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PHYTOCHEMICAL, ANTIOXIDANT AND ANTIBACTERIAL POTENTIAL OF EXTRACTS OF *CICHORIUM INTYBUS* (CHICORY)

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ABSTRACT

The Kasni, *Cichorium intybus* (*C. intybus)* is well known traditional herb included in many systems of medicine like Ayurveda, Unani and Siddha. Keeping in view its medicinal value phytochemical, antioxidant and antibacterial analysis of extracts of leaves and roots of C. intybus was carried out. Phytochemical analysis revealed the presence of diterpenes, alkaloids, flavonoids, proteins, amino acids, saponins, phenols, phytosterols and tannins in the extracts but glycosides, cardiac glycosides and carbohydrates were absent. Antioxidants were determined by total phenolic content (TPC) and DPPH free radical scavenging activity. Methanol extracts of leaves and roots exhibited maximum phenolic content and DPPH free radical scavenging activity followed by chloroform extracts and aqueous extracts of leaves and roots. As the concentration of extracts increases from 10µg/ml to 100µg/ml the scavenging effect also increases for all the extracts. The antibacterial activity against *S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa* shows that methanol and chloroform extracts of leaves and roots had significant antibacterial activity with maximum zone of inhibition against *E. coli*. But aqueous extracts of leaves and roots exhibited highest antibacterial activity against *P. aeruginosa*. It is concluded from the results that *C. intybus* has potential source of natural antioxidants and can be used for the treatment of diseases caused by the test organisms. Due to good phytochemical, antioxidant and antibacterial composition, *C. intybus* 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 defence system against endogenous free radicals and disease causing bacteria.

KEYWORDS: Alkaloids, Antibacterial, Free radicals, Hexane, Phenolic content.

1. INTRODUCTION

Plant derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Ncube *et al.*, 2008).

Higher and aromatics plants have been used traditionally in folk medicine as well as to extend the shelf life of foods, showing inhibition against bacteria, fungi and yeasts. Biologically active compounds from natural sources have always been a great interest for scientists working on infectious diseases (Nandagopal and Kumari, 2007). Plants produce a diverse range of bioactive molecules, making them rich source of different types of medicines. The most important of these bioactive compounds are alkaloids, flavonoids, tannins and phenolic compounds which are the important raw materials for drug production. A wide variety of these secondary metabolites (bioactive compounds) have been found *in vitro* to have antimicrobial properties for protection against aggressor agents, especially microorganisms (Silva and Junior, 2010; Bhalodia and Shukla, 2011).

Recently, interest has increased considerably in finding naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants, which are being restricted due to their carcinogenicity. Therefore, it is of great interest to carry out a screening of the plants in order to validate their use in folk medicine and to reveal the active principle by isolation and characterisation of their constituents (Mehmood *et al.*, 2012). The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world. Much work has been done on ethno-medicinal plants in India. In an effort to expand the spectrum of phytochemical, antioxidant and antibacterial agents from natural resources, *Cichorium intybus* (*C. intybus*) belonging to *Asteraceae* family has been selected in this study.

MATERIALS AND METHODS

Collection of Plant Material

The *C. intybus* plants were collected from agricultural fields to obtain roots and leaves and transferred to the

laboratory for further processing. Roots and leaves of *C. intybus* were separated manually with sharp knife, washed with distilled water and dried in hot air oven for one week. After one week, grinded into fine powder using an electric blender. The powder was then dried in oven at 40°C for 24 hours and stored in air tight jars for further analysis. The fine powdered plant parts were used for the preparation of extracts.

Preparation of Extracts

Water, chloroform and methanol were used as solvents for the preparation of extracts of roots and leaves. 25gram of powdered root and leaves were soaked separately in 100ml of each solvent and kept in a shaker for 3 days. After 3 days the mixtures were filtered through Whattman filter paper number1 and filtrates were stored in air tight bottles for further analysis.

Phytochemical Screening of Extracts

Extracts were tested for the presence of active principles such as diterpenes, alkaloids, flavonoids, proteins, free amino acids, glycosides, cardiac glycosides, carbohydrates, saponins, phenols, phytosterols and tannins following standard procedures (Tiwari *et al*., 2011).

Antioxidant Analysis

The *C. intybus* aqueous, chloroform and methanol extracts were further tested for presence of antioxidants by total phenolic content and DPPH free radical scavenging assay.

1. Total phenolic content (TPC)

The total phenolic content of the extract was determined by the Folin-Ciocalteu method. Briefly, 200µl of crude extract were made up to 3ml with distilled water, mixed thoroughly with 0.5ml of Folin-Ciocalteu reagent for 3 minutes, followed by the addition of 2ml of 20% (w/v) sodium carbonate. The mixture was allowed to stand for 60 minutes in dark, and absorbance was measured at 650 nm. The total phenolic content was calculated from the calibration curve, and the results were expressed as mg of gallic acid equivalent per gram dry weight of sample. Each assay was carried out in triplicate (Baba and Malik, 2015).

2. DPPH radical scavenging assay

The antioxidant activity of the samples was assessed through their ability of scavenging 2, 2-diphenyl-1-picrylhydrazyl stable radicals (DPPH). The samples (from 10 to 250µg/ml) were mixed with 1ml of 90µM DPPH solution and made up with 95% methanol, to a final volume of 4ml. After incubation of 60 minutes at room temperature, the absorbance was recorded at 515nm. All samples were analysed in triplicates and average of three results was calculated. A negative control was prepared by mixing DPPH solution to 0.1ml Methanol. Percent radical scavenging concentration was calculated using the following formula:

Radical Scavenging (%) = $100 \times (A_{Blank} - A_{Sample} / A_{Blank})$.

Here, A_{Blank} is the absorbance of the control (containing all reagents except the test sample), and A_{sample} is the absorbance of the test samples (Mehmood *et al.*, 2012).

Determination of Antibacterial Activity

Two Gram positive bacteria - *Staphylococcus aureus* (MTCC 6810) and *Bacillus subtilis* (MTCC 1427) and two Gram negative - *Escherichia coli* (MTCC 443) and *Pseudomonas aeruginosa* (MTCC 2453) were chosen for evaluation of antibacterial activity of *C. intybus* extracts of leaves and roots.

Antibacterial activity of *C. intybus* extracts was tested by using Agar well diffusion technique on Muller Hinton agar (MHA) media. The presence of zone of inhibition (mm) indicated the antibacterial activity. Each extract of *C. intybus* was tested against the test organisms in triplicates along with media control and organism control plates (Zearaha *et al*., 2013).

Minimum Inhibitory Concentration (MIC)

The least concentration of the extract that is able to inhibit the growth of the bacteria was determined by the macro broth dilution method. To approximately 2ml of prepared nutrient broth in series of sterile and well labelled test tubes, 2 drops of the test bacteria (previously diluted to 0.5 McFarland turbidity standard) and 2 drops of different concentrations (50, 100, 150, 200 and 250 500ug/ml) of extracts were added. The same procedure was repeated on the test organisms using the different concentrations (50, 100, 150, 200 and 250 500ug/ml) of the antibiotic (chloromphenicol), a test tube containing nutrient broth only seeded with the test organism was used as control. The tubes were then properly corked and incubated at 37°C for 24hours. The MIC was read and recorded as the least concentration of the extract that shows no visible bacterial growth (Ugoh and Haruna, 2013).

RESULTS AND DISCUSSION

The present study was performed to find out the phytochemical constituents, antioxidant and antibacterial activity of *Chicorium intybus* leaves and roots. For this purpose aqueous, chloroform and methanol extracts of *C. intybus* parts were prepared.

Phytochemical Screening of Extracts

Preliminary phytochemical analysis revealed that important phytochemical components such as diterpenes, alkaloids, flavonoids, proteins, amino acids, saponins, phenols, phytosterols and tannins were found to be present in almost all the extracts of leaves and roots of *C. intybus* but glycosides, cardiac glycosides, carbohydrates were absent in all the extracts (Table 1). The presence of flavonoids and tannins in *C. intybus* extract are in agreement with previous reports (Mehmood *et al.*, 2012;

Nandagopal and Ranjitha, 2007; Muthusamy *et al*. 2008).

Tannins, the high molecular weight polyphenolic compounds found naturally in many plants and have been found to play a protective role in plants against micro-organisms, unfavourable climatic conditions and damage by animals. On the other hand, they can form multiple hydrogen bonds with carboxylic groups of dietary proteins and proteolytic enzymes in the gastrointestinal tract which leads to reduced digestibility of proteins and finally the retardation of animal growth. Saponins are the glycosidic compounds found in most of the plants, possess a bitter taste and foaming properties. Saponins have been found to possess anti-carcinogenic and antifungal activity (Abbas *et al.,* 2015).

Table 1. Phytochemical Analysis of Extracts of *C. intybus*

Phytochemical Constituent	Aqueous Extract		Chloroform Extract		Methanol Extract	
	Leaves	Roots	Leaves	Roots	Leaves	Roots
Diterpenes	Positive	Positive	Positive	Positive	Positive	Positive
Alkaloids	Positive	Positive	Positive	Positive	Positive	Positive
Flavonoids	Positive	Positive	Positive	Positive	Positive	Positive
Proteins	Positive	Positive	Positive	Positive	Positive	Positive
Amino acids	Positive	Positive	Positive	Positive	Positive	Positive
Glycosides	Negative	Negative	Negative	Negative	Negative	Negative
Cardiac Glycosides	Negative	Negative	Negative	Negative	Negative	Negative
Carbohydrates	Negative	Negative	Negative	Negative	Negative	Negative
Saponins	Positive	Positive	Positive	Positive	Negative	Negative
Phenols	Positive	Positive	Positive	Positive	Positive	Positive
Phytosterols	Positive	Positive	Positive	Positive	Positive	Positive
Tannins	Positive	Positive	Positive	Positive	Positive	Positive

The flavonoids and phenolic acids are known to possess antioxidant activities due to the presence of hydroxyl groups in their structures and their contribution to defence system against the oxidative damage due to endogenous free radicals is extremely important (Saggu *et al.,* 2014). Phenolics or polyphenols are secondary plant metabolites that are ubiquitously present in plants and their products. Many of them have been shown to contain high levels of antioxidant activities (Razali *et al.,* 2008). Due to their redox properties these compounds contribute to the overall antioxidant activities of plants. Usually, the mechanisms of their antioxidant activity are neutralizing lipid free radicals and preventing decomposition of hydroperoxides into free radicals (Li *et al.,* 2009).

Antioxidant Activity

1. Total phenolic content (TPC)

Results shows that *C. intybus* is rich in phenolic content and TPC were found in the range of 23.4 to 62.5mg GAE/ 100 gm dry weight when calculated by using strandard curve of gallic acid. Methanol extract of leaves contain highest TPC (62.5mg GAE / 100 gm dry weight) and aqueous extract of roots contain lowest TPC (23.4mg GAE/ 100 gm dry weight). TPC in chloroform extracts of leaves and roots was found to 48.8 and 41.4mg GAE/100 gm dry weight respectively. Aqueous extracts of leaves and roots contain 30.2 and 23.4mg GAE/100 gm dry weight TPC respectively (Table 2).

Table 2. Total Phenolic Content in Extracts of *C. intybus*

S. No.	Extract	TPC (mg GAE/100g dry weight)
1.	Aqueous Extract of Leaves	30.2
2.	Aqueous Extract of Roots	23.4
3.	Chloroform Extracts of Leaves	48.8
4.	Chloroform Extracts of Roots	41.4
5.	Methanol Extracts of Leaves	62.5
6.	Methanol Extracts of Roots	54.6

It was observed that the methanol extract had more antioxidant activity than other extracts studied. The amounts of TPC from *C. intybus* leaves and roots in different solvent systems were in the ranges 30.2-62.5 GAE (mg /100 gm of dry plant matter) and 23.4-54.6 GAE (mg/100 g of dry plant matter), respectively (Table 2).

Methanol extract of the *C. intybus* leaves and roots showed the highest TPC respectively. These differences in the amount of TPC may be due to varied efficiency of the extracting solvents to dissolve endogenous compounds. The ability of different solvents to extracts TPC was of the order: methanol > chloroform > aqueous (Table 2 and Fig. 1).

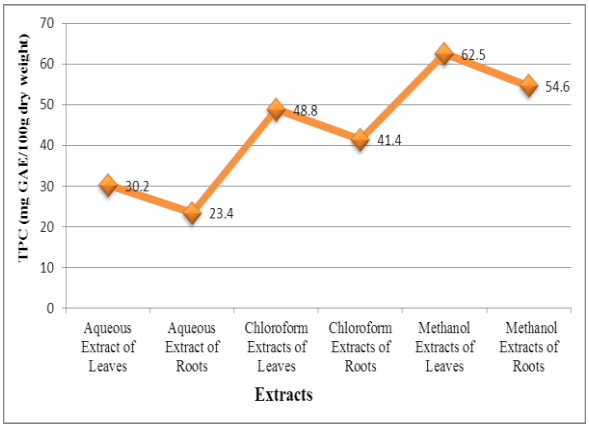

Fig. 1. Total Phenolic Content (TPC) in Extracts of *C. intybus*

Methanol is most efficient and widely used to extract anti-oxidative components including phenolic acids and other phenolic components. Although chloroform and water also extracted reasonable amounts of TPC, however, due to comparatively lower polarity, were less effective.

2. DPPH free redical scavenging assay

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 (78.62% and 71.88% respectively) followed by chloroform (68.54% and 64.21% respectively) and water (62.40% and 53.36% respectively) (Table 3 and Fig. 2).

Table 3. DPPH Free Radical Scavenging Activity of Extracts of *C. intybus*

Conc. (µg/ml)	Scavenging Activity (%) of Aqueous Extract		Scavenging Activity (%) of Chloroform Extract		Scavenging Activity (%) of Methanol Extract		Scavenging Activity (%) of Standard
	Leaves	Roots	Leaves	Roots	Leaves	Roots	Ascorbic Acid
10	6.8	5.9	7.9	6.8	9.36	8.80	22.41
20	10.60	9.69	12.05	10.45	19.12	15.40	48.18
40	21.66	18.45	21.39	20.09	25.65	20.72	64.62
60	29.48	22.79	32.84	28.76	39.12	34.74	71.44
80	48.22	39.61	55.67	50.17	64.34	57.48	74.25
100	62.40	53.36	68.54	64.21	78.62	71.88	82.10

The scavenging effect of different solvent extracts on the DPPH radical decreased in the order of methanol> chloroform> water. Among these extracts, the methanol extract showed maximum antioxidant activity followed by chloroform extract and aqueous extract (Table 3 and Fig. 2). The methanol extracts also had higher total polyphenolic content than the chloroform extracts (Leaves: 62.05 and 48.8mg/g extract respectively; Roots: 54.6 and 41.4 mg/g extract respectively) (Table 2).

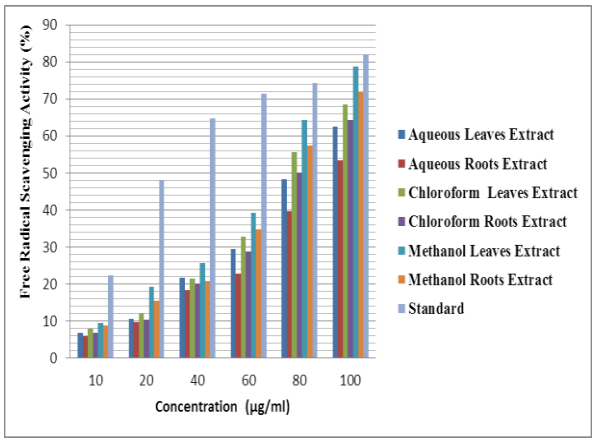

Fig.2. Comparison of Scavenging Effect (%) of Leaves and Roots of *C. Intybus*

Leaves of *C. intybus* are found to be good source of phenolic compounds. Due to high phenolic content, comparatively good reducing power and DPPH radical scavenging capacity was found in leaves. All the extracts of leaves were found to be rich in high phenolic content as compared to root extracts which may be responsible for its observed antioxidant activity. To evaluate the free radical scavenging effect of plant extracts, DPPH test is a widely used method. 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 (Table 3 and Fig. 2).

Due to the presence of phenolic compounds *C. intybus* has been found to have great medicinal value. The results show that all of the studied parts of *C. intybus* are good source of phenolic compounds. Leaves found to possess comparatively good DPPH radical scavenging capacity because of high total phenolic content. The root has been found to show comparatively low antioxidant activities due to low phenolic content (Table 2 and 3).

Antibacterial Activity and Minimum Inhibitory Concentration (MIC)

Both polar as well as non-polar solvents were used in this study for the extraction of active components from the leaves and roots of *C. intybus*. The results showed that all the solvent extracts possesses antibacterial

activity against the tested Gram positive (*S. aureus* and *B. subtilis*) and Gram negative (*E. coli* and *P. aeruginosa*) bacteria. Methanol extracts of leaves and roots showed pronounced inhibition against all the tested organisms, the maximum inhibition was observed on *E. coli* (ZOI 13.6 and 12.0mm respectively) and minimum on *S. aureus* (ZOI 10.4 and 8.2mm respectively) (Table 4).

Table 4. Zone of Inhibition (ZOI) of *C. intybus* Extracts against Test Bacteria

Test Bacteria	ZOI (mm) of Leaves Extracts			ZOI (mm) of Roots Extracts		
	Aqueous	Chloroform	Methanol	Aqueous	Chloroform	Methanol
S. aureus	5.6	9.2	10.4	5.2	6.6	8.2
B. subtilis	8.4	9.6	11.2	6.4	8.8	10.4
E. coli	9.2	12.4	13.6	8.2	11.6	12.0
P. aeruginosa	9.8	11	12.6	9.0	10.4	11.8

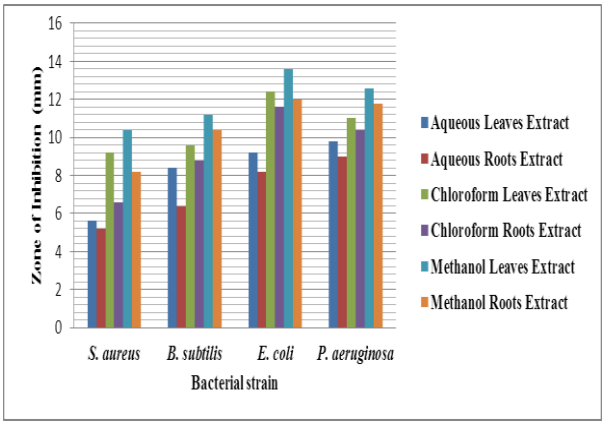

Fig.3. Comparison of Inhibition Zones (ZOI) of Extracts of Leaves and Roots of *C. Intybus*

Aqueous extracts of leaves and roots of *C. intybus* produced maximum inhibition (ZOI 9.8 and 9mm respectively) to the growth of *P. aeruginosa* whereas chloroform extracts of leaves and roots showed maximum inhibitory activity (ZOI 12.4 and 11.6mm respectively) against *E. coli*. It was found that both aqueous as well as organic extracts of the leaves and roots were successful in inhibiting the bacteria (Table 4 and Fig. 3).

Table 5. MIC (µg/ml) of *C. intybus* Extracts Against Test Bacteria

Test Bacteria	MIC (µg/ml) of Aqueous Extract		MIC (µg/ml) of Chloroform Extract		MIC (µg/ml) of Methanol Extract	
	Leaves	Roots	Leaves	Roots	Leaves	Roots
S. aureus	250	250	150	250	150	200
B. subtilis	200	250	150	200	100	150
E. coli	150	200	50	100	50	50
P. aeruginosa	150	150	100	150	50	100

The results of MIC indicated that methanol extracts of leaves and roots showed strong activity against *E. coli* evident from the lowest MIC values (50µg/ml) and least activity was against *S. aureus* with the smallest inhibition zones (10.4 and 8.2mm) and the highest MIC values (150 and 200µg/ml), respectively. Chloroform extracts of leaves and roots showed good activity against *E. coli* with inhibition zones of 12.4mm and 11.6mm and the lowest MIC values (50 and 100µg/ml), respectively. Least activity was exhibited against *S. aureus*, with the smallest inhibition zones (9.2 and 6.6 mm respectively) and the highest MIC values (50 and 100µg/ml respectively). Aqueous extracts of leaves and roots of *C. intybus* showed strong activity against *P. aeruginosa* (ZOI 9.8 and 9.0mm respectively) and the lowest MIC values (150µg/ml). Least activity was exihibited against *S. aureus* with inhibition zones 5.6 and 5.2mm respectively and the highest MIC values (250µg/ml) (Table 4 and 5). In general, the results indicated that plant extracts showed good antibacterial activity.

Antimicrobial effect of chicory leaf extract with different solvents on *S. aureus* and *E. coli* was studied by Khakzadihe et al.. *S. aureus* and *E. coli* antibiograms with different solvents had no significant difference between groups and no inhibition zone observed around different extract disks and control disks on Mueller-Hinton agar culture. They couldn't find any chicory leaves extract antibacterial effect against *S. aureus* and *E. coli* (Khakzadihe *et al*., 2014).

Ethyl acetate extract of chicory root was tested for antibacterial and anti-fungal properties. Fractionation by column chromatography of ethyl acetate extracted root powder contains the compound, inhibiting both Gram positive and Gram negative bacteria and was found to be bacteriostatic rather than bacteriocidal. The effect of chicory root extract has more bacteriostatic effect on Gram Positive bacteria than Gram negative bacteria as MIC value is more in case of Gram negative bacteria than Gram positive bacteria. The ethyl acetate fraction of root which is obtained by silica gel column

chromatography has also antifungal activity as it inhibits growth of yeast and moulds (Koner *et al*. 2011). Extract of *C. intybus* L. had mild antibacterial effect against *E. coli* and *S. aureus* and it was shown that there is a synergistic antibacterial effect between the respective medicinal plants with tetracycline, chloramphenicol and ciprofloxacin (Aqil and Ahmad, 2007). In a research conducted by Ghaderi *et al* (2012) *C. Intybus* L. had no antibacterial effect on *Streptococcus pyogen*, *Staphylococcus aureus* and *Enterococcus*. They studied comparison of antibacterial effect of *C. Intybus* L. with vancomycin, ceftriaxone, ciprofloxacin and penicillin (Ghaderi *et al.*, 2012). *In vitro* antibacterial activity of *C. intybus* against some pathogenic bacteria was studied. It was reported that plant fractions under study have great potential as antibacterial compound against *E. coli* and *P. aeruginosa* and they can be used in the treatment of infectious diseases caused by above resistant microorganisms (Verma *et al.* 2013).

CONCLUSIONS

In conclusion, all tested parts of *C. intybus* contain considerable amounts of phytochemicals and are good source of antioxidants. However, methanol extracts of *C. intybus* leaves possess comparatively higher amounts of phytochemicals, antioxidants and antibacterial compounds and also show high free radical scavenging capacity. Results contributed that, chicory contains higher concentration of organic solvent soluble compounds that may be responsible for exhibiting higher antioxidant and antibacterial activity. It may be suggested that *C. intybus* leaves, would play an important role in antioxidant defence system against endogenous free radicals because of their good antibacterial and antioxidant composition and thus improving the human health. The results confirmed the therapeutic potency of the plant scientifically and support the effectiveness of *C. intybus* to treat infectious disease locally as well as traditionally. The results also form a good basis for utilization of this plant for further pharmacological and toxicity research. The further need is to the study the different organic fractions of this plant to draw complete picture of its potential as antioxidant and antibacterial agent.

CONFLICT OF INTEREST

Authors have no conflict of interests in this study.

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