International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(6): 1734-1740

Received: 21-09-2018
Accepted: 24-10-2018

Satya Shree Jangra
Medicinal, Aromatic and Potential Crops Section, CCS Haryana Agricultural University, Hisar, Haryana, India

Vinod Kumar Madan
Medicinal, Aromatic and Potential Crops Section, CCS Haryana Agricultural University, Hisar, Haryana, India

Correspondence
Satya Shree Jangra
Medicinal, Aromatic and Potential Crops Section, CCS Haryana Agricultural University, Hisar, Haryana, India

Effect of extraction techniques on total phenolics, flavonoids and antioxidant potential of various plant parts of chicory (*Cichorium intybus* L.)

Satya Shree Jangra and Vinod Kumar Madan

Abstract
Selection of a suitable extraction technique is very important for the extraction of phytochemicals in maximum recovery and removal of undesirable constituents. Hence, the present study was undertaken to investigate the efficacy of extraction techniques viz. microwave assisted extraction, soxhlet extraction, homogenization and column chromatography towards the extraction of phytochemicals as well as on the antioxidant potential of extracts produced from different plant parts (viz. whole plant and seeds) of Chicory. Extracts prepared by using soxhlet technique had the highest extract yield, total phenolics content, flavonoids content as well as exhibited highest DPPH free radical scavenging activity and total antioxidant capacity followed by column chromatography, microwave assisted and homogenization techniques. Among plant parts of Chicory; total phenolics, flavonoids, DPPH free radical scavenging activity and total antioxidant capacity were higher of whole plant extracts in comparison to seeds extracts. Significant correlation was observed between phytochemicals (total phenolics, flavonoids) and antioxidant activity (DPPH free radical scavenging activity & total antioxidant capacity).

Keywords: chicory, extraction techniques, total phenolics, flavonoids, DPPH free radical scavenging activity, total antioxidant capacity

Introduction
Reactive oxygen species (ROS) and reactive nitrogen species (RNS) include free radicals which are usually various forms of activated oxygen and nitrogen (Halliwell and Aruoma, 1997; Ak and Gulcin, 2008; Royer *et al*., 2011) [15, 2, 30]. ROS and RNS are continuously produced in living organisms during various physiological processes. Antioxidants are compounds that can delay or inhibit the oxidation of lipid or other molecules by inhibiting the initiation or propagation of oxidizing chain reactions (Velioglu *et al*., 1998) [38]. The human body has a complex system of biological antioxidant substances which counteract the harmful effects of free radicals and other oxidants. However, this natural antioxidant system can be inefficient, and hence dietary intake of antioxidant compounds is important. Synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are often used as food additives to provide protection against oxidative degradation of foods (Gulcin *et al*., 2004; Gulcin, 2005) [14, 12]. However, they have been suspected of being responsible for liver damage and carcinogenesis (Halliwell, 1991; Cekic *et al*., 2013) [16, 6]. Therefore, interest in finding naturally occurring antioxidants has increased considerably (Moure *et al*., 2001; Oktay *et al*., 2003; Gulcin *et al*., 2006) [22, 25, 13]. There is an inverse relationship between dietary intake of antioxidant rich foods and incidence of a number of human diseases as shown by various epidemiological studies (Eberhardt *et al*., 2000; Ganesan *et al*., 2011) [8, 11].

Cichorium intybus L. is an important medicinal herb that has been used in Ayurveda, Unani and Siddha system of medicine for prevention and cure of hepatobiliary and renal system diseases (Zaman and Basar, 2013) [39]. It belongs to family Asteraceae. The plant is widely distributed in Asia, Africa, Europe, Australia and North America. It is commonly known as chicory in English and kasni in Hindi. Aerial parts, flowers, seeds and roots are the commonly used parts of this plant. All parts of this plant contain volatile oil, fatty acids, unsaturated sterols, alkaloids, triterpenes, vitamins, flavonoids, tannins, coumarins, saponins, terpenoids, cardiac glycosides, sesquiterpene lactones, anthocyanins and phenols (Nandagopal and Kumari, 2007; Shad *et al*., 2013; Abbas *et al*., 2015) [24, 32, 1]

which are responsible for its medicinal importance. Chicory plant possess various pharmacological activities like antimicrobial, anthelmintic, antimalarial, hepatoprotective, antidiabetic, gastroprotective, anti-inflammatory, analgesis, antioxidant, tumor-inhibitory, antiallergic (Street *et al*., 2013; Al-Snafi, 2016) [37, 3]. Chicory is palatable for ruminants being low in fiber and high in structural carbohydrates (Athanasiadou *et al*., 2007) [4]. Hence, chicory has been used as animal fodder and forage for livestock. Traditionally, roasted roots of chicory are used as coffee substitute with no caffeine (Schmidt *et al*., 2007) [31].

Extraction is an important step in the itinerary of phytochemical processing for isolation or separation of bioactive constituents from plant materials. Effective separation of antioxidants (high extraction yield and concentration of bioactive compounds) from a complex plant matrix is a difficult procedure due to co-extraction of various compounds which are undesirable in antioxidant extract (Bimakr *et al*., 2011) [5]. Therefore, selection of a suitable extraction technique is very important for the extraction of phytochemicals in maximum recovery and removal of undesirable constituents (Co *et al*., 2012) [7]. Thus, the objective of the present study was to evaluate the efficacy of different extraction techniques towards the extraction of phytochemicals (total phenolics, flavonoids) as well as on the antioxidant potential of extracts produced from different plant parts viz. whole plant and seeds of Chicory.

Materials and methods

Plant material

Whole plant and seeds of Chicory (*Cichorium intybus* L.) were procured from the experimental area of Medicinal, Aromatic & Potential Crops Section, Department of Genetics & Plant Breeding, CCS Haryana Agricultural University, Hisar, Haryana.

Chemicals

Commercially available and highest purity chemicals were used for various experimental procedures. Folin & Ciocalteu's phenol reagent, ammonium molybdate, sodium phosphate, L-ascorbic acid (vitamin C), sodium hydroxide and methanol were procured from SISCO Research Laboratories Private Limited (SRL). Sodium carbonate, sodium sulphate, sodium nitrite and aluminium chloride were supplied by Qualigens Fine Chemicals. 2,2′-Diphenyl-1-picrylhydrazyl (DPPH) and catechin were obtained from Sigma-Aldrich.

Preparation of extracts

Powdered samples of whole plant and seeds of Chicory were extracted using following extraction techniques:

Microwave assisted extraction

Five gram of powdered samples of Chicory whole plant and eight gram of powdered samples of Chicory seeds were weighed and placed in a 250 mL conical flask. Then, 100 mL of methanol was added to the conical flasks containing samples and left overnight. Extraction was carried out using a microwave oven (IFB, Model: 2301) with output 800 W and operating frequency 2450 MHz (2.45 GHz). The flasks containing samples were irradiated for 10 seconds in microwave oven at 40 per cent power. Solutions were not allowed to boil. After 10 seconds of irradiation, solutions were cooled to room temperature for few min. The irradiation step was repeated up to 12 times to complete the process timing of 2 min. After irradiation, the samples were cooled, filtered through Whatman no. 1 filter paper, collected and their volumes were noted.

Soxhlet extraction

Seven gram of powdered samples of Chicory whole plant and ten gram of powdered samples of Chicory seeds were weighed and placed in a filter paper (Whatman no. 1) thimble in a classical soxhlet apparatus fitted with a 250 mL round bottomed flask. Then methanol was added up to one and a half siphons i.e. approximately 150-175 mL. Extraction was performed at boiling temperature of methanol. Methanol vapours move up to the column and after getting condensed in the condenser part, floods into the chamber housing thimble filled with Chicory samples. When this chamber gets completely filled with solvent the siphon mechanism operates and the solvent containing some part of phytochemicals that got dissolved in solvent; empties this extract into round bottomed flask containing solvent. Process was continued for 6 h with completion of up to eight to nine cycles through siphon mechanism. Filtrates were collected, pooled and their volumes were noted.

Homogenization

Five gram of powdered samples of Chicory whole plant and eight gram of powdered samples of Chicory seeds were weighed and placed in a 250 mL beaker. Then, 100 mL of methanol was added to the beakers containing samples and left overnight. The samples were homogenized using an IKA T10 Basic Ultra-Turax Homogenizer for 15 min. The homogenate was filtered through Whatman no. 1 filter paper. Filterates were collected and their volumes were noted.

Column chromatography

Seven gram of powdered samples of Chicory whole plant and ten gram of powdered samples of Chicory seeds were weighed and mixed properly with appropriate amount of anhydrous sodium sulphate using pestle and mortar. For extraction, the column was filled by first putting a cotton plug at the bottom of the column; followed by a layer (about 2-3 cm) of sodium sulphate. Then the sample was put into the column with gentle tapping to get a uniformly packed column and after that again a layer of sodium sulphate was added at the top of the column. Methanol was added to the column and allowed to stand overnight. The column was run (approximately 6 h) and extract was collected at the bottom of the column in a beaker drop by drop instead of continuous flow. Extracts of each column were collected and their volumes were noted.

Estimation of total phenolics content

Total phenolics content in Chicory extracts was estimated using Folin-Ciocalteu method (Singleton and Rossi, 1965; Jangra *et al*. 2015) [35, 18]. Aliquots of extracts (0.2 mL) were mixed with 1 mol/L Folin-Ciocalteu reagent (1 mL). After that, 2.0 ml of 20% (w/v) sodium carbonate solution was added. The solutions were mixed and volume was made up to 10.0 ml with distilled water. The absorbance was measured at 730 nm using UV-Vis double beam Spectrophotometer Model 2203 (Systronics Co.). A standard (calibration) curve was prepared using gallic acid (10, 20, 30, 40, 50 and 60 µg/mL) as standard. Results were expressed as milligram gallic acid equivalent per gram (mg GAE/g).

Estimation of flavonoids content

Flavonoids content in Chicory extracts was estimated by the

aluminium chloride colorimetric assay (Marinova *et al*., 2005; Jangra *et al*. 2015) [21, 18]. Briefly, in 1.0 mL of extract, 4.0 mL of double distilled water and 0.3 mL of 5% (w/v) $NaNO_2$ were added. After 5 min, 0.3 mL of 10% (w/v) $AlCl_3$ was added. Immediately, 2.0 mL of 1 M NaOH was added and the volume was made up to 10.0 ml with double distilled water. The solutions were mixed thoroughly and the absorbance was measured at 510 nm using UV-Vis double beam Spectrophotometer Model 2203 (Systronics Co.). A standard (calibration) curve was prepared using catechin (6.25, 12.5, 25, 50, 100 and 200 µg/mL) as standard. Results were expressed as milligram catechin equivalent per gram (mg CE/g).

Evaluation of DPPH free radical scavenging activity

The antioxidant activity of Chicory extracts was evaluated by 2,2′-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method (Hatano *et al*., 1988; Jangra *et al*. 2015) [17, 18]. For evaluation of antioxidant activity, different concentrations viz. 25, 50, 100, 250, 500, 1000 and 2500 µg/mL of extracts were prepared and to each sample (0.2 mL), 3.0 mL of DPPH solution (0.1 mm in 100% methanol) was added and mixed thoroughly for 5 min. A control was also made containing 0.2 mL of solvent instead of extract. The absorbance of the sample as well as control was measured at 517 nm after 30 min of incubation in dark at room temperature using the UV-Vis double beam Spectrophotometer Model 2203 (Systronics Co.) against a blank containing respective solvent. Three replications were carried out for each sample. A graph was drawn by plotting per cent DPPH free radical scavenging activity (y-axis) against extract concentration (x-axis). Then using Microsoft Excel Software, quadratic regression equation ($y= ax^2+bx+c$) was obtained and using the quadratic equation IC_{50} (µg/mL) was calculated. The percentage of DPPH scavenged (% $DPPH^*_{sc}$) was calculated using:

$$\%\,DPPH^*_{sc} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where, $A_{control}$ is the absorbance of control and A_{sample} is the absorbance of the sample.

Evaluation of total antioxidant capacity

The total antioxidant capacity of Chicory extracts was evaluated by phosphomolybdenum method of Prieto *et al*. (1999) [29] with slight modifications. For evaluation of total antioxidant capacity of Chicory extracts, 0.3 mL of each extract (concentration: 1000 ppm) was added to test tubes and added 3 mL of phosphomolybdenum reagent (0.6 M sulfuric acid, 28 mm sodium phosphate and 4 mm ammonium molybdate). The solutions were mixed thoroughly, covered with aluminium foil and incubated at 95ºC for 90 min. After incubation, the tubes were allowed to cool down and absorbance was measured at 695 nm using UV-Vis Double beam Spectrophotometer Model 2203 (Systronics Co.) against a blank prepared similarly but containing respective solvent instead of extracts. A standard (calibration) curve was prepared using ascorbic acid (6.25, 12.5, 25, 50, 100, 200 and 400 µg/mL) as standard. The total antioxidant capacity of extracts was calculated from the standard curve and results were expressed as mg ascorbic acid equivalent per gram (mg AAE/g).

Statistical analysis

Three replications were carried out for each sample. Results were expressed as Mean ± SE. Data was subjected to one way analysis of variance (ANOVA). Relationship between phytochemicals composition and their antioxidant activities was carried out using Pearson correlation analysis in Online Statistical Analysis (OPSTAT software www.hau.ac.in).

Results and Discussion

Extract yield, total phenolics content, flavonoids content

Extract yield, total phenolics content and flavonoids content in whole plant and seeds extracts of Chicory prepared by using four extraction techniques *viz*. microwave assisted extraction, soxhlet technique, homogenization and column chromatography is given in Tables 1 and 2, respectively. In present studies, extract yield of whole plant and seeds extracts of Chicory prepared by using different extraction techniques ranged from 7.52 to 12.19 g/100g and from 5.24 to 8.54 g/100g, respectively. Total phenolics content in whole plant and seeds extracts of Chicory prepared by using different extraction techniques ranged from 4.83 to 10.12 mg GAE/g and from 2.88 to 7.40 mg GAE/g, respectively. Flavonoids content in whole plant and seeds extracts of Chicory prepared by using different extraction techniques ranged from 2.09 to 6.33 mg CE/g and from 1.57 to 3.87 mg CE/g, respectively. In present studies amongst whole plant and seeds extracts of Chicory prepared by using different extraction techniques; extract yield, total phenolics content and flavonoids content in extracts prepared by using soxhlet technique was highest followed by column chromatography, microwave assisted extraction and homogenization techniques. Among plant parts of Chicory; extract yield, total phenolics content and flavonoids content in whole plant extracts was higher in comparison to seeds extracts.

The probable reason for highest amount of total phenolics and flavonoids in extracts prepared by using soxhlet technique may be due to more efficiency of extracting solvents at higher temperatures to extract phtyoconstituents from plant matrix and may also be due to more extraction time as compared to other extraction techniques. Present findings are in agreement with Kalia *et al*. (2008) [19] who reported that extracts of aerial parts of *P. atrosanguinea* prepared by using soxhlet extraction had highest total polyphenols and flavonoids content followed by microwave-assisted extraction, ultrasound-assisted extraction and maceration. Clove buds extracts prepared by using soxhlet technique had highest extract yield; total phenolics and flavonoids content followed by refluxing, mechanical shaking and centrifugation extraction techniques (Singh *et al*., 2016; Singh *et al*., 2018) [34, 33].

Table 1: Extract yield, total phenolics, flavonoids and total antioxidant capacity of Chicory whole plant methanolic extracts prepared by using different extraction techniques

Extraction Technique / Parameter ⟶	Extract yield (g/100g)	Total phenolics (mg GAE/g)	Flavonoids (mg CE/g)	Total antioxidant capacity (mg AAE/g)
Microwave	8.88 ± 0.04	6.35 ± 0.10	3.25 ± 0.03	100.62 ± 0.18
Soxhlet	12.19 ± 0.05	10.12 ± 0.06	6.33 ± 0.05	108.07 ± 0.18
Homogenization	7.52 ± 0.02	4.83 ± 0.07	2.09 ± 0.04	65.08 ± 0.46

Column Chromatography	10.64 ± 0.06	7.43 ± 0.05	4.17 ± 0.03	101.45 ± 1.55
Mean	9.81	7.18	3.96	93.81
SE(m)	0.05	0.08	0.04	0.81
CD at 5%	0.17	0.27	0.16	2.85
CV%	0.85	1.88	1.95	1.49

Table 2: Extract yield, total phenolics, flavonoids and total antioxidant capacity of Chicory seeds methanolic extracts prepared by using different extraction techniques

Extraction Technique ↓ Parameter →	Extract yield (g/100g)	Total phenolics (mg GAE/g)	Flavonoids (mg CE/g)	Total antioxidant capacity (mg AAE/g)
Microwave	6.94 ± 0.01	3.54 ± 0.05	2.41 ± 0.03	38.70 ± 0.16
Soxhlet	8.54 ± 0.03	7.40 ± 0.06	3.87 ± 0.06	67.73 ± 0.35
Homogenization	5.24 ± 0.02	2.88 ± 0.07	1.57 ± 0.03	21.86 ± 0.13
Column Chromatography	7.97 ± 0.06	5.11 ± 0.07	2.77 ± 0.08	40.46 ± 0.30
Mean	7.17	4.73	2.66	42.19
SE(m)	0.03	0.07	0.05	0.24
CD at 5%	0.11	0.26	0.18	0.84
CV%	0.77	2.66	3.40	0.98

DPPH free radical scavenging activity

DPPH free radical scavenging method has been widely used to evaluate the antioxidant activity of compounds due to its simple, rapid, sensitive and reproducible procedure (Ozcelik *et al*., 2003) [27]. DPPH (2,2′-diphenyl-1-picrylhydrazyl) is a violet coloured stable free radical which accepts an electron or hydrogen radical to become a stable diamagnetic molecule diphenylpicrylhydrazine (Soares *et al*., 1997) [36]. When DPPH encounters proton donating substances such as an antioxidant or a radical species, the absorbance at λ_{max} (517 nm) decreases resulting in a colour change from purple to yellow. DPPH free radical scavenging activity is compared on the basis of IC_{50} values. Lower the IC_{50} values, higher will be the DPPH free radical scavenging activity.

The data of DPPH free radical scavenging activity of whole plant and seeds extracts of Chicory is given in Table 3. In present studies amongst extraction techniques, DPPH free radical scavenging activity of whole plant and seeds extracts of Chicory prepared by soxhlet technique was highest ranging from 4.56 to 92.05% and 3.85 to 91.65%, respectively followed by column chromatography (4.56 to 91.84% and 3.54 to 91.31%, respectively), microwave assisted extraction (3.30 to 91.06% and 2.78 to 91.11%, respectively) and homogenization (2.76 to 90.02% and 2.04 to 89.46%, respectively) techniques at varying concentration levels ranging from 25 to 2500 µg/mL. The corresponding IC_{50} values (µg/mL) were lowest i.e. 410.6 and 430.1, respectively of the extracts prepared using soxhlet technique followed by column chromatography (411.9 and 435.3, respectively), microwave assisted extraction (419.1 and 438.2, respectively) and homogenization (437.4 and 461.4, respectively) techniques thereby showing that extracts prepared by soxhlet technique had highest DPPH free radical scavenging activity followed by column chromatography, microwave assisted extraction and homogenization techniques. Among plant parts, IC_{50} values of whole plant extracts prepared by different extraction techniques were lower (410.6 - 437.4 µg/mL) in comparison to seeds (430.1 - 461.4 µg/mL) extracts thereby showing that whole plant extracts had higher DPPH free radical scavenging activity in comparison to seeds extracts. IC_{50} values were calculated from the quadratic regression equations which were prepared by plotting percent DPPH free radical scavenging activity on Y-axis and concentration levels on X-axis. Quadratic regression equations of various extracts of Chicory are given in Table 4.

The probable reason for highest DPPH free radical scavenging activity (i.e. lower value of IC_{50}) of the extracts prepared by soxhlet technique may be due to the presence of highest amount of antioxidant compounds i.e. total phenolics and flavonoids in the extracts prepared by soxhlet technique. Other research workers also evaluated the DPPH free radical scavenging activity of different parts of Chicory. Ozgen *et al*. (2004) [28] reported that DPPH radical scavenging activity (DPPH-RS) of *C. intybus* used in folk medicine in Eastern Turkey ranged from 3.1 to 84.9% (IC_{50} RS: 368 µg/mL) at different concentration levels ranging from 50 to 500 µg/mL. Ereifej *et al*. (2015) [9] reported 0.44 mg/mL IC_{50} value of methanolic extract of *C. intybus* leaves collected from Ajloun Mountains in Jordan as evaluated by DPPH free radical scavenging method.

Table 3: DPPH free radical scavenging activity and IC_{50} of Chicory whole plant and seeds methanolic extracts prepared by using different extraction techniques

Extraction Technique	DPPH free radical scavenging activity (%)							IC_{50} (µg/mL)
	Concentration (µg/mL)							
	2500	1000	500	250	100	50	25	
	Whole Plant							
Microwave	91.06	85.33	63.09	36.17	16.17	8.62	3.30	419.1
Soxhlet	92.05	87.29	65.68	40.57	18.75	9.32	4.56	410.6
Homogenization	90.02	83.23	62.00	34.08	15.82	6.79	2.76	437.4
Column Chromatography	91.84	86.33	63.45	36.65	17.90	9.00	4.56	411.9
	Seeds							
Microwave	91.11	83.94	60.71	33.94	16.70	7.92	2.78	438.2
Soxhlet	91.65	84.81	61.28	34.76	17.22	8.34	3.85	430.1
Homogenization	89.46	81.61	58.49	32.26	15.16	6.67	2.04	461.4
Column Chromatography	91.31	84.33	60.84	34.12	16.85	7.94	3.54	435.3

Table 4: Quadratic regression equations for DPPH free radical scavenging activity of Chicory extracts prepared by using different extraction techniques

Extraction Technique	Whole Plant	Seeds
Microwave	$y = -0.00003x^2 + 0.12183x + 4.20890$ $R^2 = 0.98658$	$y = -0.00003x^2 + 0.11865x + 3.76823$ $R^2 = 0.98895$
Soxhlet	$y = -0.00004x^2 + 0.12318x + 6.16436$ $R^2 = 0.98098$	$y = -0.00003x^2 + 0.11884x + 4.43816$ $R^2 = 0.98956$
Homogenization	$y = -0.00003x^2 + 0.11986x + 3.30804$ $R^2 = 0.98554$	$y = -0.00003x^2 + 0.11640x + 2.68049$ $R^2 = 0.98975$
Column Chromatography	$y = -0.00003x^2 + 0.12107x + 5.22347$ $R^2 = 0.98758$	$y = -0.00003x^2 + 0.11861x + 4.04859$ $R^2 = 0.98973$

Total antioxidant capacity

Total antioxidant capacity assay is a spectroscopic method for the quantitative determination of antioxidant capacity, through the formation of phosphomolybdenum complex. The assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and subsequent formation of a green phosphate Mo (V) complex at acidic pH.

In present studies amongst whole plant and seeds extracts of Chicory prepared by using different extraction techniques, total antioxidant capacity (mg AAE/g) of extracts prepared by using soxhlet technique was highest (108.07 and 67.73, respectively) followed by column chromatography (101.45 and 40.46, respectively), microwave assisted extraction (100.62 and 38.70, respectively) and homogenization (65.08 and 21.86, respectively) techniques. Among plant parts of Chicory, total antioxidant capacity of whole plant extracts was higher in comparison to seeds extracts (Table 1 and 2). The probable reason for highest total antioxidant capacity of the extracts prepared by soxhlet technique may be due to the presence of highest amount of antioxidant compounds i.e. total phenolics and flavonoids in the extracts prepared by soxhlet technique. Similar findings have also been reported by other research workers. Murugan and Parimelazhagan (2014) [23] reported that methanol extracts of *Osbeckia parvifolia* whole plant obtained by using soxhlet method showed highest total antioxidant capacity i.e. (192.3 mg AAE/g extract) followed by maceration (163.9 mg AAE/g extract) and fractionation (116.8 mg AAE/g extract) methods.

Correlation

To know the implication of polyphenolic compounds (phenolics, flavonoids) with the DPPH free radical scavenging activity & total antioxidant activity measured, correlations were evaluated. The correlative values were obtained between phytoconstituents (total phenolics & flavonoids) and antioxidant potential (IC_{50} values of DPPH free radical scavenging activity & total antioxidant capacity) (Table 5). In Chicory, the results of correlation analysis indicated that there was a negative and highly significant correlation ($R = –0.872^{**}$) at 1% level of significance between total phenolics and IC_{50} values. Similarly, flavonoids in Chicory extracts also showed negative and highly significant correlation ($R = –0.839^{**}$) at 1% level of significance with IC_{50} values. Total phenolics and flavonoids in Chicory extracts showed significant positive correlation with total antioxidant capacity. The values for correlation between total phenolics, flavonoids and total antioxidant capacity were 0.862^{**} (significant at 1% level) and 0.790^{*} (significant at 5% level), respectively.

The present findings are in agreement with studies of other research workers who reported significant correlation between total phenolics content and DPPH free radical scavenging activity of Chicory (Kostic *et al*., 2013; Ereifej *et al*., 2015) [20, 9]. The antioxidant activity of phenolic compounds is reported mainly due to their redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Osawa, 1994) [26]. Farasat *et al*. (2014) [10] reported that there were strong positive significant correlations between DPPH radical scavenging and contents of phenolics ($r = 0.889$, $p < 0.01$) and flavonoids ($r = 0.819$, $p < 0.01$) and high negative correlations between IC_{50} values and contents of phenolics ($r = –0.785$, $p < 0.01$) and flavonoids ($r = –0.804$, $p < 0.01$) in seaweeds.

Table 5: Relationship (Correlation coefficient, R) between total phenolics, flavonoids contents and antioxidant activities

	IC_{50}	Total antioxidant capacity
Total phenolics content	$–0.872^{**}$	0.862^{**}
Flavonoids content	$–0.839^{**}$	0.790^{*}

Conclusion

From the present studies, it is concluded that extracts prepared by using soxhlet technique had the highest extract yield, total phenolics content, flavonoids content as well as exhibited highest DPPH free radical scavenging activity and total antioxidant capacity followed by column chromatography, microwave assisted and homogenization techniques. Among plant parts of Chicory; total phenolics, flavonoids, DPPH free radical scavenging activity and total antioxidant capacity were higher in whole plant extracts in comparison to seeds extracts. Significant correlation was observed between phytochemicals (total phenolics, flavonoids) and antioxidant activity (DPPH free radical scavenging activity & total antioxidant capacity). Hence, total phenolics, flavonoids and antioxidant potential of chicory extracts varied with the different extraction techniques used which may be due to their different chemical structures and uneven distribution in the plant matrix.

References

1. Abbas ZK, Saggu S, Sakeran MI, Zidan N, Rehman H, Ansari, AA. Phytochemical, antioxidant and mineral composition of hydroalcoholic extract of chicory (*Cichorium intybus* L.) leaves. Saudi J Biol Sci. 2015; 22(3):322-326.
2. Ak T, Gulcin I. Antioxidant and radical scavenging properties of curcumin. Chem. Biol. Interact. 2008; 174:27-37.
3. Al-Snafi AE. Medical importance of *Cichorium intybus* - A review. IOSR Journal of Pharmacy. 2016; 6(3):41-56.
4. Athanasiadou S, Gray D, Younie D, Tzamaloukas O, Jackson F, Kyriazakis I. The use of chicory for parasite control in organic ewes and their lambs. Parasitology, 2007; 134:299-307.
5. Bimakr M, Rahman RA, Taip FS, Ganjloo A, Salleh LM, Selamat J, *et al.* Comparison of different extraction

methods for the extraction of major bioactive flavonoid compounds from spearmint (*Mentha spicata* L.) leaves. Food Bioprod Process. 2011; 89:67-72.
6. Cekic SD, Cetinkaya AN, Avan AN, Apak R. Correlation of total antioxidant capacity with reactive oxygen species (ROS) consumption measured by oxidative conversion. J Agric Food Chem. 2013; 61(22):5260-5270.
7. Co M, Fagerlund A, Engman L, Sunnerheim K, Sjoberg PJR, Turner C. Extraction of antioxidants from Spruce (*Picea abies*) bark using ecofriendly solvents. Phytochem Anal. 2012; 23(1):1-11.
8. Eberhardt MV, Lee CY, Liu RH. Antioxidant activity of fresh apples. Nature. 2000; 405:903-904.
9. Ereifej KI, Feng H, Rababah T, Almajwal A, Alu′datt M, Gammoh SI, *et al*. Chemical composition, phenolics, anthocyanins concentration and antioxidant activity of ten wild edible plants. Food Nutr Sci. 2015; 6:581-590.
10. Farasat M, Khavari-Nejad RA, Nabavi SMB, Namjooyan F. Antioxidant activity, total phenolics and flavonoid contents of some edible green seaweeds from northern coasts of the Persian Gulf. Iran J Pharm Res. 2014; 13(1):163-170.
11. Ganesan K, Kumar KS, Rao PVS. Comparative assessment of antioxidant activity in three edible species of green seaweed, Enteromorpha from Okha, Northwest coast of India. Innov. Food Sci Emerg Technol. 2011; 12(1):73-78.
12. Gulcin I. The antioxidant and radical scavenging activities of black pepper (*Piper nigrum*) seeds. Int. J Food Sci Nutr. 2005; 56(7):491-499.
13. Gulcin I, Mshvildadze V, Gepdiremen A, Elias R. The antioxidant activity of a triterpenoid glycoside isolated from the berries of *Hedera colchica*: 3-O-(β-D-glucopyranosyl)-hederagenin. Phytother Res. 2006; 20(2):130-134.
14. Gulcin I, Sat IG, Beydemir S, Elmastas M, Kufrevioglu OI. Comparison of antioxidant activity of clove (*Eugenia caryophylata* Thunb) buds and lavender (*Lavandula stoechas* L.). Food Chem. 2004; 87(3):393-400.
15. Halliwell B, Aruoma OI. Free Radicals and Antioxidants: The need for *in vivo* markers of oxidative stress. Antioxidant methodology: In vivo and In vitro Concepts (eds O. I. Aruoma & S. L. Cuppett). AOCS Press, Champaign, Illinois, 1997, 1-22.
16. Halliwell B. Reactive oxygen species in living systems: Source, biochemistry, and role in human disease. Am J Med. 1991; 91:14-22.
17. Hatano T, Kagawa H, Yasuhara T, Okuda T. Two new flavonoids and other constituents in licorice root, their relative astringency and radical scavenging effects. Chem Pharm Bull. 1988; 36:2090-2097.
18. Jangra SS, Madan VK, Singh S. Effect of solvents on extraction of various phytochemicals and antioxidant activity in carrot (*Daucus carota* L.). J Indian Chem. Soc. 2015; 92:1149-1154.
19. Kalia K, Sharma K, Singh HP, Singh, B. Effects of extraction methods on phenolic contents and antioxidant activity in aerial parts of *Potentilla atrosanguinea* Lodd. and quantification of its phenolic constituents by RP-HPLC. J Agric Food Chem. 2008; 56:10129-10134.
20. Kostic DA, Velickovic JM, Mitic SS, Mitic MN, Randjelovic SS, Arsic BB, *et al*. Correlation among phenolic, toxic metals and antioxidant activity of the extracts of plant species from Southeast Serbia. Bull Chem Soc Ethiop. 2013; 27(2):169-178.
21. Marinova D, Ribarova F, Atanassova M. Total phenolics and total flavonoids in Bulgarian fruits and vegetables. J Univ Chem. Technol Metallurgy. 2005; 40(3):255-260.
22. Moure A, Cruz JM, Franco D, Dominguez JM, Sineiro J, Dominguez H, et al. Natural antioxidants from residual sources. Food Chem. 2001; 72:145-171.
23. Murugan R, Parimelazhagan T. Comparative evaluation of different extraction methods for antioxidant and anti-inflammatory properties from *Osbeckia parvifolia* Arn. – An in vitro approach. Journal of King Saud University – Science. 2014; 26(4):267-275.
24. Nandagopal S, Kumari BDR. Phytochemical and antibacterial studies of Chicory (*Cichorium intybus* L.) - A multipurpose medicinal plant. Advan Biol Res. 2007; 1(1-2):17-21.
25. Oktay M, Gulcin I, Kufrevioglu OI (2003). Determination of in vitro antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. LWT – Food Sci Technol. 2003; 36(2):263-271.
26. Osawa T. Novel natural antioxidants for utilization in food and biological systems. Post-harvest Biochemistry of Plant Materials in the Tropics (eds I. Uritani, V. V. Garcia and E. M. Mendoza). Japan Scientific Societies Press, Tokyo, Japan, 1994, 241-251.
27. Ozcelik B, Lee JH, Min DB. Effects of light, oxygen, and pH on the absorbance of 2,2-diphenyl-1-picrylhydrazyl. J Food Sci. 2003; 68(2):487-490.
28. Ozgen U, Mavi A, Terzi Z, Coskun M, Yildirim A. Antioxidant activities and total phenolic compounds amount of some Asteraceae species. Turk J Pharm Sci. 2004; 1(3):203-216.
29. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal Biochem. 1999; 269(2):337-341.
30. Royer M, Diouf PN, Stevanovic T. Polyphenol contents and radical scavenging capacities of red maple (*Acer rubrum* L.) extracts. Food Chem Toxicol. 2011; 49(9):2180-2188.
31. Schmidt BM, Ilic N, Poulev A, Raskin R. Toxicological evaluation of a chicory root extract. Food Chem. Toxicol. 2007; 45(7):1131-1139.
32. Shad MA, Nawaz H, Rehman T, Ikram N. Determination of some biochemicals, phytochemicals and antioxidant properties of different parts of *Cichorium intybus* L.: A comparative study. J Anim Plant Sci. 2013; 23(4):1060-1066.
33. Singh I, Madan VK, Jangra SS, Dusyant. Effect of various extraction techniques and solvents on extract yield and sugars content of clove (*Syzygium aromaticum* L.). Asian J Chem. 2018; 30(4):759-761.
34. Singh I, Madan VK, Jangra SS, Singh S. Effect of extraction techniques and solvents on various phytochemicals and antioxidant activity of clove (*Syzygium aromaticum* L.) buds. Asian J Chem. 2016; 28(4):801-806.
35. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybidic-phosphotungstic acid reagents. Am J Enol Vitic. 1965; 16:144-158.
36. Soares JR, Dinis TCP, Cunha AP, Almeida LM. Antioxidant activities of some extracts of *Thymus zygis*. Free Radic Res. 1997; 26(5):469-478.
37. Street RA, Sidana J, Prinsloo G (2013). *Cichorium intybus*: Traditional uses, phytochemistry, pharmacology,

and toxicology. Evid-Based Complementary Altern. Med. 2013; 2013:1-13.
38. Velioglu YS, Mazza G, Gao L, Oomah BD. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. J Agric Food Chem. 1998; 46:4113-4117.
39. Zaman R, Basar SN (2013). A review article of Beekhe Kasni (*Cichorium intybus*) its traditional uses and pharmacological actions. Res. J Pharmaceutical Sci. 2013; 2(8):1-4.