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IN VITRO STUDY ON ANTIOXIDANT AND ANTIDIABETIC ACTIVITIES OF FLAVONOID RICH FRACTION FORM LEAVES OF *CICHORIUM ONTYBUS*

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Abstract

Traditionally, *Cichorium ontybus* leaves has been extensively used to delicacy numerous infectious diseases. The aims of this examination were to determine the total phenol and flavonoid content of this plant using spectrophotometer; and assess the antioxidant and antidiabetic possessions of the flavonoid rich fraction form leaves of *Cichorium ontybus*. The total flavonoid, and phenolic content assays showed that the flavonoid rich fraction had higher phenolic. The ABTS radical, Lipid peroxidation metal chelating, superoxide radical, nitric oxide scavenging and reducing power abilities and antibacterial activity were also evaluated. The flavonoid rich fraction fraction revealed higher antioxidant properties of ABTS radical (72.31%), Lipid peroxidation (66.34%) metal chelating (78.32%), superoxide scavenging activity (71.56%) and Nitric oxide scavenging activity (81.45%). The antibacterial properties of the polyphenol rich fraction were analyzed by the disc diffusion method, and the flower extracts had higher antibacterial activities against the four bacterial strains used in the study. This study provides information on the

synergistic antioxidant and antibacterial properties of phenolics derived from the leaf parts of *Cichorium ontybus*.

1. INTRODUCTION

Currently oxidative stress is an main issue faced by numerous diabetics patients which is triggered due to over creation of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), O_2^{-}• and OH•. These condition are engage for the injury of supreme of the important molecules such as DNA, lipids and proteins, and also cause the death of cell (Wang et al., 2018). Plant bioactive metabolites can be consumed to control the balance between free radicals and diabetics might be a slighter injurious choice when associated to synthetic chemicals (Juarez et al., 2015). Subsequently, a few modern research have been attentive on the discovery of hopeful new antioxidants plant compounds such as phenolics, and flavonoids, which have comparable possessions and fewer-toxic effect obligating clear biological mechanism. Herbal plants have contained higher antioxidant metabolites, which are good promise of bio-molecules, for example anthocyanins, flavonols and flavones. These therapeutic metabolites are phenolic hydroxyl groups, and their place is significant to nonconformity in their antiradical and other biological mechanism (Uttara et al., 2009).

Type-II diabetes mellitus is most common and thoughtful metabolic disorders with unusually high blood glucose levels (hyperglycaemia) due to defects in insulin secretion, or action, or both (El-Kaissi and Sherbeeni, 2011). Hydrolysis of dietary carbohydrates such as starch is the major source of glucose in the blood. Because α-glucosidase and α-amylase play a critical role in carbohydrate digestion and glycoprotein processing, inhibitors of these enzymes might be used to treat diabetes, human immunodeficiency virus, Gaucher's disease, cancers, and Alzheimer's disease. Some inhibitors, such as acarbose and voglibose, are widely used clinically in combination with diet to control blood glucose levels of patients (Standl and Schnell, 2012). To stop or failure the side effects of these drugs and also to deliver more candidates of drug selections, it is still important to seek new α-glucosidase controller for further drug progress. In recent years, many efforts have been made to approach glucosidase inhibitors from natural sources for antidiabetes treatment (Jin et al., 2013).

Cichorium intybus L., commonly known as chicory, is an erect fairly woody perennial herb, around 1 m in height with a fleshy taproot of up to 75 cm in length and large basal leaves. Historically, chicory was grown by the ancient Indian as a medicinal plant, coffee substitute, and vegetable crop and was occasionally used for animal forage. The juice is said to be a folk remedy

for cancer of the uterus and for tumors. In India, although it is considered a widespread weed, leaves, stems, and roots are made into a tea for jaundice and chicory syrup is used as a tonic and purifying medicine for infants (Sezik et al., 2001). In India, an ointment is made from the leaves for wound healing. Decoction refers to a preparation that is made by adding cold water to the plant material which is then boiled and allowed to simmer for 5–10 min after which it is strained (Gurib-Fakim, 2006). The present investigation was directed to ascertain the efficacy of this plant in management of diabetes as well as the oxidative stress associated with it.

2. MATERIALS AND METHODS

2.1. PLANT MATERIAL

Cichorium intybus was obtained from Herbal garden of Government Siddha Medical College, Arumbakkam, Chennai, Tamilnadu, India. A plant taxonomist authenticated the plant and samples were kept in the Medicinal Botany herbarium with voucher specimen numbers MB/GSMC-278/2021. The leaf were sufficiently air-dried in 5 days at the ambient room temperature, while the flower was cut into smaller pieces and air-dried in 7 days.

2.2. PHYTOCHEMICAL SCREENING

The aqueous extract of *Cichorium intybus* were subjected to phytochemical screening to determine the presence of secondary metabolites such as alkaloids, flavonoids, terpenoids, tannins, glycosides, saponins and polyphenols using standard procedures (Aida et al., 2001; Hess et al., 1995).

2.3. TOTAL PHENOLIC CONTENT

The total phenolic content (TPC) of aqueous extract of *Cichorium intybus* was determined using the method by Singleton, (1965). The aqueous extract (1 mL, 1 mg/mL) was mixed thoroughly with 1 mL of 50% Folin-Ciocalteu reagent and 1 mL of 2% Na_2CO_3, and centrifuged at 13400X *g* for 5 min. The absorbance of upper phase was measured using a spectrophotometer (ELICO (SL150) UV–Vis Spectrophotometer) at 750 nm after 30 min incubation at room temperature. Total phenolic content was expressed as a catechol equivalent.

2.4. ESTIMATION OF FLAVANOID

A 1ml aliquot of each aqueous extract of *Cichorium intybus* was mixed thoroughly with 1ml of 2% aluminium chloride and 0.5 ml 0f 33% acetic acid followed by the addition of 90% methanol and the content is thoroughly stirred and allowed to stand for 30 minutes (Elfalleh et al.,

2019). The absorbance was measured at 414 nm using a UV-Visible Spectrophotometer. Quercetin was used as a standard.

2.5. EXTRACTION OF FLAVONOID

Flavonoid were extracted from crushed leaves of *Cichorium intybus* (100 g), according to the method of Zhang et al. (2000). The fraction was completed twice at 20 °C in a shaking incubator. Methanol/acetone/water (3.5:3.5:3, v/v/v) containing 1 % formic acid were used extracting solvents were 100 mL at 30 min. The extract was then filtration through Whatman No.1 filter paper. The filtrates solution were evaporated under vacuum at 40 °C to remove methanol and acetone. Lipophilic colours materials were removed from the aqueous phase by two consecutive extractions in a separator funnel with a twofold volume of petroleum ether. The aqueous phase was finally collected and further extracted three times by ethyl acetate (ethyl acetate: aqueous phase = 1:1, *v/v*) in the separator funnel. The ethyl acetate phases were collected, evaporated and dried under vacuum at 35 °C to obtain polyphenol sample.

2.6. ABTS *(*2,2'-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid) RADICAL SCAVENGING ASSAY

ABTS radical scavenging activity of flavonoid rich fraction from leaves of *Cichorium intybus* was followed by Re et al. (1999). ABTS radical was newly prepared by addition 5 ml of 4.9 mM potassium persulfate solution to 5 ml of 14 mM ABTS solution and kept for 16 h in dark. This solution was diluted with distilled water to produce an absorbance of 0.70 at 734 nm and the same was used for the antioxidant activity. The final solution of standard group was made up to 1 ml with 950 µl of ABTS solution and 50 µl of Ascorbic acid. Correspondingly, in the experiment group, 1 ml reaction mixture encompassed 950 µl of ABTS solution and 50 µl of different concentration of each extracts. The reaction mixture was vortexed for 10 s and after 6 min, absorbance was recorded at 734 nm against distilled water by using a Deep Vision (1371) UV–Vis Spectrophotometer and compared with the control ABTS solution. Ascorbic acid was used as reference antioxidant compound.

ABTS Scavenging Effect (%) = $[(A_0 - A_1/A_0) \times 100]$ Where A_0 is the absorbance of the control reaction and A_1 is the absorbance of flavonoid rich fraction from leaves of *Cichorium intybus*.

2.7. INHIBITION OF LIPID PEROXIDATION ACTIVITY

Lipid peroxidation induced by Fe^{2+}ascarbate system in egg yolk was assessed as thiobarbituric acid reacting substances (TBARS) by the method of Badmus et al. (2010). The experimental mixture contained 0.1 ml of egg yolk (25% w/v) in Tris-HCl buffer (20 mM, pH 7.0); KCl (30 mM); $FeSO_4$ $(NH_4)_2SO_4.7H_2O$ (0.06 mM); and different concentrations of flavonoid rich fraction from leaves of *Cichorium intybus* flower in a final volume of 0.5 ml. The experimental mixture was incubated at 37°C for 1 h. After the incubation period, 0.4 ml was collected and treated with 0.2 ml sodium dodecyl sulphate (SDS) (1.1%); 1.5 ml thiobarbituric acid (TBA) (0.8%); and 1.5 ml acetic acid (20%, pH 3.5). The final volume was made up to 4.0 ml with distilled water and then kept in a water bath at 95 to 100 °C for 1 hour. After cooling, 1.0 ml of distilled water and 5.0 ml of n-butanol and pyridine mixture (15:1 v/v) were added to the reaction mixture, shaken vigorously and centrifuged at 4000 rpm for 10 min. The absorbance of butanol-pyridine layer was recorded at 532 nm in Deep Vision (1371) UV–Vis Spectrophotometer) to quantify TBARS. Inhibition of lipid peroxidation was determined by comparing the optical density (OD) of test sample with control. Ascorbic acid was used as standard.

Inhibition of lipid peroxidation (%) by the each extracts was calculated according to 1-(E/C) × 100, where C is the absorbance value of the fully oxidized control and E is absorbance of the test sample.

2.8. SUPEROXIDE RADICAL SCAVENGING ASSAY

This assay was based on the capacity of the flavonoid rich fraction from leaves of *Cichorium intybus* to inhibit the photochemical reduction of Nitroblue tetrazolium (NBT) in the presence of the riboflavin-light-NBT system (Tripathi and Pandey Ekta, 1999; Tripathi and Sharma, 1999). Each 3 ml reaction solution contained 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 2 µM riboflavin, 100 µM Ethylene diamine tetra acetic acid (EDTA), NBT (75 µM) and different concentration of extracts. It was kept visible in fluorescent light and absorbance was taken after 6 min at 560 nm by using a Deep Vision (1371) UV–Vis Spectrophotometer. Identical tubes with reaction mixture were kept in the dark served as blanks. The percentage inhibition of superoxide radical activity was measured by comparing the absorbance of the control with test sample solution:

$$\% \text{ Super oxide radical scavenging capacity} = [(A_0-A_1)/A_0] \times 100$$

Where A_0 was the absorbance of control and A_1 was the absorbance of flavonoid rich fraction from leaves of *Cichorium intybus*.

2.9. METAL CHELATING ACTIVITY

Metal chelating capacity of flavonoid rich fraction from leaves of *Cichorium intybus* was measured according to Dinis et al., (1994). 1 ml of different concentrations of flavonoid rich fraction was added to 0.05 ml of 2 mM ferric chloride solution. The reaction was initiated by the addition of 0.2 ml of 5 mM Ferrozine and the mixture was shaken vigorously. After 10 min, the absorbance was measured at 562 nm against blank. All readings were taken in triplicate and ascorbic acid was used as standard. The % inhibition of ferrozoine-Fe2+complex was calculated by following equation.

$$\% \text{ Inhibition of ferrozoine-Fe2+complex} = [(A_0-A_1)/A_0] \times 100$$

Where A_0 was the absorbance of control and A_1 was the absorbance of polyphenol rich fraction form flowers of *C. occidentalis*.

2.10. GLUCOSE UPTAKE IN YEAST CELLS

Glucose uptake assay by yeast cells was performed according to Cirillo et al. (1963). The yeast cell suspended in distilled water was subjected to repeated centrifugation (3000 × *g*, 5 min) until clear supernatant fluids were obtained and 10% (v/v) of the suspension was prepared in distilled water. Various concentrations of flavonoid rich fraction from leaves of *Cichorium intybus* (25 to 100 µg/ml) were added to 1 ml of glucose solution (20 mM) and incubated together for 10 min at 37 °C. Reaction was started by adding 100 µl of yeast suspension followed by vortexing and further incubation at 37 °C for 60 min. After 60 min, the tubes were centrifuged (2500 × *g*, 5 min) and amount of glucose was estimated in the supernatant. Glycomet was used as standard drug. The percentage increase in glucose uptake by yeast cells was calculated using the following formula:

Increase in glucose uptake%=Abs sample-Abs control/Abs sample×100

Where, Abs sample is the absorbance of test sample and Abs control is the absorbance of control reaction (containing all reagents except the test sample). All the experiments were carried out in triplicates.

2.11. INHIBITION OF ALPHA-AMYLASE

Inhibition of alpha-amylase method followed by Narkhede *et al.*, (2011). In this assay, added 390 µl of 0.02 M phosphate buffer (pH 7), positive control (acarbose), different concentrations of flavonoid rich fraction from leaves of *Cichorium intybus* and 10 µl of α-amylase enzyme were mixed and incubated at 37°C for 10 min. Added 10 µl of starch to this mixture and again incubated 37°C for 1 h (Megha et al., 2013). After incubation, added 0.1 ml 1% iodine

solution and 5 ml of distilled water and optical density was measured at 565 nm. Inhibition of enzyme activity was calculated as follows:

$$\text{Percentage inhibition} = (A - C) \times 100 / (B - C)$$

Where, A=Absorbance of the sample, B=Absorbance of blank (without α-amylase), and C=Absorbance of control (without starch).

2.12. INHIBITION OF ALPHA-GLUCOSIDASE

The inhibitory activity of α-glucosidase method was followed by. The first step carried out substrate of starch solution (2% w/v maltose or sucrose, 1 mL) with Tris buffer (0.2 M, pH 8) and various concentrations of flavonoid rich fraction from leaves of *Cichorium intybus* for 5 min at 37°C. The reaction was initiated by adding α-glucosidase enzyme (1 mL of 1 U/mL yeast α-glucosidase) to the reaction mixture, followed by incubation for 10 min at 37°C. The reaction was terminated by heating the contents in a boiling water bath. 3,5-dinitrosalicylic acid (1 mL) was added with the product before being incubated for 5 min and added with distilled water (9 mL). The amount of liberated glucose was measured by glucose oxidase peroxidase method.

3.13. STATISTICAL ANALYSIS

All tests were completed in triplicates ($n = 3$) and the data are accessible as the mean ± standard error. Differences between the means of the individual groups were analyzed using the analysis of variance procedure of SPSS software Version 20 (IBM). The significance of differences was defined at the $p < 0.05$ and $p < 0.01$ level.

3. RESULT AND DISCUSSION

3.1. PHYTOCHEMICAL SCREENING

The phytochemical screening of aqueous extract of *Cichorium intybus* studied presently showed the presence of alkaloids, flavonoids, phenol, Terpenoids, glycosides and saponin, and absence of glycosides and tannin (Table -1).

Table-1. Phytochemical screenings from the leaves of *Cichorium intybus*

Sl. No.	Phytochemical Constituents	Observation	Aqueous extract of *Cichorium intybus*
1	**Alkaloids** -Dragendorff's test -Mayers test	Orange / red precipitate Cream precipitate	+ +
2.	**Flavonoids** -Alkalai Reagent -Lead aceate test	Intense yellow colour Precipitate formed	+ +
3.	**Glycosides** -Keller-Killiani test	Pink colour (Ammonia layers)	+
4.	**Tannin** -$FeCl_3$ test	Blue-black colour	+
5.	**Saponins** -Frothing test	Foam	-
6.	**Terpenoids** -Salkowski test	Reddish brown colour ring formed in interface	-
7.	**Polyphenols** -Ferrozine test	Raddish blue	+
8.	**Anthocyanin** **-Ammonia test**	Pink color in ammonia layer	+

+ Positive result; - Negative result

3.2. TOTAL PHENOLIC AND FLAVONOID CONTENT FROM THE AQUEOUS EXTRACT OF *CICHORIUM INTYBUS*

In this context, the preliminary experiments revealed that from the decoction of cumin seed, flax seed and ragi at 60 °C for 60 min since it afforded a maximum yield of phenolics. The yields dry plant from the decoction of cumin seed, flax seed and ragi ranged 65.31 % (w/w) and respectively. Therefore, the total phenolic and flavonoid contents were reported as catechin and rutin equivalents respectively (Table-2).

Table-2. Yield and total phenolic and flavonoid contents of from the aqueous extract of *Cichorium intybus*

Sample	Yield of extract (g/100 g of defatted Content)	Total phenolic content (µg catechin equivalents per gram.	Total flavonoid content (µg rutin equivalents per gram.
Aqueous extract of *Cichorium ontybus*	65.31±1.89[a]	51.23±2.32[b]	43.64±1.24[b]

[a]Data are expressed as mean ± standard deviation (n = 3) on a fresh weight basis.

[b]Means in each column sharing the same letter are not significantly (P = 0.05) different from other.

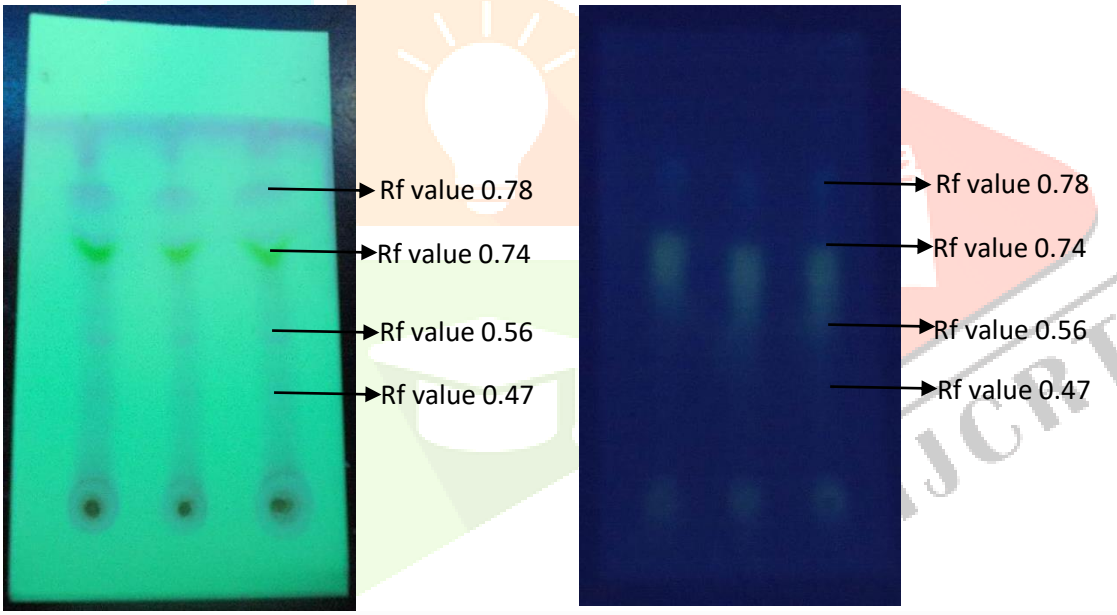
3.3. THE PARTIAL CHARACTERIZATION OF AQUEOUS EXTRACT OF *CICHORIUM INTYBUS* BY TLC

The aqueous decoction partition with decoction of cumin seed, flax seed and ragi loaded on Pre-coated TLC plates (60 F_2 54 Merck) and developed with a solvent system of petroleum ether, chloroform and methanol in the ratio of 1:0.5:0.1 were efficient to extract the antidiabetic, antioxidant and anti-inflammatory compound it is used for further studies. The developed plate was viewed under UV 240nm and 360nm (Table-3).

Table-3. Partial characterization of flavonoid rich fraction from leaves of *Cichorium intybus* TLC

S.No	Flavonoid rich fraction from leaves of *Cichorium intybus*		
	UV 240 nm Rf value	**UV 360 nm Rf value**	**Visible Rf value**
1	0.78	0.82	-
2	0.74	0.71	-
3	0.62	0.62	-
4	0.42	-	-

Fig-1. Partial characterization of flavonoid rich fraction from leaves of *Cichorium intybus* by TLC



TLC plate Viewed UV Light at 240 nm

TLC plate Viewed UV Light at 360 nm

3.4. GLUCOSE UPTAKE IN YEAST CELLS

Different concentrations of flavonoid rich fraction from leaves of *Cichorium intybus* are subjected to *in vitro* glucose uptake assay employing yeast as model. The percentage of glucose uptake in yeast cells by the extract was compared with standard drug glycomet. There was concentration dependent increase in percentage of glucose uptake with increasing in concentration of flavonoid rich fraction from leaves of *Cichorium intybus* (Table-4). Flavonoid rich fraction from leaves of *Cichorium intybus* exhibited highest percentage of glucose uptake *i.e.* 72.34 ± 0.78, which was almost near to the standard *i.e.* 68.31 ± 1.56 at 100 µg concentration. Results also indicated that flavonoid rich fraction from leaves of *Cichorium intybus* had almost same efficiency in increasing the glucose uptake by yeast cells as compared to standard drug

metronidazole. Type II diabetes characterized by deficiency of insulin causing increased level in blood glucose level and it depends on the uptake of glucose by the cells (Shori, 2015). In the present study, flavonoid rich fraction from leaves of *Cichorium intybus* were subjected to *in vitro* anti-diabetic assay by means of yeast as model.

Table-4. Glucose uptake in yeast cells of flavonoid rich fraction from leaves of *Cichorium intybus*

a

Different concentration of extract	**Percentage of Glucose uptake in yeast cells activity**	
	Flavonoid rich fraction from leaves of *Cichorium intybus*	**Standard Glycomet**
25 µl/ml	19.63 ± 0.78	16.32 ± 2.46
50 µl/ml	35.21 ± 1.89	33.32± 1.89
75 µl/ml	53.64 ± 2.78	51.34 ±2.78
100 µl/ml	72.34 ± 1.48	68.31 ± 1.56
EC_{50} value	63.31	66.31

Results are expressed as percentage of Glucose uptake in yeast cells activity with respect to control. Each value represents the mean+SD of three experiments.

3.5. INHIBITION OF α- AMYLASE

Alpha amylase is an enzyme that hydrolyses alpha-bonds of large alpha linked polysaccharide such as glycogen and starch to yield glucose and maltose. Alpha amylase inhibitors bind to alpha- bond of polysaccharide and prevent break down of polysaccharide in to mono and disaccharide. The present experimental study it was observed that flavonoid rich fraction from leaves of *Cichorium intybus* demonstrated polyphenol compounds significant Alpha amylase inhibition activity as compared to standard drug Glycomet (Table-5).

Table-5. α- amylase inhibition of flavonoid rich fraction from leaves of *Cichorium intybus*

Different concentration of extract	Inhibition Percentage of α- amylase	
	Flavonoid rich fraction from leaves of *Cichorium intybus*	**Standard Glycomet**
25 µl/ml	18.36 ± 2.36	16.34 ± 1.64
50 µl/ml	30.24 ± 1.89	27.34 ± 2.78
75 µl/ml	48.34 ± 2.14	46.34 ± 1.89
100 µl/ml	67.35± 1.78	65.34± 2.46
EC_{50} value	72.36	77.36

[a] **Results are expressed as percentage of α- amylase inhibition activity with respect to control. Each value represents the mean+SD of three experiments.**

3.6. α-GLUCOSIDASE INHIBITORY ACTIVITY

The results of *in-vitro* α-glucosidase inhibitory study are showed in Table-11. The herbal tea formulated equal amount of flavonoid rich fraction from leaves of *Cichorium intybus* showed a concentration-dependent inhibition of enzyme. The highest concentration of 100 µg/ml tested showed a maximum inhibition of nearly 72.13% herbal tea formulated equal amount of flavonoid rich fraction from leaves of *Cichorium intybus* seems to be less potent in α-glucosidase inhibitory potential compared to Glycomet (68.34%). It may be that α-glucosidase is more sensitive towards glycomet with the concentration required for 50% inhibition (EC_{50}) found to be 65.34 µl/ml (Table-6). Inhibitors of *α*-glucosidase are used to control the blood sugar levels for type 2 diabetes mellitus. Usually, *α*-glucosidase inhibitors are consumed with meals as they act to decelerate the breakdown of complex sugars into glucose resulting in a delay in glucose absorption which lowers postprandial blood sugar levels.

Table-6. α-Glucosidase inhibitory activity of flavonoid rich fraction from leaves of *Cichorium intybus*

Different concentration of extract	Inhibition Percentage of β-Glucosidase	
	Flavonoid rich fraction of *Cichorium intybus*	Standard Glycomet
25 µl/ml	20.31 ± 1.73	17.34 ± 1.34
50 µl/ml	33.64 ± 0.89	29.34 ± 0.78
75 µl/ml	53.67 ± 2.78	50.34 ± 1.78
100 µl/ml	72.13 ± 1.56	68.34 ± 2.19
EC_{50} value	65.34	71.34

[a] Results are expressed as percentage of β-Glucosidase in yeast cells activity with respect to control. Each value represents the mean+SD of three experiments.

3.7. FREE RADICAL-SCAVENGING ABILITY USING ABTS ASSAY

The radical scavenging ability was measured by ABTS assay as per given in table 3. The inhibition percentage of the ABTS radical activity was assessed on average and high free radical-scavenging values were found in flavonoid rich fraction from leaves of *Cichorium intybus.* In ABTS assay, inhibition percentage was high in flavonoid rich fraction from leaves of *Cichorium intybus* 63.21% with EC_{50} value 70.23µl/ml. The pure ascorbic acid was lower activity 61.23 with EC_{50} value 81.34µl/ml (Table-7). Nevertheless, in present study, it is showed that these activities were mainly due to anthocyanin and flavonoids compounds. It is known that vitamin C (ascorbic acid) and carotenoids are chief source of discrepancy of antioxidant/ antiradical activities in plant materials (Villano et al., 2007).

Table-7. Free radical-scavenging ability using ABTS assay of flavonoid rich fraction from leaves of *Cichorium intybus*

Different concentration of extract	Percentage of ABTS radical activity	
	Flavonoid rich fraction from leaves of *Cichorium intybus*	Standard Vitamin-C
25 µl/ml	16.34 ± 2.36	15.34 ± 1.87
50 µl/ml	28.34 ± 1.89	26.34 ± 2.13
75 µl/ml	46.32 ± 2.36	43.32 ± 1.89
100 µl/ml	63.21 ± 1.45	61.23 ± 0.89
EC_{50} value	73.69	81.34

[a]Results are expressed as percentage inhibit of ABTS ability with respect to control. Each value represents the mean+SD of three experiments

3.8. INHIBITION OF LIPID PEROXIDATION

Flavonoid rich fraction from leaves of *Cichorium intybus* also inhibited the lipid peroxidation induced by ferrous sulfate in egg yolk homogenates. Maximum inhibition was recorded in flavonoid rich fraction from leaves of *Cichorium intybus* 73.32% with EC_{50} value 65.32µl/ml and lowest inhibition percentage ascorbic acid 70.23% with EC_{50} 69.34 µl/ml (Table-8). As it is identified that lipid peroxidation is the net result of any free radical attack on membrane and other lipid components present in the system, the lipid peroxidation may be enzymatic (Fe/NADPH) or non-enzymatic (Fe/ascorbic acid). In the present study, egg yolk was used as substrate for free radical mediated lipid peroxidation, which is a non-enzymatic method. Normally, the mechanism of phenolic compounds for antioxidant activity includes neutralizing lipid free radicals and preventing decomposition of hydroperoxides into free radicals.

Table-8. Inhibition of lipid peroxidation activity of flavonoid rich fraction from leaves of *Cichorium intybus*

Different concentration of extract	Lipid peroxidation inhibition percentage	
	Flavonoid rich fraction from leaves of *Cichorium intybus*	Standard Vitamin-C
25 µl/ml	18.65 ± 2.46	16.32 ± 1.48
50 µl/ml	38.32 ± 0.89	35.64± 0.89
75 µl/ml	54.32 ± 1.89	51.23 ± 2.45
100 µl/ml	73.32 ± 2.56	70.23 ± 1.45
EC_{50} value	65.32	69.34

[a] Results are expressed as percentage inhibit of lipid peroxidation with respect to control. Each value represents the mean+SD of three experiments.

3.9. SUPEROXIDE SCAVENGING ASSAY ACTIVITY

Flavonoid rich fraction from leaves of *Cichorium intybus* exhibited powerful scavenging activity for superoxide radicals in a concentration dependent process than positive control. Flavonoid rich fraction from leaves of Cichorium intybus showed highest radical activity in the percentage of 65.32% with EC_{50} value 70.21 µl/ml when compared to positive control 61.23% with EC_{50} Value 77.65 µl/ml (Table-9). One of the standard methods to produce Superoxide radicals is through photochemical reduction of nitro blue tetrazolium (NBT) in the presence of a riboflavin-light-NBT system. These superoxide radicals are extremely toxic and may be produced either through xanthine activity or through mitochondrial reaction. Superoxide radicals are reasonably a weak oxidant may decompose to form stronger reactive oxidative species, such as singlet oxygen and hydroxyl radicals (Khattab et al., 2015).

Table-9. Superoxide scavenging assay activity of flavonoid rich fraction from leaves of *Cichorium intybus*

Different concentration of extract	Percentage of Superoxide scavenging activity	
	Flavonoid rich fraction from leaves of *Cichorium intybus*	Standard Vitamin-C
25 µl/ml	15.23 ± 1.46	14.23± 0.78
50 µl/ml	27.32 ± 0.89	25.34± 2.36
75 µl/ml	48.67 ± 2.15	45.32 ± 1.78
100 µl/ml	65.32 ± 1.78	61.23 ± 0.28
EC_{50} value	70.21	77.65

[a] Results are expressed as percentage of Superoxide scavenging activity with respect to control. Each value represents the mean+SD of three experiments.

3.10. METAL CHELATING ACTIVITY

The metal chelating property of decoction from cumin seed, flax seed and ragi was displayed as per Table-10. The formulated decoction from cumin seed, flax seed and ragi was evaluated for their ability to compete with ferrozine for ferrous iron in the solution. In this evaluation, the flavonoid rich fraction from leaves of *Cichorium intybus* hindered the formation of ferrous and ferrozine complex, signifying that they have chelating activity and are capable of capturing ferrous iron before ferrozine. Flavonoid rich fraction from leaves of *Cichorium intybus* reduced the greenish blue color complex immediately and showed the highest chelating activity 68.90% With EC_{50} Value 78.65 µl/ml than positive control Vitamin-C 65.67% with EC_{50} value 81.56 µl/ml.

Table-10. Metal chelating activity of flavonoid rich fraction from leaves of *Cichorium intybus*

Different concentration of extract	Percentage of Metal chelating activity	
	Flavonoid rich fraction from leaves of *Cichorium intybus*	Standard Vitamin-C
25 µl/ml	16.34 ± 1.54	14.89± 2.56
50 µl/ml	32.89 ± 1.67	29.78 ± 0.67
75 µl/ml	53.67 ± 0.45	51.76 ± 2.13
100 µl/ml	68.90 ± 1.65	65.67 ± 1.89
EC_{50} value	78.65	81.56

[a]Results are expressed as percentage of Metal chelating activity with respect to control. Each value represents the mean+SD of three experiments.

CONCLUSION

The current exploration presented that the flavonoid rich fraction from leaves of *Cichorium intybus* possesses significant invitro hypoglycemic and antioxidant activity. Hereafter, the synergistic combination of hypoglycemic activity and antioxidant activity may prove to be very effective in the management of diabetes and the associated oxidative stress. The quantitative estimation of total polyphenolics and flavonoids confirmed that the leaves of *Cichorium intybus* contain substantial quantity of polyphenolics and flavonoids which are the known antioxidant from plant sources. So, the activity may be due to the presence of substantial quantities of flavonoid compounds in the extract.

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