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Phytochemical Analysis and Yield Characterization of Eight *Cichorium intybus* L. Landraces

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Abstract: The field experiment was conducted on eight *Cichorium intybus* L. landraces collected from different geographical regions in Egypt and during the two successive seasons of 2013/2014 and 2014/2015 to evaluate the variation in yield and phytochemical compositions. Within the two groups of leaf midrib colors, the landraces showed white green midrib were also displayed the highest chlorophyll contents, with Qena landrace observed the most significant increase in Chlorophyll a (chl. a, 17.92 and 18.69 mg/100 g FW), Chlorophyll b (chl. b, 8.94 and 11.24 mg/100g FW) and total chlorophyll (23.94 and 29.93 mg/100g FW) in both seasons, respectively. On the other side, Behiera landrace, with red leaf midrib, recorded the highest Carotenoids (2.13 and 2.30 mg/100g FW) and anthocyanin (4.76 and 3.98 mg/g FW) contents in both seasons, respectively. Further, Chicory yield, ascorbic acid (AA), total soluble solids (TSS), total sugars, inulin and other constituents' contents have displayed significant variation among the eight landraces investigated in this study. This variability was shown to critically impact Chicory yield and phytochemical profiles. The phytochemical profiling indicated that Behiera landraces exhibited the highest nutritional compositions, particularly antioxidants such as flavonoids (7.12 and 6.54 mg/g DW) and 2, 2- diphenyl-pecrylhydrazil (DPPH, 76.44 and 87.30 mg/g DW). Furthermore, the highest total phenols were obtained from Alexandria landrace, particularly in the 1st season (4.12% of DW) and Behiera landrace, particularly in the 2nd season (4.11% of DW). Finally, the SDS-PAGE profile from Chicory seeds showed close relationships among the studied landraces with some genetic diversity. The study is highly useful as initial work to introduce the scientific community to the agronomic and nutritional attributes of one of the neglected plants grown naturally in Egypt, highlighting the necessity of integrating Chicory in future breeding programs for the development of varieties.

Key words: *Cichorium intybus* L. · Phytochemical compositions · Anthocyanins · Inulin · Flavonoids · Phenols · Antioxidants · Egyptian flora

INTRODUCTION

Cichorium intybus L. (Chicory) is a Mediterranean plant species belonging to the *Asteraceae* family. *Cichorium* includes approximately hundred genera and many hundreds of species of which some genera are used as fresh vegetables or for salad and according to their utilization [1, 2]. Historically, chicory was grown by the ancient Egyptians as early as 5000 years ago its culinary leaves nutritional values and used as a medicinal plant for therapeutic application [3-6].

Chicory plants possess excellent phytochemical compounds due to the presence of a number of medicinally important composition such as chlorophyll a, b, total chlorophyll, carotenoids, anthocyanins and vitamins [7, 8, 9]. The photosynthetic pigments like chlorophylls and carotenoids were elevated in leafy vegetables [10]. The pigments contents in plants are important, not only due to the coloration and physiological function but also due to their acknowledged roles in health [11, 12]. Carotenoids and chlorophylls have an important part in the prevention of various diseases

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associated with oxidative stress, such as cancer, cardiovascular diseases and other chronic diseases [13]. Red chicory is characterized by a high content of anthocyanin pigments [14]. The phytochemical composition of red chicories recorded a large amount of as anthocyanins, flavonoids and phenolic acids, hydroxybenzoic and hydroxycinnamic acids as well as of red anthocyanins [15, 16].

The chicory yield quantity and quality depend on several factors, including the course of weather conditions, season and method of cultivation, soil moisture, fertilization and varieties [17, 18, 19]. However, the yield potential of the currently available bolting-resistant varieties has not yet been completely exploited due to the slow youth growth that most chicory varieties display at low temperature [20]. In the field, cultivation of *Cichorium intybus* L. plants was used a modern approach on chicory yield and quality production. Also, the application of biological active substances is widely used to manage crop growth and improve crop production [21, 22]. According to United States Department of Agriculture (USDA) (2009) chicory is vegetable contains vitamin C in edible parts (8 mg /100 g of f.w.) what is in agreement with Francke and Majkowska-Gadomska [18] and Rozek [17]. Leaves of chicory are good sources of phenols, vitamins A and C as well as potassium, calcium and phosphorus [23].

Chicory is a plant whose tuberous roots and leaves store inulin, with high fructose content about 94% [24]. *Asteraceae* plant family contain high amount of inulin storage in their root, leaf, flower and seeds. Inulin is a prebiotic used as a dietary soluble fiber, low calorie sweetener and it enhances calcium absorption in the intestine [25]. Fresh chicory contain 68% inulin, 14% sucrose, 5% cellulose, 6% protein, 4% ash and 3% other compounds, while dried chicory contains approximately 98% inulin and 2% other compounds [26, 9]. Flavonoids have also been shown to act as scavengers of various oxidizing species investigated the content of phenolic compounds, mainly cinnamic acids and flavonoids in *C. intybus L. var. silvestre* [27, 28]. The total phenolic compounds and their classes varied widely among chicory cultivars [29]. Chicory leaves have a quality and quantity of flavonoids, phenolic acids, ascorbic acid and tocopherols are well-known subclass of phytochemical compounds which possess antioxidant properties and are used for the treatment of cancer [30-33].

Chicory is a rich in phenolic compounds it has antioxidant and anticancer effects are found in the leaves of chicory than other parts [5, 33, 34, 35]. The phytochemical screening confirmed the presence of tannins, saponins, flavonoids, in the chicory leaves. The chicory leaves showing good free radical scavenging capacity due to higher DPPH radical inhibition and lower IC50 value [9, 33].

Identification of plant genetic diversity and polymorphic relationships can be assessed by several markers [36]. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is widely used due to its validity and simplicity for describing genetic structure of crop germplasm [37]. The molecular characterization of *Chicorium intybus* L. can form the basis for an additional criterion of selection of phenotypically homogeneous genotypes to be used in breeding synthetic varieties and commercial hybrids. Moreover, the possibility of identifying the types of *Chicorium* in commercial through their molecular characterization can be an essential element for certifying typical local products and in the near future could represent a basic requisite for their use in a serious and consumer-oriented production and marketing context [38].

The objective of this work was to evaluate the nutritional composition of eight edible *Cichorium intybus* L. landraces which collected from different locations in Egypt. The present study was undertaken with an aim to evaluate the pigments content (chlorophylls, carotenoids and anthocyanins) of chicory landraces. In addition to determining their chemical compositions such as TSS, total sugars, inulin, ascorbic acid, flavonoids, total phenolic compounds and their antioxidant activity. The eight chicory genotypes will be identify by using molecular markers to reveal the inheritance relationships may be useful to use it during the breeding program.

MATERIALS AND METHODS

Plant Material: The present study was carried out during the two successive seasons of 2013/2014 (first season) and 2014/2015 (second season) on Chicory landraces (*Cichorium intybus* L) Asteraceae family which collected from eight geographical regions in Egypt (Table 1 and Fig. 1). Plants were grown over two years at the Horticulture farm in Faculty of Agriculture, Al-Azhar University, Cairo, Egypt.

The seeds were sown in nursery in greenhouse on September 1st each season for thirty days and then were transplanted into the field on October 1st, in both seasons. The plots were two m^2 has three lines and each line has 10 plants in each side (total 20 plants/line and 60 plants/plot). The chicory plantings were arranged in a randomized complete block design. Each plot was first harvested 50 days after transplanting followed by second

Table 1: Chicory genotypes collected from eight governorates in Egypt

Landraces	Midrib color	Leaf blade shape	Earliness of flowering	Leaves taste
Qena	White green	Serrated	late	Very good
Sohag	White green	Lobed ++	Late	Very good
Elminya	White green	Lobed +++	late	Good
Aswan	White green	Lobed +	earlier	Bitter
Tanta	Red ++	Serrated	moderate	Very good
Behiera	Red +++	Lobed ++	late	Very good
Alexandria	Red +	Lobed +++	moderate	good
Matruh	Red +	Lobed +	earlier	bitter

+++ strong, ++ middle and + light

Fig. 1: The eight leaf blade shape and their midrib color in the collection of *Cichorium intybus* L. landraces

and third sequential harvests at 30 days apart for all genotypes. The morphological characteristics and DNA fingerprint identification of these chicory landraces are arranged for another article by Helaly and others (Data not shown).

Chemical Analysis in Chicory Fresh Tissues: Chicory fresh leaves, from first harvest, were used to determine the content and characteristics of chemicals in this study. Chlorophyll a (Chl. a), chlorophyll b (Chl. b), total chlorophyll and carotenoids (mg/100g FW) were determined according to the spectrophotometric method described by Hipkins and Baker [39]. Anthocyanins content of the chicory leaves was determined according to procedures outlined by Manchinelli [40] and estimated by the method described by Helaly *et al.* [41]. The TSS content was determined by hand refractometer as a percentage according to A.O.A.C. [42]. Total titratable acidity (g citric/100 g. FW) was determined by titration with 0.01 N NaOH using phenolphthalein as indicator according to A.O.A.C [42]. Ascorbic acid (mg/100g. FW) was determined by the method of titration with 2, 6-dichlorophenol indophenol dye according to A.O.A.C. [42]. The yield of chicory landraces were harvested and weighted for three cutting times. The sums of three harvests of chicory yield were calculated per plant in gram and per plot in kilogram.

Chemical Analysis in Chicory Dry Tissues: Total sugars and reducing sugar were determined calorimetrically as grams of sugar per 100 g tissue dry weight (%) according to the method of Smith *et al.* [43]. The inulin content was determined following the method of Winton and Winton [44]. The total flavonoid contents were prepared as described by Abbas *et al.* [9] and measured by a colorimetric assay used absorbance of the mixture in pink color and determined at 510 nm according to Rohman *et al.* [45]. The total phenolic levels in the chicory leaf tissue were determined by using the method of Chandler and Dodds [46] and modified according to Helaly *et al.* [41, 47]. The total antioxidant content in methanolic extracts of leaves in *Cichorium intybus* L. was estimated according to the method described by Brand-Williams *et al.* [48]. For determination of 2, 2- diphenyl-pecrylhydrazil (DPPH), methanolic extract was prepared and determined as described by Shad *et al.* [9, 33].

SDS-PAGE Profile of Chicory Seeds: The sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of seeds of eight chicory landraces were analyzed according to the method developed by Laemmli [49].

Statistical Analysis: The number of replicates (n) and the SE are shown for most measurements. The data was statistically analyzed with the SAS version 9.1

(www.sas.com) using ANOVA ($P \leq 0.05$) on the corresponding degrees of freedom (df) followed by Tukey's-HSD (honest significant difference) procedure for multiple comparisons of all experimental data [50].

RESULTS

Chemical Characterization of Eight Chicory Landraces in Fresh Leaf Tissues

Four Classes of Pigments Namely: Chl. a, Chl. b, total chlorophyll and carotenoids were identified and quantified under the spectrophotometer conditions during seasons 2013/2014 and 2014/2015. Overall, chlorophylls and carotenoids contents in chicory leaves were varied significantly among the eight landraces examined herein (Fig. 2). The highest Chl. a content was recorded in Qena landrace (17.92 and 18.69 mg/100 g FW in both seasons, respectively), while the lowest Chl. a content was observed in Beheira landrace (7.85 and 6.88 mg/100 g FW in both seasons, respectively, Fig. 2A). Further, the highest Chl. b content was obtained from Qena landrace (8.94 and 11.24 mg/100 g FW in both seasons, respectively), while the least value was obtained from Matruh landrace (3.12 and 5.11 mg/100 g FW in both seasons, respectively, Fig. 2B). Furthermore, concerning to the total chlorophyll, Qena landrace produced the highest content (26.86 and 29.93 mg/100g FW in both seasons, respectively), whereas the lowest value was obtained from Behiera landrace (11.63 and 14.46 mg/g FW in both seasons, respectively, Fig. 2C). On the other side, the maximum value for carotenoids content was recorded in Behiera landrace (2.13 and 2.30 mg/100g FW in both seasons, respectively), while the lowest content was obtained from Qena landrace (0.99 and 0.75 mg/100g FW in both seasons, respectively, Fig. 2D).

It was observed from Fig. (3) that anthocyanins, TSS and ascorbic acid contents in chicory leaves were also differed significantly in the eight landraces during the two seasons. Anthocyanins, important phytochemical and antioxidant components were extracted and quantified from chicory leaves (Fig. 3A). Anthocyanins content was high in four out of eight landraces and was significantly increased in Behiera landrace (4.76 and 3.98 mg/g FW in both seasons, respectively). In the contrast the lowest value of anthocyanins was obtained from Qena landrace (0.45 and 0.67 mg/g FW in both seasons, respectively). The highest significant of TSS % (Fig. 3b) was obtained from Tanta landrace in first season (5.1%) and from Qena in the second season (5.45) in comparison to Aswan landrace which exhibit the lowest number 2.25 and 2.45% in both season, respectively. Titratable acidity (Fig. 3c) was not reaching the significant level among all chicory landraces. The greatest value of ascorbic acid (Figure 3d) was obtained from Tanta landrace (33.92 and 27.29 mg/100g FW in both seasons, respectively). However, the least value was obtained from Aswan landrace (22.39 and 15.33 mg/100g FW in both seasons, respectively).

Characterization of Yield of Eight Chicory Landraces: The total yield of chicory landraces leaves per plant and total yield per plot during both seasons are presented in Table 2. Data cleared that there were significant differences among chicory landraces, however the greatest value was obtained from Behiera landrace (294.3 and 319.8 g/plant and 17.66 and 19.19 kg/plot in both seasons, respectively), followed by Tanta landrace (290.0 and 247.0 g/plant and 17.40 and 14.82 kg/plot in both seasons, respectively). On the other side, the lowest value was obtained from Matruh landrace (134.6 and 146.7 g/plant and 8.08 and 8.80 kg/plot, respectively), followed by Aswan landrace (178.8 and 181.3 g/plant and 10.73 and 10.88 kg/plot in both season, respectively).

Chemical Characterization of Eight Chicory Landraces in Dry Leaf Tissues: The chemical composition in chicory leaves such as dry matter, total sugar, reducing sugar and nun reducing sugar percentage are presented in Table 3. A statistically significant difference was observed among various landraces of *C. intybus* regarding these chemical compositions. The average of both seasons showed that the highest dry matter content was found from Behiera landrace (9.83 and 10.27 % in both seasons, respectively) followed by Tanta landrace (9.21 and 7.53% in both seasons, respectively). On the other side, the least dry matter content was obtained from Matruh (5.26 and 5.37% in both seasons, respectively) and Aswan (4.87 and 6.11 % in both seasons, respectively). The highest total sugar was found in Behiera landrace (8.45 and 9.83% in both seasons, respectively) followed by Alexandria landrace (8.76 and 6.88% in both seasons, respectively). The leaves of Behiera landrace were found to be comparatively high in reducing sugars (4.86 and 6.14 % in both seasons, respectively. In contrast the least value of reducing sugars was obtained from Tanta (4.84% in the first season) and Matruh landrace (4.16% in in the second season). Qena landrace revealed the lowest recorded number of reducing sugars 2.39% in the first season, while Alexandria landrace recorded 2.11% in the second season. The less recorded number of nun reducing sugars was obtained from Tanta landrace (2.19% in the first season) and Matruh landrace (1.67% in second season).

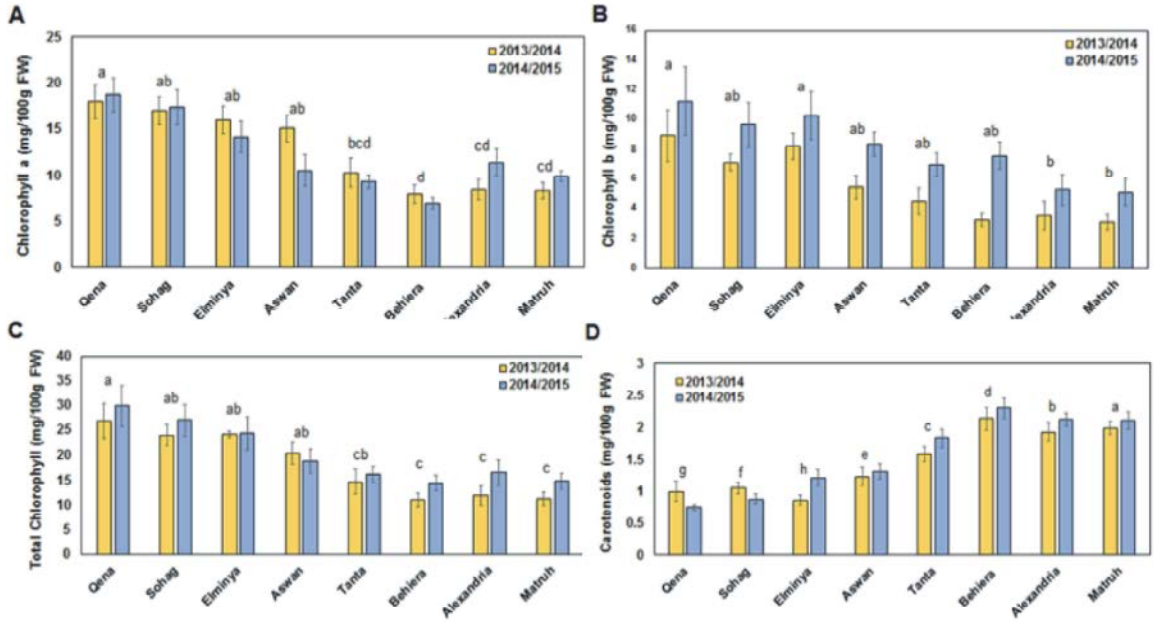

Fig. 2: Pigments contents in chicory landraces as chlorophyll a (A), chlorophyll b (B), Total chlorophyll (C) and Carotenoids (D) during 2013/2014 and 2014/2015 seasons

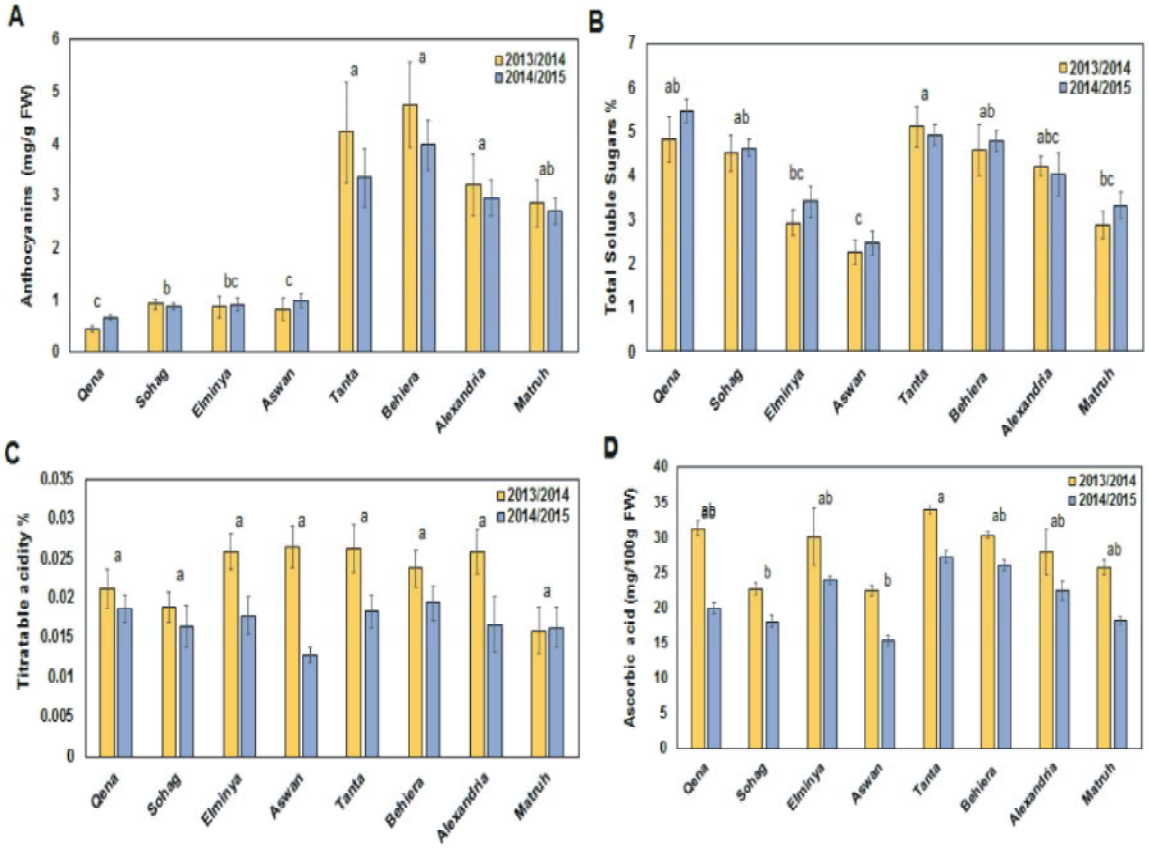

Fig. 3: Chemical characterization in chicory landraces fresh leaves as anthocyanins (A), total soluble solids (B), titratable acidity (C) and ascorbic acid (D) during 2013/2014 and 2014/2015 seasons

Table 2: Evaluation of Chicory landraces via total yield /plant (gm) and total yield/plot (Kg) during 2013/2014 and 2014/2015 seasons

Characters	Total yield (g)/plant		Total yield (Kg)/plot	
Landraces	1st season	2nd season	1st season	2nd season
Qena	234.3 ± 0.6	237.3 ± 0.92	14.06 ± 0.6	14.24 ± 0.9
Sohag	272.0 ± 1.0	246.0 ± 0.46	16.32 ± 1.0	14.76 ± 0.5
Elminya	224.2 ± 0.7	208.6 ± 0.57	13.45 ± 0.7	12.52 ± 0.6
Aswan	178.8 ± 0.8	181.3 ± 0.09	10.73 ± 0.8	10.88 ± 0.1
Tanta	290.0 ± 0.7	247.0 ± 0.80	17.40 ± 0.7	14.82 ± 0.8
Behiera	294.3 ± 0.4	319.8 ± 0.54	17.66 ± 0.4	19.19 ± 0.5
Alexandria	223.0 ± 0.9	217.0 ± 0.53	13.38 ± 0.9	13.02 ± 0.5
Matruh	134.6 ± 0.3	146.7 ± 0.62	8.08 ± 0.3	8.80 ± 0.6
LSD at 5%	4.39	3.71	1.19	0.98

Table 3: Evaluation of Chicory landraces via dry matter, total sugar, reducing sugar and nun reducing sugars contents during 2013/2014 and 2014/2015 seasons

Characters	Dry matter %		Total sugar %		Reducing sugar %		Non reducing sugars %	
Landraces	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Qena	7.23 ±0.65	7.12 ±0.32	7.46 ±0.46	8.19 ±0.17	2.39 ±0.20	3.37 ±0.22	5.07 ±0.24	4.82 ±0.18
Sohag	8.51 ±0.29	7.49 ±0.27	5.41 ±0.11	8.44 ±0.45	2.49 ±0.16	2.75 ±0.21	2.92 ±0.15	5.69 ±0.26
Elminya	6.64 ±0.49	6.21 ±0.37	6.33 ±0.28	7.93 ±0.25	3.06 ±0.21	2.32 ±0.11	3.27 ±0.24	5.61 ±0.12
Aswan	4.87 ±0.14	6.11 ±0.38	6.54 ±0.31	7.83 ±0.23	3.40 ±0.23	3.14 ±0.27	3.14 ±0.18	4.69 ±0.35
Tanta	9.21 ±0.55	7.53 ±0.29	4.8 4±0.31	7.91 ±0.34	2.65 ±0.12	3.92 ±0.28	2.19 ±0.10	3.99 ±0.19
Behiera	9.38 ±0.63	10.27 ±0.4	8.45 ± 0.1	9.83 ±0.54	4.86 ±0.24	6.14 ±0.22	3.59 ±0.16	3.69 ±0.11
Alexandria	6.6 ±0.65	6.36 ±0.41	8.67 ±0.24	6.88 ±0.57	2.77 ±0.26	2.11 ±0.18	5.9 ±0.23	4.77 ±0.27
Matruh	5.26 ±0.47	5.37 ±0.22	6.53 ±0.21	4.16 ±0.21	2.98 ±0.17	2.49 ±0.16	3.55 ±0.18	1.67 ±0.19
LSD at 5%	1.45	1.28	0.87	0.55	0.26	0.35	0.73	0.95

The biochemical compositions of inulin, flavonoids, total phenols and antioxidants were significantly differences among eight chicory landraces which presented in Fig. 4. Inulin contents (Fig. 4a) are much important component in chicory plants and the result exhibit non-significant differences were observed among all landraces. However, the elevated value of inulin was found from Behiera (0.27 and 0.21 g/100g DW in both seasons, respectively), while the least number was obtained from Aswan (0.17 and 0.10 g/100g DW in both seasons, respectively). The highest flavonoids (Fig. 4b) were obtained from Behiera landrace (7.12 and 6.54 mg/g DW in both seasons, respectively). In the contrast Aswan and Qena landraces reached the least flavonoids content (3.11 and 2.56 mg/g DW in both seasons, respectively). The elevated value of total phenols (Fig. 4c) was revealed from Behiera landrace (3.74 and 4.11 % in both seasons, respectively). On the contrast, the lowest percentage of total phenols was obtained from Sohag landrace (2.47%) and Matruh landrace (2.23%) in first and second seasons, respectively. Referring to the antioxidants contents (Fig. 4d) as 2, 2 Diphenyl -1 – picryhydrazyl (DPPH) widely used to evaluate the free radical scavenging effect of plant extract. The DPPH scavenging capacities of methanolic extract in different *Cichorium intybus* L. landraces in terms of percent inhibition of DPPH. The highest antioxidants percentage of chicory leaves extract was obtained from Behiera (67.44% and 87.30% in both seasons, respectively). However, the least percentage of antioxidants was obtained from Aswan landrace (51.18%) in the first season and Matruh landrace (48.93%) in the second season.

Identification of Chicory Genotypes by Seed Storage Protein Based on SDS-PAGE: This study focused to identify eight Egyptian chicory genotypes by using seed storage protein (SDS-PAGE). The protein content in the tested dry seeds of eight chicory genotypes was extracted and analyzed by using SDS-PAGE technique. The obtained data showed that the protein gel exhibits a maximum of 72 protein bands distributed over a wide range of molecular weights ranged from 20 to 230 kilo Dalton (KD) (Fig. 5). The data matrix of seed protein profile were coded and applied to ýcomputer software to determine the similarity between the studied chicory genotypes. The similarity from protein matrix of the eight Egyptian chicory genotypes is summarized in Tables 6 and 7. According to the tables, the obtained results exhibited that chicory genotypes relationship classified into six groups which highlighted as the following: 1) The value of similarity among the different pairs of the studied genotypes fluctuates between 0.44 and 1.00. 2) The lowest similarity value (0.44) is scored between the two genotypes Behiera X Elminya 3) The highest similarity value (1.000) is detected among the three genotypes Tanta, Alexandria, Matruh and Sohag.

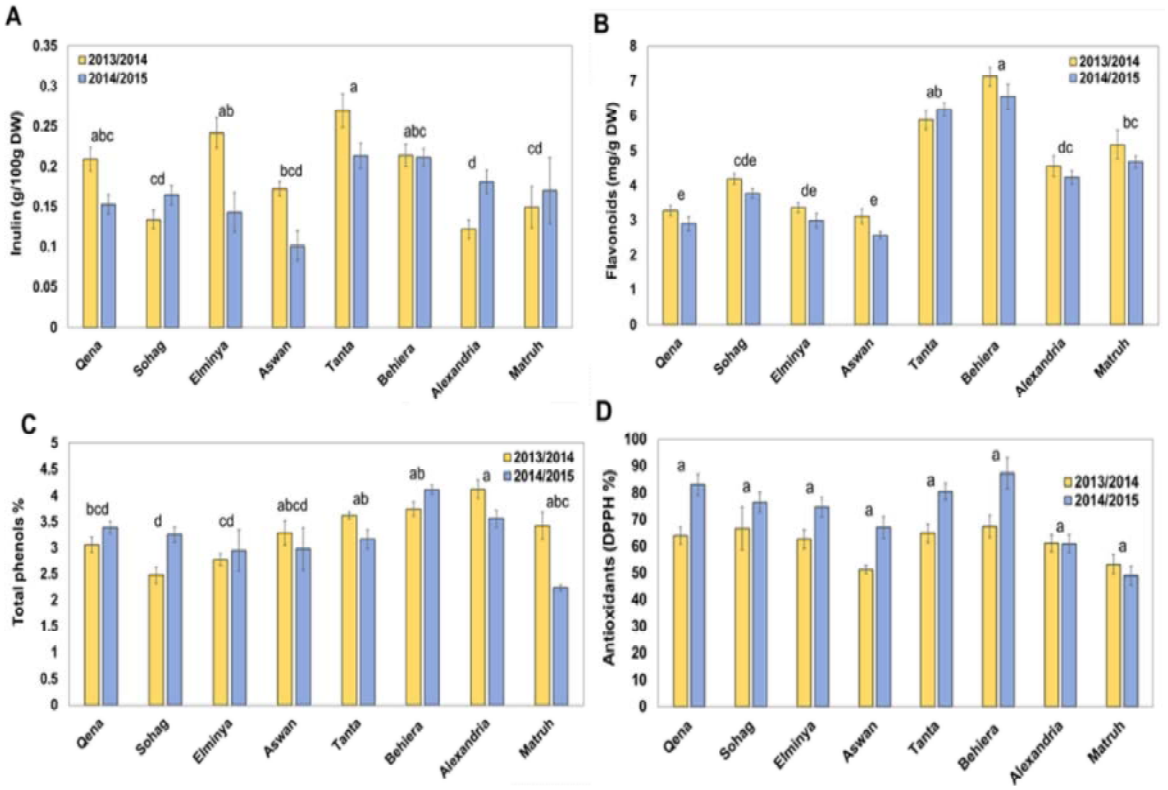

Fig. 4: Chemical characterization in chicory landraces dry leaves as inulin (A), flavonoids (B), total phenols (C) and antioxidants (D) during 2013/2014 and 2014/2015 seasons

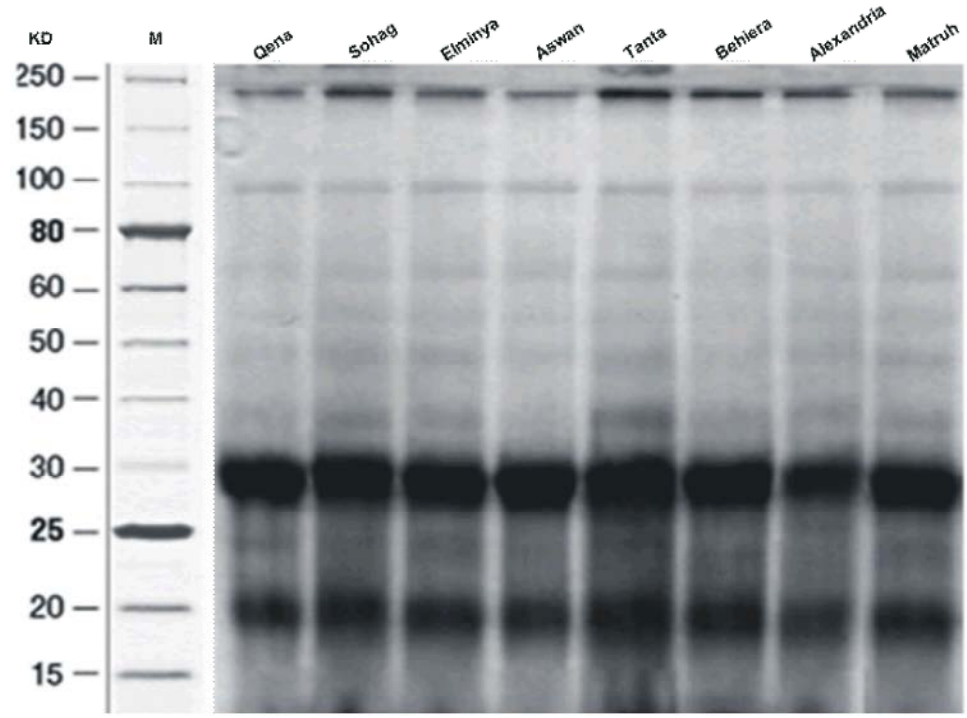

Fig. 5: Electrophoretic protein gel of the various chicory landraces

Table 6: Similarity from protein matrix of the various chicory genotypes by Unweighted Pair-Group Mathematic Average (UPGMA)

Genotypes	G1	G2	G3	G4	G5	G6	G7	G8
Qena	1.00							
Sohag	0.78	1.00						
Elminya	0.89	0.89	1.00					
Aswan	0.67	0.67	0.56	1.00				
Tanta	0.78	1.00	0.89	0.67	1.00			
Behiera	0.56	0.56	0.44	0.89	0.56	1.00		
Alexandria	0.78	1.00	0.89	0.67	1.00	0.56	1.00	
Matruh	0.78	1.00	0.89	0.67	1.00	0.56	1.00	1.00

Table 7: Relationships among the various chicory genotypes by similarity matrix based on SDS-PAGE protein (UPGMA)

Group 1 100 % similarity	Group 2 89 % similarity	Group 3 78 % similarity	Group 4 67 % similarity	Group 5 56 % similarity	Group 6 44% similarity
Tanta X Alex.*	Elminya X Qena	Qena X Tanta	Aswan X Qena	Behiera X Qena	Behiera X Elminya
Tanta X Matruh	Elminya X Sohag	Qena X Alex.	Aswan X Sohag	Behiera X Sohag	---- -----
Tanta X Sohag	Eminya X Tanta	Qena X Matruh	Aswan X Tanta	Behiera X Tanta	----- -----
Sohag X Alex.	Elminya X Alex.	----- -----	Aswan X Alex.	Behiera X Alex.	----- -----
Matruh X Alex.	Elminya X Matruh	----- -----	Aswan X Matruh	Behiera X Matruh	----- -----
----- -----	Behiera X Aswan	----- -----	----- -----	Elminya XAswan	----- -----

* Alex. = Alexandria

DISCUSSION

Wild plants in the Mediterranean basin have a long history of being utilized for food, medicine, fuel, shelter and others applications [39, 49]. *Cichorium intybus* L. is a typical Mediterranean plant, occurring also in Western Asia, Egypt and North America [50]. Multiple research papers have been published which described the phytochemical composition and several health properties of *C. intybus*, including antidiabetic reagent, wound healing and antioxidant capacities of chicory grown in various European countries [51, 52, 53].

In this study, it was evaluated the agronomic and nutritional characteristics of eight chicory landraces grown naturally, or sometime are classified as a weed in Clover (*Trifolium alexandrinum*) fields in Egypt, in order to highlight their usefulness as ingredients in food and medical applications.

The chlorophylls (chlorophyll a, b and total) are virtually essential pigments for the conversion of light energy to stored chemical energy [54]. The relative content of chlorophylls showed significant changes among the Chicory landraces evaluated in this research. The Qena landrace was rich in Chl a, b and total chlorophyll and differ significantly from the other chicory landraces. The results exhibited a positive correlation between chlorophylls content and the leaf midrib colors, that all the landraces with white green leaf midrib were rich in chlorophylls in contrast to landraces with colored leaf midrib. On the other side, there was a negative correlation between both carotenoid or anthocyanin contents and chlorophyll contents, that the landraces (i.e. Behiera) with high carotenoids or high anthocyanins contain less chlorophylls. The differences in pigments content among the chicory landraces may due to the influence of several factors, including the geographical origin, growth conditions and nutrition, environmental stresses and genetic background [55]. Like other leafy vegetables, the richness in photosynthetic pigments; chlorophylls and carotenoids, in our Chicory landraces can be an added-value to utilize this crop as a source for vitamin C and β-Carotene which of importance in food and medicine industry [56]. Chlorophyll and Carotenoids concentration correlate to the photosynthetic potential of plants, giving some indication of the physiological and phytochemical status of the plant, in addition to their vital roles in human health [11, 12, 57, 58]. The variation of photosynthetic pigments content detected herein was in consistent with the previous findings, that the content of these pigments are varied significantly among different species and/or cultivars of leafy vegetables [59].

Red chicory with a visible red leaf midrib and vein color contain high levels of phenolic compounds such as anthocyanins and this trait render Chicory useful in human health improvement, as it is involved in curing many diseases associated with visual capacity, brain cognitive function, obesity, cardiovascular risk and cancer prevention [60, 61, 62]. Also it was observed that red chicory can represent a good source of phytochemicals in terms of total anthocyanins which effectively corresponded to an increase of antioxidant, cytoprotective and antiproliferative activities [16].

Moreover, chicory leaves have been reported to contain high levels of TSS and ascorbic acid [49, 63]. Accordingly, we measured the relative content of TSS and ascorbic acid in the leaves of the eight chicory landraces herein and the results indicated major significant changes of the content of these two biochemicals among the landraces. The highest value of TSS was recorded in Qena landrace, followed by Tanta and Behiera landraces, whereas the ascorbic acid content was significantly higher in the most of landraces except in Aswan and Matruh, where the lowest value in the nutritional compositions was also recorded.

It was observed from previous studies that the leaves of wild chicory show better nutritional profile of nutrients than the cultivated ones and their content of vitamin C is approximately forty times higher than that in lettuce [17, 64]. In the context of the importance of determining the chicory yield and its nutritional values, we evaluated the eight landraces according to their yields and we detected major significant changes in yield productivity among those landraces. Accordingly, we can group the landraces into three groups based on the yield qualities; i) Aswan and Matruh landraces were produced the lowest value of yield and total sugars, on a dry matter basis, in comparison to others landraces, ii) Behira, Tanta, Qena and Sohag landraces were rich in yield quantity and quality and iii) Alexandria and Elminya landraces were on the average. Our findings of yield quality and quantity indicate that Chicory landraces grown in Egypt can be comparable to the cultivated varieties grown worldwide.

Regarding to the other constituents we measured in this research, it was observed that Behiera and Tanta landraces were exhibited the highest values of inulin, flavonoids, total phenolics and antioxidants content than other landraces. This finding can be considered an added-value to chicory since red chicory landraces (i.e. Behiera and Tanta) which are rich in anthocyanins, flavonoids and phenolic acids are known to possess antioxidant activities due to the presence of hydroxyl groups in their structures and their contribution to defense system against the oxidative damage due to endogenous free radicals [67, 68]. Based on that, Abbas *et al.* [9] have partially characterized and confirmed the high contents of total flavonoids, total phenolic and total antioxidant capacity in the red chicory leaf extracts. The variation in the above-mentioned constituents between red chicory and white-green midrib chicory varieties (landraces) is probably due to the influence of several factors, including both varietal and environmental factors, the latter being related to growing, soil and climatic conditions, on the antioxidant activities [29, 67, 68].

Leaves were also found to possess comparatively good free radical scavenging capacity due to their higher DPPH radical inhibition and lower IC50 value. However, all other parts of Chicory (i.e. stem, flower, seed) showed lower percentage of DPPH radical inhibition and higher IC50 values as compared to those of Trolox and ascorbic acid taken as standard antioxidants [33, 71].

Concerning to identification of eight *Chicorium intybus* L. by molecular marker, the data analysis indicated that the genetic relationship of these chicory genotypes was tightly associated with its origin and traits. It could be concluded that seed protein SDS-PAGE markers can be a powerful tool for identification and genetic diversity analysis in Chicory genotypes. The molecular information was used to carry out a positive selection of all of types within each population in order to maintain the most similar and distinguishable individuals. Molecular markers would be used to support field selection programs aiming to reach a degree of genetic uniformity that would probably not be achievable with the simple phenotypic evaluation performed by farmers [5, 10, 15].

This article sheds the lights on the importance of positioning the Egyptian Chicory landraces as new chicory accessions that can grow widely as a traditional seasonal crop, thus converting a neglected crop into a domestic crop. The growing of these landraces in different geographical areas in Egypt could be helpful in future breeding programs for the production of chicory varieties. To date, chicory remains an extremely versatile plant, amenable to genetic manipulation and there is interest shown in genetically engineered chicory to obtain higher yields and create new potentials [5, 15].

CONCLUSIONS

The study revealed that in all the genotypes despite of being morphologically different and divided to two groups. The first group has white green color in their leaf midrib and being rich in chlorophyll a, b and total. The second group has red color in their leaf midrib and rich in Anthocyanins, antioxidants and flavonoids. The highest yield was obtained from Sohag genotype from the first group followed by Behiera genotype from the second group. SDS-PAGE is used due to its validity

and simplicity for describing genetic structure of chicory germplasm, but its implication has been limited mainly to plants due to less polymorphism in most of the chicory genotypes. The characterizations analysis is dedicate most diversity among all genotypes and may be further utilized as potent genotypes in *Chicorium intybus* breeding program for the varietal development. The variability I morphological, chemical and molecular analysis is offering the possibility of mass selection from the existing varieties to breed superior chicory.

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REFERENCES

1. Van Stallen, N., V. Noten, M. Demeulemeester and M. De Proft, 2000. Identification of commercial chicory cultivars for hydroponic forcing and their phenetic relationships revealed by random amplified polymorphic DNAs and amplified fragment length polymorphisms. Plant Breeding, 119: 265-270.
2. Hazra, B., R. Sarkar, S. Bhattacharyya and P. Roy, 2002. Tumour inhibitory activity of chicory root extract against Ehrlich ascites carcinoma in mice. Fitoterapia, 73: 730-733.
3. El-Alfy T.S., H. El-Askary, S. Abd El-Tawab and S.M. Kamel, 2009. Apharmacognostcal study of *Cichorium intybus* L. herb growing in Egypt. Bull. Fac. Pharm. Cairo Univ., 47(2): 77-99.
4. Wang, Q. and J. Cui, 2011. Perspectives and utilization technologies of chicory (*Cichorium intybus* L.): a review. African Journal of Biotechnology, 10(11): 1966-1977.
5. Street, R.A., J. Sidana and G. Prinsloo, 2013. *Cichorium intybus*: Traditional Uses, Phytochemistry, Pharmacology and Toxicology. Evidence-Based Complementary and Alternative Medicine, pp: 1-13.
6. Das, S., N. Vasudeva and S. Sharma, 2016. *Cichorium intybus*: A Concise on its ethnomedicinal, botanical and phytopharmacological aspects. Drug Development and Therapeutics, 7(1): 1-12.
7. Muthusamy, V.S., S. Anand, K.N. Sangeetha, S. Sujatha, B. Arun and B.S. Lakshami, 2008. Tannins present in *Cichorium intybus* L. enhance glucose uptake and inhibit adipogenesis in 3T3-L1 adipocytes through PTP1B inhibition. Chem. Biol. Interact., 174(1): 69-78.
8. Atta, A.H., T.A. Elkoly, S.M. Mouneir, G. Kamel, N.A. Alwabel and S. Zaher, 2010. Hepatoprotective effect of methanolic extracts of *Zingiber officinale* and *Cichorium intybus*. Indian J. Pharm. Sci., 72(5): 564-570.
9. Abbas, Z.K., S. Saggu, M.I. Sakeran, N. Zidan, H. Rehman and A.A. Ansari, 2015. Phytochemical, antioxidant and mineral composition of hydroalcoholic extract of chicory (*Cichorium intybus* L.) leaves. Saudi Journal of Biological Sciences, 22: 322-326.
10. Kiaer, L.P., M. Philipp, R.B. Jrgensen and T.P. Hauser, 2007. Genealogy, morphology and fitness of spontaneous hybrids between wild and cultivated chicory (*Cichorium intybus*). Heredity, 99: 112-120.
11. Liu, Y.T., C.O. Perera and V. Suresh, 2007. Comparison of three chosen vegetables with others from South East Asia for theit lutein and zeaxanthin content. Food Chemistry, 101: 1533-1539.
12. Niizu, P.Y. and D.B. Rodriguez-Amaya, 2005. New data on the carotenoid composition of raw salad vegetables. Journal of Food Composition and Analysis, 18: 739-749.
13. Sangeetha, R.K. and V. Baskaran, 2010. Carotenoid composition and retinol equivalent in plants of nutritional and medicinal importance. Efficacy of b - carotene from *Chenopodium album* in retinol-deficient rats. Food Chemistry, 119: 1584-1590.
14. Rossetto, M., A. Lante, P. Vanzani, P. Spettoli, M. Scarpa and A. Rigo, 2005. Red chicories as potent scavengers of highly reactive radicals: a study on their phenolic composition and peroxyl radical trapping capacity and efficiency. Journal of Agricultural and Food Chemistry, 53(21): 8169-8175.
15. Bais, H.P. and G.A. Ravishankar, 2001. *Cichorium intybus* L. cultivation, processing, utility, value addition and biotechnology, with an emphasis on current status and future prospects. Journal of the Science of Food and Agriculture, 81(5): 467-484.

16. Devoli, L., F. Morroni, G. Lombardi-Boccia, M.O. Lucarini, P. Hrelia, G. Cantelli-Forti and A. Tarozzi, 2013. Red Chicory (*Cichorium intybus* L. cultivar) as a Potential Source of Antioxidant Anthocyanins for Intestinal Health. Hindawi Publishing Corporation Oxidative Medicine and Cellular Longevity, pp: 1-8.
17. Rozek E., 2007. Plonowanie cykorii sałatowej (*Cichorium intybus L. var. foliosum* Bisch) uprawianejw kilku terminach. Annales UMCS, Horticultura, XVII(2): 11-18.
18. Francke, A. and J. Majkowska-Gadomska, 2008. Effect of planting date and method cultivation on the chemical composition of radicchio. J. Elementol., 13(2): 199-204.
19. Biesiada, A. and E. Kołota, 2010. The effect of nitrogen fertilization on yielding and chemical composition of radicchio chicory for autumn harvest cultivation. Acta Sci. Pol., Hortorum Cultus, 9(4): 85-91
20. Baert, J.R.A., 1997. The effect of sowing and harvest date and cultivar on inulin yield and composition of chicory (*Cichorium intybus* L.) roots. Indus. Crops Prod., 6: 195-199.
21. Cerny, I., V. Pacuta, G. Villar, 2000. Influence of Atonik on sugar beet yield and technological quality, Listy Cukrovarnicke a Reparske, 116: 316-319.
22. Cerny, I., V. Pacuta and M. Kovar, 2008. Yield and quality of chicory (*Cichorium intybus* L.) in dependence on varity and foliar application of atonik and polybor 150. Journal Central European Agriculture, 9(3): 425-430.
23. Mulabagal, V., H. Wang, M. Ngouajino and M. Nair, 2009. Characterization and quantification health of beneficial anthocyanins in leaf chicory (*C. intybus*) varieties. Eur Food Res Technol., 230: 47-53.
24. Couri, S., F.S. Gomes, R.I. Nogueira, V.C. Wilberg, L.M.C. Cabra, V.V. Silva and D.L. Almeida, 2016. Determination of inulin content of chicory roots (*Cichorium intybus* L.) cultivated organically in three regions of Rio de Janeiro state. Embrapa Agroindustria de Alimentos, Av. das Americas 29501, Guaratiba-RJ, CEP- 23020-470. Retrieved on: 18 August 2016
25. Devacht, S., P. Lootens, J. Baert, J. Van Waes, E.Van Bockstaele and I. Roldan-Ruiz, 2011. Evaluation of cold stress of young industrial chicory (*Cichorium intybus* L.) plants by chlorophyll a fluorescence imaging. I. Light induction curve. Photosynthetica 49(2): 161-171.
26. Meehye, K. and H.K. Shin, 1996. The water-soluble extract of chicory reduces glucose uptake from the perfused jejunum in rats. J. Nutr., 126(9): 2236-2242.
27. Harborne, J.B and C.A. Williams, 2000. Advances in flavonoid research since 1992. Phytochemistry, 55: 481-504.
28. Mulinacci, N., M. Innocenti, S. Galori, G. Lamarca and F.F. Vincieri, 2001. Optimization of the chromotografic determination of polyphenols in the aerial part of *Cichorium intybus* L. Chromotographia, 54: 455- 461.
29. Sinkovic, L., L. Demsar, D. Znidarcic, R. Vidrih, J. Hribar and D. Treutter, 2015. Phenolic profiles in leaves of chicory cultivars (*Cichorium intybus* L.) as influenced by organic and mineral fertilizers. Food Chemistry, 166: 507-513.
30. Shahidi, F., 2000. Antioxidant in food and food antioxidants. Nahrung., 44: 158-163.
31. Bergman, M., L. Varshavsky, H.E. Gottlieb and S. Grossman, 2001. The antioxidant activity of aqueous spinach extract: chemical identification of active fractions. Phytochem., 58: 143-152.
32. Barnes, S., 2001. Role of phytochemicals in prevention and treatment of prostate cancer. Epidemiol. Rev., 23(1): 201-205.
33. Shad, M., H. Nawaz, T. Rehman and N. Ikram, 2013. Determination of some biochemicals, phytochemicals and antioxidant properties of different parts of *Cichorium intybus* L.: A comparative study. J. Anim. Plant Sci., 23: 1060-1066.
34. Rozpądek, P., K. Wêżowicz, A. Stojakowska, J. Malarz, E. Surowka, T. Anielska, R. Ważny and Z.K. Miszalski, 2014. Turnau Mycorrhizal fungi modulate phytochemical production and antioxidant activity of *Cichorium intybus* L. (*Asteraceae*) under metal toxicity. Chemosphere, 112: 217-224.
35. Yook, J., M. Kim, P.B.T. Pichiah, S. Jung, S. Chae and Y. Cha, 2015. The Antioxidant Properties and Inhibitory Effects on HepG2 Cells of Chicory Cultivated Using Three Different Kinds of Fertilizers in the Absence and Presence of Pesticides. Molecules, 20(7): 12061-12075.
36. Afzal, M.A., M.M. Haque and S. Shanmugasundaram, 2004. Random Amplified Polymorphic DNA (RAPD) Analysis of Selected Mungbean [*Vigna radiata* (L.) Wilczek] Cultivars. Asian Journal of Plant Sciences, 3: 20-24.
37. Buckseth, T. and Y.V. Singh, 2016. Singh Seed storage Protein profiling of Pea (*Pisum sativum* L.) Genotypes using SDSPAGE. Int. Res. J. Biological Sci., 5(1): 37-41.

38. Barcaccia, G., M. Lucchin, R. Lazzarin, P. Parrini, 2003. Relationships among radicchio (*Cichorium intybus* L.) types grown in Veneto and diversity between local varieties and selected lines as assessed by molecular markers. Eucarpia Leafy Vegetables, pp: 105 -110.
39. Hipkins, M.F. and N.R. Baker, 1986. Photosynthesis Energy Transduction. In: M.F. Hipkins and N.R. Baker (eds.). Spectroscopy. IRL, Oxford, UK, pp: 51-105.
40. Manchinelli, A.L., 1990. Interaction between light quality and light quantity in the photoregulation of anthocyanin production. Plant Physiol., 92: 1191-1195.
41. Helaly, A.A., J.P. Baek, E. Mady, M.H.Z. Eldekashy and L. Craker, 2015. Phytochemical Analysis of Some Celery Accessions. Journal of Medicinally Active Plants, 1(4): 1-7
42. A.O.A.C., 2000. Official methods of Analysis of A.O.A.C. (Association of Official Analytical Chemists) International (17th ed.). A.O.A.C. International, Gaithersburg, MD.
43. Smith, F.M.A., G.D.K Hamilton and P.A. Geeds, 1956. Colorimetric method for determination of sugars and related substances. Anal Chem., 28: 550.
44. Winton, A.L. and K.B. Winton, 1958. The Analysis of Foods. John Wiley and Sons. Inc, London, pp: 857.
45. Rohman, A., S. Riyanto, N. Yuniarti, W.R. Saputra, R. Utami and W. Mulatsih, 2010. Antioxidant activity, total phenolic and total flavaonoid of extracts and fractions of red fruit (*Pandanus conoideus* lam) Int. Food Res. J., 17: 97-106.
46. Chandler, S.F. and J.H. Dodds, 1983. The effect of phosphate, nitrogen and sucrose on the production of phenolics and solasidine in callus cultures of *Solanum laciniatum*. Plant Cell Reports, 2(4): 205-208.
47. Helaly, A.A., A.A. Alkharpotly, E. Mady and L.E. Craker, 2017. Characterization of Four Molokhia (*Corchorus olitorius*) Landraces by Morphology and Chemistry. Journal of Medicinally Active Plants. In press.
48. Brand-Williams, W., M.E. Cuvelier and C. Berset, 1995. Use of free radical method to evaluate antioxidant activity. Lebensm. Wiss. Technol., 28: 25-30.
49. Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 277: 680-685.
50. Yuan, Y., 2011. Multiple imputations using SAS software. Journal of Statistical Software, 45(6): 1-25.
51. Helaly, A.A., M. Maray, A.S.A Abo El Hamd and A.A. Mohamed, 2016. Physical and Chemical Changes in the Endive Plants (*Cichorium endivia L. var. crispum*) During Developmental Stages. Adv. Plants Agric. Res., 5(1): 1-8
52. Dalar, A. and I. Konczak, 2014. *Cichorium intybus* from Eastern Anatolia: Phenolic composition, antioxidant and enzyme inhibitory activities. Industrial Crops and Products, 60: 79-85.
53. Azay-Milhau, J., K. Ferrare, J. Leroy, J. Aubaterre, M. Tournier, A.D. Lajoix and D. Tousch, 2013. Antihyperglycemic effect of a natural cichoric acid extract of chicory (*Cichorium intybus L.*): A comparative in vitro study with the effects of caffeic and ferulic acids. J. Ethnopharmacol., 150: 755-760.
54. Carazzone, C., D. Mascherpa, G. Gazzani and A. Papetti, 2013. Identification of phenolic constituents 1in red chicory salads (*Cichorium intybus*) by high-performance liquid chromatography with diode array detection and electrospray ionization tandem mass spectrometry. Food Chem., 138: 1062-1071.
55. Morales, P., I.C.F.R. Ferreira, A.M. Carvalho, M.C. Sanchez-Mata, M. Camara, V.M. Fernandez-Ruiz, Pardo-de-Santayana and J. Tardío, 2014. Mediterranean non-cultivated vegetables as dietary sources of compounds with antioxidant and biological activity. LWT-Food Sci. Technol., 55: 389-396.
56. Gitelson, A.A., Y. Gritz and M.N. Merzlyak, 2003. Relationships between leaf chlorophyll content and spectral reflectance and algorithms for non-destructive chlorophyll assessment in higher plant leaves J. Plant Physiol., 160(3): 271-282.
57. Van Den Berg, H., R. Faulks, H.F. Granado, J. Hirschberg B. Olmedilla and G. Sandmann, 2000. The potential for the improvement of carotenoid levels in foods and the likely systemic effects. Journal of the Science of Food and Agriculture, 80: 880-912.
58. Kimura, M. and D.B. Rodriguez-Amaya, 2002. A scheme for obtaining standards and HPLC quantification of leafy vegetable carotenoid. Food Chemistry, 78: 389-398.
59. Gamon, J.A. and J.S. Surfus, 1999. Assessing leaf pigment content with a reflectometer. New Phytologist, 43: 105-117.
60. Xue, L. and L. Yang, 2009. Deriving leaf chlorophyll content of green-leafy vegetables from hyperspectral reflectance. ISPRS Journal of Photogrammetry and Remote Sensing, 64: 97-106.

61. Znidarcic, D., D. Ban and H. Sircelj, 2011. Carotenoid and chlorophyll composition of commonly consumed leafy vegetables in Mediterranean countries. Food Chemistry, 129: 1164-1168.
62. Espin, J.C., M.T. Garcia-Conesa and F.A. Tomas-Barberan, 2007. Nutraceuticals: facts and fiction. Phytochemistry, 68(22-24): 2986-3008.
63. Spencer, J.P.E., D. Vauzour and C. Rendeiro, 2009. Flavonoids and cognition: the molecular mechanisms underlying their behavioral effects. Archives of Biochemistry and Biophysics, 492(1-2): 1-9.
64. Tsuda, T., 2012. Dietary anthocyanin-rich plants: biochemical basis and recent progress in health benefits studies. Molecular Nutrition and Food Research, 56(1): 159-170.
65. Kowalczyk, K., J. Gajc-Wolska, M. Marcinkowska, E. Jabrucka-Pioro, 2015. Assessment of quality attributes of endive (*Cichorium endivia* L.) depending on a cultivar and growing conditions. Acta Sci. Pol. Hortorum Cultus, 14(2): 13-26.
66. Jancic, D., V. Todorovic, Z. Basic and S. Sobajic, 2016. Chemical composition and nutritive potential of *Cichorium intybus* L. leaves from Montenegro. J. Serb. Chem. Soc., 81(10) 1-14.
67. Saggu, S., M.I. Sakeran, N. Zidan, E. Tousson, A. Mohan and H. Rehman, 2014. Ameliorating effect of chicory (*Chichorium intybus* L.) fruit extract against 4-tert-octylphenol induced liver injury and oxidative stress in male rats. Food Chem. Toxicol., 72C: 138-146.
68. Razali, N., R. Razab, S. Mat Junit and A. Abdul Aziz, 2008. Radical scavenging and reducing properties of extracts of cashew shoots (*Anacardium occidentale*). Food Chem., 111: 38-44.
69. Carratu, B. and E. Sanzini, 2005. Sostanze biologicamente attive presenti negli alimenti di origine vegetale. Annali dell, Istituto Superiore di Sanita, 41(1): 7-16.
70. Montefusco, A., G. Semitaio, P.P. Marrese, A. Iurlaro, M. De Caroli, G. Piro, G. Dalessandro and M.S. Lenucci, 2015. Antioxidants in varieties of chicory (*Cichorium intybus* L.) and wild poppy (*Papaver rhoeas* L.) of Southern Italy. Journal of Chemistry, pp: 1-8.
71. Al-Snafi, A.E., 2016. Medical importance of *Cichorium intybus* - A review. Journal of Pharmacy 6(3): 41-56.