Original Article

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Antioxidant and Antibacterial Properties of Chicory (*Cichorium intybus* L.) Root and Leaves Extract- An Experimental Study

Nutrition Section

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ABSTRACT

Introduction: *Cichorium intybus (C. intybus)* is a scientific term used for chicory plant. The medicinal plants have immensely contributed to health needs of humans throughout their existence.

Aim: To study the phytochemical constituents, antioxidant and antibacterial properties of the methanolic root and leaf extract of *C. intybus*.

Materials and Methods: This experimental study was conducted at Sharda University, Noida, Uttar Pradesh, India from January 2022 to March 2022. Phytochemical, antimicrobial and antioxidant activities of methenolic extract of *C. intybus* both leaves and roots were assessed using different methods. Antibacterial activity was done using Well Diffusion Method against *Staphylococcus aureus, Pseudomonas aeruginosa, E. coli* and *Salmonella* Typhimurium.

Results: The results for antioxidant activity was found better in leaves of *C. intybus* when compared with its root. The IC50 value for the leaves was found to be 63.8±1.4 µg/mL whereas root showed 76.1±1.2 µg/mL. Further, the samples were tested for antibacterial activity against both gram positive and gram negative bacteria using Well-diffusion method against given microorganism *Staphylococcus aureus* (MTCC 87), *Pseudomonas aeruginosa* (MTCC 424), *E. coli* (MTCC 68) and *Salmonella* Typhimurium (MTCC 68). With zone of inhibition 25.24±0.34 mm, 18.78±1.12 mm, 20.1±0.4 mm and 24.8±0.5 mm respectively, while on comparison with standard drug.

Conclusion: The methanolic extract of both roots and leaves have good antioxidant and antibacterial properties, thus, will help to protect against many diseases and will enhance the immune system for maintaining good health.

Keywords: Immune system, Medicinal plant, Nutrition

INTRODUCTION

Natural products assumed to be a distinct part in old traditional medicine systems such as Chinese, Ayurveda, Unani and Egyptian and are being used even today. The World Health Organisation (WHO) reported that 75% of the world population still depends on plant-based traditional medications for primary healthcare [1]. Nature has been a source of therapeutic agents for thousands of years, and a large number of modern important medications have originally been obtained from natural sources. *Cichorium intybus* can be used for medicinal purposes and it has blue and white flowers that can be easily grown anywhere. The plant grows under the wide range of cultivation conditions, in North West India (Punjab, Kashmir, Andhra Pradesh, Karnataka, Gujarat and Maharashtra), Baluchistan, Belgium, France, Germany, Persia, Netherlands, Switzerland, South Africa, Waziristan, West Asia, and the United Kingdom [2]. The medicinal plants have immensely contributed to health needs of humans throughout their existence. Microorganisms cause a number of deleterious diseases like *Mycobacterium tuberculosis, Candida auris, Streptococcus pneumoniae*, in human being. Synthetic drugs and antibiotics do not completely cure these diseases because the microorganisms develop resistance against these compounds. Therefore, work is going on the extraction of anti-inflectional compounds including bioactive from natural sources like plants and animals. The antimicrobial compounds show broad-spectrum bioactivity against infection causing agents such as fungi, bacteria, protozoon, viruses and yeasts [3].

C. intybus best known for the use of its roots as coffee substitute or additives to coffee as it provides bitterness in taste without having any caffeine [4]. However, its leaves, flowers and roots have been customarily utilised as homegrown solution for various ailments since ancient times. Although commercialised as coffee substitute, *C. intybus* is also used in indigenous system of medicine to treat different ailments from wounds to diabetes [4]. Roots of the plant have an important role in food industries and in formulation of herbal medicine. The plant has also been used as vegetables for human consumption. Both roots and leaves are used for many years as a good nutritional source while, as coffee substitutes, only roots are used [5].

Different studies have showed many therapeutic components in chicory plant such as caffeic acid, quercetin, flavonols and inulin which helps to prevent from the diseases [6,7]. It has been shown to have antidiabetic [8,9], anti-inflammatory, antioxidant and antihepatotoxic activities [10,11]. Several beneficial chemical components like inulin, flavonoids, lactones, vitamins and minerals have been identified in chicory [12] and a significant number of these constituents have not been fully investigated for their pharmacological potential. The present research paper targets on analysis of antioxidant and phytochemical activity of chicory roots. Medicinal plant extracts and its products, exhibit a good antioxidant activity, which is used to treat severe illness like liver diseases. Herbal plants have been used from ancient times for the prevent and cure of life style disease like diabetes, hyperlipidaemia to other complicated diseases like cancer as well. Phytochemical, also known as phytonutrient, is an important plant derived compound, plays an essential role in various important body functions. Plants contain various potential natural compounds that possess antioxidant ability due to the presence of hydroxyl compounds.

These antioxidants are classified in two groups: natural or primary antioxidants and synthetic or secondary antioxidants. Primary antioxidants are mostly phenolic compounds, and include, polyphenols, vitamins and minerals. Secondary antioxidants include, Butylated Hydroxyl Toluene (BHT), Propyl Gallate (PG), Butylated Hydroxyl anisole (BHA), Tertiary Butyl Hydroquinone (TBH) etc. [13]. These antioxidants, protects the body from free radical damage produced by oxidative reactions from lipid, carbohydrates

and protein metabolism. In addition to this, the antioxidants, also enhances the stability of food products, and prevent rancidity. Although, secondary antioxidants are used as food additives in food industry however, their use needs to be reassessed due to the presence of toxic compounds, after degradation. For that purpose, the need of more natural antioxidants is emerging in food market, with advancement of new technologies and innovative products. Natural antioxidants like bioflavonoids, vitamins, phenolic acid and tocopherols, are used to treat various ailments [14]. Thus, the aim of the present study was to determine the antioxidant and antibacterial properties of *C. intybus* leaves and root.

MATERIALS AND METHODS

The present study was an experimental study conducted at Sharda University, Noida, Uttar Pradesh, India from January 2022 to March 2022.

Extract Preparation

Roots and leaves of *C. intybus* were dried and grinded to fine powder and stored at room temperature. About 100 gm of powdered material were taken for Soxhlet extraction process by using methanol as solvent at 50-70°C for about 7-8 hours. After this, the solvent was allowed to evaporate at room temperature and supernatant was stored at 4°C for further analysis.

Analysis of Phytochemicals

Phytochemicals screening was done quantitively using standard procedures given by Sofowora A [15]. Methanolic extract of both root and leaves were identified for different phytochemicals like phenolic, alkaloids, phytosterols, tannins, flavonoids, proteins, terpenoids and glycosides etc., as reported by Shad MA et al., [16].

Tannins

About 2 mL of extract of both root and leaves were boiled in distilled water and filtration was done using waterman filter paper. In the filtrate 2-3 drops of ferric chloride was added and colour change was observed. The emergence of brownish green or a blue-black colour indicated that tannins were present.

Saponins

About 2 mL of sample (leaves and roots of *C. intybus*) was boiled in water bath using distilled water and then filtered. The filtrate was shaken vigorously with distilled water until having persistent froth. Olive oil was mixed with the froth and shaken vigorously. If emulsion was formed, that indicated the presence of saponins.

Flavonoid

In the methanolic extract of root and leaves of *C. intybus*, 2-3 drops of 1% aluminum solution were added. The solution turned in yellow colour showed the presence of flavonoids.

Phytochemical Quantitative Analysis

Total Flavonoids Content (TFC): The TFC of methanolic extract of roots and leaves of *C. intybus* were estimated by Colorimetric assay as suggested by Rohman A and Man YC [17]. Absorbance was taken at 510 nm for the mixture. Rutin was taken as standard for the plotting of calibration curve. Total flavonoid content of the samples were articulated as mg rutine equivalents/gram of sample and calculation was done using calibration curve (R^2=0.997).

Total Phenolic Content (TPC): Quantitative estimation of TPC of methanolic extract of both root and leaves of *C. intybus* done by the method as suggested by Jindal KK and Singh RN [18]. Gallic acid was used as standard for the formation of standard curve at different concentrations, linear callibration curve of gallic acid, with coefficient of determination (r^2) value of 0.998, was obtained. For the estimation of TPC standard curve was used. The estimated values were expressed as (mg/g gallic acid equivalent).

Antioxidant Activity

Antioxidant property of methanolic extract of both root and leaves of *C. intybus* was estimated using DPPH (2, 2'-diphenyl-1-picrylhydrazyl) reagent. Scavenging activity was determined by the method as described by Brand-Williams W et al., [19]. The control solution was prepared using 0.2 mmol/L of DPPH in methanol; control (500 mL) was added in the methanolic extract solution at different concentration (100-500 µg/mL). Vigorous shaking of mixtures was done to make a homogenous solution and then allowed standing for 30 minutes at room temperature. The readings was taken at different concentration in ultraviolet-visible spectrophotometer at the absorbance of 515 nm, the concentration of DPPH is reduced as the concentration of antioxidant increases. Ascorbic acid was taken as standard and absorbance was determined under similar condition. All the readings were taken in triplicates for lowering the error. The antioxidant activity was calculated by using the formula:

$$I\%=(Ac-As)/Ac\times100$$

Where, I is inhibition, Ac is the absorbance of control and As is the absorbance of extract solution, respectively.

Antibacterial Assay

The agar well diffusion method [20] was used to conduct antimicrobial activity tests. The microorganisms used for the study were: *Staphylococcus aureus* (MTCC 87), *Pseudomonas aeruginosa* (MTCC 424), *E. coli* (MTCC 68) and *Salmonella* Typhimurium (MTCC 68). Petri dishes were filled with 15 mm of the nutrient agar medium. Petri dishes were inoculated and tempered at temperature 40°C with target microorganism. Newly produced suspensions were made by diluting target strain microbial cultures to a microbial concentration of 10^8 colony forming unit (cfu)/mL. Agar plates were solidified for 1 hour before wells of 8 mm diameter were formed and filled with 100 µL of diluted stock solutions using a sterile cylinder (plant extracts). Wells were pored with pure extract (100 µL) and used as negative control, positive control was streptomycin (50 µg/mL). Petri dishes were incubated for 24 hours at 37°C for bacterial strain. The antimicrobial activities of *C. intybus* leaf and root extracts were calculated by measuring diameter of zone of inhibition around the well using ruler and the value represent the diameter of the well. The values were then compared with standard antibacterial drug (ampicillin) and accordingly zone of inhibition was calculated.

STATISTICAL ANALYSIS

For analysing the result statistically, readings were taken in triplicates and data were given with standard deviation. Antioxidant activity was analysed using correlation and p-value take as 0.05.

RESULTS

Phytochemical Analysis

The non nutritive plant chemicals are called as phytochemicals, they have disease preventive properties, thus sometimes also known as phytonutrients. They have preventive properties against chronic diseases which include heart disease, cancer, diabetes, osteoporosis and vision diseases. The selected qualitative phytochemical analysis of the methanolic extracts of both root and leaves of *C. intybus* showed the presence of following phytochemicals; phenolics, tannin, flavonoids, proteins/amino acids, carbohydrates, fats/oils, sterols, alkaloids. The results of phytochemical study is depicted in [Table/Fig-1].

Total Phenolic Compound and Total Flavonoids Analysis

The results of quantitative analysis of phytochemicals are show in [Table/Fig-2]. Total phenolic content and flavonoid content of *C. intybus* leaves and seed extract is from (15.2±0.5 to 25.2±0.3). The chicory leaves extract was found to have significant amount of flavonoids and phenolic contents in comparison to root.

Phytoconstituents	*Cichorium intybus* root	*Cichorium intybus* L. Leaves
Phenolics	+	+
Tannin	+	+
Flavonoids	+	+
Proteins/amino acids	+	+
Carbohydrates	+	+
Fats/oils	+	+
Sterols	+	+
Terpenoids	-	-
Glycosides	-	-
Alkaloids	+	+

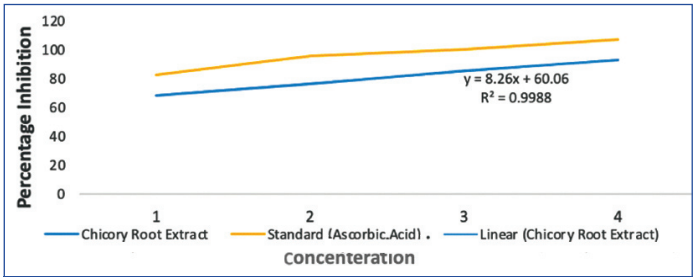
[Table/Fig-1]: Preliminary phytochemical analysis of *C. intybus* root and leaves extract *Cichorium intybus* L.
(+) confirms presence and (-) confirms absence of above shown category of phytoconstituents

Methanolic extract of root and leaves of *C. Intybus*		
	Root extract	Leaves extract
Total Phenolic Content (TPC)	20.5±0.1 (mg/100 gm of gallic acid equivalent per gram)	25.2±0.3 (mg/100 gm of gallic acid equivalent per gram)
Total Flavonoid content (TFC)	15.2±0.5 mg quercetin equivalents/gm dry weight	18.1±0.0 mg quercetin equivalents/gm dry weight

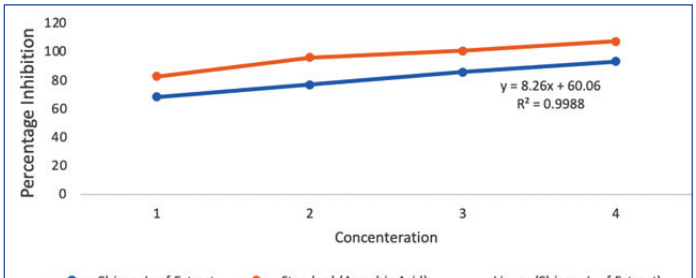
[Table/Fig-2]: Total phenolic compounds and total flavonoids of root and leaves extract of *Cichorium intybus*.

Antioxidant Analysis

The results for antioxidant activity of both root and leaves are depicted in [Table/Fig-3,4] and both showed good antioxidant properties.



[Table/Fig-3]: Antioxidant property of chicory root extract.



[Table/Fig-4]: Antioxidant property of chicory leaf extract.

Antibacterial Assay

Antibacterial effect of *C. intybus* leaf and root extract was studied against different microorganism strains and results are revealed in [Table/Fig-5]. Results indicated that *C. intybus* root extract was found more effective against these bacteria i.e., *Staphylococcus aureus, Pseudomonas aeruginosa, E. coli* and *Salmonella* Typhimurium with zone of inhibition 25.24±0.34 mm, 18.75±1.12 mm, 20.1±0.4 mm and 24.8±0.5 mm, respectively, while on comparison with standard drug, ampicillin its was found effective. While for leaf extract, zone of inhibition was found less effective against *Salmonella* Typhimurium and *E. coli*, on low concentration when compared with standard antibacterial drug. The IC50 value of both extracts was found to be 299.98 µg/mL as IC50 value indicates concentration required to reduce 50% of DPPH.

Plant Extract	Concentration mg/mL	Zone of inhibition diameter (mm)			
		Staphylococcus aureus	*Escherichia coli*	*Pseudomonas aeruginosa*	*Salmonella* typhimurium
Methanolic root extract	2.5	13.45±0.12	5.9±0.9	7.2±0.7	6.3±0.34
	4.5	20.67±0.78	10.6±0.67	15.8±1.1	12.4±3.1
	5.5	25.24±0.34	18.78±1.12	20.1±0.4	24.8±0.5
Methanolic leaf extract	2.5	10.64±0.38	ND	4.9±2.4	ND
	4.5	14.83±0.84	4.3±0.23	10.2±2.5	ND
	5.5	20.56±0.26	10.52±0.62	16.3±0.82	12.53±0.56
Ampicillin		22±0.02	24±0.39	21±0.14	26.34±0.34

[Table/Fig-5]: Antibacterial activity of methanolic extracts of *C. intybus* root and leaf.

DISCUSSION

In recent years, plants and its different parts are widely used for herbal formulations in treatment of many ailments. The presence of flavonoid and phenolic compounds are known to have antioxidant properties because of the presence of hydroxyl groups in their chemical structures, thus they contribute defense system to against oxidative stress [21]. In the present study, *C. intybus* roots and leaves extracts were studied for the presence of phytochemicals and studies have reported that phytochemicals have a wide range of importance in different biological activities like antimicrobial, antioxidant, antiallergic and anti-inflammatory [22,23]. Antioxidant compound in food play vital role and helps to enhance immune system. Most widely used procedure employed to assess antioxidant potency of plant and biological samples were DPPH radical scavenging activity. Any substance at low concentrations compared to that of an oxidisable substrate that significantly delays or prevents oxidation of that substrate is called as antioxidant [22]. The screening of phytochemicals of leaves and roots of *C. intybus* showed the presence of tannins, saponins, flavonoids. These results were in line with the report by Shad MA et al., [16]. Tannins are polyphenolic compounds with high molecular weight and present in almost every plant and showed protective effects against unfavourable conditions [24]. While saponins are the glycosidic compounds present in almost all the plant compounds, having bitter taste and form foams. A study reported that the saponins have the properties of anticarcinogenic and antifungal. Presence of carbohydrate, proteins indicate its nutritional composition *C. intybus* have been found for great medicinal importance as it has been a source of both phenolic and flavonoid content [25]. Antioxidant properties of flavonoid and phenolics acids is because of presence of hydroxyl group in their chemical structure. Thus, it helps in scavenging free radicals and contributes to defense system and oxidative damage [26]. DPPH radical is widely used to determine antioxidant activity of many plants and biological samples. It was found out that the free radical scavenging activity of leaf extract is more effective when compared with the standard in current study. The IC_{50} value of both extracts was found to be 299.98 µg/mL as IC_{50} value indicates concentration required to reduce 50% of DPPH. Antioxidant activity of the extract was due to the presence of phenolic and flavonoid compounds [27]. Further, the antimicrobial activities of *C. intybus* extract was evaluated using standard well diffusion method, the result obtained showed that the extract of the root and leaves exhibit antimicrobial potential against tested microorganism. Diameter of zone of inhibition increases with increase in concentration of the extract. Antimicrobial properties of *C. intybus* root and leaf extract was due to the presence of tannins, as they deprecate substrate required for microbial growth and also have direct action on microbial metabolism [28].

Limitation(s)

Further investigations are required to understand the mechanism behind the therapeutic activities of *Cichorium intybus*.

CONCLUSION(S)

C. intybus is grown and employed for a variety of applications throughout the world. It is frequently used as a therapeutic and preventative measure or to preserve overall health. It is a very adaptable plant that provides advantages to both animals and people because of the significant amounts of proteins, carbohydrates, minerals, and phytoconstituents components it contains. From the present study it has been concluded that roots and leaves possess good antioxidant and antimicrobial quality, thus, can be used in therapeutic formulations.

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