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ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF ROOT LEAVES AND FLOWERS OF *CICHORIUM INTYBUS*

Ammara Ijaz [*1], Shreez Ahmad [1], Hina Gul [1], Nyla Jabeen [2] and M. Gulfraz [3]

Department of Biochemistry [1], PMAS Arid Agriculture University, Rawalpindi, Pakistan.
Department of Biotechnology [2] and Bioinformatics Islamic International University, Islamabad, Pakistan.
Department of Chemistry [3], Comsat Institute of Information Technology, Abbottabad, Pakistan.

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Correspondence to Author:
Ammara Ijaz

Department of Biochemistry,
PMAS Arid Agriculture University,
Rawalpindi, Pakistan.

E-mail: ammaraijaz018@gmail.com

ABSTRACT: A study was conducted to assess root, leaves and flowers extracts of *Cichorium intybus* for their antimicrobial activities (against four bacterial and fungal strains), antioxidants activities (DPPH, ABTS H_2O_2 and reducing power assays) and cytotoxicities assays. The results indicate remarkable inhibition of plant extracts for the growth of all bacterial and fungal strains as well as appropriate MIC values were observed as compared to standard antibiotics used. The activities of these extracts against four assays like DPPH, H_2O_2, ABTS, and reducing powder was promising. Whereas the cytotoxic activity of extracts study against brine shrimp was reliable. The phytochemical analysis of the various parts of *Cichorium intybus* revealed that activities of these extracts might be due to the presence of various secondary metabolites like phenolics and flavonoids those were analyzed with higher quantities from these extracts. Therefore such a study could be useful for the development of new pharmaceuticals that could lead such compounds for the preparation of new medicines required for human and animals disorders.

INTRODUCTION: The World Health Organization estimated that about three-quarter population relies on plant-based preparations which have been used in their traditional medicinal system and as the basic needs for human primary health care [42]. About 25% of the pharmacological drugs were isolated from plants in the developed countries [1], those are being used against many human infections.

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A large number of medicinal plants have been investigated to get active secondary metabolites, and recent interest in these substances has been stimulated by potential health benefits arising from the antioxidant activities of these compounds [12].

Secondary metabolites are the chemical components produced by the plants which are classified in different groups due to their divergence in structure, composition, and solubility. These metabolites are remarkably significant and highly considered for their medicinal importance [2]. Although flavonoids possess many biochemical properties the best-described property of flavonoids is their capability to act as antioxidants. Natural antioxidants either in the form of raw extracts or their chemical

constituents are very effective to prevent the destructive processes caused by oxidative stress [31, 46]. Natural antioxidants have a wide range of biochemical activities, including inhibition of ROS generation, direct or indirect scavenging of free radicals, and alteration of intracellular redox potential [41]. Antioxidants protect living organisms from the damage caused by uncontrolled production of reactive oxygen species, concomitant lipid peroxidation, protein damage, and DNA strand breakage. An antioxidant, which can quench reactive free radicals, can prevent the oxidation of other molecules and may, therefore, have health-promoting effects in the prevention of degenerative diseases.

The frequency of life-threatening infections has increased worldwide and is becoming an important cause of morbidity and mortality in immune-compromised patients in developed countries. Since few decades, the health benefits of synthetic antibiotics are under threat as most of the commonly used antibiotics become less effective against certain human infections not, only because many of them produce toxic reactions, but also due to the emergence of drug-resistant bacteria. Thus it is essential to investigate newer drugs with lesser resistance. Drugs derived from natural sources play a significant role in the prevention and treatment of human diseases [17, 21]. Herbs are widely exploited in traditional medicine, and their curative potentials are well documented in the literature.

Cichorium intybus commonly known as chicory is an important medicinal plant that belongs to the family Asteraceae [42]. Plant family Asteraceae consists of about 1000 genera and have more than 25000 species, most of them are used in medicines, edible oils, rubber industry, vegetables and pesticides preparation [2]. Significant activities of Chicory roots as Antimicrobial, anti-diabetic, immune enhancement, anti-hepatotoxic, anti-hyperuricemia, and anti-hypertriglyceridemia were reported by Alloush *et al.* [4]

Different studies exposed that water extract of *Cichorium intybus* L. have provided remarkable effects on low-density lipoprotein (LDL), degradation of the fatty acids of LDL and inhibitory effects on the production of thiobarbituric acid [28]. The phytochemical screening reported in the literature indicates the presence of tannins, saponins, flavonoids, terpenoids, cardiac glycosides and anthocyanins in each part of this plant [29].

Therefore, keeping in view the medicines values, Antimicrobial, Antioxidants and Cytotoxic effects of root, leaves and flowers extracts of *Cichorium intybus* were assessed during present experimental work.

MATERIALS AND METHODS:

Collection of Samples: The root, flowers and leaves samples of *Cichorium intybus* were collected from areas of Gujrat (Pakistan) in fine plastic bags duly labeled with names and location of collection and were transported to Agriculture lab of Department of Biochemistry PMAS Arid Agriculture Rawalpindi. The samples were identified by expert taxonomist from the Department of Botany and registered as a voucher specimen for future reference.

Preparation of Samples: Samples were washed thoroughly to remove unwanted materials, then shadow and sun-dried followed by oven drying at 60°C for overnight. The dried samples were converted into powdered form and passed through sieves (80 mash). The samples were saved in fine plastic bags and stored at a lower temperature until further uses. Total 50 grams each of root, leaves and flower samples were dissolved in distilled water, methanol, chloroform, and n-hexane and extracted by using soxhlet and rotary evaporator techniques followed by shaking for overnight and then filtrated. The extracts were used for qualitative and quantitative estimation and percentage yield of the extract was calculated and dried extracts were stored in air tight vials for further processes.

Qualitative and Quantitative Estimation of Phytochemicals: Qualitative evaluation of flavonoids, alkaloids, phenols, tannins, and saponins were carried out from various plant extracts by the specific methods described by Harborne (1998)) and AOAC (2003) [5, 20]. Whereas for quantitative estimation of plant samples were analyzed for total phenolic, flavonoid and tannins. Total phenolic contents were determined by using the Folin-Ciocalteu reagent method as described by Kim *et al.* [22]

Whereas flavonoids contents were estimated by using the method reported by Hussain *et al.* [25] Determination of tannins was carried out using Folin-ciocalteu method as reported by Makkar *et al.* [27]

Antioxidant Activity of Plant Extracts: The capacity of the different plant extracts to reduce Fe^{+3} ions into Fe^{+2} ions was assessed [8]. The scavenging ability of the plant extracts was determined by using 1, 1 diphenyl 1-2 - picrylhydrazyl (DPPH) assay, following method reported by Moon and Shibamoto (2009). Free radical scavenging assays were performed by using 2, 2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) [7]. Whereas the capacity of plant extracts to scavenge H_2O_2 was determined by using the procedure described Ruch *et al.* [35].

Estimation of Antimicrobial Activity of Plant Extracts: The plant extracts were screened to determined antibacterial potential by using agar well diffusion assay against four bacterial strain, *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC15224), *Klebsiella pneumonia* (MTCC618) and *Bacillus subtilis* (ATCC6633) and for comparison standard antibiotics (Cefixime and Roxithromycin) were used in this study [10] and OD was determined at 420 nm with UV/visible spectrophotometer. The MIC was estimated as the lowest concentration of the extracts that blocked the bacterial growth after 24 h of incubation period [15]. The antifungal activity of plant extracts was estimated by using agar tube dilution method against four strains of fungus (*Aspergillus niger*, 0198; *Aspergillus flavus*, 0064; *Aspergillus fumigates*, 66 and *Fusarium solani*, 0291) as reported by Ettebong and Nwafor [15].

Cytotoxic Activity of Plant Extracts:

Brine Shrimps Cytotoxicity Assay: Brine shrimps cytotoxicity assay was carried out to evaluate the cytotoxic effects of plant extracts by using the method reported by [35].

Analysis of the Plant Extracts with HPLC: About 1% of plant extract was prepared in HPLC grade methanol. The samples were sonicated for 10-12 min followed by filtration and adjustment of 200 ml of volume with HPLC grade methanol. An aliquot of 5 ml was filtered through a C18 column and was eluted with 4 ml of methanol. The volume was further increased to 10 ml followed by filtration, and 20 µl of which was injected into HPLC column by adjusting isocratic flow rate of 1 ml/min mobile phase acetonitrile and 0.1% phosphoric acid (36:64) respectively, retention time 7 and absorbance was measured at 200 nm wavelength by using UV/visible detector.

Statistical Analysis: After triplicate analysis of all parameters data was statistically analyzed by using ANOVA as reported by Steel and Torrie (1980) [36].

RESULTS AND DISCUSSIONS: The root, leaves and flower samples of *C. Intybus* were analyzed for their proximate composition and results are represented in **Table 1**. Proximate analysis of plants samples gives valuable information regarding the quality of the plant extracts.

TABLE 1: BIOCHEMICAL COMPOSITION (%) OF DIFFERENT PARTS OF *C. INTYBUS* AFTER ANALYSIS OF 100 g DRY WEIGHT OF SAMPLE

Sample	Dry matter	Moisture	Crude protein	Crude fat	Crude fiber	Total ash
Root	98.01±0.1	42±0.1	5.94±0.01	17±0.1	28.28±0.1	7.99±0.1
Leaves	94.11±0.01	17±0.1	14.16±0.01	19±0.1	17.59±0.5	19.2±0.5
Flower	92.1±0.1	67±0.1	15.6±0.01	27±0.1	20.2±0.1	12.5±0.1

Results are mean of 3 values ± Standard deviation

Qualitative analysis of *C. intybus* extracts indicates the presence of various secondary metabolites like Phenols, flavonoids, alkaloids, tannins, and saponins **Table 2**.

TABLE 2: PHYTOCHEMICAL COMPOSITION OF DIFFERENT EXTRACTS OF *C. INTYBUS*

Extracts	Phenols	Flavonoids	Tannins	Alkaloids	Saponins
Root					
Methanol	+	+	+	+	+
Ethanol	+	+	+	+	-
n-hexane	+	+	+	+	+
Chloroform	+	+	+	+	+

Aqueous	+	+	+	+	+
Leaves					
Methanol	+	+	+	-	+
Ethanol	++	++	++	+	+
n-hexane	+	+	+	-	+
Chloroform	+	+	+	-	+
Aqueous	+	+	+	-	+
Flower					
Methanol	+	+	+	-	-
Ethanol	++	++	+	+	+
n-hexane	+	+	+	-	-
Chloroform	+	+	+	-	-
Aqueous	+	+	+	-	-

+ Present; - Absent

The presence of such metabolites indicates the importance of plant extracts; for examples, flavonoids are considered important due to their antioxidant and antimicrobial activities [33]. The phenols act as immune enhancer' anti-inflammatory, anti-clotting, and hormone modulators whereas saponins have anti-hemolytic and cholesterol binding properties. Tannins are soluble polyphenols and have many functions in the living organism, probably due to these reasons, many researchers suggested multiple utilization of *C. intybus* [19, 40, 44].

Quantification of Flavonoid from Plant Extracts: a Higher amount of flavonoids was found in ethanolic flower extracts of *C. intybus* extracts (3.162 ± 0.51 mg/g) followed by ethanolic leaves extracts of *C. intybus* (2.862 ± 0.15 mg/g) **Table 2**. Flavonoids have been shown to possess several biological properties many of which may be related, to their antioxidant and free-radical-scavenging ability [14, 32].

The amount of flavonoids found in the present study was lower than the amount reported by other [44] when they analyzed leaves samples of *C. intybus* by using a different method of analysis, however, amount of flavonoids found in the present study were comparable with results reported by other authors.

TABLE 3: QUANTIFICATION OF IMPORTANT PHYTO CHEMICALS FROM VARIOUS EXTRACTS OF *C. INTYBUS*

Extracts	Total phenols (mg GAE/ g dry weight)	Total flavonoids (QE mg/g dry weight)	Tannins (mg GAE/g dry weight)	Yield (%)
Root				
Methanol	1.139±0.02	1.1616±0.06	0.187±0.04	2.31
Ethanol	2.681±0.23	2.712±0.04	1.812±0.05	2.09
n-hexane	0.242±0.07	0.816±0.13	0.893±0.14	1.34
Chloroform	2.312±0.34	2.231±0.07	2.748±0.19	2.18
Aqueous	1.082±0.23	1.232±0.05	0.436±0.05	1.04
Leaves				
Methanol	2.176±0.08	2.862±0.15	1.361±0.08	1.12
Ethanol	2.986±0.05	2.912±0.06	1.481±0.04	2.15
n-hexane	0.817±0.15	1.765±0.04	1.124±0.05	0.58
Chloroform	2.241±0.08	2.714±0.05	2.263±0.06	1.65
Aqueous	1.202±0.02	1.816±0.03	1.932±0.04	1.78
Flower				
Methanol	1.864±0.07	2.781±0.62	0.286±0.04	1.98
Ethanol	2.376±0.08	3.162±0.51	1.391±0.07	2.81
n-hexane	0.163±0.11	0.764±0.05	0.193±0.02	0.89
Chloroform	1.178±0.06	1.863±0.03	0.545±0.20	1.84
Aqueous	0.432±0.14	1.732±O.01	0.302±0.02	1.23

Values are expressed as mean ± Standard deviation (n=3); % age yield of all three parameters from total extract used

Total Phenolic Contents: The phenolic contents of plant extracts were determined, and results were compared with a standard curve of Gallic acid **Table 3**. The ethanolic leaves extracts showed a higher amount of total phenol (2.986 ± 0.05mg GAE/g) as compared to other solvents extracts while a lower amount of total phenol was found in n-hexane and aqueous extract of *C. intybus* **Table 3**. Phenols are the secondary metabolites and are present in plants in wider range and can scavenge

free radicals and are considered as anticancer agents. Amount of total phenols found in the current study were higher than reported by Ashafa *et al.* [7] after worked on some other plant extracts.

Total Tannin Contents (TTC): According to results higher amount of tannins was found in chloroform root extracts (2.748 ± 0.19 mg GAE/g) followed by chloroform leaves extracts (2.263 ± 0.06 mg GAE/g) of *C. intybus*. Plant tannins are a distinctive group of polyphenolic polymers of relatively high molecular weight. Tannins prevent the body from free radical-induced RBC hemolysis and involved in decreasing rate of nutrients absorption; those are required to synthesise new substances for body function [11].

Antimicrobial Activity of Plant Extracts The methanol extract of *C. intybus exhibited* active inhibitory potential against *S. aureus*, *E. coli*, *K. pneumonia* and *B. subtilis* strains as compared to all other extracts and cefixime and DMSO; however, lower zone of inhibition was observed as compared to roxithromycin (standard) **Table 3**.

All plant extracts showed a remarkable antibacterial potential against both Grams +ve and Gram-ve strains which indicate that extracts of *C. intybus* are composed of antimicrobial components (flavonoids, *etc*.,) that can be used to treat infectious disorders caused by the severe resistant pathogenic microorganisms. The current findings support the results reported by other authors with slight variations [16]. Antimicrobial resistance (AMR) is a major threat to global public health. Medicinal plants have long been used as remedies for infectious diseases by native cultures around the world [39] and have the potential for providing effective treatments for antibiotic-resistant infections [9, 18].

TABLE 3: ANTIBACTERIAL ACTIVITIES OF VARIOUS EXTRACTS *C. INTYBUS;* ZONE OF INHIBITION

Extracts	*S. aureus*	*E. coli*	*K. pneumonia*	*B. subtilis*	Roxithromycin	Cefixime	DMSO
Root							
Methanol	21.6±0.6	21.4±0.9	19.5±0.3	22.6±0.4	14.6±0.9	13.5±0.3	0.0
n-hexane	17.2 ±0.5	17.3±0.8	17.6±0.4	16.5±0.7	12.3±0.9	11.4±0.8	0.0
Chloroform	18.3±0.6	19.2±0.3	14.2±0.4	15.8±0.5	13.6±0.8	12.7±0.9	0.0
Aqueous	12.4±0.8	15.6±0.6	11.3±0.5	11.6±0.3	10.2±0.7	9.4±0.8	0.0
Leaves							
Methanol	22.5±0.3	20.8±0.4	23.5±0.7	16.8±0.6	14.3±0.7	15.8±0.9	0.0
n-hexane	19.6±0.5	19.2±0.9	19.2±0.6	14.5±0.5	14.5±0.7	15.3±0.8	0.0
Chloroform	13.4±0.4	15.7±0.8	21.6±0.5	15.7±0.9	13.6±0.7	14.8±0.5	0.0
Aqueous	11.5±0.7	13.2±0.7	13.5±0.4	11.2±0.7	9.7 ±0.8	8.2±0.3	0.0
Flower							
Methanol	18.3±0.6	23.6±0.5	18.3±0.6	21.7±0.4	15.6±0.6	17.9±0.8	0.0
n-hexane	16.5±0.6	21.5±0.1	17.6±0.4	18.2±0.4	14.2±0.5	16.8±0.6	0.0
Chloroform	13.5±0.5	18.4±0.4	15.4±0.5	17.6±0.3	14.7±0.6	15.4±0.5	0.0
Aqueous	11.7±0.6	12.6±0.5	13.2±0.6	13.2±0.7	12.8±0.6	11.2±0.9	0.0

Results mean ± SD after triplicate analysis (n=3).

TABLE 4: MINIMUM INHIBITORY CONCENTRATION (µg/ml) FOR VARIOUS BACTERIAL STRAINS

Extracts	*S. aureus*	*E. coli*	*K. pneumonia*	*B. subtilis*	Roxithromycin	Cefixime	DMSO
Root							
Methanol	0.1±0.6	2.1±0.7	0.8±0.5	1.4±0.6	1.6±0.8	1.5±0.5	0.0
n-hexane	1.7 ±0.1	1.6±0.9	0.7±0.4	1.6±0.7	1.2 ±0.3	1.1±0.8	0.0
Chloroform	1.8±0.9	1.9±0.5	1.2.2±0.4	1.5±0.5	1.3±0.5	1.2±0.5	0.0
Aqueous	1.9±0.3	1.6±0.7	1.7±0.5	1.6±0.3	1.2±0.6	1.4±0.3	0.0
Leaves							
Methanol	0.5±0.6	0.8±0.1	0.3±0.9	1.1±0.4	0.2±0.7	0.8±0.3	0.0
n-hexane	1.6±0.5	1.9±0.8	1.9±0.5	1.5±0.5	1.5±0.9	1.1±0.8	0.0
Chloroform	1.4±0.4	1.5±0.9	1.6±0.7	1.4±0.6	1.6±0.4	0.4±0.6	0.0
Aqueous	1.5±0.7	1.3±0.7	1.8±0.4	1.9±0.3	1.7 ±0.8	2.2±0.3	0.0
Flower							
Methanol	0.3±0.7	0.6±0.4	1.3±0.6	1.7±0.4	0.6±0.6	0.9±0.2	0.0
n-hexane	1.5±0.5	1.5±0.1	17.6±0.4	18.2±0.4	1.2±0.5	1.3±0.5	0.0
Chloroform	1.3±0.4	1.4±0.4	15.4±0.5	17.6±0.3	1.4.±0.5	1.2±0.7	0.0
Aqueous	1.7±0.7	1.6±0.1	13.2±0.6	13.2±0.7	1.8±0.2	1.8±0.3	0.0

Results are Means ± SD, (n = 3)

Minimum inhibitory concentration indicates the significant antimicrobial potential of *C. Intybus* extracts and data obtained, through the determination of MIC of various extracts and antibiotics are presented in **Table 4**. The results revealed variability in the inhibitory concentrations of each extract for given bacteria. The lowest MIC was observed for methanol extracts perhaps due to its purity or solubility of plant materials in this solvent **Table 4**. Results found in the present study were comparable to results report by other authors including Guessan *et al*., [30] and Kaur *et al.* [25]

TABLE 5: ANTIFUNGAL ACTIVITIES OF VARIOUS EXTRACTS OF *C. INTYBUS*; ZONE OF INHIBITION IN mm

Extracts	*Aspergillus niger*	*Fusarium salani*	*Aspergillus flavous*	*Aspergillus fumigates*	Terbinafine	DMSO
Root						
Methanol	25.8±0.5	21.8±0.4	18.3±0.3	21.5±0.4	18.±0.3	0.0
n-hexane	18.3 ±0.5	17.2±0.5	17.8±0.4	15.4±0.8	15.3±0.5	0.0
Chloroform	16.3±0.4	18.2±0.3	16.3±0.4	15.8±0.5	14.6±0.3	0.0
Aqueous	11.4±0.2	9.6±0.3	11.6±0.2	11.6±0.3	10.1±0.6	0.0
Leaves						
Methanol	21.5±0.1	19.8±0.3	21.1±0.5	19.6±0.3	16.3±0.6	0.0
n-hexane	18.6±0.3	17.2±0.9	17.2±0.9	18.5±0.6	15.5±0.4	0.0
Chloroform	16.4±0.2	16.1±0.9	16.6±0.3	15.7±0.9	14.6±0.7	0.0
Aqueous	10.5±0.6	11.2±0.4	11.5±0.4	9.2±0.5	9.7 ±0.3	0.0
Flower						
Methanol	28.1±0.6	25.6±0.5	19.3±0.5	22.5±0.3	15.6±0.6	0.0
n-hexane	26.5±0.4	21.1±0.6	17.3±0.6	19.2±0.2	16.2±0.4	0.0
Chloroform	23.5±0.5	18.4±0.1	16.4±0.1	16.6±0.4	15.7±0.3	0.0
Aqueous	11.7±0.3	12.6±0.8	11.2±0.5	10.2±0.7	11.8±0.4	0.0

Results are Means ± SD, (n = 3).

Data in **Table 5** represents the antifungal activity of *C. intybus* extracts, according to results higher fungal activity was represented by methanolic flower extracts of *C. Intybus* extracts (28.1±0.6 mm) followed by n-hexane extracts (26.5 ±0.4mm) and lowest zone of inhibition by aqueous leaves extracts (11.7±0.5 mm) **Table 5**.

Extracts prepared in organic solvents showed activity against fungal strains and our results are comparable to results of plant extracts reported by Fawole *et al.* (2008) [18], Parekh and Chanda, (2005) [33], who found out that water extracts showed no/poor fungus toxicity than organic solvents, which is also indicated by present study as date given in **Table 5**.

It was assumed that due to less solubility of the active substance in aqueous media, has shown lower antimicrobial activity whereas it was remarkable in organic solvents.

***In-vitro* Antioxidant Assay:** To check the antioxidant potential of *C. intybus* extracts, five different assays *i.e.* DPPH, H_2O_2, ABTS Phosphomolybdate and reducing power assays were performed, and ascorbic acid was used as positive control.

All solvents extracts (methanol, n-hexane, chloroform, and water) showed a remarkable quantity of antioxidant activity, although the values were quite less than the positive control (ascorbic acid) and standard rutin **Table 6**, which is indicated by correlation of antioxidants potential of phenolics and flavonoids **Table 7**.

It was reported by Aqil *et al*., (2006) [6] that majority of the antioxidant activities of plants extracts are due to the flavones, isoflavones, flavonoids, anthocyanin, coumarin lignans, catechins, and isocatechins. Analysis of plant extracts with HPLC revealed the presence of a reliable quantity of quercetin **Table 8, Fig. 2 - 3**, that was responsible for the majority of biological activities of these extracts as reported by Javanmardi *et al*., (2003) [23] and Rathee *et al*., (2009) [35]. Furthermore, antioxidant-based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer [13]. Phenolics act as antioxidants in some ways such as reducing agents, hydrogen donors, free radical scavengers, and singlet oxygen quenchers and, therefore, act as cell saviors [15, 19].

TABLE 6: ANTIOXIDANT POTENTIAL OF VARIOUS *C. INTYBUS* extracted AT CONCENTRATION OF 100 µg/ml AND IC_{50} VALUES (µg/ml) OF RADICAL SCAVENGING WHEN ABSORBANCE WAS MEASURED AT 700 nm

Extracts	DPPH	H_2O_2	ABTS	Reducing Power assays	Ascorbic acid	Rutin
Root						
Methanol	0.214±0.01	3.13±0.05	1.32±0.08	0.19±0.01	1.91±0.8	3.77±1.8
n-hexane	0.311±0.01	2.45± 0.06	2.25±0.07	0.13±0.01	1.2 ±0.3	1.4±0.8
Chloroform	0.023±0.01	1.92±0.71	7.65± 0.15	0.16±0.01	1.8±0.4	1.6±0.5
Aqueous	3.08±0.01	8.13±0.2	9.13±0.26	0.18±0.05	1.7±0.5	1.8±0.3
Leaves						
Methanol	0.019±0.00	0.85±0.01	0.38±0.19	0.11±0.01	0.4±0.8	0.9±0.2
n-hexane	0.025±0.01	1.19±0.81	1.91±0.52	0.19±0.0	1.3±0.5	1.1±0.3
Chloroform	0.023±0.01	1.25±0.93	1.65±0.74	0.09±0.00	1.5±0.3	1.4±0.5
Aqueous	0.318±0.01	1.43±0.75	1.86±0.43	0.18±0.01	1.6 ±0.7	2.2±0.3
Flower						
Methanol	0.016±0.0	0.66±0.04	1.37±0.65	0.19±0.01	0.8±0.5	0.9±0.1
n-hexane	0.012±0.01	1.57±0.15	1.76±0.42	0.12±0.03	1.7±0.3	1.6±0.7
Chloroform	0.010±0.0	1.46±0.04	1.54±0.56	0.18±0.01	1.8.±0.2	1.2±0.7
Aqueous	0.028±0.01	4.63±0.12	1.82±0.63	0.19±0.02	2.9±0.2	3.8±0.6

Results are Means ± SD, (n = 3).

TABLE 7: CORRELATION BETWEEN PHYTOCHEMICALS AND ANTIOXIDANT POTENTIALS OF VARIOUS ASSAYS

Assays	Phenolic	Flavonoids
IC_{50} DPPH radical scavenging potential	1.35±0.5	1.34±0.6
IC_{50} of reducing power capacity	1.16 ±0.5	1.17±0.2
IC_{50} of hydrogen peroxide scavenging potential	1.51±0.8	1.58±0.6
IC_{50} of ABTS radical scavenging potential	1.43±0.4	0.151±0.01

Values are Mean ±SD, (n=3).

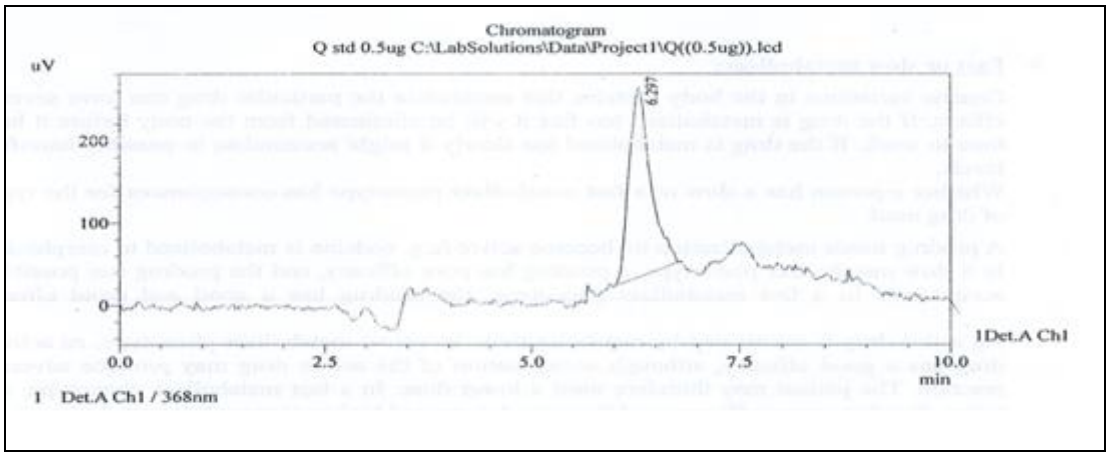

FIG. 2: CHROMATOGRAM OF STANDARD (QUERCETIN)

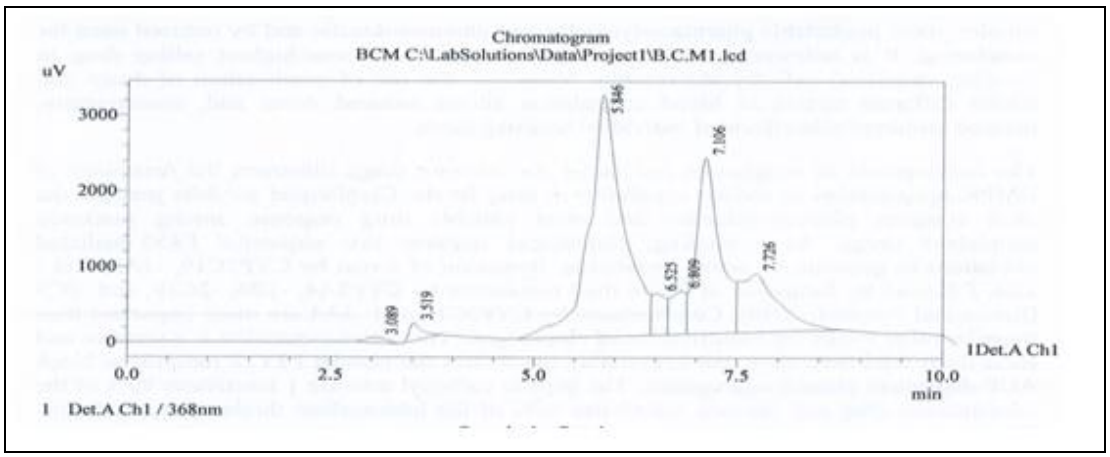

FIG. 3: CHROMATOGRAM OF FLAVONOID ANALYZED FROM VARIOUS *C. INTYBUS* EXTRACTS BY USING HPLC

TABLE 8: QUANTIFICATION OF FLAVONOID FROM *C. INTYBUS* EXTRACTS THROUGH HPLC

Solvents used	Area	Concentration(mg/g)
Methanol	90200	122.627
Ethanol	89871	138.509
n-hexane	69800	100.101

Various extractions are showing the presence of flavonoids.

TABLE 9: CYTOTOXICITY SCREENING OF VARIOUS CONCENTRATION (µg/ml) OF *C. INTYBUS* EXTRACTS

Extracts	10	100	1000	LD_{50}
Root				
Methanol	35.1±0.6	42.1±0.6	50.8±0.3	<1000
n-hexane	34.6 ±0.5	41.6±0.5	49.7±0.3	700
Chloroform	39.8±0.3	46.9±0.3	49.2±1.4	100
Aqueous	47.4±0.3	51.6±0.7	59.7±0.3	270
Leaves				
Methanol	36.5±0.6	41.7±0.1	52.3±0.7	<1000
n-hexane	31.6±0.5	36.9±0.8	39.1±0.5	665
Chloroform	33.4±0.5	36.5±0.9	39.6±0.3	100
Aqueous	51.5±0.7	54.3±0.7	56.3±0.4	260
Flower				
Methanol	33.5±0.6	35.6±0.5	39.3±0.5	<1000
n-hexane	31.5±0.5	31.9±0.1	37.5±0.1	668
Chloroform	39.3±0.4	41.5±0.4	43.5±0.6	1000
Aqueous	41.7±0.7	44.6±0.8	43.2±0.5	265

Values are Mean ±SD, (n=3) and significantly different (P<0.05); positive control are saline sea salt

Brine Shrimps Lethality Assay: Three different dilutions of *C. intybus* extracts (10,100 and 1000 µg/ml) were made to check brine shrimps cytotoxicity assay. The results revealed the better brine shrimps larvicidal potential and lethality was maximum at the maximum concentration of *C. intybus* extracts as the lethality of brine shrimps was concentration dependent. It was assumed that plant extracts might be composed of antitumor components in the form of essential phytonutrients. The plant extracts whose value, *i.e.* LD_{50} <1000 µg/ml was considered as biologically active while LD_{50} > 1000 µg/ml was biologically inactive (non - toxic), while higher mortality rate was shown by n-hexane and chloroform extracts of *C. intybus* **Table 9**. Results of brine shrimp lethality obtained in the current study were comparable with results reported by Sandeep *et al*. (2012) [38].

The study revealed that phytochemicals present in *C. intybus* extracts exhibited wonderful biological properties. The various extracts of *C. intybus* have shown antibacterial, antifungal, antioxidants, and cytotoxic activities.

It is concluded that the results obtained in the present study are in agreement to a certain degree with the traditional uses of *C. intybus* extracts against many human ailments. The obtained results could form a good basis for selection of various *C. intybus* extracts for further investigation in the potential discovery of new natural bioactive compounds.

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CONFLICT OF INTEREST: Nil

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