulin; 2. nystose 3. sucrose, 4 fructose

are rich source of sugars and short chain fructooligosacharides. Sucrose was presented in both extracts. The content of 1-kestose and nystose, which possessed well pronounce probiotic effect (Van Loo, 2004), reached above 2 % dw. Moreover, the content of inulin in the investigated plant samples was relatively high - 16 % dw. HPLC chromatograms of carbohydrates in collected root are presented in Figure 3. The obtained results for inulin content in November taproots were with 2 % higher than reported by Milala et al. (2009). Therefore, the investigated plants contained constant level of inulin type fructans independently from harvest time and location. Chicory roots can be applied as a rich source of soluble dietary fibers in preparation of healthy food and nutrition formula.

The obtained extracts from taproots of common chicory harvest during the autumn showed the highest total phenolic contents: 7.9 ± 0.9 (in 95 % (v/v) ethanol) and 6.7 ± 0.9 (subsequent water extraction) mg GAE/g DW, respectively (Table 3). The similar results for 95 % ethanol were reported by Jurgonbski et al. (2011) and Özgen et al., (2004). Koleva et al. (2012) obtained 70 % EtOH extract from *Cichorium intybus L.* with flavonoid content less than 1 mg/ml. However, until now the detailed results for quantitative evaluation of total flavonoids in 95 % v/v EtOH and water extracts for roots of common chicory have not been reported. In our study the highest level of total flavonoids was observed in water extracts obtained after ethanol pre-treatment for both plants (2.8 ± 0.2, mg EQ/g dw) (Table 3).

Table 3. Total phenolic contents and total flavonoids contents of *Cichorium intybus* L. extracts.

| Harvest time | Extracts | Total polyphenols, mg GAE[1]/g dw | Total flavonoids, mg EQ[2]/g dw |
|---|---|---|---|
| | | Mean±SD[3] | |
| May | 95% EtOH | 4.3 ± 0.5 | 0.6 ± 0.1 |
| | water | 3.7 ± 0.4 | 2.7 ± 0.2 |
| November | 95% EtOH | 7.9 ± 0.9 | 1.0 ± 0.1 |
| | water | 6.7 ± 0.9 | 2.8 ± 0.2 |

[1]Expressed as milligram of gallic acid per gram dry material, [2]Expressed as milligram of quiercetin per gram dry extract, [3]SD – standard deviation (n=6)

To evaluate the antioxidant activities of obtained ethanol and subsequent water extracts from common chicory roots, their abilities to scavenge the synthetic DPPH and ABTS radicals, as well as their power to reduce ferric (FRAP) and cupric (CUPRAC) ions were

investigated. The analysis were performed in triplicates and expressed as mM Trolox equivalents (mM TE) by dry weight (Table 4).

Table 4. Antioxidant activity of the extracts obtained from roots of common chicory (*Chicorium intubus* L.)

| Harvest time | Extracts | DPPH | ABTS | FRAP | CUPRAC |
|---|---|---|---|---|---|
| May 2012 | EtOH | 29.2±0.5 | 8.6±0.6 | 1.2±0.2 | 64.6±2.0 |
| | $H_2O$ | 16.1±2.8 | 1.8±0.3 | 1.5±1.5 | 58.5±3.3 |
| November 2012 | EtOH | 31.3±0.1 | 9.9±0.7 | 8.7±0.2 | 123.6±0.6 |
| | $H_2O$ | 29.1±0.8 | 6.1±0.5 | 5.7±0.5 | 114.2±4.3 |

Until now, not detailed information was available for antioxidant activity of root from common chicory grown on territory of Bulgaria. Only radical scavenging activities of methanol and water extracts from *Chicorium intubus L.* evaluated by DPPH method were reported (Özgen et al., 2004; Ilaiyaraja and Khanum, 2010; Nikolova et al., 2011).

For the first time we evaluated the antioxidant potential of 95 % (v/v) EtOH and subsequent water extracts by four methods DPPH, ABTS, FRAP and CUPRAC. Among the results obtained during our investigation, the 95 % (v/v) EtOH extract showed the highest antioxidant activities (31.3±0.1, 49.9±0.7, 28.7±0.2, 123.6±0.6 mM TE/g dw for methods DPPH, ABTS, FRAP and CUPRAC, respectively). In addition the ethanol extracts also had the highest total phenolic content (Table 3) 4.3 mg/g and 7.9 mg/g GAE, that may be the possible explanation about their enhanced antioxidant activity. The most common explanation of the observed tendency was the presence of phenolic acids and flavonoids in them (Ivanov et. al., 2013). According to Ilaiyaraja and Khanum (2010) the hydrogen donating ability of these compounds is responsible for their effective antioxidant property and used for protecting against cellular oxidative damage. It has been reported that not only phenolic compounds can be responsible for the *in vitro* antioxidant activity of chicory preparations. Jurgonbski et al. (2011) proposed that sugars themselves, especially sucrose and fructans, could also act as radical scavengers in plant cells. The presence of inulin and FOS in roots of common chicory additionally improve their biological activity.

## CONCLUSIONS

The current report is the first comprehensive study that presented detailed information for inulin, FOSs, phenolic, flavonoids content and antioxidant activity of edible taproot from common chicory (*Cichorium intybus* L.) grown in Bulgaria. The antioxidant potential of extracts positively correlated with their phenolic contents and flavonoids contents, respectively. Except important source of inulin-type fructans the determined metabolites profile of its roots revealed their potential application as radical scavengers due to the presence of polyphenols. Therefore, this complex of biologically active substance in their roots offers many future applications in field of herbal medicine and nutrition for production of healthy food with well-pronounced healthy effect.

## REFERENCES


Ahmed B., Tawfeq A., Howiriny A., Siddiqui B., 2003, Antihepatotoxic activity of seeds of Cichorium intybus. J. Ethnopharmacol., 87(2-3), p. 237-240.

Alexieva I., Mihaylova D., Popova A., 2013. Evaluation of the antioxidant capacity of aqueous extracts of fresh samardala (*Allium bulgaricum* L.) leaves, Scientific works, vol. LX, Plovdiv, p. 826-831

Benzie F., Srain J., 1996. Ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Anal. Biochem., 239, p. 70-76

Bagaoutdinova R. I., Fedoseyeva G. P., Okoneshnikova T. F., 2001. Fructose-containing carbohydrates in plants of different families localization and content, Chemistry and Computational Simulation. But. Com., Vol. 2, No.5.

Gazzani G, Daglia M, Papetti A, Gregotti C., 2000. In vitro and ex vivo anti- and prooxidant components of Cichorium intybus. J.Pharm Biomed Anal., 23, p. 127-133

Ilaiyaraja N, Khanum F., 2010, Evaluation of Antioxidant and Toxicological properties of Chicory leaves. International Journal of Pharmaceutical & Biological Archives, 1(2), p. 155 – 163

Ivanov I., Vrancheva R., Marchev A., Petkova N., Aneva I., Denev P., Georgiev G., Pavlov A., 2014.Antioxidant activities and phenolic compounds in Bulgarian Fumaria species, Int. J. Curr. Microbiol. App.Sci., 3(2) p. 296-306

Jung G., Shaffer J., Varga, Everhart G., 1996. J. Agron., 88, p. 104 - 111

Jurgonbski A., Milala J., Jusbkiewicz J., Zdunbczyk Z. and Kryl B., 2011. Composition of Chicory Root, Peel, Seed and Leaf Ethanol Extracts and Biological Properties of Their Non-Inulin Fractions. Food Technol. Biotechnol., 49 (1), p. 40–47

Kim J., Mun Y., Woo W., Jeon K., An N., Park J., 2002. Effects of the ethanol extract of Cichorium intybus on the immunotoxicity by ethanol in mice. Int Immunopharmacol., 2, p. 733-744

Kivrak I., Duru M., Öztürk M., Mercan N., Harmandar M., Topçu G., 2009. Antioxidant, anticholinesterase and antimicrobial constituents from the essential oil and ethanol extract of Salvia potentillifolia. Food Chem., 116, p. 470-479

Koleva V., Chilev Ch., Penchev I., Simeonov E., 2012. Extracting of biologically active substances from Cichorium intybus, Scientific works of UFT: "Food science, engineering and technologies". UFT Academic Publishing House, Plovdiv, p. 198-200

Kocsis I., Hagymasi K., Kery A., Szoke E., Blazovics A., 2003. Effects of chicory on pancrease status of rats in experimental dyslipidaemia. Acta Biol. Szeged., 4, p. 143-146

Kumari B., Velayutham P., Anitha S., 2007. Comparitive study on inulin and esculin content of in vitro and in vivo plants of Chicory (Cichorium intybus L. Cv. Lucknow Local). Adv Biol Res; 1 (1-2): 22-25.

Lingyun W., Jianhua W., Xiaodong Zh., Da I., Yalin Y., Chenggang C., Tianhua F., Fan Zh., 2007. Studies of the extraction technical conditions of inulin from Jerusalem artichoke tubers. J. of Food Engineer, 79, 1087-1093

Marchev A., Petrova A., Nedelcheva D., Lazarova I., Trucheva B., Kostova N., Bankova V., Pavlov A., 2011. GS/MS profiles and antioxidant activity of extract from Lavandula vera MM and Rosa damascene Mill. Cell suspension cultures. Scientific works-UFT. Vol. LVIII, 2, p. 183-188.

Mihaylova, D., Georgieva, L., Pavlov, A. 2013**.** *In vitro* antioxidant activity and phenolic composition of *Nepeta cataria* L. extracts. International Journal of Agricultural Science and Technology, 1(4), p.74-79

Milala J., Grezelak, Król B., Juśkiewicz J., Zduńczyk Z., 2009. Composition and properties of chicory extracts rich in fructans and polyphenols. Pol. J. Food Nutr. Sci., 59 (1), p. 35-43

Nikolova M., Evstatieva L., Nguyen Th., 2011. Screening of plant extracts for antioxidant properties. Botanica Serbica, 35 (1), p. 43-48

Özgen U., Mavi A., Terzi Z., Coflkun M., Yildirim A., 2004. Antioxidant activities and total phenolic compounds amount of some asteraceae species, Turkish J. Pharm. Sci., 1 (3), p. 203-216

Ozurk, D., Bal M., Erol A., Sahin M., Ozkan C., Karakas E., Ata M., Karabay P., 2006. Determination of nutritive value of wild chicory forage harvest different maturity stage using in vitro and in situ measurements. Pakistan J. of Biol. Science 9 (2), p. 253-259

Papetti A, Daglia M, Gazzani G., 2002. Anti- and pro-oxidant activity of water soluble compounds in Cichorium intybus var silvestre (Treviso red chicory). J Pharm Biomed Anal., 30, p. 939-945.

Petkova N., Denev P., 2013. Evaluation of fructan content of the taproots of Lactuca serriola L. and Sonchus oleraceus L. Scientific Bulletin, Series F "Biotechnologies", Volume XVII, Bucharest, p. 117-122

Petkova N., Vrancheva R., Denev P., Ivanov I., Pavlov A., 2013, HPLC-RID method for determination of inulin and fructooligosacharides. Acta Scientifica Naturalis. (in press)

Petkova, N., Vrancheva R., Ivanov I., P. Denev P. Pavlov, A., Aleksieva J., 2012. Analysis of biologically active substances in tubers of Jerusalem artichoke (Helianthus tuberosus L.). 50 years FoodRDI International Scientific-Practical Conference" Food, Technologies & Health" Proceedings Book, p.

Petrovic J, Stanojkovic A, Comic L, Curcic S., 2004. Antibacterial activity of Cichorium intybus. Fitoterapia, 75, p. 737-739

Pushparaj P., Low K., Manikandan J, Tan B., Tan C.. 2007. Anti-diabetic effects of Cichorium intybus in streptozotocin-induced diabetic rats. J Ethnopharmacol; 111 (2), p. 430-434.

Rastogi R.P., Mehrotra B.N., 1994. Compendium of Indian Medicinal Plants. Rastogi, R.P. (Ed.). Orient Longman Ltd., Madras, India, p. 74

Tiwari S., 2008. I Jour. of Nat. Products, Vol. 1 p. 27-35

Van Loo, J., Coussement P., De Leenheer L., Hoebregs H., Smits, G., 1995). On the presence of inulin and oligofructose as natural ingredients in the Western diet. Critical Reviews in Food Science and Nutrition, 35, 525–552

Vrancheva R., Petkova N., Ivanov, I., Denev, P., Pavlov, A., Aleksieva, J., 2012. Carbohydrate composition and antioxidant activity of root extracts of Inula Helenium L., Youth Scientific conference "Klimentovi dni", 2012. Book of Abstracts, 3, p. 62.

Zafar R., Ali S., Anti-hepatotoxic effect of root and root callus extracts of Cichorium intybus L. J Ethnopharmacol., 1998, 63, p. 227