1	Chicory (<i>Cichorium intybus</i> L.) as a food ingredient –
2	nutritional composition, bioactivity, safety, and health
3	claims: A Review
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15	Abstract
16	Chicory (Cichorium intybus L.) is a perennial herb from the Cichorium genus, Asteraceae family,
17	and is worldwide cultivated. So far, chicory has been used mainly in animal feed, but also in several cases
18	in the food industry: as salad, for teas and tea blends, for coffee supplementation, and as a source for the
19	inulin production. Nowadays there is an increasing interest in chicory utilization for food production and

supplementation. Some compounds present in chicory, such as polyphenols, inulin, oligofructose and
 sesquiterpene lactones may be considered as potential carriers of food functionality. This review
 describes nutritional, mineral and bioactive composition of the chicory plant and summarized the main

biological activities associated with the presence of bioactive compounds in the different plant parts.
Finally, the review explores possibilities of uses of chicory and its implementation in food products, with
intention to design new functional foods.

26 Keywords: chicory, nutritional composition, bioactive compounds, biological activity, food

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28 **1. Introduction**

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30 Since ancient times, plants have been studied for the purpose of their application in traditional 31 medicine as well as in the food sector in order to improve the health benefit and sustainability of different 32 food products. Particular attention was drawn to bioactive components present in such plants, mainly to 33 secondary metabolites as prominent constituents which are mostly the cause of biological activity and 34 functionality of these plants. Numerous studies have proven that many of these plants possess various 35 biological activities (Akhtar, Ismail, Fraternale, & Sestili, 2015; Al-Snafi, 2016). Plants, seaweeds, 36 microalgae and food by-products are the most important sources of functional compounds such as: dietary 37 fiber, phenolic compounds, flavonoids, oils, plant sterols, proteins, prebiotics, probiotics, anthocyanins, 38 carotenoids and many others (Da Silva, Barreira, & Oliveira, 2016; Herrero, Sánchez-Camargo, Cifuentes, 39 & Ibáñez, 2015). 40 Previous scientific interest has resulted in the implementation of various plant species (such as 41 fruits and algae) or their ingredients in different types of food products and beverages in order to find novel

42 bioactive molecules which can bring them functionality (Mocan et al., 2017; Admassu, Zhao, Yang,

Gasmalla, & Alsir, 2015). As a result, numerous scientific reviews summarized nutritional, mineral and
bioactive composition of food and health potentially beneficial plants such as: lettuce (*Lactuca sativa* L.),
true morels (*Morchella*) and carob pods (*Ceratonia silique* L.) (Kim, Moon, Tou, Mou, & Waterland, 2016;
Tietel & Masaphy, 2018; López-Sánchez, Moreno, & García-Viguera, 2018).

According to our knowledge, only a few scientific publications investigated chicory in the context of food ingredient, and among these chicory was considered as a source of inulin, oligofructose and sesquiterpene lactones as potential carriers of functionality (Ferioli, Manco, & D'Antuono, 2015; Shoaib et al., 2016; Jeong et al., 2017).

51 Chicory (*Cichorium intybus* L.) is a perennial herb from the *Cichorium* genus, *Asteraceae* family, 52 and is worldwide cultivated. The origin of this species is Europe (Mediterranean region), but it also may be 53 cultivated in all other temperate regions and semi-arid areas (mid-Asia, northern Africa, eastern USA, 54 Australia) (Al-Snafi, 2016; Wang & Cui, 2011). Chicory has rich nutritional composition and is potentially a 55 rich source of bioactive substances for human food fortification: inulin, sesquiterpene lactones (especially 56 lactucin, lactucopicrin, 8-deoxy lactucin, guaianolid glycosides, including chicoroisides B and C, sonchuside C), caffeic acid derivatives (chiroric acid, chlorogenic acid, isochlorogenic acid, dicaffeoyl 57 58 tartaric acid), fats, proteins, hydroxycoumarins, flavonoids, alkaloids, steroids, terpenoids, oils, volatile compounds, vitamins (α-tocopherol, γ-tocopherol), β-carotene, zeaxanthin and minerals (Al-Snafi, 2016; 59 60 Petropoulos et al., 2017). In addition to its important nutritive profile, chicory shows many types of 61 biological activity: hepatoprotective, anti-inflammatory, antioxidant, sedative. immunological. 62 cardiovascular, hypolipidemic, antidiabetic, anticancer, gastro-protective, antimicrobial and many others 63 (Bahmani et al., 2015; Al-Snafi, 2016).

The aim of this review is to consolidate the nutritional composition and bioactivity of the chicory plant, describe its health beneficial properties and demonstrate *Cichorium intybus* L. plant as a potential food ingredient. The importance of plant composition information is reflected in the ability to evaluate the intake of nutrients by consuming chicory enriched food products. Furthermore, this review collects literature regarding the relationship between nutritional components and their health-related bioactivity, which is the main point of food functionality.

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71 **2. Nutritional composition of chicory**

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Various nutritionally important compounds detected in chicory plant indicate its rich and versatile nutritional composition. Carbohydrates, phenolic compounds, flavonoids, fatty and amino acids, sesquiterpene lactones, vitamins, and minerals have been present in the *Cichorium intybus* L. Nutritional composition of raw chicory plant (*Cichorium intybus* L.) is presented in Table 1. Many scientific papers investigated the nutritional composition of individual chicory plant parts, and that information is summarized in Table 2.

- 79
- 80 Table 1
- 81 Nutritional composition of raw chicory (*Cichorium intybus* L.) per 100 g fresh weight (f.w.)
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Proximates Water 94.52 g Energy 17 kcal/ 71kJ Protein 0.9 g Total lipid (fat) 0.1 g Ash 0.47 g Carbohydrate 4 g Total dietary fiber 3.1 g Minerals 0.24 mg Ca 19 mg Fe 0.24 mg Mg 10 mg P 26 mg K 211 mg Na 2 mg Zn 0.16 mg Cu 0.051 mg Mn 0.1 mg Se 0.2 µg Vitamins 2.8 mg Thiamin 0.062 mg	Nutrient	Amount
Energy 17 kcal/ 71kJ Protein 0.9 g Total lipid (fat) 0.1 g Ash 0.47 g Carbohydrate 4 g Total dietary fiber 3.1 g <i>Minerals</i> 0.24 mg Ca 19 mg Fe 0.24 mg Mg 10 mg P 26 mg K 211 mg Na 2 mg Zn 0.051 mg Mn 0.1 mg Se 0.2 µg Vitamins 0.2 µg Vitamin C 2.8 mg Thiamin 0.062 mg	Proximates	
Protein0.9 gTotal lipid (fat)0.1 gAsh0.47 gCarbohydrate4 gTotal dietary fiber3.1 gMinerals0.24 mgCa19 mgFe0.24 mgMg10 mgP26 mgK211 mgNa2 mgZn0.16 mgCu0.051 mgMn0.1 mgSe0.2 µgVitamins0.1 mgVitamin C2.8 mgThiamin0.062 mg	Water	94.52 g
Total lipid (fat)0.1 gAsh0.47 gCarbohydrate4 gTotal dietary fiber3.1 gMinerals19 mgFe0.24 mgMg10 mgP26 mgK211 mgNa2 mgZn0.16 mgCu0.051 mgMn0.1 ggVitamins2.8 mgVitamin C2.8 mgThiamin0.062 mg	Energy	17 kcal/ 71kJ
Ash0.47 gCarbohydrate4 gTotal dietary fiber3.1 gMinerals19 mgCa19 mgFe0.24 mgMg10 mgP26 mgK211 mgNa2 mgZn0.16 mgCu0.051 mgMn0.1 mgSe0.2 µgVitamins2.8 mgThiamin0.062 mg	Protein	0.9 g
Carbohydrate4 gTotal dietary fiber3.1 gMinerals19 mgCa19 mgFe0.24 mgMg10 mgP26 mgK211 mgNa2 mgZn0.16 mgCu0.051 mgMn0.1 mgSe0.2 µgVitamins2.8 mgThiamin0.062 mg	Total lipid (fat)	0.1 g
Total dietary fiber3.1 gMinerals19 mgCa19 mgFe0.24 mgMg10 mgP26 mgK211 mgNa2 mgZn0.16 mgCu0.051 mgMn0.1 mgSe0.2 µgVitaminsVitaminsVitamin C2.8 mgThiamin0.062 mg	Ash	0.47 g
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Ca 19 mg Fe 0.24 mg Mg 10 mg Mg 10 mg P 26 mg K 211 mg Na 2 mg Zn 0.16 mg Cu 0.051 mg Mn 0.1 mg Se 0.2 µg Vitamins 2.8 mg Thiamin 0.062 mg	Total dietary fiber	3.1 g
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Vitamins Vitamin C 2.8 mg Thiamin 0.062 mg	Mn	0.1 mg
Vitamin C 2.8 mg Thiamin 0.062 mg	Se	0.2 µg
Thiamin 0.062 mg	Vitamins	
J. J	Vitamin C	2.8 mg
Riboflavin 0.027 mg	Thiamin	0.062 mg
	Riboflavin	0.027 mg

Niacin	0.16 mg
Pantothenic acid	0.145 mg
Vitamin B-6	0.042 mg
Total folate	37 µg
Folic acid	0 µg
Folate, food	37 µg
Folate, DFE	37 µg
Vitamin B-12	0 µg
Vitamin A, RAE	1 µg
Retinol	0 µg
Vitamin A, IU	29 IU
Vitamin D (D2+D3)	0 µg
Lipids	
Fatty acids, total saturated	0.024 g
14:0	0.001 g
16:0	0.021 g
18:0	0.001 g
Fatty acids, total monounsaturated	0.002 g
18:1	0.002 g
Fatty acids, total polyunsaturated	0.044 g
18:2	0.037 g
18:3	0.006 g
Fatty acids, total trans	0 g
Cholesterol	0 mg
Amino acids	
Tryptophan	0.016 g
Threonine	0.025 g
Isoleucine	0.054 g

	Leucine	0.039 g
	Lysine	0.035 g
	Methionine	0.005 g
	Phenylalanine	0.022 g
	Valine	0.041 g
	Arginine	0.066 g
	Histidine	0.015 g
83	DFE, dietary folate equivalents;	RAE, retinol activity equivalent;
84	IU, international unit; data adopted fror	n http://ndb.nal.usda.gov/ndb/search;
85	accessed on Octo	ober 28th, 2019;
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89	Table 2	
90	Nutritional composition of chicory (Cichorium intybus L) plant parts, per 100 g of dry weigh
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Content g/100g	Dry mater	Moisture	Ash	Crude fiber	Insoluble dietary fiber	Soluble dietary fiber	Total dietary fiber	Carbo- hydrates	Total soluble sugar	Inulin	Crude protein	Crude fat	Ether extract	Nitrogen free extract	Reference
	96.08	58	8.12	27.32							5.54		1.04	57.98	Jan et al. (2011)
		75.63 ±	4.25 ±	5.12 ±	30.73 ±	0.42 ±	31.15	89.41 ±	11.06 ±	44.69 ±	4.65 ±		1.69 ±		Nwafor, Shale, and Achilonu
		0.39	0.11	1.55	0.33	0.07		1.07	1.00	0.88	0.25		0.71		(2017)
ot		87.57	8.96			66.93		70.43	12.33		5.17	3.01			Zarroug, Abdelkarim, Dorra,
Root		±	±			±		±	±		±	±			Hamdaoui, Felah, and
		0.05	0.07			0.01		0.05	0.04		0.17	0.5			Hassouna (2016)
		76.32	4.45					90.77			3.83	0.95			Khalaf, El-Saadani, El-
		±	±					±			±	±			Desouky, Abdeldaiem, and
		0.2	0.32					0.17			0.35	0.04			Elmehy (2018)
		83.06	10.91	16.78				70.71	7.80	10.95	14.70		3.68		Nwafor, Shale, and Achilonu
		±	±	±				±	±	±	±		±		(2017)
		1.55	1.86	2.20				3.08	1.45	2.56	1.03		0.19		(2011)
af	95.1		18.6	22.4				38.2			14.0	1.9			Ereifej et al. (2015)
Leaf	93.0	22.64	18.65	17.61									0.33	49.31	Jan et al. (2011)
		85.77	15.13					72.11			10.22	2.54			Khalaf, El-Saadani, El-
		±	±					±			±	±			Desouky, Abdeldaiem, and
		0.15	0.74					0.11			0.26	0.07			Elmehy (2018)
		6.40	6.91	25.68				31.66			19.57	22.89			Wen Ying and Jin Gui (2012)
		±	±	±				±			±	±			Puna chicory
eq		0.16	0.15	0.19				0.42			0.17	0.67			r und emeery
Seed		6.65	6.80	25.68				34.72			19.20	22.56			Wen Ying and Jin Gui (2012)
		±	±	±				±			±	±			Commander chicory
		0.23	0.20	0.17				0.23			0.13	0.23			commander emeery

95.0 ± 0.20		8.4 ± 0.05				1.79 ± 0.04	10.7 ± 0.3	14.4 ± 0.2			Jurgoński, Milala, Jusbkiewicz, Zduńczyk, and Bogusław (2011)
94.74	5.26	11.55	36.63				18.55		13.58	19.69	Jan et al. (2011) Wild chicory seeds
95.76	4.24	17.19	38.12				14.45		10.28	19.69	Jan et al. (2011) Chicory seeds from the market

98 2.1. Carbohydrates

99 Carbohydrates, proteins and lipids are structural units and the main reserve materials in plants. 100 Furthermore, carbohydrates express important role in plant defense response. Their content depends on 101 plant species, cultivation method, weather conditions and other conditions which will be discussed in the 102 following paragraphs. 103 Carbohydrates content in chicory parts decreases in the following order: root (70.43 - 90.77%) > leaf (38.2 -72.11%) > seed (31.66 - 34.72%) (Table 2). (Jan et al., 2011; Nwafor, Shale, & Achilonu, 2017; Zarroug, 104 105 Abdelkarim, Dorra, Hamdaoui, Felah, & Hassouna, 2016; Khalaf, El-Saadani, El-Desouky, Abdeldaiem, 106 and Elmehy, 2018; Ereifej et al., 2015; Wen Ying & Jin Gui, 2012; Jurgoński et al., 2011). Among them, the 107 most present are fibers and sugars (Table 2). Some studies noted variations in soluble dietary fibers (0.42 -108 66.93%) and crude fiber content (5.12 - 27.32%) in chicory root (Nwafor et al., 2017; Zarroug et al. 2016). 109 Similar crude fiber content was found in chicory leaves (16.78-22.4 g/100g dry weight (d.w.)), while crude 110 fiber content in seeds ranged from 25.68 to 38.12 g/100 g d.w. (Table 2). Slight differences in nutritional 111 composition may be observed between various chicory varieties and cultivation technique such as organic or mineral fertilization or soil salinization (Wen Ying & Jin Gui, 2012; Jurgoński et al., 2011; Jan et al., 112 2011; Sinkovič, Demšar, Žindarčič, Vidrih, Hribar, & Treutter, 2015., Petropoulos et al., 2017.). 113 114 There is a lack of information about nutritive composition of chicory stem. Shad, Nawaz, Rehman, 115 and Ikram (2013) reported presence of 2.12±0.10 g/100g d.w. of total sugar content in chicory stem. The 116 similar content was reported in chicory root (2.03±0.02 g/100g d.w.) while leaves and seeds contained 117 higher amounts of total sugars (4.50±0.37 g/100g d.w. and 3.05±0.06 g/100g d.w. respectively). 118 Compering the carbohydrate contents in chicory to Jerusalem artichoke tubers (that varied from 55-65% depending on harvesting date) (Kocsis, Liebhard, & Praznik, 2007), and to cereals such as millet 119 120 (total carbohydrates ranged 71.54-83.75 % depending on the set applied) (Chen, Ren, Zhang, Diao, & 121 Shen, 2013), it can be concluded that chicory (especially root and leaves) may be considered as a rich 122 and competitive source of carbohydrates. Leaves of Cichorium intybus L. showed higher content of crude 123 protein, fiber, carbohydrates and lower fat content in comparison with some wild plants which could be 124 considered as natural sources for antioxidants and valuable natural resources (Ereifej et al., 2015). 125 Compared to chicory root, stem and seeds, leaves also showed higher contents of sugars (4.50 g/100g d.w.), and non-reducing sugars (4.27 g/100 g d.w.) (Shad et al., 2013). Fructose seems to be the most
abundant sugar present in chicory seeds, 22.5% d.w. (Jurgoński et al., 2011). Some studies reported
presence of saccharides in chicory flowers (*Cichorii flos*) (Judžentienė & Būdienė, 2008).

129 Chicory cultivar also showed a great impact on root yield and the amount of carbohydrates. 130 Among the sugars, fructose was the most abundant compound with the content of 0.6-11.5%, then 131 glucose (0.3-10.6%) and sucrose (2.6-11.1%) during three different experiments. Experiment 1 was devoted to determination of optimum planting and harvesting dates, experiment 2 was aimed to examine 132 133 additional chicory cultivars harvested during a longer time period, while experiment 3 studied a chicory 134 root development from the first of September through mid-November (Wilson, Smith, &Yonts, 2004). 135 Fructan DP (degree of polymerization) was also influenced by different planting and harvesting dates, but 136 the biggest share had fructans with DP 3-10 (28.4-60.3%), then DP 11-20 (11-31.9%) and DP>20 (1.9-137 25.3%) depending on planting date, cultivar, and harvest date (Wilson et al., 2004).

138 Robert, Happi, Wathelet and Paquot (2008) investigated yield of pectin and soluble sugars in 139 chicory root. They concluded that extraction temperature, time, protease pretreatment, water purity, and 140 water washing of pulps significantly affected yield and pectin composition with an increase of yield and purity of pectin in rough extraction conditions. Pectin and galacturonic acid yield varied due to extraction 141 142 conditions from 35.0±03 to 242±1.0 mg pectin/g d.w. and 24-182 mg GAE/g d.w. The following sugar 143 components dominated in the pectin extract: galacturic acid (671 to 782 mg/g d.w.), galactose (21-60 144 mg/g d.w.), rhamnose (10-24 mg/g d.w.), while xylose, arabinose and glucose were present in lower 145 quantities (0-1 mg/g d.w., 5-18 mg/g d.w. and 0-3 mg/g d.w. respectively). Robert et al. (2008) also 146 mentioned that harvest date caused the major changes in terms of neutral sugar content, while 147 galacturonic acid content was constant during the harvest period.

According to results obtained by Jurgoński et al. (2011) chicory leaves contained 62.4 g of monoand disaccharides on 100 g f.w., higher than in all other chicory plant parts (root, seed and peel).

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151 *2.1.1. Inulin*

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153 Chicory root is one of the major natural sources of inulin. This water-soluble storage polysaccharide belongs to a group of non-digestible carbohydrates called fructans. It is a fructose polymer 154 155 with β -(2-1)-glycosidic-linkage which is a long-chain carbohydrate, consisting of 2–60 fructose molecules 156 with a terminal glucose molecule. Inulin can play a role of fat and sugar replacer, texture modifier and as 157 compound in functional food development due to its prebiotic properties. Many studies investigated inulin 158 provided health benefits including: regulation of blood lipids (LDL-cholesterol and triacylglycerol) 159 concentration, positive effect on constipation, bifidogenic effect with bacteria in the colon, decreasing the 160 risk of many gastrointestinal diseases (ulcerative colitis, Crohn's disease, colon cancer), enhancing 161 mineral absorption (especially Ca, Mg and Fe), regulates appetite by affecting gastrointestinal hormones, 162 immune-modulating effects and others (Shoaib et al., 2016). Many studies reported different amounts of 163 inulin presented in chicory root. That content varies from 11-20 g inulin/100 g in the fresh root (Figueira, 164 Jin, Brod, & Honorío, 2004) to 44.69% inulin on dry root weight basis (Nwafor et al., 2017). Chicory root 165 (11-20 g/100 g) possesses similar or higher amount of inulin compared to inulin rich plants: barley grains 166 (18-20 g/100 g), Jerusalem artichoke tubers (12-19 g/100 g), asparagus roots (15 g/100 g), agave stem 167 (12-15 g/100g), dandelion roots (12-15 g/100 g) and dahlia tuber (10-12 g/100 g) (Shoaib et al., 2016). 168 Milala, Grzelak, Król, Juśkiewicz and Zduńczyk (2009) investigated inulin and total fructan content in 169 chicory peel, root, leaves and seed. They obtained 14.5% inulin in the root (calculated on the dry matter) 170 and 61.8% of total fructans. These values were the highest in comparison with other chicory plant parts. 171 Chicory root yield and inulin content is significantly influenced by year's weather conditions, as reported in 172 the study of Černý, Pačuta and Kovár (2008). They reported 18.13-22.93 % of inulin in dry chicory root. 173 The main factors which affect root yield, the content of inulin and the length of the inulin chains are the 174 sowing date, the harvest date and the genotype. The significant reduction of the average inulin chain 175 length may be noticed in the autumn when degrading fructan enzymes are the most active. This leads to 176 the conclusion that early harvested plants (in September) are rich in longer-chain inulin molecules 177 (Roustakhiz & Majnabadi, 2017).

There are different reports about the DP of inulin from chicory root. Roberfroid and Delzenne (1998) stated that chicory inulin is a long chain fructan with DP 2-60. Vandoorne et al. (2014) investigated flooding stress impact on root growth, inulin content and inulin DP. Control samples showed 10.6 to 15.1

mean DP and 67.1 to 85.7 g inulin/ 100g d.w., depending on number of weeks after sowing. Samples obtained after flooding noted 10.1 to 12.3 mean DP and inulin amount of 76.4 to 85.0 g/100g d.w. in root. Monti, Amaducci, Pritoni and Venturi (2005) explained that the fructan average DP of single chicory root (commonly 10–20) is the average of short (fewer than 10 units) and long fructan chains (about 60). Chicory seeds contain the smallest amount of total fructans (1.9% d.w.) in relation to chicory root, peel and leaves (61.8% d.w., 47.7% d.w., 3.3% d.w., respectively), with inulin content of 0.3% d.w. (Milala et al., 2009).

- 188
- 189 2.2. Volatile compounds
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The composition of volatile organic compounds in plants is species specific and affected by 191 192 external conditions, including the availability of environmental factors such as light, nutrients and water 193 (Kegge & Pierik, 2010). Their composition is also influenced by method of extraction. Extraction 194 methodologies for volatile compound isolation from chicory applied organic solvents such as petroleum 195 ether, chloroform, hexane and ethyl acetate (Nandagopal & Kumari, 2007). In the study of Judžentienė 196 and Būdienė (2008), the volatile fractions from chicory root and chicory areal parts were isolated by 197 hydrodistillation for 2 h with the Clevenger type apparatus with a mixture of pentane and diethyl ether 198 (1:1). Solid-phase micro extraction (SPME) may also be used for isolation of volatile compounds from 199 chicory according to Bais, Dattatreya, and Ravishankar (2003).

200 Volatile oils are found in all parts of the plant, with the highest concentration in the roots which 201 have been found to be effective at eliminating intestinal worms in humans (Athanasiadou, Githiori, & Kyriazakis (2007). Gol, Noghani and Chamsaz (2014) reported 48 different compounds from chicory root 202 203 essential oil with camphor (20.74%), cymene (15.06%), gamma-terpinene (13.24%) and cuminal (10.79%) as major components. It can be noted that chicory root contains higher amount of camphor, gama-204 terpinene and cymene (20.74%, 13.24% and 15.06%, respectively) compared to Salvia officinalis 205 206 (11.25%, 0.08% and 0.07%, respectively) (Radulescu, Chiliment, & Oprea, 2004). Other volatile 207 compounds found in chicory root are octane (34.3-69.8%), nonadecane (0.3-3.9%), aliphatic compounds 208 and their derivates (64.1-81.3%) and tentatively identified compounds (4.8-22.7%) (Judžentienė & 209 Būdienė, 2008). Hexadecanoic acid (32.9%), nonadecane (26.1%) and trans-α-bergamotene (14.0%) were the most abundant volatile oil compounds from chicory areal parts reported in study of Rustaiyan, 210 211 Masoudi, Ezatpour, Danaii, Taherkhani, and Aghajani (2011). Besides that, the oil contained also three 212 sesquiterpene hydrocarbons, two oxygenated sesquiterpenes and nine aliphatic compounds with share of 213 17.1%, 4.8% and 78.1 %, respectively. Bais et al. (2003) identified volatile aromatic compounds (propyl 214 isovalerate, undecanal, nonanol, isoamyl nonanoate and 2-decene-1-ol) in hairy chicory root after impact 215 of fungal elicitors. Cichorium intybus L. leaves showed 7.43% oil yield, with better essential oil antifungal 216 activity against Ganoderma lucidum than against Bacillus subtilis (Hanif, Bhatti, Jamil, Anjum, Jamil, & 217 Khan, 2010). The p-cymol (17.12%), y-terpinene (15.18%), cuminal (10.53%) and thymol (9.38%) were 218 the most abundant compounds present in chicory leaves essential oil in study of Gol et al. (2014).

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220 2.3. Phenolic compounds

Phenolic compounds are one of the most important groups of compounds responsible for various plant bioactivities. With its great morphological diversity, chicory presents the rich source of phenolic compounds, especially phenolic acids and flavonoids. Phenolic composition of chicory is highly influenced by cultivar and fertilizer administration. Moreover, the content of phenolic components varies depending on the part of the plant, with chicory seed and leaf as the richest parts.

226

227 2.3.1. Total phenolic content

228

229 Total phenolic content of 20.0±0.90 mg gallic acid equivalent (GAE)/g for dry methanolic chicory 230 root extract was reported by Nwafor et al. (2017). Denev, Petkova, Ivanov, Sirakov, Vrancheva, and 231 Pavlov (2014) noted different amounts of total phenolic content depending on the chicory culture, the 232 harvest date and extraction solvent. According to these data total polyphenolic content varied from 3.7 to 233 7.9 mg GAE/ g d.w. Total phenolic contents were significantly different for wild and cultivated chicory root 234 (22.4 and 35.1 mg GAE/100 g, respectively) (Spina et al., 2008). Total phenolic content seems to be 235 noticeable higher in chicory leaves, compared to other plant parts. According to Khalaf et al. (2018), total 236 phenolic content in chicory leaves was 865.91 mg GAE/100 g (d.w.). Heimler, Isolani, Vignolini and 237 Romani (2009) determined contents of total phenolics in chicory leaves and noted amounts of 404.6 to 238 696.3 mg GAE/100 g f.w. By comparing results obtained by Lin and Tang (2007) and previously 239 mentioned studies devoted to the determination of total phenolic content in chicory leaves, it seems that 240 chicory leaves contain a higher amount of total phenolics when compared to different fruits (strawberry, 241 oriental plum, loquat) and vegetables (green, yellow, and red paper, red and white onion, ceylon spinach, 242 bitter melon, beetroot). In study of Milala et al. (2009), lyophilized extract obtained from chicory seeds was 243 the richest in polyphenol compounds compared to root, peel and leaves, and contained more than 10% of 244 total phenolics.

245

246 2.3.2. Phenolic acids

247 Milala et al. (2009) determined 0.3% (d.m.) caffeoylquinic acids and 0.2% (d.m.) dicaffeoylquinic 248 acids in root of Cichorium intybus L. Juśkiewicz, Zduńczyk, Żary-Sikorska, Król, Milala, and Jurgoński 249 (2011) stated content of caffeoylquinic acids (0.50 g/100 g), monocaffeoylquinic acids (0.30 g/100 g) and 250 dicaffeoylquinic acids (0.20 g/100 g) in lyophilized chicory root. By analyzing ethanol extract of chicory 251 seeds, Jurgoński, Juśbkiewicz, Zdunczyk and Bogusław (2012) determined 9.6 g phenolic 252 compounds/100 g fresh mass including mono- and dicaffeoylquinic acids (caffeoylquinic acids (CQA), 253 2.8 g/100 g f.w. and 6.8 g/100 g f.w. respectively), with antioxidant activity of 505.1 nmol Trolox equivalent 254 (TE)/g. They also confirmed that CQA-rich extract from chicory seeds in improving diet-induced metabolic 255 disturbances. Pandino, Courts, Lombardo, Mauromicale, and Williamson (2010) reported total 256 caffeoylquinic acid content in wild cardoon (0.0021% d.w.) and in globe artichoke (0.0071-0.2514% d.w.), 257 which indicates that chicory possesses higher contents of these acids. On the other side, leaves of 258 Acanthopanax trifoliatus (Asian vegetable and medical plant), harvested at different seasons contained 259 mono and dicaffeoylquinic acids varied from 0.25-0.90% and 0.10-1.36% (Sithisarn, Muensaen, & 260 Jarikasem, 2011).

Willeman et al. (2014) reported the presence of chlorogenic acid in chicory root, flour and roasted grains at levels of 100.2 μ g/g of d.w., 1547 μ g/g, and 822.5 μ g/g, respectively. Khalaf et al. (2018) reported 272.48 mg/100 g d.w. chlorogenic acid, while Bahri et al. (2012) quantified hidroxycinnamic acids

in leaf tissue of *Cichorium intybus* L. and noted average concentrations of 130 mg/kg f.w. for chlorogenic
acid. In study of Nwafor et al. (2017), chlorogenic acid content was 17.84% in chicory root.

According to results obtained by Jurgoński et al. (2011) fresh chicory contained 2.13 g/100 g of chicoric acid. *Cichorium intybus* L. leaves are also rich in chicoric acid with content of 370 mg/kg f.w. reported by Bahri et al. (2012). Moreover, chicoric acid in leaves (0.87 to 6.14 mg/g) was determined by Heimler et al. (2009).

Caffeic acid is present in chicory root with content of 35.22% (Nwafor et al., 2017), as well as 1.27
mg/100 g d.w. in leaves (Khalaf et al., 2018).

272 Protocatechuic (2.50%, 7.98 mg/100 g d.w.), p-hydroxybenzoic (11.04%), iso vanillic (1.97%, 273 30.66 mg/100 g d.w.) and p-coumaric acids (9.65%, 22.84 mg/100 g d.w.) were also present in chicory 274 root and leaves, respectively (Nwafor et al., 2017; Khalaf et al., 2018). Khalaf et al. (2018) identified some 275 phenolic acids in chicory leaves including gallic acid (1.52 mg/100 g d.w.), 4-amino-benzoic (8.61 mg/100 276 g d.w.), p-OH-benzoic (11.93 mg/100 g d.w.), caffene (68.76 mg/100 g d.w.), ferulic acid (5.85 mg/100 g 277 d.w.), iso-ferulic acid (49.10 mg/100 g d.w.), e-vanilic acid (131.02 mg/100 g d.w.), benzoic acid (77.50 278 mg/100 g d.w.), ellagic acid (91.19 mg/100 g d.w.), alpha-cumaric (7.58 mg/100 g d.w.), 3,4,5-methoxy-279 cinnamic (6.37 mg/100 g d.w.), salycilic acid (15.76 mg/100 g d.w.) and cinnamic acid (0.70 mg/100 g 280 d.w.). Bahri et al. (2012) guantified hidroxycinnamic acids in leaf tissue of Cichorium intybus L. and noted 281 average caftaric acid concentrations of 246 mg/kg fresh weight (f.w.).

282

2 Shad et al. (2013) determined total phenolic acids in chicory stems, and reported content of

- 283 2.09±0.21g/100g d.w.
- 284

285 2.3.3. Flavonoids

The flavonoid content in leaves was found at 112.38 mg quercetin equivalent (QE)/100 g d.w. in study of Khalaf et al. (2018), apropos 4.96 to 21.80 mg catechin/100g f.w. (Heimler et al., 2009). The range of these values was conditioned by first and second sampling, as well as the way of chicory farming (conventional or biodynamics). Total flavonoids (0.08±0.03 g/100g d.w.) were also determined in chicory stems (Shad et al., 2013). In their study of weed seeds, Abbas, Rana, Shahid, Mahmood-ul-Hassan and Hussain (2012) reported the presence of flavonoids in *Cichorium intybus* L. seeds, in content of 14.13%). 292 By comparing total flavonoid content in strawberry (14.6 mg QE/100 g f.w.), oriental plum (37.6 mg 293 QE/100 g f.w.), mulberry (250.1 mg QE/100 g f.w.), loquat (14.2 mg QE/100 g f.w.), green (7.5 mg QE/100 294 g f.w.), yellow (4.1 mg QE/100 g f.w.), red (10.4 mg QE/100 g f.w.) paper, red (36.5-56.4 mg QE/100 g 295 f.w.) and white (30.6 mg QE/100 g f.w.) onion, ceylon spinach (133.1 mg QE/100 g f.w.), bitter melon (15.0 296 mg QE/100 g f.w.), beetroot (62.8 mg QE/100 g f.w.), with total flavonoid content in different parts of 297 chicory plant, it may be concluded that chicory leaves are promising source of flavonoids (Lin & Tang, 298 2007).

299 Quercetin glucuronide (0.74±0.02 g/100g f.w.) as well as luteolin glucuronide (0.98±0.216 to 300 3.33±0.05 mg/g) were detected only in chicory leaves (Jurgoński et al., 2011, Heimler et al., 2009).

301 According to Mulabagal, Wang, Ngouajio, and Nair (2009) the most abundant anthocyanin (>95) 302 in leaves from five different Cichorium intybus L. cultivars was cyanidin-3-O-(6"-malonyl-β-303 glucopyranoside). They also noted that anthocyanin from chicory leaf in its pure form presents in vitro 304 activities similar to aspirin and may show anti-inflammatory activity in vivo. Nørbæk, Nielsen, and Kondo 305 (2002) investigated anthocyanins from flowers of Cichorium intybus L. and reported the presence of four anthocyanins: delphinidin 3,5-di-O-(6-O-malonyl-β-D-glucoside), delphinidin 3-O-(6-O-malonyl-β-D-306 307 qlucoside)-5-O- β -D-qlucoside, delphinidin $3-O-\beta-D$ -glucoside- $5-O-(6-O-malonyl-\beta-D-glucoside)$ and 308 delphinidin 3,5-di-O- β -D-glucoside. 3-O-*p*-coumaroyl quinic acid has been identified also, for the first time. 309 Khalaf et al. (2018) identified some phenolic compounds in chicory leaves including catechein

310 (96.41 mg/100 g d.w.), catechol (5.13 mg/100 g d.w.), epicatachin (13.12 mg/100 g d.w.) and coumarin 311 (11.10 mg/100 g d.w.). In the study of Spina et al. (2008) the content of epigallocatechin gallate in wild and 312 cultivated chicory root was similar (16.5 µg/g and 17.53 µg/g respectively). Tannins are also present in 313 Cichorium intybus L. seeds in percent of 14.53±0.02 (Abbas et al., 2012).

314

315 2.4. Amino acids and proteins

316 Amino acids are organic compounds containing amino and carboxyl functional groups, that are 317 building blocks of life. They are present in plants, animals and humans and they have an important role in 318 human health maintenance. The levels of amino acids vary and many plant defense responses against

biotic or abiotic stress involve metabolic adjustments in amino acid metabolism and their presence. Someof them are determined in chicory and their presence is described.

Foster, Fedders, Clapham, Robertson, Bligh, & Turner (2002) reported different amounts of amino acids present in chicory rossete leaves differed by cultivation date and cultivar. They noticed similarities in amino acid composition among the cultivars and decrease of cisteine and hydroxyproline as well as decrease in total N concentration with latter harvested period. The major amino acids contained in rossete leaves were glycine (72.2-91.7 μmol/g d.w.), alanine (61.6-84.7 μmol/g d.w.), leucine (64.0-93.2 μmol/g d.w.), aspartate plus asparagine (60.9-83.5 μmol/g d.w.) and glutamate plus glutamine (69.5-96.2 μmol/g d.w.).

Glutamine, glutamate, asparate, asparagine, glycine, serin and arginin are the main amino acids
in chicory root and leaves, while ornithin, proline, threonine, isoleucine, valine, leucine, alanine, histidine,
lysine, phenylalanine and γ-butiric acid are present in lower amounts (Druart, Goupil, Dewaele, Boutin, &
Rambour, 2000). Salty soil conditions may improve proline accumulation both in wild chicory root and
leaves as plant response to stress (Sergio et al., 2012).

333 Total content of amino acids in two varieties of chicory seeds was in the range of 10.61-14.91 %, with glutamic acid (2.14-3.58%), aspartic acid (1.11-1.58%), arginine (1.04-1.54%), glycine (0.79-0.99%) 334 335 and leucine (0.63-0.99%) as the most abundant acids (Wen Ying & Jin Gui, 2012). Chicory seeds may be 336 considered as good source of amino acids when compared to oil seeds and legumes such as sunflower (4.889-5.083% glutamic acid, 2.201-.3.002% aspartic acid, 1.586-2.194% arginine, 0.934-1.332% glycine 337 338 and 1.490-1.511% leucine), safflower (0.021-0.363% glutamic acid, 0.201-0.247% aspartic acid, 1.559-339 1.665% arginine, 0.857-1.022% glycine and 1.002-1.023% leucine) and ground nut (1.397% glutamic acid, 3.459% aspartic acid, 2.795% arginine, 1.232% glycine and 1.622% leucine) (Ingale & Shrivastava, 340 341 2011).

The water-soluble proteins are the most abundant in chicory, followed by approximately similar contents of a salt soluble protein and free amino acids. Leaves have higher content of free amino acids and water-soluble protein content while roots contain appreciable amount of salt soluble protein content, taking into account their contents in chicory root, leaves, stem and seeds (Al-Snafi, 2016).

346	Water soluble proteins in stems (9.43±1.77 g/100g d.w.) where higher than in chicory roots and
347	seeds (5.57±0.58 g/100g d.w. and 8.35±1.82 g/100g d.w, respectively), while chicory leaves contained
348	14.13±1.50 g/100g water soluble proteins. Salt soluble proteins were abundant in stems at amount of
349	6.85±0.38 g/100g d.w., similar as in leaves and seeds (6.91±0.53 g/100g d.w. and 6.81±0.51 g/100g d.w,
350	respectively), while root contained 7.94±0.30 g/100g d.w. Free amino acids in stems were present in
351	content of 5.98±0.31% g/100g d.w more than in roots and seeds (a 1.23±0.07 g/100g d.w. and 2.03±0.05
352	g/100g d.w, respectively) while leaves were the richest in free amino acids content (8.46±0.24) (Shad et
353	al., 2013).
354	According to Table 2, chicory seeds are the richest source of crude protein (11.26 to 19.57%),
355	followed by leaves (10.22 to 14.70%) and root (3.83 to 5.54%). In the study of Akeel, Al-Sheikh, Mateen,
356	Syed, Janardhan, and Gupta (2014) concentration of protein in Cichorium intybus L. seeds was reported
357	to be in the range 110.8-146.6 μg/ml depending on pH (6.5 and 7.4). When compared to crude protein
358	content in Datura innoxia (17.21, 13.90, 2.09% in leaf, seed and root respectively) (Ayuba, Ojobe, &
359	Ayuba, 2011), and buckwheat (22.7, 13.1, 5.6% in leaf, seed and root respectively) (Vojtíšková,
223	
360	Kmentová, Kubáň, & Kráčmar, 2019), chicory plant represents good source of proteins.
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360 361	Kmentová, Kubáň, & Kráčmar, 2019), chicory plant represents good source of proteins. The RDA (Recommended Daily Allowance) for adult men and women is 0.80 g of protein/ kg of
360 361 362	Kmentová, Kubáň, & Kráčmar, 2019), chicory plant represents good source of proteins. The RDA (Recommended Daily Allowance) for adult men and women is 0.80 g of protein/ kg of body weight/day. According to this statement a 70-kilogram woman should eat 56 grams of protein each
360 361 362 363	Kmentová, Kubáň, & Kráčmar, 2019), chicory plant represents good source of proteins. The RDA (Recommended Daily Allowance) for adult men and women is 0.80 g of protein/ kg of body weight/day. According to this statement a 70-kilogram woman should eat 56 grams of protein each day. The intake range of 10 to 35 percent set for protein provides a reasonable guideline for how much
360 361 362 363 364	Kmentová, Kubáň, & Kráčmar, 2019), chicory plant represents good source of proteins. The RDA (Recommended Daily Allowance) for adult men and women is 0.80 g of protein/ kg of body weight/day. According to this statement a 70-kilogram woman should eat 56 grams of protein each day. The intake range of 10 to 35 percent set for protein provides a reasonable guideline for how much protein should be part of a balanced and healthy diet (Lupton, 2002). Due to this recommendation, chicory
360 361 362 363 364 365	Kmentová, Kubáň, & Kráčmar, 2019), chicory plant represents good source of proteins. The RDA (Recommended Daily Allowance) for adult men and women is 0.80 g of protein/ kg of body weight/day. According to this statement a 70-kilogram woman should eat 56 grams of protein each day. The intake range of 10 to 35 percent set for protein provides a reasonable guideline for how much protein should be part of a balanced and healthy diet (Lupton, 2002). Due to this recommendation, chicory seeds and leaves containing more that 10% of crude proteins are good sources of proteins from a dietary
360 361 362 363 364 365 366	Kmentová, Kubáň, & Kráčmar, 2019), chicory plant represents good source of proteins. The RDA (Recommended Daily Allowance) for adult men and women is 0.80 g of protein/ kg of body weight/day. According to this statement a 70-kilogram woman should eat 56 grams of protein each day. The intake range of 10 to 35 percent set for protein provides a reasonable guideline for how much protein should be part of a balanced and healthy diet (Lupton, 2002). Due to this recommendation, chicory seeds and leaves containing more that 10% of crude proteins are good sources of proteins from a dietary
360 361 362 363 364 365 366 367	Kmentová, Kubáň, & Kráčmar, 2019), chicory plant represents good source of proteins. The RDA (Recommended Daily Allowance) for adult men and women is 0.80 g of protein/ kg of body weight/day. According to this statement a 70-kilogram woman should eat 56 grams of protein each day. The intake range of 10 to 35 percent set for protein provides a reasonable guideline for how much protein should be part of a balanced and healthy diet (Lupton, 2002). Due to this recommendation, chicory seeds and leaves containing more that 10% of crude proteins are good sources of proteins from a dietary
360 361 362 363 364 365 366 367 368	Kmentová, Kubáň, & Kráčmar, 2019), chicory plant represents good source of proteins. The RDA (Recommended Daily Allowance) for adult men and women is 0.80 g of protein/ kg of body weight/day. According to this statement a 70-kilogram woman should eat 56 grams of protein each day. The intake range of 10 to 35 percent set for protein provides a reasonable guideline for how much protein should be part of a balanced and healthy diet (Lupton, 2002). Due to this recommendation, chicory seeds and leaves containing more that 10% of crude proteins are good sources of proteins from a dietary point of view.
360 361 362 363 364 365 366 367 368 369	Kmentová, Kubáň, & Kráčmar, 2019), chicory plant represents good source of proteins. The RDA (Recommended Daily Allowance) for adult men and women is 0.80 g of protein/ kg of body weight/day. According to this statement a 70-kilogram woman should eat 56 grams of protein each day. The intake range of 10 to 35 percent set for protein provides a reasonable guideline for how much protein should be part of a balanced and healthy diet (Lupton, 2002). Due to this recommendation, chicory seeds and leaves containing more that 10% of crude proteins are good sources of proteins from a dietary point of view.
360 361 362 363 364 365 366 367 368 369 370	Kmentová, Kubáň, & Kráčmar, 2019), chicory plant represents good source of proteins. The RDA (Recommended Daily Allowance) for adult men and women is 0.80 g of protein/ kg of body weight/day. According to this statement a 70-kilogram woman should eat 56 grams of protein each day. The intake range of 10 to 35 percent set for protein provides a reasonable guideline for how much protein should be part of a balanced and healthy diet (Lupton, 2002). Due to this recommendation, chicory seeds and leaves containing more that 10% of crude proteins are good sources of proteins from a dietary point of view. 2.5. Minerals

are essential in human metabolism and body functions. *Cichorium intybus* L. mineral diversity is detailedin Table 3.

Chicory root is a good source of minerals including calcium, potassium, sodium and magnesium which are distinguished by content compared to other minerals in the root (Nwafor et al., 2017; Jan et al. 2011; Harrington, Thatcher, & Kemp, 2006; Foster, Clapham, Belesky, Labreveux, Hall, & Sanderson, 2006). Harrington et al. (2006) noticed the benefits of mineral composition of chicory root along with some common pasture weeds such as perennial ryegrass, white clover, and dandelion, due to the content of P, S, Mg, Na, Cu, Zn and B.

Rosa et al. (2017) reported also the presence of Cr (0.0736 mg/100 g), Al (14.602 mg/100 g), Cd (0.019 mg/100 g), Ni (0.0574 mg/100 g), Co (0.0149 mg/100 g) and Si (6.78 mg/100 g), in chicory leaves. Abbas, Saggu, Sakeran, Zidan, Rehman, and Ansari (2015) noted high amount of mineral elements especially Ca (3.5%) and Mg (0.28%), while Se content was 3.2 mg/100 g. Reported silicon content is within limit of the daily intake from the British diet, even though a safe recommended dietary allowance as well as tolerable upper intake level for Al, Cd, Ni, Co and Si hasn't been set yet for human diet.

Wen Ying and Jin Gui (2012) compared chemical composition of two varieties of chicory seeds (Puna chicory and Commander chicory) with alfalfa and corn seeds. Chicory seeds proved to be more dominant in the appearance of the mineral composition with higher contents of K (5.92 to 6.49 mg/g), Ca (19.52 to 20.09 mg/g), P (9.43 to 9.45 mg/g), Mg (3.59 to 3.96 mg/g), Cu (22.33 to 23.00 µg/g), Zn (55.50 to 60.83 µg/g) and Mn (32.83 to 37.66 µg/g).

By comparing wild and cultivated chicory seeds offered at the markets, Jan et al. (2011) noted slight differences in Mn and Ca content, as it is shown in Table 3. They reported that seeds are especially rich in Ca, K, Mg and Na.

The mineral composition of the flower was not much studied. According to Szentmihàlyi, Marczal and Then (2006), chicory flower is rich in Fe (15.52 mg/100 g d.w.), Al (10.26 mg/100 g d.w), Mn (4.316 mg/100 g d.w), Zn (4.20 mg/100 g d.w) and B (3.66 mg/100 g d.w), as presented in Table 3. Compared to leaves, shoots root and herb, flowers seem to contain the smallest content of mineral elements. Al, As, B, Ba, Cr, Cu, Fe, Mn, Ni, Ti and Zn were present at significantly different concentrations in these plant parts.

401

- 402 Table 3
- 403 Mineral composition of chicory plant parts

Mineral mg/ 100g	Са	к	N	Р	S	Mg	Na	Fe	Cu	Mn	Zn	Pb	Co	Se	Мо	Reference
	181.26 ± 4.40	103.7 ± 4.62				20.14 ± 1.69	67.42 ± 2.45	1.77 ± 0.21	0.36 ± 0.02	0.31 ± 0.10	0.39 ± 0.03	0.04 ± 0.003				Nwafor, Shale, and Achilonu (2017)
	360	1700				300	50		2.7	7.5	4					Jan et al.(2011)
Root	540 ± 0.00	380 ± 0.00					140 ± 0.00	0.74 ± 0.01	0.07 ± 0.01	0.26 ± 0.01	0.17 ± 0.1	0.79 ± 0.01	0.09 ± 0.01			Zarroug, Abdelkarim, Dorra, Hamdaoui, Felah, and Hassouna (2016)
	1180	3800	4350	663	627	393	591	16.7	1.86	16.1	5.77		0.027	0.004	0.042	Harrington, Thatcher, and Kemp (2006)
	1280	2860		280	410	270		17100	1100	8700	3100					Foster, Clapham, Belesky, Labreveux, Hall, and Sanderson (2006)
	248.16	4550.96		842.82		124.02	51.08	19.18	1.045	6.28	2.28		0.0149		0.0152	Rosa et al. (2017)
Leaf	3500					280	80.0		3.2	7.1	4.72			0.032		Abbas, Saggu, Sakeran, Zidan, Rehman, and Ansari (2015)
	2500	2500				750	60		2.5	10.0	5.0					Jan et al. (2011)
	292.61 ± 13.35	166.57 ± 3.43				6.94 ± 5.86	88.84 ± 2.58	9.17 ± 0.85	0.60 ± 0.06	0.90 ± 0.01	0.91 ± 0.03	0.03 ± 0.01				Nwafor, Shale, and Achilonu (2017)
Seed	2000	1170				500	560		1.5	2.5	6.5					Jan et al.(2011) Wild seed

	3000	1100		650	510		1.3	6.5	6.0			Jan et al.(2011) Market seed
Flower						15.52 ± 1.2	2.23 ± 0.2	4.316 ± 0.14	4.20 ± 0.44			Szentmihàlyi, Marczal, and Then (2006)

405 2.6. Fatty acids and derivatives

Lipids are mostly present in plant cell membranes and may act as plant energy stores. Applied cultivation practice may affect lipid profile of plant. Fatty acids, especially omega-3, 6, 9 express important health benefits in human body. Many fatty acids and their derivatives were described in chicory.

Average total lipid content in chicory leaves was 0.13 g/100g wet weight (w.w.), lower than in other three wild edible Mediterranean *Asteraceae* plants: *Taraxacum obovatum, Chondrilla juncea* and *Sonchus oleraceus* (García-Herrera et al., 2014). Chicory plant contains significant quantities of the next fatty acids in descending order: linoleic, palmitic, linolenic, stearic, behenic and eicosanoic acids, and 2.5 % of oil (Kam & Kanberoglu, 2019).

Fathalla, Bishr, Singab, and Salama (2015) investigated fatty acid methyl esters of Cichorium 414 415 intybus L. seeds and reported the presence of lauric acid methyl ester (0.13 %), myristic acid methyl ester 416 (0.06 %), palmitoleic methyl ester (0.38 %), palmitic acid methyl ester (19.77%), methyl dihydromalvalate 417 (0.44 %), 9,12- linoleic methyl ester (58.98 %), stearic acid methyl ester (9.07 %), methyl linolelaidate 418 (0.59 %), linolenic acid methyl ester (0.81 %), 11-eicosenoic acid methyl ester (0.65 %) and eicosanoic 419 acid methyl ester (1.69 %). Najib, Ahamad, Ali and Mir (2014) reported the presence of five fatty acid esters in methanolic extract of the seeds of Cichorium intybus L .: n-hexadecanyl hexadecanoate, n-420 421 pentadecanyl octadec-9-enoate, n-hexadecanyl octadec-9-enoate, n-hexadecanyl octadecanoate and n-422 octadecanyl octadecanoate.

423 The total fatty acid content in leaves of nine chicory cultivars ranges from 104 to 644 mg/100 g 424 f.w., where α-linolenic acid (64%) is dominant. Chicory leaves are not so rich in fatty acids when 425 compared to coriander (Coriandrum sativum L.) where the highest total fatty acid content was found in 426 basal leaves with 61.21 mg/g d.w., followed by upper ones (41.8 mg/g d.w.) depending on different salt 427 conditions during cultivation (0, 25, 50 and 75 mM of NaCl) (Neffati & Marzouk, 2008). The most abundant 428 fatty acid in chicory leaves seems to be α -linolenic acid, followed by linoleic and palmitic acids. An n-6/n-3 429 polyunsaturated fatty acids ratio of forced chicory is 1.38 and differs among cultivars. Among unsaturated 430 fatty acids, oleic acid is the only one determined in chicory samples. Significant differences in content of 431 fatty acids present in wild and cultivated chicory leaves was reported, while chicory leaves contains more 432 α-linoleic acid than lettuce (20%) and spinach (40%) (Elgersma, Søegaard, & Jensen, 2013; Sinkovič,
433 Hribar, Vidrih, Ilin, & Žnidarčič, 2015; Jančić, Todorović, Basić, & Šobajić, 2016).

434

435 2.7. Sesquiterpene lactones

436 Sesquiterpene lactones are secondary plant metabolites mostly known from 437 the *Asteraceae* family. *Cichorium intybus* L. is a rich source of sesquiterpene lactones, which have role in 438 plant defense (Rees & Harborne, 1985). They are also considered as potential carriers of functionality with 439 many positive bioactivities reported.

440 Chicory root is a rich source of bitter-tasting sesquiterpene lactones, which promote appetite and 441 digestion. Sesquiterpene lactones are soluble accumulating metabolites in Asteraceae, especially in 442 chicory, mainly present in the latex (Wulfkuehler, Gras, & Carle, 2013; Willeman et al., 2014). The most 443 abundant sesquiterpene lactones isolated from the root of Cichorium intybus L. are lactucin, 8-444 deoxylactucin, 11(S),13-dihydro-8-deoxylactucin, lactucopicrin, 11(S),13- dihydrolactucopicrin, jacquinelin, 445 crepidiaside B, lactuside A (Ripoll et al., 2007b; Beharav et al., 2010; Wulfkuehler et al., 2013; Willeman et 446 al., 2014). Kisiel and Zielińska (2001) reported that lactucin-like guaianolides and their glycosides are the 447 most abundant lactones present in roots and aerial parts of *Cichorium* species. Furthermore, roots of the 448 plants produce a small number of germacrane- and eudesmane-type sesquiterpene lactones and their 449 glycosides. Poli et al. (2002) mentioned that sequiterpene lactone content depends on the harvest date. 450 Despite the bitter taste, sesquiterpene lactones derived from chicory root have many important activities 451 such as: anti-inflammatory, anti-tumour, anti-leukaemic, cytotoxic, antimicrobial, allergenic, antioxidant, 452 antibiotic, anti-cancerigenous, antiprotozoal, anthelmintic, antimalarial, sedative and anti-diabetic activity, 453 inhibits egg hatching of Haemonchus contortus, promots appetite and digestion in humans, protects plants 454 against herbivore attacks and helps signaling between plants (Schmidt, Ilic, Poulev, & Raskin, 2007; 455 Ferioli & D'Antuono, 2012; Ferioli et al., 2015; Verma et al., 2013; Zhang, Yan, Wang, & Liu, 2016; Fan et 456 al., 2017; Peña-Espinoza, Williams, Thamsborg, Simonsen, & Enemark, 2017; Giambanelli, D'Antuono, 457 Ferioli, Garrido Frenich, & Romero-González, 2018).

Ferioli and D'Antuono (2012) detected various sesquiterpene lactones in leaves of two different cultivars of chicory. They quantified the presence of free and bounded forms of sesquiterpene lactones,

460 and their content was: 11(S), 13-dihydrolactucin (437.4 mg/kg d.w.), lactucin (350.3 mg/kg d.w.), 8-461 deoxylactucin (598.8 mg/kg d.w.), 11(S),13-dihydro-8-deoxylactucin (613.9 mg/kg d.w.), 11(S),13-462 dihydrolactucopicrin (69.5 mg/kg d.w.) and lactucopicrin (315.7 mg/kg d.w.). According to Ferioli et al. 463 (2015) chicory is richer in total sesquiterpene lactones (383-2497 mg/kg d.w.) when compared to endive 464 (128-2045 mg/kg d.w.). Foster, Cassida and Sanderson (2011) investigated variation in sesquiterpene 465 composition and concentration in chicory leaves during growth. They stated that concentration of total 466 sesquiterpene lactone generally decreases or remains constant, while composition of sesquiterpene 467 lactones during the growing period varied among the forage chicory cultivars.

468

469 2.8. Vitamins

470 Only a few studies mentioned vitamin content in chicory. Chicory leaves are rich in B_1 , B_2 , B_6 and 471 C, with average amounts of 133,4 to 156.7 µg/ 100 g f.w., 112.4 to 116.1 µg/100 g f.w., 162.8 to 168.7 µg/ 472 100 g and 3.5 to 4.0 µg/ 100 g f.w. in wild and cultivated chicory plants, respectively, while the content of 473 vitamin A in leaves was reported to be 0.45 mg/100 g f.w. (Jančić et al., 2016).

474

475 **3. Biological activity**

476 In recent years, many studies reported diverse bioactivity of compounds isolated from the chicory. 477 Phenolic compounds including gallic acid, protocatechic acid, chicoric acid, chlorogenic acid, caftaric acid, 478 (Heimler et al., 2009), hexoside (Dalar & Konczak, 2014), flavonoids (Khalaf et al., 2018), inulin (Shoaib et 479 al., 2016), sesquiterpene lactones (Schmidt et al. 2007), coumarins (Khalaf et al., 2018) or other 480 compounds present in chicory extracts were considered as responsible for positive impact on human 481 health. Some of the proven bioactivities from compounds detected in chicory are antiradical (Kagkli, 482 Corich, Bovo, Lante, & Giacomini, 2016), antimicrobial (Verma et al., 2013), antioxidant (Abbas et al., 483 2015), hepatoprotective (Neha et al., 2014), antibacterial (Liu, Wang, Liu, Chen, & Cui, 2013), antidiabetic 484 (Abdel-Rahim, Rashed, El-Hawary, Abdelkader, Kassem, & Mohamed, 2016), anthelmintic (Peña-485 Espinoza, Williams, Thamsborg, Simonsen & Enemark, 2017) and anti-parasitic activity (Peňa-Espinoza,

486 Boas, Williams, Thamsborg, Simonsen, & Enemark, 2015). Bioactivity of chicory compounds is presented

- in Table 4.
- 488
- 489 Table 4

490 Summarized bioactivity of *Cichorium intybus* L.

Bioactivity/Health condition	Plant part	Extract type	Compound/s	Reference
Anti-hepatotoxic activity	Chicory plant	Ethyl-acetate extracts		Li, Gao, Huang, Lu, Gu, and Wang (2014)
	Leaf	Water extract	Cichoric acid	Zhang et al. (2014)
	Leaf	Ethanolic extract	Flavonoids	Neha et al. (2014)
	Seed	Alcoholic extract	Phytoconstituents from extract such as flavonoids, saponins and their glycosides	Fathalla, Bishr, Singab, and Salama (2015)
Anti-diabetic	Plant (root, leaves, and stems)	Ethanolic extract	Potentially inulin	Pushparaj, Low, Manikandan, Tan, and Tan (2007)
	Leaf		Potentially fibers (inulin)	Abdel-Rahim, Rashed, El-Hawary, Abdelkader, Kassem, and Mohamed (2016)
	Seed	Aqueous extract	Phytochemicals such as anthocyanins, tannins, coumarins, chicoric acid, chlorogenic acid, and caffeic acid	Ghamarian, Abdollahi, Su, Amiri, Ahadi, and Nowrouzi (2012)
Antimicrobial	Root	Ethyl acetate extract		Koner, Ghosh, and Roy (2011)
	Root and leaf	Methanolic extract	Potentially inulin, sesquiterpene lactones, coumarins, flavonoids.	Verma et al. (2013)
	Root		Sesquiterpenoid lactones from chicory root: cichoralexin, 10 α- hydroxycichopumilide and 8 α- angeloyloxycichoralexin; terpenoids and phenolics.	Nishimura and Satoh (2006)
	Leaf		Phenolic compounds - gallic acid, protocatechic acid, chicoric acid and chlorogenic acid.	Kagkli, Corich, Bovo, Lante, and Giacomini (2016)

	Leaf and root	Ethanolic and methanolic extracts of γ-irradiated chicory	Phenolic compounds and flavonoids	Khalaf, El-Saadani, El-Desouky, Abdeldaiem, and Elmehy (2018)
	Seed	Methanolic extract and ethylacetate fraction	Alkaloids, flavonoids, saponins, tannins, steroids, anthraquinone.	Mehmood, Zubair, Rizwan, Rasool, Shahid, and Ahmad (2012)
	Seed	Aqueous and organic (ethanol and ethyl acetate) extracts	Watersoluble compounds (inulin, flavonoids, etc)	Shaikh, Rub, and Sasikumar (2016)
	Flower	Ethyl acetate extract		Petrovic, Stanojkovic, Comic, and Curcic (2004)
	Root	Ethanolic extract	Caffeoylquinic acid	Liu et al. (2013)
	Flower	Methanolic extract	Phenolic compounds	Derakhshani, Hassani, Pirzad, Abdollahi, and Dalkani (2012).
Antioxidant	Leaf	The ethanolic and methanolic extracts of γ-irradiated chicory	Phenolic compounds and flavonoids	Khalaf, El-Saadani, El-Desouky, Abdeldaiem, and Elmehy (2018)
	Seed	The methanolic extract and ethylacetate fraction	Alkaloids, flavonoids, saponins, tannins, steroids, anthraquinone.	Mehmood, Zubaır, Rızwan, Rasool, Shahid, and Ahmad (2012)
Anti-inflammatory	Root	Polar solvent extract	Sesquiterpene lactones	Ripoll, Schmidt, Ilic, Poulev, and Raskin (2007a) Ripoll et al. (2007b)
		Water extract	Chicoric acids: Caffeic and ferulic acids	Azay-Milhau et al. (2013)
Antihyperglycemic	Root	Methanolic extract partitioned with ethyl acetate	Caffeic acid moiety, di-O- caffeoylquinic acids suppressed and derivates (3-caffeoylquinic, caffeic, and quinic acids)	Jackson, Rathinasabapathy, Esposito, and Komarnytsky (2017)
Antimalarial activity	Root	Aqueous root extracts further extracted with Et ₂ O or EtOAc	Sesquiterpene lactons: lactucin and lactucopicrin	Bischoff, Kelley, Karchesy, Laurantos, Nguyen-Dinh, and Arefi (2004)
Antifungal activity	Root	Aqueous phase of acetone extract purified with hexan	Sesquiterpene lactons from root: 8-deoxylactucin and 11β,13- dihydrolactucin.	Mares, Romagnoli, Tosi, Andreotti, Chillemi, and Poli (2005)
Anti-cancer activity	Root	Methanolic extract	Lactucin, β-sitosterol, quinic acid, succinic acid and polyphenols like	Mehrandish, Mellati, Rahimipour, and Nayeri (2017)

			flavonoids.	
Tumour inhibitory activity	Root	Ethanolic extract	Inulin, cichoriin, esculin, esculetin, caffeine, polyacetylenes, organic acids, gums, proteins and vitamins	Hazra, Sarkar, Bhattacharyya, and Roy (2002)
		Hydroalcholic extracts	Phenolic compounds and flavonoids	Heimler, Isolani, Vignolini and Romar (2009)
Antiradical activity	Leaf		Gallic acid, protocatechic acid, chicoric acid and chicoric acid and chlorogenic acid.	Kagkli, Corich, Bovo Lante, and Giacomir (2016)
Antiosteoporotic effect	Leaf	Aqueous extract	Saccharides and flavonoids	Hozayen, El- Desouky, Soliman, Ahmed, and Khaliefa (2016)
Enzyme (α- glucosidase, α- amylase, pancreatic lipase and angiotensin converting enzyme) inhibitory activities, prevention of metabolic syndrome.	Leaf	Lyophilised hydrophilic extract	Hydroxycinnamic acids and flavonoids, with isomers of chlorogenic acid, caftaric acid, cichoric acid,and luteolin hexoside	Dalar and Konczak (2014)
Anti-parasitic activity (Os <i>tertagia</i> ostertagi)	Leaf	Ethyl acetate extract of primal methanol/water extract	Sesquiterpene lactones: lactucin, 11β, 13- dihydrolactucin, 8- deoxylactucin, 11β, 13- dihydro-8-deoxylactucin, lactucopicrin, 11β, 13- dihydro-lactucopicrin.	Peňa-Espinoza, Boas, Williams, Thamsborg, Simonsen, and Enemark (2015)
Anthelmintic effects	Leaf	Methanol extract	Sesquiterpene lactones	Peña-Espinoza, Williams, Thamsborg, Simonsen, and Enemark (2017)
Radioprotective effect	Seed	Methanolic extract	Phenolic compounds such as chlorogenic acid which act as antioxidant agents.	Hosseinimehr, Ghaffari-Rad, Rostamnezhad, Ghasemi, Pourfallah and Shahani (2015)
Improvement of glycemia, atherogenic index and antioxidant status	Seed	Ethanolic extract	Caffeoylquinic acid-rich extract	Jurgoński, Juśbkiewicz, Zdunczyk and Bogusław (2012)

494 3.1. Anti-hepatotoxic activity

Li et al. (2014) noted the hepatoprotective effect of ethyl-acetate extract of chicory plant against 495 496 carbon tetrachloride-induced hepatic fibrosis in rats. Oral doses of 6, 18, and 54 g/kg per day were 497 investigated for hepatoprotective effect in Wistar albino rats, with a dose of 54 g/kg per day as the most 498 effective. Significant decreasing of serum levels of aspartate aminotransferase (149.04±34.44, P<0.01), 499 alanine aminotransferase (100.72±27.19, P<0.01), hexadecenoic acid (548.50±65.09, P<0.01), laminin 500 (28.69±3.32, P<0.01) and hydroxyproline (263.33±75.82, P<0.01) in liver was noted. Fathalla et al. (2015) suggested that defatted extract showed lower hepatoprotective activity than total extract, and connected 501 502 this with potential bioactivity of lipid fraction from chicory seed extract. Zhang et al. (2014) linked anti-503 hepatitis B effect of chicory leaves with chicoric acid and its anti-hyaluronidase, phagocytosis stimulatory 504 and antioxidative properties. Dose of 10–100 µg/ml chicoric acid from chicory leaves reduced significantly 505 the hepatitis B surface and envelope antigen levels with the maximum inhibition rates of 79.94% and 506 76.41%, respectively. Flavonoids present in hydroalcoholic leaf fractions also may be responsible for 507 hepatoprotective role of chicory (Neha et al., 2014). Kostic et al. (2013) mentioned the role of chicory 508 flower in treatment of skin diseases, diabetes and anti-hepatotoxic activity.

509

510 3.2. Anti-diabetic effect

511 Daily administration of 125 mg/kg ethanolic chicory plant extract for 14 days to Sprague–Dawley 512 diabetic rats reduced serum glucose by 20%, triglycerides by 91% and total cholesterol by 16%. Inulin 513 potentially express anti-diabetic effect in tested animals. The possibility that this extract induces insulin 514 secretion from pancreatic β -cells was also highlighted in the study of Pushparaj, Low, Manikandan, Tan, 515 and Tan (2007). Aqueous extract of chicory seed prevented body-weight loss and decreased fasting blood 516 sugar in four-week trial in rats. Chicory appeared to have short-term (about 2 hours) and long-term (28 517 days) effects on Type 2 diabetes mellitus as well as to be a natural dietary supplement for slowing down 518 the pace of diabetes progress (Ghamarian et al., 2012). Chicory seed ethanolic extract containing 9.6% of 519 caffeoylquinic acids improved glycemia, decreased the atherogenic index and increased blood antioxidant 520 status during 28-day treatment of Wistar rats (Jurgoński et al., 2012).

521

522 3.3. Antimicrobial effect

Ethanolic, methanolic, ethyil-acetate and aqueous extracts of chicory root, leaves and seeds 523 524 expressed antimicrobial activity due to presence of phenolic compounds, flavonoids and sesquiterpene 525 lactones depending on the plant part (Table 4). Koner et al. (2011) tested ethyl acetate extract of chicory 526 root for anti-bacterial and anti-fungal properties and stated that chicory root has great ability to inhibit both 527 Gram-positive and Gram-negative bacteria (Staphylococcus aureus, Bacillus subtilis, Pseudomonas 528 fluorescens, Rhizobium leguminosarum, Escherichia coli, Vibrio cholera), yeast (Sachharomyces 529 cerevisiae) and Aspergillus niger. Verma et al. (2013) reported in vitro antibacterial activity of Cichorium 530 intybus L. against some pathogenic bacteria as well as antibacterial activity of chicory leaves extract 531 against Gram-negative pathogenic bacteria (Escherichia coli and Pseudomonas aeruginosa) considering 532 inulin, sesquiterpene lactones, flavonoids and coumarins responsible for this activity. Petrovic, 533 Stanojkovic, Comic and Curcic (2004) connected antibacterial activity of areal parts of chicory with 534 presence of cichorin in its flowers. Shaikh, Rub and Sasikumar (2016) proved antimicrobial activity of 535 chicory seed extract against Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans and 536 Escherichia coli. Sesquiterpene lactones isolated from chicory leaves using ethyl-acetate and methanol 537 showed important anti-parasitic activity against Ostertagia ostertagi and anthelmintic effect against free-538 living and parasitic stages of Cooperia oncophora (Peňa-Espinoza et al., 2015; Peña-Espinoza, Williams, 539 Thamsborg, Simonsen, & Enemark, 2017).

540

541 3.4. Antioxidant activity

542 Antioxidant activity of chicory plant was the subject of investigation in numerous scientific studies. 543 Liu et al. (2013) linked antioxidant and antibacterial activities of chicory root extracts with the bioactive substances primarily identified as caffeoylquinic acids. Rub, Siddiqui, Ali, Shaikh, and Mukadam (2014) 544 545 investigated the antioxidant activity of the polyphenol rich fraction of chicory roots and also reported its 546 strong hypoglycemic potential. Dalar and Konczak (2014) studied the antioxidative activity of roots, stems, 547 leaves, flowers, and the whole plant of Cichorium intybus L. As it is reported in their study, the leaves 548 contained the highest level of total phenols (22.6 mg GAE/g d.w.) and exhibited the highest antioxidant 549 capacities. The FRAP (ferric reducing antioxidant capacity) value of leaf was 251.6 μ mol Fe⁺²/g d.w. The

550 ORAC (oxygen radical absorbance capacity) value of chicory plant parts was reported to decline in the 551 following order: flower ≥ leaf > root > stem. They also identified many important phenolic compounds in 552 ethanol based lyophilized extract of Cichorium intybus L. leaf: apigenin, hydroxybenzoic acid-O-hexoside, 553 caftaric acid, isorhamnetin, 4-O-caffeoylguinic acid, 5-O-caffeoylguinic acid, apigenin glucoside, luteolin 554 hexoside, guercetin/hesperitin glucoside, cichoric acid and guercetin rutinoside (rutin). Heimler et al. 555 (2009) reported 19.30 to 48.60 mg sample f.w./mg DPPH, for antiradical activity IC50 (the antiradical dose 556 required to cause a 50% inhibition) in chicory leaves. Khalaf et al. (2018) found that irradiation treatment 557 was superior for improving the phytochemical, antioxidant and antimicrobial activities and the most 558 indicated doses to maintain phytochemicals content, and to increase antioxidant activity, as well as 559 antimicrobial, were 4 and 12 kGy for roots and leaves of chicory, respectively. They also reported that 560 DPPH (1,1-diphenyl-2-picrylhydrazyl radical) radical scavenging activity of leaves was $80.95 \pm 0.39\%$. 561 Antioxidant capacities of chicory flower expressed as FRAP (240.8 µmol Fe⁺²/g d.w.) seem to be stronger 562 than for root or stem. Furthermore, flowers expressed the highest ORAC (1307.7 µmol TE/g d.w.) 563 compared to leaf, root and stem. Jurgoński et al. (2011) reported that chicory leaves extract showed 564 higher antioxidant activity (210.1 nmol/g of f.w.), compared to the seed extract (505.1 nmol/g of f.w.). 565 Chicory leaves and flowers possess valuable oxygen radical scavenging and total reducing capacities, 566 comparable to those of Chinese medicinal plants (Dalar & Konczak, 2014). According to the results 567 obtained by Montefusco et al. (2015) who investigated the hydrophilic and lipophilic antioxidant activities, 568 contents of lutein (8.0 to 30.1 μ g/g f.w.), β -cryptoxanthin (0.13 to 0.41 μ g/g f.w.) and β -carotene (3.3 to 569 14.1 µg/g f.w.) varied in cultivated and wild chicory cultivars. Antioxidant activities in chicory cultivars 570 ranged between 352 and 1056 µmol TE/100 g f.w., depending on the varietal and environmental factors, 571 related to growing, soil, and climatic conditions. Total phenolic content from 30.1 to 101.7 mg GAE/100 g 572 f.w. was reported in edible parts of cultivated and wild chicory. The flavonoid content evaluated from 11.1 573 to 66.2 mg catechin equivalents (CE)/100 g f.w., correlated with total antioxidant activity in chicory leaves. 574 They also concluded that the hydrophilic antioxidant activity was higher than the lipophilic antioxidant 575 activity in different chicory varieties. Comparing dietary supplementation with rutin and extract of chicory 576 seeds rich in caffeoylquinic acid in rats, Jurgoński et al. (2012) concluded that chicory seed extract 577 decreases serum atherogenicity and increases blood antioxidant status, contrary to rutin.

578

579 3.5. Other bioactivities

580 Many studies investigated and demonstrated a wide range of different chicory bioactivities.

581 Sesquiterpene lactones rich alcoholic extract of chicory root showed anti-inflammatory effect in 582 rats and mouses. Inflammation was reduced for 76% at 100 mg/kg dose in rats, and 71% for 200 mg/kg 583 dose in mouses (Ripoll et al., 2007b).

584 Chicoric acids, especially caffeic and ferulic acids, promote a decrease in hepatic glycogenolysis, 585 increase insulin release and reduce hepatic glycogenolysis. From the other side, natural chicoric acid 586 aqueous extract from chicory root improve intraperitoneal glucose tolerance in a dose-dependent manner 587 considering *Cichorium intybus* L. as an antihyperglycemic agent (Azay-Milhau et al., 2013). Similar 588 conclusions were noted in study of Jackson, Rathinasabapathy, Esposito, and Komarnytsky (2017), where 589 the caffeic acid moiety was responsible for antihyperglicemic effect.

Sesquiterpene lactones (lactucin and lactucopicrin) from chicory root cause antimalarial activity against HB3 clone of strain *Honduras-1* of *Plasmodium falciparum* (Bischoff, Kelley, Karchesy, Laurantos, Nguyen-Dinh, & Arefi, 2004). On the other hand, sesquiterpene lactones 8-deoxylactucin and 11β ,13dihydrolactucin, showed antifungal activity against zoophilic and anthropophilic dermatophytes (Mares, Romagnoli, Tosi, Andreotti, Chillemi, & Poli, 2005).

A pilot work of chicory anti-cancer activity has been conducted. In the study of Mehrandish, Mellati, Rahimipour, and Nayeri (2017), methanolic extract of chicory root showed cytotoxic effect on human breast cancer SKBR3 cell line with IC50 value of 800, 400 and 300 after 24, 48 and 72 hours of

598 treatment.

599 Ethanolic (80%) extract of chicory root was studied in terms of tumour-inhibitory effect against 600 Ehrlich ascites carcinoma in Swiss mice and showed significant results at doses from 300 to 700 601 mg/kg/day (Hazra et al., 2002).

Phenolic compounds from chicory seed, such as chlorogenic acid, are well-known as antioxidant
 agents, but may act as radioprotective agents also. Methanolic extract of chicory seeds was investigated
 against genotoxicity induced by ionizing radiation in human lymphocytes. All doses of chicory extract (10,
 50, 100, and 200 µg/mL) significantly reduced the frequency of micronuclei in binucleated lymphocytes

606 from human peripheral blood samples (Hosseinimehr, Ghaffari-Rad, Rostamnezhad, Ghasemi, Pourfallah,

607 & Shahani, 2015)

Aqueous chicory leaves extract rich in inulin and flavonoids may be considered as bone protection agent against glucocorticoid-induced osteoporosis in rats, more than parsley and basil extracts. These results were obtained during 8-week long treatment and indicated antiosteoporotic effect of chicory (Hozayen, El-Desouky, Soliman, Ahmed, & Khaliefa, 2016).

Dalar and Konczak (2014) evaluated the ability of lyophilized hydrophilic leaf extract to inhibit
 selected digestive enzymes related to metabolic syndrome (α-amylase, α-glucosidase, pancreatic lipase
 and angiotensin converting enzyme).

In traditional medicine, chicory flowers were used for curing common ailments, as a tonic and appetite stimulant, and in treatment of gallstones, gastro-enteritis, sinus problems, cuts and bruises (Judžentienė & Būdienė, 2008). According to Bahmani, Zargaran and Rafieian-Kopaei (2014), chicory root, leaves and flowers were traditionally used for aiding the function of the gallbladder. Zolfaghari, Adeli, Mozaffarian, Babaei and Habibi Bibalan (2012) reported laxative and anticholesterol effect of chicory. Kratchanova, Denev, Ciz, Lojek and Mihailov (2010) summarized utilization of areal chicory parts in treatment of liver diseases, cholagogue, and digestive tract.

622

623 4. Safety and Health claims

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625 Chicory extract, as well as inulin, is Generally Regarded as Safe (GRAS) by the FDA and appears 626 on the Everything Added to Food in the United States (EAFUS) list (Food and Drug Administration, 2018). 627 The safety of a chicory root extract was confirmed in study of Schmidt et al. (2007), during Ames test and 628 four-weeks sub-chronic toxicity study in male and female Sprague-Dawley rats. They reported that 629 chicory root extract containing sesquiterpene lactones is non-toxic and non-mutagenic at 70, 350, or 630 1000 mg/kg/day, administered orally. Toxicological profiling (subacute and chronic toxicity) of aqueous 631 chicory seed extract has been confirmed that has no ill effect and the dose of 200 mg/kg body weight 632 resulted in significant reduction in serum glucose (52.7%) and triglycerides levels (65.3%) and decreased 633 the oxidative burden in high-fat-diet-induced diabetic rats (Chandra, Khan, Jettey, Ahmad, & Jain, 2018).

634 The claim of chicory products short-term and/or long-term effects on diabetes has been proven by the 635 glucose tolerance test and HbA1c measurement (Nowrouzi, Mazani, Rezagholizadeh, & Banaei, 2017). 636 Furthermore, there are some clinical studies on human subjects which have demonstrated potential role of 637 bioactive chicory root extract in the management of osteoarthritis (Olsen, Branch, Jonnala, Seskar, & 638 Cooper, 2010), a positive effect of chicory inulin or oligofructose to reduce the postprandial blood glucose 639 response (Lightowler, Thondre, Holz, & Theis, 2018), as well as oligofructose-enriched chicory inulin 640 positive effects on the improvement of the glucose and calcium homeostasis, liver function tests, blood 641 pressure and reduction in hematologic risk factors of diabetes in female patients with type 2 diabetes 642 mellitus (T2DM) (Farhangi, Javid, & Dehghan, 2016).

EFSA (European Food Safety Authority) confirmed health claim that non-digestible carbohydrates from chicory (fructo-oligosaccharides (FOS, oligofructose) obtained from chicory inulin), which should replace sugars in foods or beverages reduce post-prandial glycemic responses (EFSA Panel on Dietetic Products Nutrition and Allergies, 2014). EFSA and Commission Regulation (EU) 2015/2314 confirmed that the effect on bowel function demonstrated by the intake of native chicory inulin is a beneficial physiological effect with dosage of 12 g/day for this claim (Theis, 2018).

649

9 5. Current and potential applications

650

651 Currently, there are several known chicory applications: baked and ground chicons or roots for 652 coffee substitute and supplement, utilization of leaves as a vegetable and cultivation of chicory as a forage 653 plant for poultry and animal feed (Saeed et al., 2017). Chicory, as a great source of dietary fibers such 654 as inulin and FOS and many functional food ingredients can assist in maintaining good health and in 655 preventing diseases. Inulin is mostly applied into food as low-calorie sweetener, fat substitute and texture 656 modifier, in order to increase dietary fiber content, improve technological and sensory value of the 657 products (Drabińska, Zieliński, & Krupa-Kozak, 2016). Many studies investigated inulin bioactivity: tumor 658 inhibitory, antidiabetic, anti-hepatotoxic and antioxidative activity (Table 4). Chicory powdered roots finds 659 another application in successfully replacing wheat flour (10%) and fat (25%) in cracker production after 660 debittering by soaking the roots in water or citric acid solution (Massoud, Amin, & Elgindy, 2009). Chicory 661 fibers were also incorporated in restructured sausages in order to replace fat, with conclusion that addition 662 of chicory fiber significantly reduced the moisture, fat, hardness and pH values of sausages (Choi et al., 663 2016). Bossard, Leveque, and Marboutin (2006) obtained flour from dehydrated chicory root in order to 664 prepare food dough for baking. Wang & Cui (2011) also mentioned utilization of chicory for tea production, 665 such as tea for keeping fit. Also, implementation of chicory root extracts (1, 2 and 3%) in yogurt due to 666 health promoting effect of chicory inulin as well as its ability to form creamy emulsions with aqueous liquid 667 were investigated (Jeong et al., 2017). Dried chicory root extract (2-6 %) was implemented into yogurt-ice 668 cream formulation with intention to decrease production costs by replacing dairy ingredients with other low 669 cost alternatives, such as inulin and buttermilk. By increasing the amount of dried chicory root extract in 670 voghurt-ice cream formulation increased the textural and flavor properties and overall acceptability score 671 while melting rate was decreased (Kumar, DC, Alam, & Sawant, 2018). Future research should be 672 devoted more closely to the application of chicory in food products on an industrial scale, according to its 673 important benefits and potential.

674

675 6. Conclusive remarks

676

677 *Cichorium intybus* L. is a valuable source of bioactives for new food products especially because 678 of the presence of inulin, sesquiterpene lactones and phenolic compounds in roots, leaves, seeds, and 679 flowers. Among them, inulin as the most abundant compound determines chicory as one of the richest 680 source of this prebiotic which can be used for development of added value food products.

The health-promoting characteristics of the chicory-derived applications are well backed by the experimental data available from bioactivity studies especially anti-inflammatory, anti-diabetic, antihepatotoxic, antihyperglycemic and antimicrobial activity. This data suggest that food enriched with chicory can provide a potential health effect determining it as a desirable ingredient in the diet.

685 Chicory root seems to be a promising raw material for the enrichment of functional food products 686 according to its high mass yield and diverse and rich composition of biologically important compounds 687 (inulin, sesquiterpene lactones, and minerals) compared to other parts of the plant. Chicory root inulin 688 possesses wide application possibilities in food industry: as prebiotic, fat replacer, sugar replacer, texture 689 modifier and for the development of innovative foods in order to improve health due to its beneficial role in 690 gastric health. Also, sesquiterpene lactones from the chicory root could be utilized as a source for the 691 development of functional foods with anti-inflammatory activity. Additionally, the utilization of all parts of 692 chicory may contribute to reducing food waste and improvement of food system sustainability.

- 693 Usually, processing practices of chicory plant includes roasting for production of coffee substitutes
- 694 or surrogates (Oliveira et al., 2012), slicing and drying for tea production (Lee, Kang, & Hsieh, 2004), as
- 695 well as hot water continuous extraction for inulin isolation (Zhu, Bals, Grimi, & Vorobiev, 2012) at industrial
- 696 utilization. Inulin-containing waste may be reused in industrial fermentations to produce biofuels and bio-
- 697 based chemicals, as well as for production of single-cell protein, single cell oil, citric acid, and other
- 698 chemicals (Hughes et al., 2017). On the other side, the industrial processing of chicory uses mainly the
- 699 roots, resulting in large amounts of unused chicory by-products (leaves and peels) which may be
- 700 investigated as a source for isolation of bioactive compounds such as sesquiterpene lactones and

701 polyphenols. Future developments in the optimization of the extraction of economically important bioactive 702 compounds from chicory and their implementation from pilot to industrial scale are needed in order to 703 supply industry and ensure the valorization of chicory plant parts and the sustainability of the final

- products enriched in the nutritional and functional bioactives of chicory.
- In summary, chicory plant may be considered as rich and promising raw material in terms of
 carbohydrates, proteins, phenolic compounds, flavonoids, and minerals content, making chicory as a
- _____
- 707 desirable ingredient in the development of innovative and functional food products.
- This review clearly reveals chicory as a good potential valuable resource for food ingredients and
- functional foods, beverages and dietary supplements.
- 710
- 711 Conflicts of interest
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- 713 The authors have declared no conflict of interest.

- 715 Acknowledgements
- 716

- 717 This work was funded by Ministry of Education, Science and Technological Development of 718 Republic of Serbia (451-03-68/2020-14/200222).
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