ABS ACTA BIOLOGICA SIBIRICA
A journal on the biodiversity of Siberia and the adjacent lands

Acta Biologica Sibirica 9: 265–277 (2023)
doi: 10.5281/zenodo.7865780
http://journal.asu.ru

RESEARCH ARTICLE

Primary phytochemical screening and spectroscopic assessment of chicory (*Cichorium intybus* L.)

Elizaveta P. Zotova[1, 2], Mikhail Yu. Cherednichenko[2], Elena A. Kalashnikova[2]

1 *ITMO University, 9 Lomonosova st., Saint-Petersburg, Russia*
2 *Russian State Agrarian University – Moscow Timiryazev Agricultural Academy, 49 Timiryazevskaya st., Moscow, Russia*

Corresponding author: Mikhail Yu. Cherednichenko (michael.tsch@gmail.com)

Academic editor: A. Matsyura | Received 5 May 2022 | Accepted 17 February 2023 | Published 29 April 2023

http://zoobank.org/3C082420-12F5-4BC0-8798-46C8FABBC7BD

Citation: Zotova EP, Cherednichenko MYu, Kalashnikova EA (2023) Primary phytochemical screening and spectroscopic assessment of chicory (*Cichorium intybus* L.). Acta Biologica Sibirica 9: 265–277. https://doi.org/10.5281/zenodo.7865780

Abstract

Chicory (*Cichorium intybus* L.) is a key source of valuable primary and secondary metabolites (inulin, vitamins, essential oil). The spectrum of medicinal action of chicory includes antimicrobial, anti-inflammatory, choleretic, diuretic, sedative, and appetite stimulating activities. Plant tissue and cell culture allow the production of high yields of plants, biomass, and valuable metabolites throughout the year. In our research, we produced aseptic plants from two Russian breeding (*Yaroslavsky* and *Petrovsky*), performed their primary phytochemical screening, and established the quantitative content of particular elements and substance groups using IR spectroscopy. It has been found that a high yield of viable aseptic plants of the *Yaroslavsky* cultivar can be achieved by seed treatment with 8% sodium hypochlorite for 10 minutes or 37% hydrogen peroxide for 5 minutes. For the *Petrovsky* cultivar, it is advisable to germinate nonsterile seeds and then introduce them into the culture by washing the seedlings in a slightly pink solution of potassium permanganate, followed by sterilization with 8% sodium hypochlorite for 5 minutes and washing with plants the antibiotic solution (0.8% gentamicin) for 15 minutes. All studied classes of compounds (tannins and phenols, flavonoids, saponins, alkaloids, glycosides, reducing sugars, and proteins) are present in aseptic plants of *C. intybus*; however, differences appear depending on the extractants (water and ethanol). IR spectroscopy has shown a higher content of moisture, fiber, and starch in seeds and a higher content of macronutrients, fat, ash, and proteins in aseptic plants.

Keywords
Chicory, *in vitro* culture, aseptic plants, phytochemical screening, IR spectroscopy

Introduction

Chicory is considered one of the oldest known plants. Its first references were found in the works of ancient scientists, like Theophrastus and Pedanius Dioscorides. The healing properties of chicory are well known and have been used as a medicinal plant in Europe, Asia, Africa, India, Indonesia and America. Since the 18th century, chicory has become one of the most common coffee substitutes. Today, chicory grows in the temperate and subtropical zones of both hemispheres and has ten species. *Cichorium intybus* L. is mainly cultivated in Russia. It is an upright biennial herb, a representative of the Asteraceae family (Varotto et al. 2000).

Chicory is a good honey plant that produces a large amount of nectar and pollen. Chicory roots are valuable food products, especially for people with diabetes, due to the presence of easily digestible substances. It is also widely used in the preparation of sweets and cakes and in the production of coffee and tea drinks, providing a specific taste, aroma6 and color (Juśkiewicz et al. 2006).

The whole plant is used for medicinal purposes. Roots contain bitter substances, cichorin, water-soluble inulin, choline, proteins, fats, pectin, vitamins C, B1 and E, fat, gum, essential oil, resins, tannins, mineral salts, and a large number of trace elements. Cichorin is also found in chicory flowers. Lactucin and lactucoperin, as well as inulin, ascorbic, and chicoric acids, were found in the latex of the stems and leaves. The seeds contain inulin and protocatechuic aldehyde (Innocenti et al. 2005; Heimler et al. 2009).

Chicory preparations have antimicrobial, anti-inflammatory, choleretic, diuretic, sedative, astringent and appetite stimulating effects. They have a regulatory effect on human metabolism, somewhat improve cardiac activity, and reduce sweating (Egwaikhide et al. 2009; Kagkli et al. 2016; Ning et al. 2017). Several studies have examined the antibacterial properties of chicory. Therefore, the introduction of chicory, inulin, fructose, and shredded chicory into the nutrient medium positively affects the accumulation of biomass of *Bifidobacterium bifidum* and contributes to its increase (Roberfroid et al. 1998; Azimi et al. 2013). Different groups of scientists have conducted studies on the inhibitory effect of chicory on various strains of microorganisms. For example, the inhibitory effect of chicory herb against the *Candida albicans* strain was discovered as a research result (Khodaii et al. 2020).

There are relatively few *in vitro* studies of *C. intybus* related to cultivars of Russian selection. Similar studies around the world have mainly investigated the effect of phytohormones and growth regulators on morphophysiological processes in the culture of chicory tissues and cells. For example, in 2006, a group of scientists from India studied the effect of growth regulators on the induction of the *C. intybus* callus (Velayutham et al. 2006). For plant regeneration, explants (cotyledons, leaves, hy-

pocotyls, and roots) were cultivated in nutrient medium with different concentrations of phytohormones or growth regulators in various combinations. Indol-3-acetic acid (IAA), indol-3-butyric acid (IBA), α-naphthaleneacetic acid (NAA) and 2,4-dichlorphenoxyacetic acid (2,4-D) were used to induce callus formation and the callusogenesis efficiency increased in the following order: NAA→IBA→IAA→2,4-D. Research fellows have also obtained similar results (Wagner and Gailing, 1996). After two weeks of culture in Murashige-Skoog (MS) medium with the addition of 0.5...10 mg/l IAA, IBA, NAA and 2,4-D + 2 mg/l 6-benzylaminopurine (BAP), callus formation was observed in all types of explants. In terms of callus formation, the following dependence was found among explants: leaf > cotyledons > hypocotyl > root (Park and Lim 1999; Rehman et al. 2003; Velayutham et al. 2006).

The best results (in terms of evaluating the effectiveness of callusogenesis) were achieved by adding 7.5 mg/l NAA + 2 mg/l BAP for leaf and cotyledon explants and 7.5 mg/l IBA + 2 mg/l BAP for hypocotyl and root explants in MS medium. Further subculturing of callus cells in optimal nutrient media increased the potential for shoot regeneration (Velayutham et al. 2006).

The value of representatives of the *Cichorium* L. genus determines the scientific interest in the genus demonstrated by the pharmaceutical and food industries. Introducing chicory into *in vitro* culture will allow for year-round plant cultivation, resulting in a higher biomass yield. Modern biotechnology methods will provide the opportunity to synthesize valuable sugars and secondary chicory metabolites on an industrial scale.

Materials and methods

Plant material

We used *C. intybus* seeds from *Yaroslavsky* and *Petrovsky* cultivars, harvested in 2017 and 2019, respectively, and kindly provided by the Rostov Experimental Station for Chicory.

Production of aseptic plants

The seeds of *C. intybus* from the *Yaroslavsky* and *Petrovsky* cultivars were sterilized using various sterilizing agents to obtain aseptic plant material. We started our research with the *Yaroslavsky* cultivar and continued with the *Petrovsky* cultivar. This explains the choice of sterilizing agent and sterilization mode. The seeds of the *Yaroslavsky* cultivar were sterilized using the following agents: 5% sodium hypochlorite, 8% sodium hypochlorite, 0.1% mercury (II) chloride, 70% ethanol, followed by treatment with 8% sodium hypochlorite, 10% hydrogen peroxide and 37% hydrogen peroxide. Before being introduced into *in vitro* culture, the seeds of the Petrovsky

cultivar were treated with the following sterilizing agents: 5% sodium hypochlorite, 8% sodium hypochlorite, 0.1% mercury (II) chloride, slightly pink solution of potassium permanganate followed by treatment with 8% sodium hypochlorite and 70% ethanol followed by treatment with 8% sodium hypochlorite. The seeds were sterilized for 5, 10, and 15 minutes with 5% and 8% sodium hypochlorite solutions. The exposure time was 2, 5 and 7 minutes for sterilizing the plant material with 0.1% mercury (II) chloride. In one of the variants, seeds were sterilized for 30 seconds in 70% ethanol before using 8% sodium hypochlorite (the exposure of seed sterilization in sodium hypochlorite remained the same – 5, 10 and 15 minutes).

For the *Yaroslavsky* cultivar, additional sterilization was performed in 10% and 37% hydrogen peroxide. Exposures were 2.5 and 7.5 minutes. For the *Petrovsky* cultivar, additional seed sterilization was performed in a slightly pink solution of potassium permanganate on a rotary shaker at 100 rpm for 20 minutes. The seeds were sterilized with 8% sodium hypochlorite for 5, 10, and 15 minutes. After disinfection, the seeds were thoroughly washed twice in distilled water. Under sterile conditions, the seeds were seeded in 24-well plastic plates (four replicates for each sterilization mode; 12 seeds per one replicate) in a hormone-free MS medium containing sucrose at a concentration of 3%. To control germination, the seeds germinated under nonsterile conditions on wet filter paper. The control seeds were watered daily with a small amount of distilled water. The seeds were left in a light room (16 hours of daylight and a constant temperature of 21°C) for ten days and germination data was recorded every two days.

We chose two sterilization modes for the introduction of non-sterile seedlings into aseptic cultures. Using the first, the seedlings were sterilized under aseptic conditions for 7.5 minutes with 8% sodium hypochlorite and washed twice in distilled water. Exposure was selected based on seed germination data. Using the second, the seedlings were sterilized under aseptic conditions for 5 minutes with 8% sodium hypochlorite, washed twice in distilled water and then placed in an antibiotic solution (0.8% gentamicin) for 15 minutes. On the basis of the results of the first variant, we decided to reduce the exposure to sodium hypochlorite solution. In both cases, the seedlings were preliminarily washed in a slightly pink solution of potassium permanganate for 20 minutes. After sterilization, non-sterile seedlings were placed one at a time in a glass culture tube on MS medium (ten seedlings for each variant).

The efficiency of the sterilization mode was subject to the highest yield of viable healthy explants and the proportion of plants susceptible to bacterial and fungal infection.

Primary phytochemical screening of aseptic plants

Ethanol and water extracts were obtained from dry and fresh plants to determine secondary metabolites in *C. intybus* plants. The plant material was ground in a ceramic plate to a grueling state and diluted with alcohol or distilled water in a 1: 2.5 (w / v) ratio to prepare the extracts. After that, the slurry was stirred and left for

infusion for 24 hours at room temperature. The extract was then filtered through two layers of filter paper and centrifuged at 14,000 g for 15 minutes. The resulting supernatant was used for further analysis of the content of the main groups of primary and secondary metabolites using standard methods: tannins and phenolic compounds, flavonoids, saponins, alkaloids, reducing sugars, proteins, and glycosides (Kumar et al. 2009; Baghel and Ray 2017; Choudhury et al. 2017).

IR spectroscopy

Infrared (IR) spectroscopy is based on the absorption of infrared electromagnetic radiation by molecules of the studied substance in which the vibrational and rotational states are activated. IR spectroscopy is a fundamental method to study the chemical structure of various compounds (Al-Tameme et al. 2015).

In this work, infrared spectroscopic analysis was performed on a SpectraStar XT infrared analyzer using the scientific and technical base of the Department of Chemistry of the Russian State Agrarian University – Moscow Timiryazev Agricultural Academy. During the analysis of *C. intybus* seeds and dried aseptic plants of the *Petrovsky* cultivar, the following parameters were determined: water, fat, ash, cellulose, starch, protein, as well as calcium and phosphorus. The dry plants were thoroughly ground on a ceramic plate to a dusty state. The seeds and shredded plants were placed in special cuvettes which were subsequently placed on the analyzer. The results of the analysis were obtained in graphical and digital formats in the UScan program; additional data processing was required.

Result

Production of aseptic plants

Obtaining *C. intybus* plant material free from external contamination required studying the effect of the sterilization mode on the germination efficiency and the further growth of *C. intybus* germinants. In seed experiments, the germination and susceptibility to bacterial and fungal contamination were estimated. The reported values were compared with the data for the germination of the control seeds on filter paper under nonsterile conditions (Tables 1 and 2).

The numerical data in Table 1 illustrate a significant decrease in seed germination at $\alpha = 0.05$, compared to control germination, after treatment with the following sterilizing agents: (1) 0.1% mercury chloride (II) for 5 and 7 minutes; (2) 70% ethanol followed by treatment with 8% sodium hypochlorite in the three exposures; and (3) 37% hydrogen peroxide for 2.5 and 7.5 minutes. Thus, the above sterilization modes are not recommended for obtaining aseptic plants, regardless of the contamination data.

Treatment of *Yaroslavsky* seeds with 5% sodium hypochlorite (in three exposures), 8% sodium hypochlorite (for 5 and 15 minutes), 0.1% mercury (II) chloride (for 2 minutes) and 37% hydrogen peroxide (for 5 minutes) led to an insignificant decrease in the germination rate compared to the control. Sterilization of seeds with 8% sodium hypochlorite for 10 minutes resulted in an insignificant increase in the germination rate. Therefore, these modes of sterilization can be considered optimal under the condition of low seedling contamination.

Table 1. Germination efficiency of the *C. intybus* seeds of the *Yaroslavsky* cultivar in different sterilization modes

Sterilizing agent	Exposure, min.	Germination efficiency, %	Common difference, %	LSD_{05}
Control (non-sterile seed germination)	-	60.00	-	-
5% sodium hypochlorite	5	43.33	-16.66	
	10	53.33	-6.66	
	15	33.33	-26.66	
8% sodium hypochlorite	5	50.00	-10.00	
	10	73.33	13.33	29.88
	15	36.67	-23.33	
0.1% mercury (II) chloride	2	50.00	-10.00	
	5	3.33*	-56.66	
	7	26.67*	-33.33	
70% ethanol + 8% sodium hypochlorite	5	10.00*	-50.00	42.26
	10	10.00*	-50.00	33.41
	15	15.00*	-45.00	
10% hydrogen peroxide	2.5	23.33*	-36.67	29.88
	5	10.00*	-50.00	33.41
	7.5	15.00*	-45.00	
37% hydrogen peroxide	2.5	30.00*	-30.00	
	5	46.66	-13.33	29.88
	7.5	10.00*	-50.00	

Table 2 presents the numerical data for seed contamination after applying different sterilization modes. We estimate the yield of aseptic plants when an insignificant decrease or increase in germination was observed. When seeds were treated with 8 % sodium hypochlorite for 10 minutes (in this variant, an insignificant increase in germination was observed), the same level of seedling infection was observed as in the control variant. Sterilization with 8% sodium hypochlorite for 5 and 15 minutes led to an insignificant increase and decrease in infection, respectively (in both cases, an insignificant decrease in germination was observed). Treatment with 5% sodium

hypochlorite resulted in an insignificant increase (5 and 15 minutes of treatment) and a significant increase (10 minutes of treatment) in seed contamination (an insignificant decrease in germination in all three exposures). Sterilization of seeds with 0.1 % mercury (II) chloride for 2 minutes (an insignificant decrease in germination) also led to a significant increase in seed infection compared to the control. After treatment with 37% hydrogen peroxide for 5 minutes, aseptic seedlings were obtained (an insignificant decrease in germination).

Table 2. Contamination of the *C. intybus* seeds of the *Yaroslavsky* cultivar in various sterilization modes

Sterilizing agent	Exposure, min.	Contamination, %	Common difference, %	LSD_{05}
Control (non-sterile seed germination)	-	23.33	-	-
5% sodium hypochlorite	5	40.00	+16.67	
	10	53.33*	+30.00	
	15	26.67	+3.33	
8% sodium hypochlorite	5	46.67	+23.33	
	10	23.33	0.00	
	15	13.33	-10.00	
0.1% mercury (II) chloride	2	53.33*	+30.00	
	5	43.33	+20.00	24.06
	7	13.33	-10.00	
70% ethanol + 8% sodium hypochlorite	5	46.67	+23.33	
	10	36.67	+13.33	
	15	20.00	-3.33	
10% hydrogen peroxide	2.5	26.67	+3.33	
	5	33.33	+10.00	
	7.5	30.00	+6.67	
37% hydrogen peroxide	2.5	6.67	-16.67	26.90
	5	0.00	-23.33	0.00
	7.5	0.00	-23.33	

A similar analysis was performed for the *C. intybus* seeds of the *Petrovsky* cultivar (Tables 3 and 4).

Based on the results presented in Table 3, it can be seen that all sterilization modes considered at $\alpha = 0.05$ led to a significant decrease in germination compared to the control.

The data in Table 4 show that the use of 70% ethanol followed by sterilization with 8% sodium hypochlorite and 5% sodium hypochlorite for 5 minutes led to a significant increase in the level of seed contamination compared to the control. The

following sterilization modes led to an unreliable decrease in the contamination level: potassium permanganate + 8% sodium hypochlorite (5 and 10 minutes), 0.1 % mercury (II) chloride (7 minutes) and 70% ethanol + 8% sodium hypochlorite (10 minutes). The following sterilization modes led to an insignificant increase in the level of contamination: 5% sodium hypochlorite (10 and 15 minutes), 8% sodium hypochlorite (5 and 10 minutes), potassium permanganate + 8% sodium hypochlorite (15 minutes), 70% ethanol + 8% sodium hypochlorite (15 minutes) and 0.1% mercury (II) chloride (2 and 5 minutes).

Table 3. Germination efficiency of the *C. intybus* seeds of the *Petrovsky* cultivar under different sterilization modes

Sterilizing agent	Exposure, min.	Germination efficiency, %	Common difference, %	LSD_{05}
Control (non-sterile seed germination)	-	56.25	-	-
5% sodium hypochlorite	5	18.50*	-37.75	18.79
	10	16.50*	-39.75	
	15	25.00*	-31.25	17.15
8% sodium hypochlorite	5	29.25*	-27.00	
	10	16.50*	-39.75	
Potassium permanganate + 8% sodium hypochlorite	5	16.50*	-39.75	18.35
	10	12.50*	-43.75	17.15
	15	16.33*	-39.92	
70% ethanol + 8% sodium hypochlorite	5	24.75*	-31.50	18.53
	10	16.50*	-39.75	
	15	22.00*	-34.25	17.15
0.1% mercury (II) chloride	2	16.75*	-39.50	
	5	11.00*	-45.25	18.53
	7	10.25*	-46.00	18.79

Table 4. Contamination of the *C. intybus* seeds of the *Petrovsky* cultivar under various sterilization modes

Sterilizing agent	Exposure, min.	Contamination, %	Common difference, %	LSD_{05}
Control (non-sterile seed germination)	-	33.25	-	-
5% sodium hypochlorite	5	68.75*	+35.50	
	10	56.00	+22.75	
	15	51.75	+18.50	29.40
8% sodium hypochlorite	5	39.50	+6.25	
	10	43.50	+10.25	

Sterilizing agent	Exposure, min.	Contamination, %	Common difference, %	LSD_{05}
Potassium permanganate + 8% sodium hypochlorite	5	16.50	-16.75	
	10	8.00	-25.25	
	15	62.25	+29.00	29.40
70% ethanol + 8% sodium hypochlorite	5	89.50*	+56.25	
	10	16.75	-16.50	
	15	55.67	+22.42	31.70
0.1% mercury (II) chloride	2	62.50	+29.25	36.00
	5	52.00	+18.75	46.40
	7	0.00	-33.25	0.00

Primary phytochemical screening of aseptic plants

Qualitative reactions for basic primary and secondary metabolites demonstrate that extracts from fresh and dry plants contain all classes of compounds studied: tannins, phenols, flavonoids, saponins, alkaloids, reducing sugars, proteins and glycosides. However, we should mention the different strengths of the extractants (water and ethanol).

IR spectroscopy

C. intybus is a valuable medicinal plant because of its ability to accumulate various biologically active chemicals. Separation and spectral methods are widely used to identify them, whereas identification involves the isolation of the target fraction. The identification of *C. intybus* raw materials and dried *Petrovsky* cultivar was performed using IR spectroscopy. The quantitative content of chemical components, such as calcium, phosphorus, cellulose, proteins, starch, and fat, was analyzed; the moisture and ash content was also determined in the seeds and plants. The results of the IR analysis are listed in Table 5.

Table 5. IR spectroscopy results of *C. intybus* seeds and shredded dried plants

Sample	Content of substances and elements, %							
	Ca	P	Water	Fat	Ash	Cellulose	Protein	Starch
Seed	1.08	0.27	12.26	0.95	0.9	46.53	10.92	76.32
Shredded dried plants	1.38	0.41	9.66	2.75	9.12	28.04	18.47	37.69

Discussion

Compared to the germination efficiency of the seeds and their contamination, the optimal options are those that provide the maximum yield of aseptic plants with minimum contamination. Therefore, the treatment of *Yaroslavsky* seeds with 8% sodium hypochlorite (10 minutes) and a solution of 37% hydrogen peroxide (5 minutes) can be considered the optimal sterilization conditions that provide the highest yield of aseptic plants of this cultivar.

The situation with the *Petrovsky* cultivar was more complicated. Therefore, data on seed germination and contamination have shown that none of the selected sterilization modes can be considered optimal, since the goal of our experiments was to obtain the maximum number of aseptic plants. In this regard, we decided to introduce non-sterile seedlings into *in vitro* culture after sterilization.

When *Petrovsky* cultivars were introduced into *in vitro* culture after washing them in a slightly pink solution of potassium permanganate for 20 minutes, followed by sterilization in 8% sodium hypochlorite (7 minutes), 40% of the plants survived the effects of the sterilizing agents and continued to grow, and contamination was not observed. At the same time, 20% of the plants successfully tolerated the sterilizing agents and continued growing; contamination was observed. Thirty percent of the plants died from treatment with a sterilizing agent and contamination. Ten percent of the plants died from treatment with a sterilizing agent, but no contamination was observed.

Most seedlings introduced into *in vitro* culture using the above sterilization modes were susceptible to bacterial infections. Therefore, we decided to add seedling treatment with an antibiotic solution for 15 minutes and reduce the exposure time of 8% sodium hypochlorite to 5 minutes. As a result of this treatment, it was possible to obtain 100% of the viable plants that successfully underwent the sterilization effect. Seventy percent of these plants were viable and not contaminated, and 30% were viable but contaminated. Therefore, it is possible to recommend washing the seedlings in a slightly pink potassium permanganate, followed by sterilization in 8% sodium hypochlorite (5 minutes) and treatment with antibiotic gentamicin (0.8% for 15 minutes) to obtain the largest number of viable aseptic *C. intybus* plants of the *Petrovsky* cultivar.

Earlier, Indian authors also recommended the introduction of chicory into *in vitro* culture using the green parts of vegetative plants (Velayutham et al. 2006).

As already noted, differences in the results were revealed during primary phytochemical screening depending on the extractant. Thus, in aqueous extracts from fresh plants, all classes of substances were found, except for saponins and glycosides. The ethanol extract positively reacted with saponins, alkaloids, reducing sugars, and glycosides. The aqueous extracts of dry plants contained tannins, phenols, flavonoids, reducing sugars, and proteins. An ethanol extract of dried plants showed the presence of all classes of biologically active substances studied, but without saponins or reducing sugars.

The data from the IR analysis reflect the general chemical composition of the samples, which differs in quantitative content. Therefore, a higher content of moisture, cellulose and starch was found in the seeds of common chicory, which is explained by the presence of endosperm. The dried plants that are shredded contain more calcium, phosphorus, fat, ash, and protein. Therefore, dried shredded plants are used in various industrial applications.

The results achieved in qualitative reactions and infrared spectroscopy correspond to the data indicated in other studies (Nandagopal and Ranjitha Kumari 2007; Abbas et al. 2015).

Conclusion

Seed treatment with 8% sodium hypochlorite for 10 minutes or 37% hydrogen peroxide for 5 minutes is recommended to obtain viable aseptic viable *C. intybus* plants of the *Yaroslavsky* cultivar. The seeds of the *Petrovsky* cultivar should be germinated non-sterile and then introduced into the culture by washing the seedlings in a slightly pink solution of potassium permanganate, followed by sterilization with 8% sodium hypochlorite solution for 5 minutes and washing in an antibiotic (0.8% gentamicin) for 15 minutes.

Primary phytochemical screening showed the presence of all the classes of elements (tannins, phenols, flavonoids, saponins, alkaloids, glycosides, reducing sugars and proteins) in *C. intybus* of the *Yaroslavsky* cultivar. However, the differences depended on the extractant (water and ethanol).

In infrared spectroscopy, the quantitative content of calcium, phosphorus, water, fat, ash, cellulose, proteins and starch in the seeds and plants of the *Petrovsky* cultivar was also determined. Here, the seeds showed a higher content of water, cellulose and starch, while the microplants had a higher content of macronutrients, fat, ash and proteins.

References

Abbas ZK, Saggu S, Sakeran MI, Zidan N, Rehman H, Ansari AA (2015) Phytochemical, antioxidant, and mineral composition of hydroalcoholic extract of chicory (*Cichorium intybus* L.) leaves. Saudi Journal of Biological Sciences 22 (3): 322–326. https://doi.org/10.1016/j.sjbs.2014.11.015

Al-Tameme HJ, Hameed IH, Idan SA, Hadi MYa (2015) Biochemical analysis of Origanum vulgare seeds by Fourier-transform infrared (FT-IR) spectroscopy and gas chromatography-mass spectrometry (GC-MS). Journal of Pharmacognosy and Phytotherapy 7 (9): 221–237. https://doi.org/10.5897/JPP2015.0362

Azimi A, Marhamatizadeh MH, Azadneya P (2013) The effect of *Cichorium intybus* on the activities of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* in probiotic milk and

yoghurt. International Journal of Advanced Biological and Biomedical Research 1 (9): 1016–1021.

Baghel PS, Ray S (2017) Preliminary phytochemical screening of certain aphrodisiac plants used in traditional system of medicine. International Journal of Botany Studies 2 (5): 33–36.

Choudhury J, Majumdar S, Roy S, Chakraborty U (2017) Antioxidant activity and phytochemical screening of two edible wetland pteridophytes *Diplazium esculentum* (Retz) Sw and *Marsilea minuta* L. World Journal of Pharmaceutical and Medical Research 3 (9): 195–203.

Egwaikhide PA, Okeniyi SO, Gimba CE (2009) Screening for antimicrobial activity and phytochemical constituents of some Nigerian medicinal plants. Journal of Medicinal Plants Research 3 (12): 1088–1091.

Heimler D, Isolani L, Vignolini P, Romani A (2009) Polyphenol content and antiradical activity of *Cichorium intybus* L. from biodynamic and conventional farming. Food Chemistry 114 (3): 765–770. https://doi.org/10.1016/j.foodchem.2008.10.010

Innocenti M, Gallori S, Giaccherini C, Ieri F, Vinicieri FF, Mulinacci N (2005) Evaluation of the phenolic content in the aerial parts of different varieties of *Cichorium intybus* L. Journal of Agricultural and Food Chemistry 53 (16): 6497–6502. https://doi.org/10.1021/jf050541d

Juśkiewicz J, Głązka I, Król B, Zduńczyk Z (2006) Effect of chicory products with different inulin content on rat caecum physiology. Journal of Animal Physiology and Animal Nutrition 90 (5–6): 200–207. https://doi.org/10.1111/j.1439-0396.2005.00591.x

Kagkli DM, Corich V, Bovo B, Lante A, Giacomini A (2016) Antiradical and antimicrobial properties of fermented red chicory (*Cichorium intybus* L.) by-products. Annals of Microbiology 66: 1377–1386. https://doi.org/10.1007/s13213-016-1225-3

Khodaii Z, Eslami S, Kamalinejad M, Mirzaei A, Natanzi MM (2020) Evaluation of aqueous extracts from four aromatic plants for their activity against *Candida albicans* adhesion to human HEp-2 epithelial cells. Gene Reports 18: 100554. https://doi.org/10.1016/j.genrep.2019.100554

Kumar A, Ilavarasan R, Jayachandran T, Decaraman M, Aravindhan P, Padmanabhan N, Krishnan MRV (2009) Phytochemical investigation on a tropical plant, *Syzygium cumini* from Kattappalayam, Erode District, Tamil Nadu, South India. Pakistan Journal of Nutrition 8: 83–85. https://doi.org/10.3923/pjn.2009.83.85

Nandagopal S, Ranjitha Kumari BD (2007) Phytochemical and antibacterial studies of chicory (*Cichorium intybus* L.) – A multipurpose medicinal plant. Advances in Biological Research 1 (1–2): 17–21.

Ning C, Wang X, Gao S, Mu J, Wang Yu, Liu S, Zhu J, Meng X (2017) Chicory inulin ameliorates type 2 diabetes mellitus and suppresses JNK and MAPK pathways in vivo and *in vitro*. Molecular Nutrition & Food Research 61 (8): 1600673. https://doi.org/10.1002/mnfr.201600673

Park E-J, Lim H-T (1999) Establishment of an efficient *in vitro* plant regeneration system in chicory (*Cichorium intybus* L var sativus). Acta Horticulturae 483: 367–370. https://doi.org/10.17660/ActaHortic.1999.483.42

Rehman RU, Israr M, Srivastava PS, Bansal KC, Abdin MZ (2003) *In vitro* regeneration of witloof chicory (*Cichorium intybus* L) from leaf explants and accumulation of esculin. *In Vitro* Cellular Developmental Biology – Plant 39: 142–146. https://doi.org/10.1079/IVP2002381

Roberfroid MB, Van Loo JAE, Gibson GR (1998) The bifidogenic nature of chicory inulin and its hydrolysis products. The Journal of Nutrition 128 (1): 11–19. https://doi.org/10.1093/jn/128.1.11

Varotto S, Lucchin M, Parrini P (2000) Immature embryos culture in Italian red chicory. Plant Cell, Tissue and Organ Culture 62: 75–77. https://doi.org/10.1023/A:1006468229414

Velayutham P, Ranjithakumari BD, Baskaran P (2006) An efficient *in vitro* plant regeneration system for *Cichorium intybus* L. – An important medicinal plant. Journal of Agricultural Technology 2 (2): 287–298.

Wagner GM, Gailing O (1996) The influence of different carbon sources on callus formation and shoot proliferation of chicory (*Cichorium intybus* L var sativum). Landbauforschung Volcanoes 46: 1–4.