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Determination of antioxidant activities in various extracts of *Cichorium intybus*

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Abstract
Cichorium intybus, commonly known as chicory, has a long history of medicinal use due to its diverse phytochemical composition. This study investigated the antioxidant activities of various extracts of *C. intybus*, focusing on the leaves as they are reported to have the highest concentration of bioactive compounds. Methanol extracts of *C. intybus* leaves exhibited significantly higher total antioxidant activity compared to aqueous extracts, indicating their importance as a potential source of natural antioxidants. The leaves also demonstrated superior free radical scavenging capacity (IC50 = 127.22±2.24 µg/ml) and reduced power compared to other parts of the plant. These findings suggest that *C. intybus* leaves, particularly methanol extracts, possess promising antioxidant properties, warranting further exploration of their potential applications in pharmaceutical and nutraceutical fields. The study highlights the importance of investigating different organic fractions of *C. intybus* for their diverse therapeutic potential, aligning with the growing demand for natural and herbal remedies.

Keywords: *Cichorium intybus*, antioxidants, free radical scavenging

Introduction
In recent times, there has been a growing interest in exploring plant-derived substances for their diverse applications, ranging from traditional medicine to pharmaceutical intermediates and food supplements [1]. Medicinal plants, in particular, stand out as rich bio-resources that offer a wide array of compounds with therapeutic potential for various health conditions [2, 3]. Among these plants, *Cichorium intybus*, commonly known as chicory, has garnered attention for its extensive applications in traditional systems of medicine such as Ayurveda, Unani, and Siddha [4, 6].

Belonging to the Asteraceae family, *C. intybus* has a long history of medicinal use, with its roots and leaves being especially valued for their diuretic, laxative, antibilious, antipyretic, and blood-purifying properties [5]. The plant contains a plethora of bioactive compounds, including alkaloids, inulin, sesquiterpenes, lactones, coumarins, vitamins, chlorophyll pigments, unsaturated sterols, flavonoids, saponins, and tannins, making it a comprehensive reservoir of therapeutic agents [7].

Phytochemical analysis of *C. intybus* reveals the presence of salts, glycosides, and various minerals in its leaves, while the roots are known for their stomachic, diuretic, and blood-purifying qualities [8, 9]. The genus Cichorium, consisting of six species, is primarily distributed in Europe and Asia, with *C. intybus* being prevalent in regions such as North-West India, Punjab, Kashmir, Andhra Pradesh, Karnataka, and Maharashtra. Additionally, chicory is cultivated in countries like Belgium, France, Germany, Switzerland, West Asia, and the United Kingdom [9].

Despite its widespread use, *C. intybus* is not officially described in the European Pharmacopoeia or any official Pharmacopoeia of a European Union member state [9, 10]. Nevertheless, the plant's prevalence has led to its incorporation into traditional medicines globally. Notably, different parts of the plant, especially the root, have been utilized in addressing various health concerns [10, 11, 13].

This study aims to explore the antioxidant activities of various extracts of *C. intybus*, considering its rich phytochemical composition and medicinal significance [1, 4, 7].

The plant's historical uses, ranging from treating digestive disorders to ailments like jaundice, rheumatism, and headaches, underscore its potential as a valuable candidate for pharmaceutical formulations and as a contributor to antioxidant defense systems against free radicals [8, 9]. The focus on antioxidant activities aligns with the broader goal of tapping into natural resources to expand the spectrum of phytochemicals with potential health benefits [10, 17].

Materials and Methods

Total Antioxidant Activity of extract was estimated by Phoshphomolybdenum method described by [1]. This method is based on the reduction of phosphomolybdic acid to phosphomolbdenum blue complex by sodium sulphide. The obtained Phosphomolbdenum blue complex is oxidized by the addition of nitrite and this causes reduction in intensity of the blue colour [3].

An aliquot of 0.5 ml (40µg/ml conc.) of sample solution was combined with 1ml of reagent solution (0.6M sulphuric acid, 28mM Sodium Phosphate and 4 mM of Ammonium molybdate). In case of reaction blank, 0.5 ml of methanol was used in place of sample [9, 15, 16]. The tubes were incubated in a boiling water bath at 95 °C for 90 min. After the sample containing test tubes were cooled in room temperature and the absorbance was measured at 695nm against blank in UV-spectrophotometer [10-12] The higher absorbance value indicates higher antioxidant activity. Experiment was done in three replicate. The ascorbic acid in the range of 20-100 µg was used for standard curve [20].

DPPH Radical Scavenging activity

The free radical scavenging capacity of the extract was determined using DPPH method described by [14]. 1, 1 Diphenyl 2- Picryl Hydrazyl is a stable (in powder form) free radical with red colour which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity. The scavenging reaction between (DPPH) and an antioxidant (HA) can be written as, [13].

$$(DPPH) + (H\text{-}A) \longrightarrow DPPH\text{-}H + (A)$$

% radical scavenging activity = (absorbance of blank-absorbance of sample) / (absorbance of blank) x 100

Antioxidants react with DPPH and reduce it to DPPH-H and as consequence the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability. It was measured by a decrease in absorbance at 517 nm of a solution of coloured DPPH in methanol brought about by the sample.

IC50 values (mg/mL) were calculated from the linear response curve of percent inhibition of DPPH at various concentrations of extracts.

IC50=We can calculate the IC50 values by plotting the curve of inhibitions and corresponding concentrations following the formula y= ax + b where y= 50 and we calculate X which is the value of IC50. A lower IC50 value indicated a greater antioxidant activity.

Total Reducing Power

The Total reducing power of extract was determined by the method of Oyaizu (1986) with slight modification [18]. The potassium ferricyanide react with ferric chloride in the present of anti oxidant, potassium ferrocyanide and ferrous chloride are formed as a product [20]. Presence of reducers causes the conversion of the Fe3+/ferricyanide complex used in this method to the ferrous form that has maximum absorption at 700nm.

Different concentrations (20, 40, 60, 80, 100 µg) of the extract were mixed with 2.5 ml of phosphate buffer (0.2M, pH 6.6) and 2.5 ml potassium ferric cyanide (1%) followed by incubation at 50 °C in water bath for 20 min. After incubation, 2.5 ml of TCA (10%) was added to terminate the reaction. Finally 0.5 ml $FeCl_3$ solution (1%) was added and the absorbance was measured at 700nm against an appropriate blank solution. Ascorbic acid at various concentrations (20-100µg) was used as standard. Experiment was done with three replicate. Increase in absorbance indicated the increasing reducing power of respective concentration of extract [14-16].

Results

The antioxidant activity of the samples was assessed through their ability of scavenging 2, 2_-diphenyl-1-picrylhydrazyl stable radicals (DPPH) at maximum absorbance of 517nm. Results shows that as the concentration of phenolic compounds or degree of hydroxylation of the phenolic compounds increases, DPPH scavenging activity also increases, hence does the antioxidant activity. *C. intybus* leaves and roots showed maximum DPPH free radical scavenging activity in methanol (127.22±2.24 and 241.54±12.65 respectively) and water (416.66±1.14 and 303.03±8.03 respectively). The antioxidant ability of the extracts was screened by DPPH assays and IC50 values as reported in Table 1.

IC50 value is the inhibitory concentration of the antioxidant at which 50% inhibition of DPPH radical occurs. The IC50 values were calculated by linear regression of plots, where the abscissa represented the concentration of the tested plant extracts and the ordinate the average percent of scavenging capacity from three replicates. Due to high Phenolics content, comparatively good reducing power and DPPH radical scavenging capacity was found in leaves [2] In this method, methanolic DPPH solution is reduced in the presence of antioxidant to form non-radical DPPH-H. The degree of discolouration shows the scavenging potential of the extract. The extracts had dose dependent activity, i.e. DPPH scavenging activity increased proportionately to the increase in concentration of the extracts. *C. intybus* leaf and root extracts showed excellent radical scavenging activity, with IC50 (the extract concentration providing 50% of inhibition).

The statistical analysis of DPPH antioxidant activity was held taking the leaf and root part of *C. intybus* L, plant under consideration. While working with two different solvents i.e., methanolic and aqueous, both leaf and root extracts of *C. intybus* shows slight variability regarding IC50 value. Leaves extracts tends to show great radical scavenging activity in respect to the root part.). When compared with the synthetic antioxidants BHT (IC50=55.43 µg/mL) and Ascorbic Acid (IC50=43.74 µg/mL), both methanolic and aqueous extracts offered slightly lower antioxidant activity.

Here, IC50 value is inversely proportional to radical scavenging activity. Thus, lower the IC50 value more will be the inhibitory power and more will be the radical scavenging activity and hence enhancement in the

antioxidant activity. Leaves of *C. intybus* were found to possess comparatively good free radical scavenging capacity due to higher DPPH radical inhibition and lower IC50 value. Irrespective of the solvent extraction the leaves of *C. intybus* possess more antioxidant property than root parts.

Table 1: Antioxidant activity: Total Antioxidant Activity (TAO) and DPPH IC_{50} value of methanolic and aqueous extracts of *C. intybus.*

Antioxidant Activity						
Antioxidant assay	**Ascorbic Acid(Standard)**	**BHT (Standard)**	**Methanolic Leaf extract**	**Methanolic root extract**	**Aqueous leaf extract**	**Aqueous root extract**
Total Antioxidant Content(mg Ascorbic acid/g extract)	---------	--------	76.54±0.73	49.89±0.00	48.62±6.34	32.13±0.00
DPPH scavenging activity, IC50 (µg/mL)	43.74±0.34	55.43±1.14	127.22±2.24	241.54±12.65	416.66±1.14	303.03±8.03

Values are IC_{50} ± SE of three parallel replicates

Table 2: Reducing power (absorbance at 700 nm) of methanolic and aqueous extracts of *C. intybus* as compared to that of BHT taken as standard antioxidant.

Total Reducing Activity					
Concentration (700nm)	**BHT (Standard)**	**Methanolic Leaf extract**	**Methanolic Root extract**	**Aqueous Leaf extract**	**Aqueous Root extract**
20	1.56±0.06	0.24±0.004	0.17±0.002	0.10±0.001	0.11±0.001
40	2.54±0.07	0.36±0.004	0.20±0.003	0.13±0.006	0.12±0.002
60	3.08±0.07	0.50±0.005	0.22±0.002	0.20±0.01	0.13±0.001
80	3.97±0.07	0.71±0.01	0.26±0.004	0.23±0.02	0.13±0.001
100	3.99±0.08	0.83±0.01	0.30±0.005	0.75±0.03	0.14±0.003

Values are means ± SE of at least three determinations.

Considerably, the methanolic extracts of leaf and root have higher radical scavenging activity as compared to aqueous extracts and the order is as follows -

Methanolic leaf > Methanolic root > Aqueous root > Aqueous leaf

Overall the methanolic extracts of *C. intybus* leaves possess comparatively higher amounts of free radical scavenging capacity (C_{50} = 127.22±2.24 µg/ml) as compared to aqueous extracts (IC_{50} =416.66±1.14 µg/ml). Thus, would play an important role in antioxidant defence system against endogenous free radicals and thus improving the human health as per compared to the standard synthetic antioxidants ascorbic acid and BHT. The values of IC_{50} of leaves and roots are high if compared with Ascorbic acid and BHT and this is not surprising since analyzed samples are complex extracts and they most likely contain pro-oxidants agents which may compete with the antioxidants in the reaction with DPPH radicals. Thus IC_{50} values are the results of the balance between pro-oxidant and antioxidant molecules.

All part of *C. intybus* showed lower percentage of DPPH radical inhibition and higher IC_{50} values as compared to those of ascorbic acid taken as standard antioxidants [19, 20]. The order obtained for gained antioxidant property is as methanolic extract (IC_{50}=127.22±2.24 µg/ml) > aqueous extract (IC_{50}=416.66±1.14 µg/ml) of leaves and very low in root part (IC_{50}=241.54±12.65 and 303.03±8.03 µg/ml of methanolic and aqueous extract respectively) [4, 17].

On comparison the IC50 value of chicory leaves extract was found to be 67.2 ±2.6 l µg/ml and hence too showed the similar tendency in leaves showing good free radical scavenging capacity due to higher DPPH radical inhibition and lower IC_{50} value [20, 19]. However, indicating the high perspective of antioxidants concerning *C. intybus* leaves possessing to have higher inhibitory power [12].

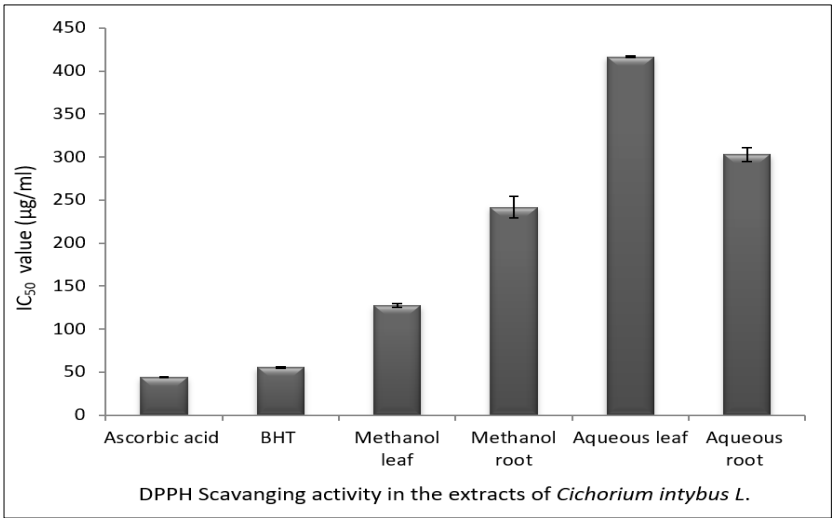

Fig 1: DPPH radical scavenging activity, IC_{50} value of Leaves and Roots extracts of *C. intybus*

As we know, a balance between free radicals and antioxidants is necessary for proper physiological function. Synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have recently been reported to be dangerous for human health [3] Thus, the search for effective, nontoxic natural compounds with antioxidative activity has been intensified in recent years to which medicinal aromatic plants like *C. intybus* relates the best and can prove a good replacement to the synthetic antioxidants like BHT and BHA.

Total Reduction Capability: Like the antioxidant activity, the reducing power increased with increasing amount of the extract. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. For the estimation of the reductive ability, we investigated the Fe^{3+} to Fe^{2+} transformation using the method of Oyaizu, where the change in the optical density of the final mixture is measured at 700nm. Increase in optical density indicates higher reductive ability. The reduction capability of all tested plant extracts increased dependently with increasing concentration (Figure 1) and these increments were statistically significant (p<0.01). All tested extracts found to have lower reducing power as compared to standard materials (BHT) at all concentrations (20, 40, 60, 80 and 100 µg/mL).

Reducing agents hinder lipid peroxidation as they donate a hydrogen atom and stop the chain reaction that causes membrane lipid damage. Reducing power of extracts and standard at high concentration followed the order: BHT>*C. intybus* leaf> *C. intybus* root. *C. intybus* has slightly high absorbance value that indicates its reductive potential and electron donor ability for stabilizing free radicals.

The statistical analysis of results showed a significant difference in the reducing abilities of leaf and root part of *C. intybus.* Leaves were found to possess comparatively higher value of reducing power ability than the root ones [17, 19, 13]. Solvents such as methanolic and aqueous plays a comparable role in reducing power capability of *C. intybus* leaf and root extracts. However, the reducing powers of all parts of *C. intybus* were found to be low as compared to that of BHT taken as standard antioxidant [17, 18].

A statistically significant difference in reducing activity was observed in the extracts of *C. intybus* concerning the standard taken, where optical density of the extract is directly proportional to the reducing activity of the extract. High absorbance high would be the reductive potential. While taking BHT as standard, as per the concentration (20, 40, 60, 80, 100 µg/ml) increases the absorbance too increases and hence the reducing activity shown in Table 2. BHT possess more reducing activity than any other extracts of the plant i.e., from 1.56±0.06 to 3.99±0.08 with the increase in the absorbance at 700nm. Whereas, the methanolic extracts of leaf and root tends to show less reducing activity then the standard from 0.24±0.004 to 0.83±0.01 and 0.17±0.002 to 0.30±0.005 in leaf and root extract respectively. On comparison of methanolic extract to aqueous extract, the latter shows less reducing activity i.e., from 0.10±0.001 to 0.75±0.03 and 0.11±0.001 to 0.14±0.003 in leaf and root part respectively.

Overall the methanolic leaf extract possess comparatively good reducing activity with the increase in the absorbance (700nm) and thus due to their redox properties these compounds contribute to the overall antioxidant activities of plant *C. intybus.* The below given fig.2. shows the significant differences of the leaf and root extracts of *C. intybus* in respect to BHT taken as standard antioxidant.

The overall results indicated that *C. intybus* exhibited the best antioxidant activity determined as total content of free radical scavenging, and total reducing activity. The leaves extract was found to show comparatively good phytochemical as well as antioxidant property i.e., low value of IC_{50} for DPPH inhibition and high reducing power. Due to excellent biochemical, phytochemical and antioxidant composition, *Cichorium intybus* leaves would be valuable candidate in pharmaceutical formulations and play an important role in improving the human, livestock and poultry health by participating in the antioxidant defense system against endogenous free radicals.

Several reports have suggested that compounds yielding antioxidant properties could also serve as potential antityrosinase agents capable of blocking melanin synthesis [13-15]. Compounds isolated from *C. intybus* methanol extract as dihydroquercetin 7-4'-dimethyl ether and blumeatin exhibited competitive inhibitor on tyrosinase activity [17-14]. Dietary phenolic have been shown to possess antityrosinase and antioxidant properties as Quercetin compound that was isolated from our plant extract exhibited antityrosinase and antioxidant effects. Therefore, the presence of antioxidant molecules and tyrosinase inhibitors in *C. intybus* make this plant a promising source of biomolecules for applications in the pharmaceutical field.

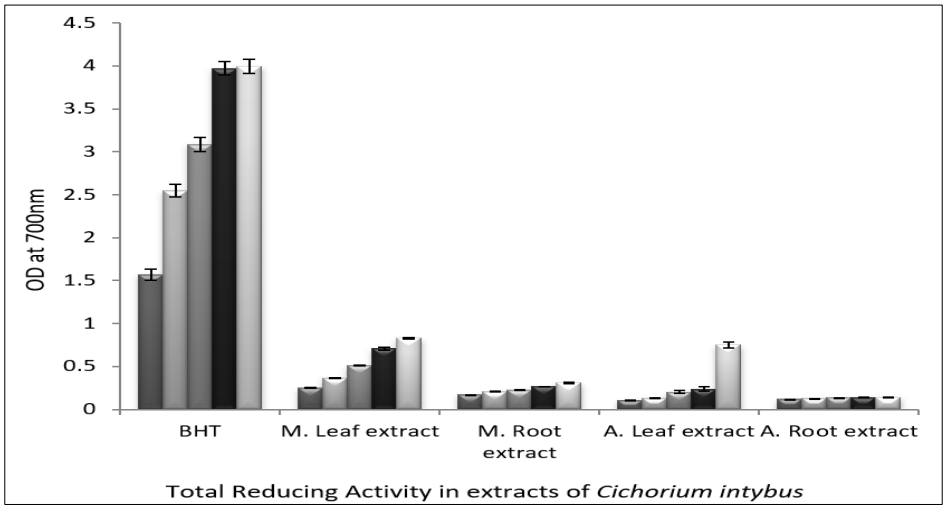

Fig 2: Reducing power (absorbance at 700 nm) of methanolic extracts of different parts of *C. intybus* as compared to that of BHT taken as standard antioxidant.

Conclusion

In conclusion, the study on various extracts of *Cichorium intybus* has provided valuable insights into its antioxidant activities and phytochemical composition. The investigation encompassed the assessment of total antioxidant activity using different extraction solvents. The findings underscore the medicinal significance of *C. intybus*, particularly its leaves, as a potent source of antioxidants.

The results revealed that all tested parts of *C. intybus* contain substantial amounts of phytochemicals, positioning the plant as a promising natural resource for antioxidant compounds. Methanol extracts of *C. intybus* leaves exhibited notably higher concentrations of antioxidants, indicating a robust free radical scavenging capacity. The strong correlation between the concentration of antioxidant activity further supports the contribution of these compounds to the observed effects.

Notably, the study demonstrated that methanol extracts of *C. intybus* leaves possessed superior free radical scavenging capacity (IC50 = 127.22±2.24 µg/ml) compared to aqueous extracts (IC50 = 416.66±1.14 µg/ml), emphasizing their potential role in antioxidant defense systems. The reducing power of the extracts also followed a similar trend, with methanol leaf extracts exhibiting higher abilities than aqueous root extracts.

The observed results provide a foundation for further pharmacological and toxicity research, urging the exploration of different organic fractions of *C. intybus.* The complex phytochemical profile of dry leaves offers promising applications in herbal medicine and nutrition for the production of health-promoting foods [13]. Given the global trend towards herbal drugs and natural products, the study highlights the urgent need to evaluate the therapeutic potentials of such plants, aligning with WHO guidelines, and recognizing that a significant portion of worldwide drug sales is derived from natural products.

In conclusion, the secondary metabolites identified in *C. intybus* contribute significantly to its various biological activities, including antioxidant, hypoglycemic, antidiabetic, antimicrobial, anti-inflammatory, anticarcinogenic, antimalarial, anti-cholinergic, and anti-leprosy activities. This comprehensive exploration of *C. intybus* reaffirms its potential as a valuable herbal remedy with multifaceted health benefits.

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