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In vitro Antioxidant Activity and Phytochemical Screening of the Methanolic Extract of *Cichorium intybus*

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ABSTRACT

Oxidants or reactive oxygen species are formed in our carcass at some stage in aerobic metabolism foremost to numerous diseases like as cancer, cardiovascular diseases and so forth. The chemicals that counterbalance these oxidants are Antioxidants. Natural antioxidants are the inferior metabolites of phytochemicals moreover are ideal over synthetic antioxidants, which are originate to inflict side possessions. Plants are a foundation of bulky quantity of drugs comprising to dissimilar groups such as anti-cancer, antimicrobials, antioxidants etc. A huge amount of the vegetation are claimed to acquire the antioxidant properties in the habitual system as well as are also used extensively by the ethnic people universal. It is now alleged that nature has given the cure of ailment in one way or another. This study has been done on *Cichorium intybus* for the achieve of oxidants and their neutralization by natural antioxidants. Methanolic extract of *Cichorium intybus* was investigated for its Free radical scavenging property by different *in-vitro* models i.e. 1,1-diphenyl-2 picryl hydrazyl (DPPH) and reducing power. The methanol extract initiate to have additional free radical scavenging activity in the midst of all extracts. Present study has also evaluated for phytochemical screening of methanolic extract.

Key words: Antioxidants, Free radicals, DPPH, *Cichorium intybus* and Phytochemical Screening.

1. INTRODUCTION

In recent times, Plant-derived substances has develop into of enormous interest due to their multitalented applications. Medicinal vegetation are the richest bio-resource of drugs of conventional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates along with chemical entities for synthetic drugs [1].

Phytochemicals are the chemicals extracted as of plants. These chemicals are classify as primary or else secondary constituents, depending on their role in plant metabolism. Primary constituents consist of the general sugars, amino acids, proteins, purines as well as pyrimidines of nucleic acids, chlorophyll's and all that. Secondary constituents are the outstanding plant chemicals consist of alkaloids i.e. imitative from amino acids, terpenes i.e. group of lipids furthermore phenolics which are derived from carbohydrates [2].

Antioxidants are secondary constituents or metabolites which are initiate in nature in the body along with in plants like as fruits plus vegetables. An antioxidant be capable of defined in effortless provisions as anything so as to inhibits or prevents oxidation of a vulnerable substrate. Plant life fabricate a very striking array of antioxidant compounds that comprises of carotenoids, flavonoids, cinnamic acids, benzoic acids, folic acid, ascorbic acid, tocopherols furthermore tocotrienols to thwart oxidation of the disposed substrate [3]. Widespread antioxidants take account of vitamin A, vitamin C, vitamin E, moreover certain compounds called carotenoids (like lutein and beta-carotene) [4]. These plant-based dietary antioxidants are thought to have an chief role in the continuation of human health for the reason that our endogenous antioxidants endow with insufficient protection beside the steady and inevitable challenge of reactive oxygen species (ROS; oxidants) [5].

Invention of free radicals or reactive oxygen species (ROS) through metabolism and erstwhile activities further than the antioxidant capacity of a natural system gives rise to oxidative stress [6]. Oxidative stress plays a function in heart diseases, malaria, neurodegenerative diseases, AIDS and cancer [7]. This conception is supported by escalating indication that the oxidative damage plays a significant role in the improvement of chronic, age-related degenerative diseases, as well as that dietary antioxidants combat this also subordinate the risk of disease [8,9] and therefore arises a inevitability to extract these antioxidants

from the plant matrix. In the recent study the extraction technique such as Soxhlet extraction have been used to segregate antioxidants from the plants. Therefore, the present study designed to regard the free radical scavenging behavior of the methanolic extract of *Cichorium intybus,* which is a perennial herb of the Asteraceae family, with blue, lavender, or intermittently white flowers, is also known as blue sailors, endive, succory, and coffeeweed, Kashen'na or Kasini [10] and is native to the Mediterranean region, mid Asia and northern Africa. In olden times, chicory was grown by the ancient Egyptians as a medicinal plant, coffee substitute, vegetable crop, and occasionally for animal forage [11]. Further, it was screened for the presence of phyto-chemicals viz. alkaloids, flavonoids, tannins, saponins and terpenoids.

2. MATERIALS AND METHODS

2.1. Collection

Authentic samples: Various market samples of *Cichorium intybus* were procured from Chunnilal Attar Ayurvedic Store, Ghat Gate, Jaipur in the month of March, 2010.

2.2. Identification

All the samples were authenticated and were given identification number. The identification was as follows:

These samples were authenticated and submitted in Ethnomedicinal Herbarium, Centre of Excellence funded by DST, MGiaS, Jaipur (Rajasthan).

2.3. Processing of plant materials

During the course of the study each sample was screened for its foreign matter and milled, before use.

2.4. EXPERIMENTAL

Present studies were performed on *Cichorium intybus* for the following studies-.

1. Phytochemical test of plant extract
2. Antioxidant Potentials of Methanolic extract of plant

2.4.1. Phytochemical Screening

Phytochemical screening was performed using standard procedure:

2.4.1.1. Test for Reducing Sugars (FEHLINGS TEST)

The aqueous ethanol extract (0.5gm in 5 ml of water) was added to boiling fehling's solution (A and B) in a test tube. The solution was observed for a colour reaction.

2.4.1.2. Test For Terpenoides (SALKOWSKI TEST)

To 0.5 gm each of the extract was added to 2ml of chloroform. Concentrated sulphuric acid (3ml) was carefully added to form a layer. Reddish brown coloration of the interface indicates the presence of terpenoides.

2.4.1.3. Test For Flavonoides

4ml of extract solution was treated with 1.5ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated Hydrochloride acid was added and red colour was observed for flavonoids and orange color for flavons.

2.4.1.4. Test For Tannins

About 0.5 g of the extract was boiled in 10ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

2.4.1.5. Test For Saponins

To 0.5 g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously. And observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

2.4.1.6. Test For Alkaloids

Alkaloids solutions produce white yellowish precipitate when a few drops of Mayer's reagents are added. Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's regent.

The alcoholic extract was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of mayer's reagent. The sample was then observed for the turbidity or yellow precipitation.

2.4.2. ANTIOXIDANT ACTIVITY

2.4.2.1. Preparation of test extracts

All the test plant sample and their adulterants were milled and refluxed in ethanol for 36 h, filtered, concentrated to dryness *in vacuo*. A portion of ethanolic extract was further successively extracted in pet. ether, benzene, chloroform, alcohol and water, concentrated and stored at minimum temperature, until used.

2.4.2.2. Preparation of DPPH

DPPH (2, 2'-diphenyl-1-picrylhydrazl, $C_{18}H_{12}N_5O_6$; Hi media) 0.8 mg was dissolved in 10 ml methanol to obtain a concentration of 0.08

mg/ml for antioxidative (qualitative and quantitative) assay.

2.4.2.3. Qualitative Assay

Each successive extract (10 mg) was dissolved in 10 ml of its suitable solvent to get a concentration of 1 mg/ml and from this, 0.25µl was taken with the help of micropipette, applied on silica gel G coated plates. These circular spots were sprayed with DPPH solution, allowed to stand for 30 min. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced, and the changes in colour (from deep- violet to light- yellow on white) were recorded at 517 nm on a UV spectrophotometer (Varian Cary PCB 150, Water Peltier System).

2.4.2.4. Quantitative Assay

A concentration of 1 mg/ml of ethanolic extract of each test sample was prepared to obtain different concentrations (10^2µg to 10^{-3} µg/ ml). Each diluted solution (2.5 ml each) was mixed with DPPH (2.5ml). The samples were kept in the dark for 15 min at room temperature and then the decrease in absorption was measured. Absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured. The UV absorbance was recorded at 517 nm. The experiment was done in triplicate and the average absorption was noted for each concentration. Data were processed using EXCEL and concentration that cause 50% reduction in absorbance (RC_{50}) was calculated. The same procedure was also followed for the standards- quercetin and ascorbic acid.

3. RESULTS AND DISCUSSION

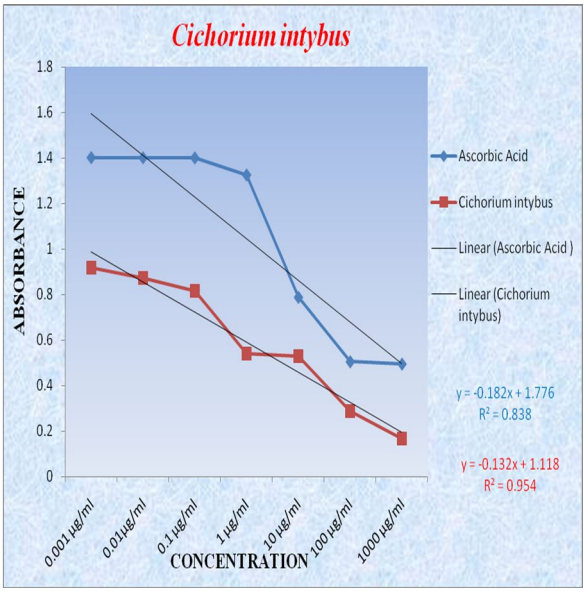
In the present investigation it was showed that the maximum optical density comes out to be 0.917 nm which is at the concentration 10^{-3} µg/ml and the smallest optical density is 0.168 nm which is at the concentration 10^3 µg/ml where as the other shows comparable O.D at different concentrations i.e. 0.872 nm at 10^{-2} µg/ml, 0.814 nm at 10^{-1}µg/ml, 0.541 nm at 1 µg/ml, 0.530 nm at 10^1 µg/ml, 0.289 nm at 10^2 µg/ml (table-1.)

In the present investigations antioxidant activity of *Cichorium intybus* showed appreciable activity against the DPPH assay method where the regression line clear cut showed the effectiveness of it as it's have potentials which are comparable to ascorbic acid. The antioxidant activity of *Cichorium intybus* in methanolic extract using DPPH assay method (Tahao, 1994) shows appreciable activity comparable to standard ascorbic acid. The straight line showed Y= -0.182x+1.776 & regression = 0.838 whereas, in above drug the straight line is Y= -0.132x+1.118 & regression = 0.954 (Fig-1.)

Table -1: Showing Optical density of *Cichorium intybus* on different concentrations.

CONCENTRATION (µg/ml)	O.D (nm)
0.001	0.917
0.01	0.872
0.1	0.814
1	0.541
10	0.530
100	0.289
1000	0.168

Fig -1: Graph showing Antioxidant Activity of *Cichorium intybus* at different concentration.



The phytochemical screening of *Cichorium intybus* shows the occurrence of alkaloids, flavonoids and tannin whereas it shows the absence of saponin respectively. The screening of the *Cichorium intybus* shows only a small amount of differences in the constituents of the medicinal plants. This drug shows the authentication of broad-shouldered antioxidant activity more or in a less important amount. The presence of alkaloids, flavonoids and saponin in this plant is convincing to be conscientious for the free radical scavenging effects observed. (Table-2.)

Table -2: Showing phytochemical screening results of *Cichorium intybus.*

Cichorium intybus	
TEST	Observations
Reducing Sugar	-
Saponin	-
Tannin	+
Terpenoides	-
Flavonoides	+
Alkaloides	+

4. CONCLUSION

Reactive oxygen species (ROS) or oxidants produced in our body owing to exogenous in addition to endogenous factor are establish to be conscientious for various diseases. Day to day follow a line of investigation is enlightening the potential of phytochemical antioxidants as health benefactors. This is owed to their capability to counterbalance the free radicals or reactive oxygen species or oxidants dependable for the onset of cell damage. Synthetic antioxidants are bring into being to be hurtful to the health. For the most part of the natural antioxidants from plant source are safer to health and enclose enhanced antioxidant activity. Various processes of extraction are used such as Soxhlet extraction for antioxidants from plant matrix. In vitro antioxidant study results of DPPH and reducing power method has cleared that all extracts possesses significant antioxidant properties and be correlated to this property. Phytochemical investigations clears that *Cichorium intybus* contain saponins, flavonoids and alkaloids.

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5. REFERENCES

1. Ncube NS, Afolayan AJ and Okoh AI. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. African Journal of Biotechnology., 2008; 7 (12): 1797-1806.
2. Walton JR. Varietal Innovation and the Competitiveness of the British Cereals Sector, 1760-1930, Agricultural History Review., 1999; 47(1): 29-57.
3. Hollman PCH. Evidence for health effects of plant phenols: local or systemic effects?. J. Sci. Food Agric., 2001; 81: 842–852.
4. Hayek MG. Dietary vitamin E improves immune function in cats. In Reinhart G. A and Carey D. P. Eds. Recent Advances in Canine and Feline Nutrition, Iams Nutrition Symposium Proceedings. Wilmington, OH, Orange Frazer Press., 2000; 3: 555-564.
5. Fridovich I. Oxygen toxicity, a radical explanation, The J. Experimental Biol., 1998; 201: 1203–1209.
6. Mikulikova L and Popov P. Oxidative stress, metabolism of ethanol and alcohol-related diseases, J. Biomed. Sci., 2001; 8: 59–70.
7. Sian BA. Dietary antioxidants—past, present and future?, Trends in Food Sci.Technol., 2003; 14: 93-98.
8. Atoui AK, Mansouri A, Boskou G and Panagiotis K. Tea and herbal infusions Their antioxidant activity and phenolic profile, Food Chem., 2005; 89: 27-36.
9. Alasalvar CM, Al-Farsi PC, Quantick F, Shahidi R and Wiktorowic Z. Effect of chill storage and modified atmosphere packaging (MAP) on antioxidant activity, anthocyanins, carotenoids, phenolics and sensory quality of ready-to-eat shredded orange and purple carrots, Food Chem., 2005; 89: 69–76.
10. Uigur-Pharmacopoeia-Compile-Committee. Uigur pharmacopoeia (Book I, 2nd edition) Xinjiang Science and Technology Sanitation Book Concern, Urumchi, China.1999.
11. Munoz CLM. Spanish medicinal Plants: *Cichorium intybus* L. Boletin de la Real Sociedad Espanola de Historia Natural, 2004; 99: 41-47.