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PHYTOCHEMICAL ANALYSIS, ANTIBACTERIAL AND WATER PURIFICATION PROPERTIES OF *CHICHORIUM INTYBUS* LEAVES

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Abstract

Chicory (*Cichorium intybus L.*) belongs to the family Asteraceae and it is a small aromatic biennial or perennial herb. The whole plant contains a number of medicinally important compounds such as inulin, esculin, volatile compounds (monoterpenes and sesquiterpenes), coumarins, flavonoids and vitamins. In the present study, we evaluated the phytochemical analysis for the presence of various secondary metabolites and antibacterial activity of the leaf extracts of chicory against pathogenic bacteria like gram positive (*Bacillus subtilis*) and gram negative (*Escherichia coli*) bacteria by in vitro agar well diffusion method. The aqueous leaf extract of chicory showed pronounced inhibition than ethanol extract. Leaf extracts showed more inhibitory action on *Bacillus subtilis* than *Escherichia coli.* The water purification property was carried out using the herbal beads prepared by the *Cichorium intybus* leaves.

Keywords: *Cichorium intybus L.*, esculin, secondary metabolites, in vitro agar well diffusion, herbal beads.

1. Introduction

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 [1]. Biologically active compounds from natural sources have always been a great interest for scientists working on infectious diseases [2]. Cichorium intybus L. is a medicinally important plant that belongs to the family Asteraceae. The tuberous root of this plant contains number of medicinally important compounds such as inulin, bitter sesquiterpene lactones, coumarins, flavonoids and vitamins [3]. The plant root is used as antihepatotoxic, antiulcerogenic, anti-inflammatory, appetizer, digestive, stomachic, liver tonic, cholagogue, cardiotonic, depurative, diuretic, emmenagogue, febrifuge, alexeteric and also as tonic. It is useful in vitiated conditions of kapha and pitta, cephalalgia, heapatomegaly, inflammations, anorexia, dyspepsia, flatulence, colic, gout, burning sensation, allergic conditions of skin, jaundice, splenomegaly, hyperdipsia, skin diseases, leprosy, strangury, amenorrhoea, chronic and bilious fevers, ophthalmia, pharyngitis, vomiting, arthralgia, lumbago, asthma and general debility [4, 5]. This plant is also used to treat AIDS, cancer, diabetes, dysmenorrhoea, impotence, insomnia, splenitis and tachicardia [6]. Inulin is used to replace fat or sugar and reduce the calories of food. It is suitable for consumption by diabetics [7] and is also used in inulin clearance test to measure glomerular filtration rate-GFR [8]. Recent pharmacological investigation of the root extract of this plant revealed immunomodulator, antitumor and anticancer properties [9, 10].

The root is rich in alkaloids, which forms an ingredient or adulterant in coffee. The deep purple flower heads yield blue dye. The flowers are also used in floral clocks by Linnaeus [11]. The sesquiterpene lactones such as lactucin and lactucopicrin were isolated from chicory and reported for its antibacterial and antimalarial activity [12]. The antifungal activity of chicory was also reported [13-16]. Based on the studies carried out in chicory, worldwide report shows that the roots and leaves of this plant possess strong antibacterial and nematicidal effect [17]. However, to the best of our knowledge, very few reports are available on antibacterial properties of chicory leaf against the important human pathogenic bacteria so far. In the present study we reported the antibacterial activity of Cichorium intybus L. against pathogenic bacteria.

The study confirms that both aqueous as well as ethanol leaf extracts possess strong antibacterial properties against pathogens such as gram positive (Bacillus subtilis) and gram negative (Escherichia coli) bacteria. Followed by that water purification potential of prepared herbal beads was checked.

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2 Experimental Methods

2.1 Collection of plant sample

The plant sample Chichorium intybus leaves were commercially available in the vegetable market, Tirupattur. Tamilnadu, India. The leaf was cleaned properly and shadow dried. After complete drying, the separated leaves were powdered. It was stored in an airtight container.

2.2 Preparation of Leaf Extracts

A mixture of 250 ml distilled water, ethanol, and 25 grams of finely powdered leaf was used. After that, the suspension is allowed to incubate for 48 hours, or roughly two days, with periodic shaking. The contents are filtered via Whatman No. 1 filter paper following a 48-hour incubation period. The filtrate was then subjected to analysis.

2.3 Qualitative Phytochemical

Screening Phytochemical Analysis

The plant extract solutions were assessed for the existence of the phytochemical analysis by using the following standard methods.

2.3.1 Test for proteins

Millon's test: Crude extract when mixed with 2ml of Millon's reagent, white precipitate appeared which turned red upon gentle heating that confirmed the presence of protein.

Ninhydrin test: Crude extract when boiled with 2 ml of 0.2% solution of Ninhydrin, violet color appeared suggesting the presence of amino acids and proteins.

2.3.2 Test for carbohydrates

Fehling's test: Fehling's test involves mixing equal volumes of Fehling A and Fehling B reagents, adding 2 ml to crude extract, and gently boiling it. The presence of reducing sugars is indicated by the appearance of a brick-red precipitate at the bottom of the test tube.

Benedict's test: As 2 ml of Benedict's reagent was added to crude extract and heated, a reddish-brown precipitate developed, signifying the presence of carbohydrates.

Molisch's test: 2ml of Molisch's reagent were combined with crude extract, and the mixture was thoroughly shaken. Next, a cautious 2 ml of concentrated H_2SO_4 was gently poured along the test tube's side. The presence of carbohydrates was suggested by the appearance of a violet ring during the interphase.

Iodine test: There was a mixture of 2 ml of iodine solution and crude extract. The presence of the carbohydrate was identified by a dark blue or purple coloring.

2.3.3 Test for phenols and tannins

Crude extract was combined with 2ml of a 2% $FeCl_3$ solution. Phenols and tannins were characterized by a blue-green or black coloring.

2.3.4 Test for flavonoids

Shinoda test: Concentrated HCl and a few pieces of magnesium ribbon were combined with crude extract was added drop wise. Pink, scarlet color appeared after a few Crude extract was mixed minutes which indicated the presence of flavonoids.

Alkaline reagent test: 2ml of a 2% NaOH solution was used to cure. When a few drops of diluted acid were added, the bright yellow color that had formed went colorless, indicating the presence of flavonoids.

2.3.5 Test for saponins

In a test tube, 5 ml of distilled water was combined with crude extract, and the mixture was agitated vigorously. It was believed that the production of stable foam indicated the presence of saponins.

2.3.6 Test for glycosides

Liebermann's test: 2ml of acetic acid and chloroform were combined with the crude extract. Ice was used to chill the concoction. A precise concentration of H_2SO_4 was introduced. When the hue changed from violet to blue to green, it meant that the glycone part of the glycoside, or the steroidal nucleus, was present.

Salkowski's test: Chloroform (2 ml) was combined with crude extract. After that, 2 ml of concentrated H_2SO_4 was added and given a gentle shake. The existence of a steroidal ring, or the glycone portion of the glycoside, was indicated by a reddish-brown colour.

Keller-Kilani test: Crude extract was mixed with 2 ml of glacial acetic acid containing 1-2 drops of 2% solution of $FeCl_3$. The mixture was then poured into another test tube containing 2 ml of concentrated H_2SO_4.The presence of cardiac glycosides was indicated by a brown ring at the interphase.

2.3.7 Test for steroid

Additions of concentrated H_2SO_4 were made after the crude extract and 2 ml of chloroform were combined. The presence of steroids was identified by a red hue formed in the bottom chloroform layer. Another experiment involved combining 2 millilitres of chloroform with crude extract. Next, the mixture was added to 2 millilitres of concentrated $H_2SO_4$and acetic acid, respectively. Steroid presence was revealed by the greenish color was developed.

2.3.8 Test for terpenoids

After dissolving the crude extract in 2ml of chloroform, it was dried out. After adding 2 ml of concentrated H_2SO_4, this was boiled for approximately 20 minutes. Terpenoids were indicated by a greyish tint.

2.3.9 Test for alkaloids

2ml of 1% HCl was combined with crude extract and heated gradually. The mixture was then supplemented with Wagner's and Mayer's reagents. The ensuing precipitate's turbidity was seen as proof that alkaloids were present [18].

2.4. Preparation of Herbal beads

For bead production, 100 ml of an aqueous calcium chloride solution were agitated at 4000 rpm while 50 ml of

2% sodium alginate was added dropwise from a glass syringe with a size 22 needle. $CaCl_2$ and were present in the solution at a 1% concentration total [19].

2.5 Characterization Studies

2.5.1 UV-Visible spectroscopy analysis

The prepared herbal beads from Chichorium intybus leaves loaded alginate beads were characterised using UV-Visible spectroscopy by its extreme absorbance and wavelength under UV- Visible spectrophotometer (Shimadzu-2700) [19].

2.5.2 Fourier-transform infrared spectroscopy (FTIR)

The FTIR of synthesised herbal beads loaded alginate beads and interacted samples with varied pH was taken by Nicolet Impact 400 using 500 mg of KBr pellet [19].

2.6 Antibacterial Activity

Determination of antibacterial activity:

The antibacterial activity of the leaf extracts and prepared herbal beads was determined using agar well diffusion method by following the published procedure with slight modification [22]. Nutrient agar was inoculated with the given microorganisms by spreading the bacterial inoculums on the media. Wells (8 mm diameter) were punched in the agar and filled with leaf extracts and beads. Control wells containing DMSO (negative control) and standard antibiotic disc (positive control) viz., gentamicin and tetracycline were also incubated in the same plate [23]. The plates were incubated at 37°C for 24 hr and the antibacterial activity was assessed by measuring the diameter of the zone of inhibition. The relative antibacterial potency of the given preparation was calculated by comparing its zone of inhibition with that of the standard antibiotic disc.

Statistical analysis:

The resultant clear zones around the discs were measured in mm. The antibacterial activity of chicory leaf extracts was indicated by clear zones of growth inhibition. Data of three independent experiments represented as the mean values.

2.7 Water purification property

Stock solution of standard was prepared by dissolving 10 mg of 1M potassium permanganate (KMnO4) in 100ml double distilled water. By diluting the stock solution, the required concentration of standard was prepared [20-21].

3 Results and Discussion

3.1 Phytochemical screening of leaf extracts:

The preliminary phytochemical screening of the leaf extracts using two different solvents was reported (Table 1). All the results obtained listed below.

Table 1: The Preliminary Phytochemical Analysis

S.NO	PHYTOCHEMICALS	AQUEOUS EXTRACT	ETHANOL EXTRACT
1.	Carbohydrates	+	+
2.	Tannins	+	+
3.	Saponins	-	+
4.	Alkaloids	+	+
5.	Flavonoids	+	-
6.	Glycosides	-	-
7.	Quinones	-	+
8.	Phenols	+	+
9.	Terpenoids	+	+
10.	Steroids	+	+

Indicates: + Present and – Absent

Qualitative Phytochemical analysis was performed on the aqueous and ethanolic extract of Chichorium intybus leaf to detect the presence of several Phytochemicals, namely carbohydrates, phenols, flavonoids, quinones, steroids, tannins, saponins, terpenoids and alkaloids. The results are given in Table 1. Carbohydrates, tannins, alkaloids, flavonoids, phenols and terpenoids, steroids were present in the aqueous leaf extract. The aqueous extract doesn't contain the saponins, glycosides and quinones. Whereas the ethanolic leaf extract contains carbohydrates, tannins, saponins, alkaloids, quinones, phenols, terpenoids and steroids. Flavonoids and glycosides were not present in the ethanol leaf extract. Based on the findings the aqueous and ethanolic leaf extract of plant studied, the ethanolic leaf extract contains many Phytochemicals (Fig 1 and 2).

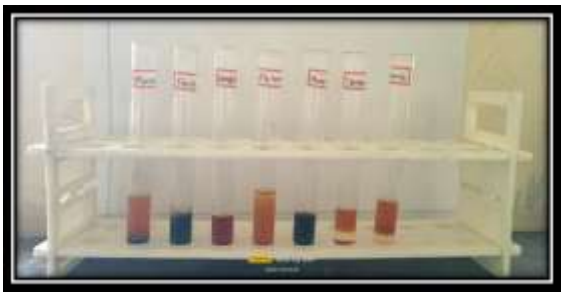


Fig.1. Qualitative Phytochemical Analysis of Aqueous Leaf Extract

Fig.2. Qualitative Phytochemical Analysis of Ethanolic Leaf Extract

3.2 PREPARATION OF HERBAL BEADS USING CHICHORIUM INTYBUS LEAVES

Enough research has been done in the previous few years to improve the decontamination and remediation capabilities of aquatic ecosystems using Herbal based materials. *Chichorium intybus* leaves encapsulation were created in the current work and combined with alginate beads in a unique way [24-27]. The professionals can readily handle the remediation agent in the shape of beads, and these innovative herbal beads are anticipated to be a good replacement for currently available materials. It was discovered that the Herbal beads had an average size of 4 mm (Fig 3 and Fig 4).

Fig.3 Freshly prepared beads

Fig 4 Size of a bead – 4 mm.

3.3 UV- Visible Absorption Spectroscopy of *Chichorium intybus* Herbal beads

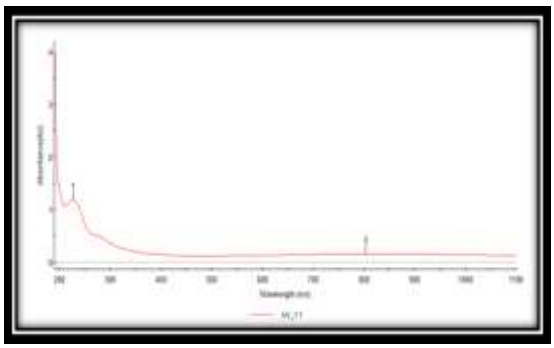


Fig 5: UV-visible Spectroscopy Analysis

From Figure 5 shows a UV-visible absorption spectroscopy is a prominent technique for analysing the optical properties of plant material. The Chichorium intybus beads absorption spectra shown in graph. It has a noticeable absorption band at about 226.7 nm and 804 nm (Table 2).

Table 2- UV– Visible absorption spectrum of herbal beads.

Name	Peak No.	Peak(nm)	Peak (AU)
Herbal-Beads	1.	226.7	1.19
	2.	804.0	0.16

3.4 FTIR Spectrum of *Chichorium intybus* Herbal beads

The Fourier-transform infrared spectroscopy analysis documented the chemical compounds and its coordination in the prepared *Chichorium intybus* Herbal beads.

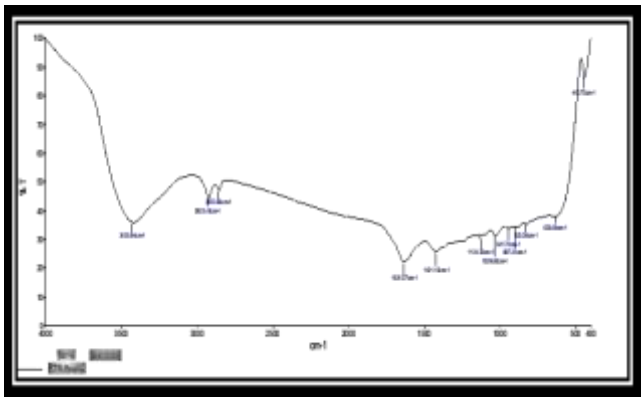


Fig 6: FTIR Spectroscopy graph

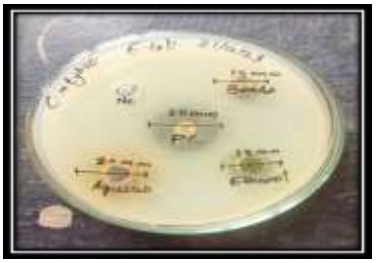
Table-3 FTIR Spectrum of *Chichorium intybus* Herbal bead

S.No	Absorption Band (cm^{-1})	Indication
1.	3420.94 (cm^{-1})	O-H stretching
2.	2923.48 (cm^{-1})	N-H stretching
3.	2853.96 (cm^{-1})	N-H stretching
4.	1631.57 (cm^{-1})	C=C stretching
5.	1421.15 (cm^{-1})	O-H bending
6.	1124.33 (cm^{-1})	C-O stretching
7.	1029.80 (cm^{-1})	C-N stretching
8.	897.24 (cm^{-1})	C-H bending
9.	823.38 (cm^{-1})	C-Cl stretching
10.	630.09 (cm^{-1})	C-I stretching

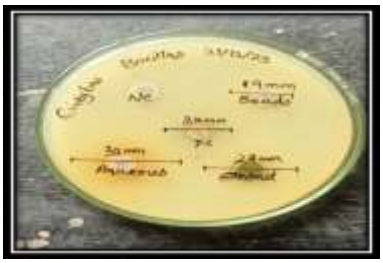
The IR spectra of samples of particles typically depend on the particle size and shape shows the FT-IR spectra of the generated herbal beads. The peak at 3420 cm^{-1}, which is most likely generated by ambient moisture, indicates the presence of -OH residue (Table 3 and Fig 6)

3.5 Antibacterial Activity

Antibacterial activity was assessed using the conventional agar well diffusion method to determine the Chichorium intybus leaf extracts and herbal beads. Using 2% DMSO (dimethyl- sulphoxide) as negative control, same concentrations of the extracts (Extracts 1, 2, 3) were created. By using the spread plate approach, test microorganisms Escherichia coli and Bacillus subtilis were seeded onto the appropriate Mueller-Hinton agar medium. After that, the plate was incubated for 24 hours at 37°C. By measuring the diameter of the inhibitory zone that formed around the well, the antibacterial activity was evaluated (Fig 7).



Escherichia coli



Bacillus subtillis

Fig.7. Anti-bacterial activity

Extract 1 – Aqueous extract of *Chichorium Intybus* + DMSO
Extract 2 – Ethanolic extract of *Chichorium Intybus* + DMSO
Extract 3 – Herbal beads of *Chichorium Intybus* + DMSO

It was found that the aqueous, ethanolic and prepared herbal beads of the leaves were successful in inhibiting the bacteria in a dose dependent manner. The aqueous extract is showing more inhibition compared to ethanol and beads extracts as shown in (Table – 4). The antibacterial activity of leaf extracts of chicory at 150µl Concentration was documented.

Table-4 Antibacterial activity of *Chichorium intybus* Herbal bead

Particulars	*E.Coli*	*Bacillus subtilis*
***Chichorium intybus* Beads**	15 mm	19 mm
***Chichorium intybus* Aqueous extract**	20 mm	32 mm
***Chichorium intybus* Ethanolic extract**	12 mm	27 mm
Positive control (Gentamicin, Tetracycline)	22 mm	22 mm

*Each value represents mean of triplicate.

3.6 Water Purification Property

The water purification was done by following the standard protocol referring to previous studies. According to the procedure the standard $KMnO_4$ was acted as contaminant and the prepared herbal beads were added accordingly. Then after two days there was observed some color changes in the pink color solution (Fig 8). This notifies the reduction of contamination in the water. This was confirmed by measuring it in Colorimetrically. Hence the prepared herbal beads contain the water purification property (Table 5).

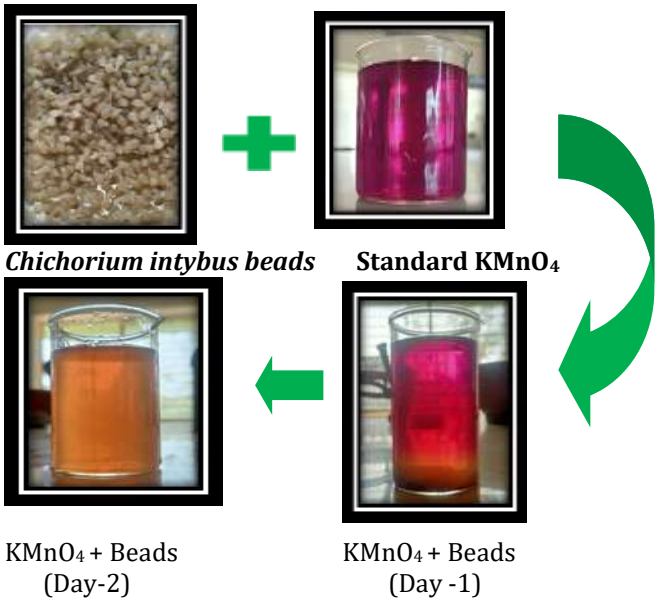


Fig 8: Water Purification

Table -5: Colorimetric Analysis

Optical density at 540 nm	Standard $KMnO_4$	$KMnO_4$ + Beads (Day 1)	$KMnO_4$ + Beads (Day 2)
	0.91	0.66	0.25

4. Conclusion

As per the available literature *Chichorium intybus* aqueous and ethanol extract was prepared. Phytochemical analysis was completed and herbal beads was prepared for further research investigation. UV - visible spectroscopy confirmed the characteristic absorption peaks at 226.7 nm and 804 nm. FTIR spectroscopic analysis given a detailed information about the chemical compounds present in the prepared herbal beads. The microorganism namely *E. coli* and *bacillus subtillis* documented good antibacterial activity when compared to positive control- Gentamicin, Tetracycline respectively. The water purification property of *Chichorium intybus* herbal beads was proven by the standard $KMnO_4$ solution. The Day 2 interaction studies highlighted the maximum absorption of $KMnO_4$Thus the phytochemical analysis, herbal beads preparation, UV-visible spectroscopy, FTIR spectroscopy, antibacterial and water

purification activity of *Chichorium intybus* leaves was analyzed.

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