Vol.62: e19180370, 2019
http://dx.doi.org/10.1590/1678-4324-2019180370
ISSN 1678-4324 Online Edition

BRAZILIAN ARCHIVES OF BIOLOGY AND TECHNOLOGY

Article - Biological and Applied Sciences

Cichorium intybus from India: GC-MS Profiling, Phenolic Content and *in vitro* Antioxidant Capacity of Sequential Soxhlet Extracted Roasted Roots

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Received: 2018.07.20; Accepted: 2019.06.22

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HIGHLIGHTS

- The chicory roots possessed antioxidant property even in roasted form.
- The roasted roots are rich source of total phenolics and flavonoids
- Antioxidant activity show a positive relation with total phenolics and flavonoids content
- Methanol was best solvent for extraction of chicory roots for studied parameters

Abstract : Research on the bio-activities and chemical composition of roasted C. intybus roots from India is very little. In present studies GC-MS analysis of volatile components of roasted C. intybus roots, phenolics and flavonoid content estimation and antioxidant potential of roasted C. intybus roots was carried out. Antioxidant potential was also evaluated using FRAP, DPPH, hydroxyl radical, nitric oxide and superoxide free radical scavenging method. Extracts were prepared by sequential Soxhlet extraction. GC-MS analysis of volatile components of roasted C. intybus root extracts revealed that 5-hydroxymethyl furfural was major volatile component in dichloromethane and methanol extract whereas lupeol and its derivative compounds were major constituents of hexane extract. Quantitative estimation for total phenols and flavonoids showed that the methanol extract of C. intybus roots contained highest phenolic and flavonoid content as compared to other extracts and also showed strong radical scavenging activities which were comparable with ascorbic acid used as standard. All extracts showed IC_{50} values less than 0.6 mg/mL furthermore, extracts of roasted C. intybus showed the high total antioxidant potential for the reduction of Fe^{3+} to Fe^{2+}. The C. intybus roots possess good antioxidant capacity even after roasting and all the extracts showed good activities.

Keyword Antioxidant potential; Chicory; Chemical Composition; Solvent extracts.

INTRODUCTION

Cichorium intybus L. also known as chicory, is a plant belonging to Asteraceae family. Roasted *C. intybus* roots are used as coffee substitute in many coffee-like beverages [1]. Roasted *C. intybus* powder has been widely used in coffee blends, since many coffee drinkers prefer its distinct flavour[1]. It enhances the colour, flavour and aroma of beverages [2]. In Germany it is sold as a constituent of mixtures with roasted barley malt. Roots of *C. intybus* are used for the production of inulin and as ingredients in certain roasted products [3]. *C. intybus* also have an economic importance in many regions of the world [4]. The crop has potential as a biomass crop for industrial use as the plant bears tuberous roots which store inulin that can easily be converted to alcohol [5]. Hui et al. [6] reported use of inulin as a food ingredient for fat and sugar replacement as a low calorie bulking and texturizing agents. *C. intybus* also possess importance as forage crop, raw material for fructose and as a feed additive. *C. intybus* roots are also used in salads, as a chewing gum and in spice production [7]. *C. intybus* (100 g) could provide good amount of total polyphenols i.e. up to 400 mg, to the human diet [8]. *C. intybus* gained attention due to its phytochemical contents having nutraceutical potential [9]. It contained phytochemicals like flavonoids, cinnamic and quinic acid derivatives, coumarin, and anthocyanins. In addition to these *C. intybus* was reported contained compounds such as alkaloids, inulin, sesquiterpene lactones, vitamins, chlorophyll pigments, unsaturated sterols, saponins, and tannins with putative health benefits also [10].

C. intybus plant possess a wide variety of bioactivities and multiple research papers have been published describing the phytochemical composition and several health properties of *C. intybus*, including antidiabetic, hyperglycemic, wound healing and antioxidant capacities of different parts of *C. intybus* plant [11-15]. Change in antioxidant potential of chicory leaves after treatment with different drying method is recently reported by Li et al. [12]. Most of the work was carried out on fresh plant parts or their water extracts only. But the evaluation of solvent extracts especially of roasted parts of *C. intybus* plant is less. The study of literature revealed that work regarding bioactivity and composition of widely consumed roasted roots of *C. intybus* is very less. In present studies GC-MS analysis, estimation of total phenolic and flavonoid contents of different extracts of roasted *C. intybus* root powder and their antioxidant activity using various *in vitro* models was undertaken.

MATERIAL AND METHODS

Chemicals and reagents

2,2-diphenyl-1-picrylhydrazyl, 2,4,6-tripyridyl-s-triazine, acetic acid, aluminium chloride, ascorbic acid, deoxyribose, ferric chloride, dichloromethane, ethylene diamine tetraacetic acid, ferrous sulfate, Folin-Ciocalteu reagent, gallic acid, Hydrogen-peroxide, hexane methanol, monobasic and dibasic potassium phosphate, naphtyl ethylene diamine dihydrochloride, nicotinamide adenine dinucleotide, nitro blue tetrazolium, phenazine methoxy sulphate, phosphoric acid, potassium acetate, quercetin, sodium acetate, sodium carbonate, sodium nitroprusside, sulphanilamide and thiobarbituric acid.

Preparation of extracts

C. intybus roots (1kg) were cut into small pieces and oven dried at 150˚C until it turn brown. Extracts were prepared using sequential Soxhlet extraction method. The roasted pieces were powdered in grinder and extracted with different solvents. The roasted *C. intybus* root powder (100 g) was packed in a thimble and subjected to Soxhlet apparatus and extracted sequentially with solvents (250 mL) [16] i.e. hexane, dichloromethane and then

methanol. Refluxing was carried out for 12 h for each solvent. Distilling off the solvents in round bottom flask gave crude extracts. The extracts were stored at 4˚C.

Gas chromatography-mass spectrometry analysis

Roasted *C. intybus* root extracts were analysed using gas chromatography-mass spectrometry (QP2010 Plus, Shimadzu, Japan), equipped with a Rtx-5 MS capillary column (30.0 m x 0.20 mm i.d., 0.25 µm film thickness) for the separation of the components. Injector, kept at 290 °C, was operated in split injection mode and the split valve was closed for 1 min. Carrier gas used was helium gas at a constant pressure of 69 kPa. The column oven was maintained at 100 °C for 1 min initially and then elevated at 2.5 °C/min to 220 °C, then at 5 °C/min to 300 °C. The ionization mode was electron impact (70 eV) and interface temperature was 260 °C. The mass selective detector worked in the scan mode from 40 and 800 m/z. After 3.0 min of injection data acquisition was started. Parameters used in mass spectrometer were; Ionization voltage (EI) which was 70 eV, peak width was 2s, mass range was 40–800 amu and detector voltage was 0.89 kV. Identification of peaks was done by comparing the mass spectra with mass spectra data available on database of NIST08, WILEY8, Perfumery and Flavor and Fragrance libraries.

Estimation of total phenolic and flavonoid contents

Total phenolic content was determined according to Follin-Ciocalteu method [17]. In 100 µl of each concentration of standard solution, 100 µl of Folin-Ciocalteu reagent and 2 ml of 2% sodium carbonate (Na_2CO_3) were mixed. The resulting mixture was allowed to stand at room temperature for 30 min and the absorbance was measured at 743 nm using UV-Visible Spectrophotometer against a blank prepared similarly but containing distilled water instead of standard solution of gallic acid. A standard curve was obtained by plotting absorbance against amount of gallic acid. A standard (calibration) curve was obtained by plotting the absorbance at 743 nm against various concentration of gallic acid.

Estimation of total flavonoids in extracts of *C. intybus* roots was done by method prescribed by Eom et al. [18]. In extracts (0.5 ml) 0.1 ml of 10% aluminium chloride and 0.1 ml of potassium acetate (1M) were added. In this mixture 4.3 ml of 80% methanol was added to make 5 ml volume. The absorbance was measured against a blank containing respective solvent without extracts at 415 nm using UV-VIS double beam spectrophotometer. The amount of total flavonoids present in the extracts was calculated from the standard curve of quercetin and results were expressed as milligrams of quercetin equivalent per gram (mg QE g^{-1}).

Antioxidant capacity analysis

The antioxidant capacity was evaluated using five different *in vitro* models involving ferric reducing antioxidant power assay (FRAP), 2, 2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$), nitric oxide ($NO^{\bullet}$), hydroxyl ($OH^{\bullet}$), Superoxide radical ($O_2^{\bullet}$) scavenging methods.

Ferric reducing antioxidant power

Ferric reducing antioxidant power (FRAP) of various extracts were determined using method given by Benzie & Strain [19]. The fresh working FRAP solution was prepared by mixing 25 mL acetate buffer (300 mM, pH -3.6), 2.5 mL 2, 4, 6-tripyridyl-s-triazine (10 mM) and 2.5 mL hydrated ferric chloride (20 mM). The FRAP solution was heated to 37°C before use. Extracts (0.2 mL) were mixed with 2.8 mL of the FRAP solution and allowed to react for 30 min in the dark condition. Coloured product was read at 593 nm. Results were expressed in terms of µmol of Fe (II)/mL of extract.

DPPH radical scavenging method

2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging of all the extracts were

determined [20]. To 1mL of various concentrations (0.05-1.0 mg /mL) of test extracts dissolved in methanol, 1 mL DPPH solution (0.1 mM) in methanol was added. The colour of reaction mixture was read at 517 nm using UV-spectrophotometer after keeping the reaction mixture in dark 30 min at room temperature. Ascorbic acid was used as a reference material. All tests were performed in triplicate. The percentage inhibition was determined by comparing the absorbance values of tested components and control using the formula

$$\text{Percentage inhibition (\%)} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Hydroxyl radical (•OH) scavenging method

Hydroxyl radical ($^{\bullet}$OH) scavenging activity was determined by deoxyribose-degradation method [21]. A mixture containing ascorbic acid (0.1 mM), deoxyribose (2.8 mM), ethlene diamine tetraacetic acid (0.1 mM), ferric chloride (0.1 mM), hydrogen peroxide (1 mM), phosphate buffer (20 mM, pH 7.4) was various concentrations of extracts in a final volume of 1mL. The reaction mixture was incubated for 1hr at 30 ºC. For this, 3mL thiobarbituric acid (0.67%) was added to each test tube and kept for 1hr in boiling water. The absorbance of the chromophore formed was read at 532 nm against blank prepared in similar way. Control was also run parallel, in which no compound was added. The percentage inhibition was calculated using same formula given above.

Nitric oxide ($NO^{\bullet}$) scavenging method

Nitric oxide radical scavenging of all the extracts of *C. intybus* roots were determined [22]. In a test tube 0.5 mL volume of various concentrations (0.05-1g /mL) of extracts, 0.5 mL of sodium nitroprusside (10 mM) and 0.5 mL phosphate buffered saline were added followed by incubation at 30ºC for 2.5 hrs. After incubation, 1 mL of freshly prepared Gries reagent was added. The absorbance of chromophore formed was read at 548 nm against blank after making the final volume of 3 mL with distilled water. The percentage inhibition of nitric oxide generation was calculated using same formula given above.

Superoxide radical (O_2•) scavenging activity

Superoxide radical scavenging was based on the method described by Liu et al. [23]. Tested extract (1 mL) was mixed with 1 mL of phosphate buffer (pH 7.4) containing 1 mL of NBT (50 µM) solution, 1 mL of NADH (78 µM) solution. To this mixture 1 mL of phenazine methoxy sulfatenicotinamide solution (60 µM) was added to initiate the reaction. The colour of reaction mixture was read after incubating it at 25°C for 5 min at 560 nm against blank samples using UV-spectrophotometer. The percentage inhibition was calculated using same formula given above.

Statistical analysis

Experimental results were mean ± standard deviation of three parallel measurements and analysed using software SAS 9.4. Differences between mean were determined using Tukey multiple range test and correlations were obtained by Pearson correlation coefficient. P values less than 0.05 were regarded significant.

RESULTS AND DISCUSSION

Roots of *C. intybus* were extracted sequentially with solvents of increasing polarity viz hexane, dichloromethane, methanol in order to characterise all type of volatile components present in roots. The hexane soluble components present in root powder were extracted with hexane. Further same powder was extracted with dichloromethane thus component which were not soluble in hexane but soluble in dichloromethane were extracted and the components not soluble in hexane or dichloromethane were extracted out with methanol which was the most polar solvent used for extraction.

Gas chromatography-mass spectrometry analysis

Gas chromatography-mass spectrometry analysis of volatile components revealed that maximum number of volatile compounds present in *C. intybus* root powder were hexane soluble i.e. 35 compounds that were present in hexane extract. Dichloromethane soluble compounds were 25 and while methanol extract contained only 20. Components present in different extracts were mostly different except a few e.g. 5-Hydroxymethyl furfural (HMF). HMF was found to be the major component in dichloromethane and methanol extract whereas lupeol, a bioactive triterpene and its derivatives were found to be major constituents of hexane extract. GC-MS analysis of all the extracts is tabulated in Table 1. HMF, the major component have a strong caramellic to burnt sugar fragrance. Many studies showed the presence of 5-hydroxyl methyl furfural in coffee powder. HMF was reported to form during the roasting of coffee and the concentration in different commercially available roasted coffee was found to be different [24]. Presence HMF in *C. intybus* extract can be attributed to the strong and coffee like flavour of *C. intybus*. HMF was not reported as major constituent of roasted *C. intybus* root powder by some researchers. Majcher et al. [25] showed 2-methoxy phenol, 2,3-butanedione, 2-furfurylthiol, 2-thenylthiol as major volatile component of roasted *C. intybus*. Whereas 1-octene-3-one, 2-ethyl-3,5-dimethylpyrazine, 3-methylbutanal and 2,3- butanedione were reported as the most odour active compounds of roasted *C. intybus* using using dynamic headspace and simultaneous steam-distillation-solvent extraction [2]. *C. intybus* roots are well-known to contain inulin, a polymer of sugar molecules. Fachri et al. [26] reported the estimation of thermal conversion of inulin of *C. intybus* roots to HMF and derivatives.

HMF is practically present in every food containing free carbohydrates with amount up to 0.08 mg/g. But presence of high content of HMF in roasted *C. intybus* is a big concern as roasted *C. intybus* powder is widely consumed in India as coffee substitute as well as an adulterant in coffee and various studies on the metabolic activation of HMF showed a genotoxic potential of this compound [27, 28]. However, the acute toxicity of HMF was relatively low and the LD_{50} was shown to be 3.1 g/kg bw in rats [29]. The genotoxic and carcinogenic potential reviewed by Janzowski et al. [30] showed that HMF was not a highly dangerous compound. HMF was metabolised in the kidneys to 5-Hydroxy-methyl-2-furoic acid and other compounds which was then excreted with the urine. This was shown for rats and mice [31] as well as humans [32]. Thus some reported HMF as genotoxic molecule and other not thus it is not clear whether HMF is genotoxic or not but still it is a good starting material for the synthesis of precursors of various pharmaceuticals, thermo-resistant polymers and complex macrocyclic molecules. Due to the natural source, roasted *C. intybus* roots are good and cheap source for HMF.

Estimation of total phenolic and flavonoid content

Total phenolic and flavonoid contents in extracts of *C. intybus* roots were also evaluated (Table 2). The amount of total phenolic content was found to be highest in methanol extract and least in hexane extract. Similar trend was observed in case of flavonoid content also.Total phenolic contents in extracts varied from 0.68 to 10.13 mg GAE/g of root powder. The amount of flavonoids in extract varied from 0.73 to 8.8 mg QE/g of root powder. As roasted *C. intybus* root powder was sequentially extracted so actual total phenolic and flavonoid content was sum of content in all extracts i.e. phenolic content of *C. intybus* root powder was 15.01 mg GAE/g powder and flavonoid content was 13.82 QE/g powder. As content of both components was maximum in methanol extract thus it can be concluded that maximum phenolics and flavonoid present in roasted *C. intybus* roots were methanol soluble. The results of present studies were found to be in agreement with Felhi et al. [33] who reported methanol as the best solvent for extraction of total phenolics and flavonoid content. Baeza et al. [34] reported total phenolic content in *C. intybus* coffee was aprox. 22 mg GAE/g of *C. intybus* coffee which was near to content observed in present study.

Table 1. GC-MS analysis of different solvent extracts of *C. intybus* roots

Sr.No.	Hexane extract			Dichloromethane extract			Methanol extract		
	Compounds	**Retention time**	**Area %**	**Compounds**	**Retention time**	**Area %**	**Compounds**	**Retention time**	**Area %**
1	Undec-3-en-2-ol	2.145	0.51	2-furancarboxaldehyde	2.378	0.56	Glyceraldehyde	2.353	9.60
2	2-Nitrohexane	2.945	0.56	Caprolactone	3.058	0.84	4-Hydroxy-4-methyl-2-pentanone,	2.422	3.20
3	2,2-Dimethyl-pentan-1-ol	3.148	0.40	4-oxo-5-methoxy-2-penten-5-olide	3.516	1.39	β-Ketoglutaric acid	2.725	2.41
4	5-Methyl-2-furancarboxaldehyde	3.646	0.81	Maltol	6.774	0.70	Dihydroxyacetone	2.879	4.35
5	3-Hexen-2-one	3.698	0.54	3-hydroxy-2,3-dihydromaltol	6.845	1.64	Hydracrylic acid	2.972	1.10
6	5-Hexen-2-one	4.888	0.42	methyl 2,4-heptadienoate	6.957	0.65	Glycerine	3.724	5.65
7	Maltol	6.171	0.45	2 4-hydroxydihydro-2(3h)-furanone	7.432	0.87	2,4-Dihydroxy-2,5-dimethyl-3(2h)-furan-3-one	3.916	1.50
8	5-Hydroxymethylfurfural	8.673	2.69	2-methyl-2h-pyran-3,4,5(6h)-trione	7.757	5.92	1,3,5-Triazine-2,4,6-triamine	5.470	3.13
9	2-Acetyl-resorcinol	9.671	0.45	4-(1-hydroxy-ethyl) .gamma. butanolactone	8.031	0.74	Unknown	5.659	1.00
10	5-Acetoxymethyl-2-furaldehyde	10.328	1.42	5-hydroxymethylfurfural	8.853	62.35	1, 2-Epoxy-3-hydroxypropane	6.728	3.47
11	Hexadecanoic acid	23.636	4.69	1,2-cyclohexanedicarboxylic acid, 4-methoxyphenyl nonyl ester	9.180	0.71	2,3-Dihydro-3,5-dihydroxy-6-methyl-4h-pyran-4-one	6.835	4.29
12	Ethyl hexadecanoate	23.887	0.75	4-[(2,6-dichlorophenyl)methoxy]-3-ethoxy- benzaldehyde,	9.699	1.34	5-Hydroxymethylfurfural	8.598	40.50
13	Methyl linoleate	25.543	0.47	5-(hydroxymethyl)-2-(dimethoxymethyl) furan	10.041	8.99	Unknown	13.329	6.08

14	Ambrosial	26.385	1.04	5-acetoxymethyl-2-furaldehyde	10.328	0.63	Unknown	13.410	1.33
15	Ethyl octadec-9,12-dienoate	26.620	5.91	dimethyl 2,5-thiophenedicarboxylate	10.861	2.13	Unknown	22.056	1.57
16	Unknown	26.725	1.44	1,2-cyclohexane dicarboxylic	12.578	0.67	Alantolactone	22.526	2.12
17	5-formyl-2-furyl)methyl acetate	37.215	0.43	2,4-di-tert-butylphenol	14.740	1.16	Isoalantolactone	23.238	1.37
18	Squalene	38.582	0.44	1-octadecene	16.450	0.69	Hexadecanoic	23.368	2.99
19	2-[1-(Hhydroxymethyl)cyclohexyl] ethanol	39.570	0.59	nonadecyl alcohol	20.355	0.81	Dehydrocostus lactone	23.987	3.31
20	Stigmasterol	44.543	0.81	4-(acetylamino)-1h-imidazole-5-carboxamide	21.686	0.59	Methyl linoleate	25.541	1.04
21	δ-Friedoolean-14-en-3-one	45.488	0.99	palmitic acid	23.395	0.72			
22	Stigmast-5-en-3-ol	45.695	1.53	5,5'-(oxydimethylene)di- 2-furaldehyde	23.849	3.55			
23	β-amyrin	46.574	8.96	1-octadecene	23.910	0.92			
24	Unknown	46.785	0.70	8-ethenyl-3,4,4a,5,6,7,8,8a-octahydro-5-methylene-2-naphthalenemethanol	24.000	0.78			
25	Unknown	47.601	13.09	Lignocerol	27.156	0.64			
26	α-amyrin	47.771	2.05						
27	Stigmast-4-en-3-one	48.320	1.37						
28	Unknown	48.797	2.88						
29	Lup-20(29)-en-3-yl acetate	49.599	18.59						
30	Clerodol	49.909	6.54						
31	Unknown	50.745	0.98						
32	Unknown	50.966	1.74						
33	Unknown	51.263	1.62						

34	Lupan-3-yl- acetate	51.700	8.18
35	Lupeol acetate	52.038	5.99

Gorjanovic et al. [35] reported unusually high content of phenolics i.e. 5.31 g GAE/litre in brew prepared from roasted *C. intybus* powder purchased from local market of Serbia which was quite high. Thus roasted *C. intybus* powder is a good source of phenolics and flavonoids.

Table 2. Total phenolic and flavonoid content of various extracts

Components	Yield (%)	Total phenols mg GAE/g of root	Total flavonoids mg QE/g of root
Methanol extract	10.1 ± 1.2	10.13±2.01	8.8±1.97
Dichloromethane extract	5.6 ± 0.8	4.20±0.42	4.24±1.62
Hexane extract	1.3 ± 0.5	0.68±0.05	0.73±0.28

Results were expressed as mean± SD.

Antioxidant activity

In order to obtain information regarding antioxidant capacity of activity of roasted *C. intybus* root powder multiple antioxidant assays were performed. Antioxidant capacity of different *C. intybus* extracts prepared by sequential Soxhlet extraction was evaluated by FRAP (Table 3), DPPH, hydroxyl, nitric oxide and super oxide radical scavenging (Table 4). Statistical analysis of data showed that there is significant difference in activities of different extracts. Methanol extract was found to be significantly more active as compared to other extracts. There is no significant difference in activity of methanol extract and ascorbic acid which used as standard.

Table 3. Ferric reducing antioxidant power of various extracts of *C. intybus* roots

Components	Concentrations(mg/mL)	Frap value (µmol /mL)
Methanol extract	0.05	169.08 ± 3.21
	0.1	284.95 ±6.36
	0.25	716.81 ±12.8
	0.5	1005.45 ±9.64
	1.0	1605.45 ±9.64
	Mean	756.35[a]
Dichloromethane extract	0.05	173.63 ±3.21
	0.1	266.805 ±6.44
	0.25	557.725 ±6.42
	0.5	848.64 ±6.42
	1.0	1475.90 ±6.43
	Mean	664.54[b]
Hexane extract	0.05	87.27±3.21
	0.1	191.82±3.22
	0.25	337.275±9.65
	0.5	758.155±12.24
	1.0	1126.35±12.23
	Mean	500.17[c]

Results were expressed as mean± SD.
Mean values followed by same letter are non-significantly different (p> 0.05) according to Tukey multiple range test

In ferric reducing antioxidant power (FRAP) assay it was found that there is significant positive correlation between FRAP value and concentration of extract. Statistical analysis revealed that methanol extract is significantly more active than other tested extracts. Rajukar & Hande [36] reported a positive correlation between FRAP value and total phenolic content. Thus high FRAP value of methanol extract can be related to the high phenolics and flavonoid content of methanol extract. Antioxidant agents with high scavenging activity showed a low IC_{50} value [37]. The overall order of activity of tested extracts on the basis of

IC_{50} values in decreasing order is as follows: Ascorbic acid > Methanol extract > Dichloromethane extract > Hexane extract

Table 4. Antioxidant activities of *C. intybus* root extracts using various *in vitro* models

Components	Concentrations (mg/mL)	DPPH radical scavenging		Hydroxyl radical scavenging		Nitric oxide radical scavenging		Superoxide radical scavenging	
		Inhibition (%)	IC_{50} (mg/mL)	Inhibition (%)	IC_{50} (mg/mL)	Inhibition (%)	IC_{50} (mg/mL)	Inhibition (%)	IC_{50} (mg/mL)
Methanol extract	0.05	39.75± 0.11	0.085	31.87± 0.16	0.088	33.62±0.51	0.087	18.23±0.41	0.258
	0.1	51.21± 0.11		58.43± 1.10		55.58±0.23		30.14±0.21	
	0.25	72.50± 0.11		75.62± 0.91		79.63±0.04		43.08±0.21	
	0.5	95.32± 0.23		88.65± 0.52		89.63±0.04		65.14±0.41	
	1.0	99.98± 0.21		99.25± 0.22		99.70±0.23		80.88±1.04	
	Mean	71.75±23.65[a]		70.76± 23.74[a]		71.63±23.99[a]		47.49±22.81[b]	
Dichloromethane extract	0.05	23.05± 0.22	0.148	25.62± 1.26	0.168	23.67±0.04	0.167	11.25±0.10	0.511
	0.1	41.26± 0.23		40.31± 0.86		40.35±0.14		21.6±0.14	
	0.25	66.02± 0.22		55.1± 1.12		59.28±0.28		31.98±0.31	
	0.5	75.40± 0.23		73.43± 1.76		73.23±0.46		49.77±0.11	
	1.0	95.21± 0.11		86.56± 0.88		91.93±0.09		64.48±0.11	
	Mean	60.18±25.41[b]		56.20±23.97[b]		57.69±23.97[b]		35.82±19.16[c]	
Hexane extract	0.05	11.7± 0.22	0.257	15.31± 0.78	0.221	4.92±0.79	0.306	5.36±0.31	1.45
	0.1	25.26± 0.23		33.43±1.26		33.80±0.14		13.67±0.21	
	0.25	43.87± 0.23		52.18± 1.15		52.04±0.23		23.60±0.31	
	0.5	63.5± 0.22		69.22± 1.96		72.17±0.46		30.88±1.04	
	1.0	90.7± 0.07		83.75± 0.36		90.68±0.18		44.63±0.31	
	Mean	47.00±27.96[c]		50.77±24.44[c]		50.72±29.80[c]		23.63±13.61[d]	
Ascorbic acid (Standard)	0.05	38.42± 1.06	0.066	32.89±1.80	0.082	31.50±0.96	0.081	29.10±0.47	0.156
	0.1	62.23± 1.28		60.47±1.25		58.42±1.81		46.27±0.74	
	0.25	82.34± 1.99		77.50±1.90		75.54±0.53		53.09±0.53	
	0.5	95.36± 0.87		89.35±1.65		89.49±1.22		68.90±0.46	
	1.0	99.48± 0.11		98.83±2.05		99.89±0.93		88.19±0.52	
	Mean	75.56±22.65[a]		71.81±23.29[a]		70.96±24.14[a]		57.11±20.11[a]	

Results were expressed as mean± SD.

Comparison were only amongst extracts for each methods and mean values in each method followed by same letter are non-significantly different (p> 0.05) according to Tukey multiple range test

The results of percentage scavenging of free radicals in present study were in consonance with the Stankovic [38] which reported that alcoholic extract of *P. cattleianum* exhibited the highest antioxidant activity and showed scavenging percentage of 94.57 %, followed by the aqueous extract 92.62 %. Also Chang et al. [39] reported that ethanol extract of *S. lappa* showed 95.02% inhibition which was comparable to that of their ascorbic acid positive control. In present studies methanolic extract was most active and showed comparable activity to that of standard in all the assays. The IC_{50} values of methanol were usually lower. Jananie et al. [40] also reported the lower IC_{50} hydro-alcoholic extract of *Cynodon doctylon* as compared to other solvent extract. Lower the IC_{50} values of treatment better is activity. Results were also in agreement with Sonawane et al. [41] who reported the positive correlation between radical scavenging activities with phenolic content. Similar trend was observed in present study as trend of activity was similar as the trend observed in case of total phenolic and flavonoidal content of extracts. It was observed that the total phenolic and flavonoid content varied with polarity of solvent used for making extract. The antioxidant activity also varied with phenolic and flavonoid content. Antioxidant potential can be related to the amount of phenols and flavonoids. Yingming et al. [42] reported that the antioxidant activity of phenolic compounds was due to their redox properties, which played an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides and the antioxidant activity of flavonoid compounds was reported due to their scavenging or chelating process [43]. Also antioxidant activity of chickpea (*Cicer arietinum* L.) increased during roasting and was also related to Maillard reaction products formation [44]. Thus high antioxidant activity of roots could be related to the Maillard reaction products as well as high phenolics and flavonoid content.

CONCLUSION

It was found that even roasted roots of chicory were rich source of phenolics and flavonoids an still possess antioxidant potential. The roasted *C. intybus* roots extracts contained good amount of phenolic and flavonoid content and methanol extract exhibited antioxidant activity equivalent to ascorbic acid. The content of phenols, flavonoids and antioxidant activity of extracts were found to be greatly affected by type of solvent used for extraction, similarly it affected the antioxidant potential of extracts. It was found that most of phenols and flavonoids present in *C. intybus* roots are methanol soluble. The higher antioxidant capacity of methanol extract of *C. intybus* roots can be related to its higher phenolic and flavonoid content. An extract is considered to be active against free radicals if $IC_{50} < 5$ mg/mL [45]. All the tested extracts showed IC_{50} values less than 0.6 mg/mL, therefore roasted *C. intybus* root powder is also a very good source of antioxidants. Although GC-MS analysis showed that among volatile components 5-hydroxymethyl furfural was major component in extracts of roasted *C. intybus* root from India. HMF might be responsible for the strong flavour and aroma of roasted root powder. HMF was also reported to be genotoxic in some reports while not in others thus it could be a point of concern. Overall it could be concluded that roasted roots of chicory also possess phenolic and flavonoidal content and good antioxidant potential. Methanol is the best solvent for extraction of roasted roots for further search of bioactive components.

Conflict of interest: The authors declared that they have no conflict of interest.

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