Research paper

In vitro antibacterial activity of *Cichorium intybus* against some pathogenic bacteria

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ABSTRACT

The root and leaf extracts of *Cichorium intybus* were investigated for antibacterial activity against gram negative pathogenic bacteria viz. *Escherichia coli* and *Pseudomonas aeruginosa*. The sensitivity was analyzed using Disk diffusion method at various concentrations where zone of inhibition was compared with the standard drug Cephotaxime. The extracts showed a wide spectrum of inhibition against the test pathogens. Methanolic extract of root and leaf proves to have the strongest antibacterial activity. Antibacterial activity of the test extracts at different inhibitory concentration varied significantly at 0.05 level of significance. The maximum activity was recorded at 200mg/ml concentration, the activity decreased with the decrease in the concentration of the extract. The present study reveals that the root and leaf extracts of *Cichorium intybus* would exert several beneficial effects by virtue of their antibacterial activity and could potentially be exploited as a source of natural antibacterial.

Keywords: Antibacterial activity, Cefotaxime, *Cichorium intybus,* disk diffusion, sensitivity.

1. INTRODUCTION

Nature has been a source of medicinal plants for thousands of years and since the beginning of man. Extraction of bioactive compounds from medicinal plants permits the demonstration of their physiological activity. It also facilitates pharmacological studies leading to synthesis of a more potent drug with reduced toxicity [1, 2, 3, 4]. Furthermore, the active components of herbal remedies have the advantage of being combined with many other substances that appear to be inactive. However, these complementary components give the plant as a whole a safety and efficiency much superior to that of its isolated and pure active components [5].

The potential of higher plants as a source for new drugs is still largely unexplored. Among the estimated 25000–500,000 Plant species, only a small percentage has been investigated phytochemically. Historically pharmacological screening of compound of natural or synthetic origin has been the source of innumerable therapeutic agents. Random screening as tool in discovering new biologically active molecules has been most productive in the area of antibiotics [6]. Even now, contrary to common belief, drug from higher plants continue to occupy an important niche in modern medicine. On a global basis, at least 150 drugs all single or modified further synthetically are currently in use, though some of them have economic reasons [7].

Cichorium intybus is a medicinally important plant that belongs to the family Asteraceae. The tuberous root of this plant contains number of phytochemicals like sesquiterpene, lactones, coumarins, flavonoids and vitamins [8]. The plant root is used as antithepatotoxic, antialcerogenic, anti-inflammatory, appetizer, digestive, stomachic, liver tonic, cholagogue, febrifuge, alexeteric and also as tonic.

The plant is also used to treat AIDS, Cancer, Diabetes, Dysmenoorhoea, insomnia, splenitis and tachycardia [9]. Recent pharmacological investigation of the root and leaf fraction of this plant revealed immunomodulator, antitumor and anticancer properties [10]. The sesquiterpene lactones such as lactucin and lactucopicrin were isolated from Chicory and reported for its antibacterial and antimalarial activity [11]. 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 [12]. However to the best of our knowledge, very few reports are available on antibacterial properties of Chicory root and leaf against the important human pathogens so far. The present study reports the antibacterial activity of root and leaf extracts of *Cichorium intybus* against some pathogenic bacteria.

2. Materials and methods

2.1 Plant material:

The healthy roots and leaves of *Cichorium intybus* were collected from Hindustan Uniliver Pvt. Ltd., Etah Kasganj Road, Etah and its nearby areas.

2.2 Extraction of active principles:

The collected roots and leaves were shade dried, crushed and their weighed amount was extracted with methanol, distilled water, Petroleum ether, Chloroform and Acetone using a Soxhlet apparatus. The solvent was evaporated to obtain the crude extract using a rotary evaporator and the yield was measured.

2.3 Test Organism:

The pure cultures of test bacterial strains used in the study were *Pseudomonas aeruginosa* (MTCC 429) and *Escherichia coli* (MTCC 443). The strains were obtained from the culture collection of the Institute of Microbial Technology (IMTECH), Chandigarh, India. The typed culture of bacteria were maintained on Nutrient agar slants and stored at 4^0C prior to use.

2.4 Antibacterial Activity Assay:

In vitro antibacterial activity of selected plant extracts were tested by disc diffusion method [13].

For susceptibility testing, crude extract was made into a suspension using suitable solvent. The concentration of the material was made 200mg/ml and the further concentrations were prepared by serial dilution. Sterile discs having a diameter of 6 mm were impregnated with 25 µl of each serial dilution of extracts and dried in an incubator to remove the solvent. The plates were inoculated with the bacterial cell culture of concentration 10^8 CFU/ml by using 0.5 McFarland turbidity standards. Sterile discs loaded with extracts were placed on inoculated surface of agar plate with the help of sterile forceps. These plates were incubated for 24 hours at 37ºC. The diameter of the zone of inhibition around each of the disc was taken as measure of the antibacterial activity. Each experiment was carried out in triplicate and mean diameter of the inhibition zone was measured in millimeter.

2.5 Statistical analysis:

Data are expressed as mean ± standard deviation (SD) of triplicates. One-way analysis of variance (ANOVA) was used to analyze the effect of different concentration of test extracts on antimicrobial activity. The statistical analysis was conducted with PAST software at a significance level of 0.05.

3. Results and discussion

3.1 Antibacterial activity of different root and leaf extracts:

The antibacterial activity of the chicory root and leaf extracts was assessed using the disc diffusion method by measuring the diameter of inhibition zones. The study revealed that all the five fractions have

considerable antibacterial activity against the test bacteria. An examination of [Table 1-4] reveals that the methanol and Acetone root and leaf fractions of Chicory showed pronounced inhibition than other organic fractions. The maximum zone of inhibition 13.3 and 12.8mm was exhibited by methanol root and leaf fractions respectively against *Pseudomonas aeruginosa. Escherichia coli* was found to be less sensitive test organism to all the root and leaf fractions of *Cichorium intybus* [Fig. 1-4]. The relative antibacterial ability to either kill or inhibit the growth of bacteria has been compared with the standard antimicrobial agent Cefotaxime.

It is evident from the results that *Pseudomonas aeruginosa* was the most sensitive test organism to all the root and leaf extracts of *Cichorium intybus*. It is also clear that methanol was the best extractive solvent for the antibacterial activity against the pathogens used. This is in accordance with the results reported by [14] in *Cichorium intybus.* The activity shown by chicory root and leaf extracts may be due to the presence of many potent compounds such as inulin, sesquiterpene, lactones, coumarins, flavonoids etc. The antibacterial activity was expressed at varying degree in accordance to dose used against the bacteria. Results also indicated that inhibitory effects of chicory root and leaf extracts against both the bacterial strains decreased with the decrease in inhibitory concentration. Similar results were also reported by [15] in *Holoptelea integrifolia.* The inhibitory effects of *H. integrifolia* leaf extract against all the four bacterial strains increased with an increase in inhibitory concentration, however, degree of toxicity of different concentration of plant extract may differ from one microorganism to another.

Based on these results, we may conclude that the active phytocompounds present in Chicory (*Cichorium intybus*) should certainly find place in treatment of various bacterial infections. The results of this study are very encouraging and indicate that this herb should be studied more extensively to explore its potential in the treatment of many infectious diseases.

Table 1. Zone of inhibition of different root fractions of *Cichorium intybus* against *E. coli*.

Plant part	Solvent	Concentration (mg/ml)	Zone of inhibition (mm)
Root	Methanol	200	11.1 ± 1.00
		100	10.3 ± 0.08
		50	9.9 ± 0.12
	Aqueous	200	8.0 ± 0.47
		100	7.8 ± 0.12
		50	7.5 ± 0.12
	Chloroform	200	8.6 ± 0.34
		100	8.4 ± 0.08
		50	8 ± 0.81
	Petroleum ether	200	10.5 ± 0.18
		100	10 ± 0.47
		50	9.9 ± 0.08
	Acetone	200	12 ± 0.81
		100	11.5 ± 0.10
		50	11.2 ± 0.04
Cephotaxime		30 mcg	18.9

±: Standard Deviation

The different concentration of methanol extract has significant effect at 0.05 level of significance ($p < 0.05$)

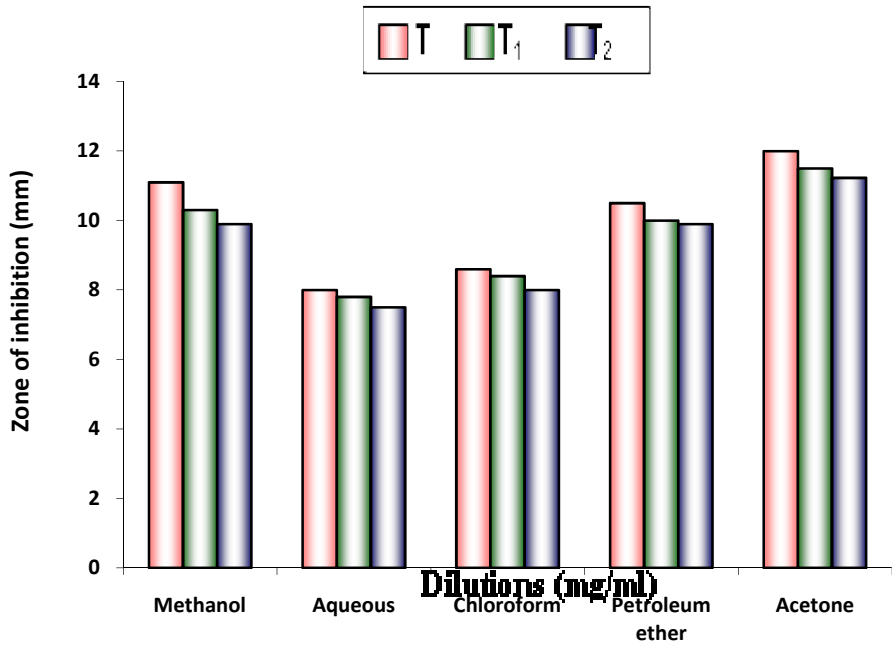

Fig. 1: Graphical representation of zone of inhibition of different root fractions of *Cichorium intybus* against *E. coli*.

Table 2. Zone of inhibition of different leaf fractions of *Cichorium intybus* against *E. coli*.

Plant part	solvent	Concentration (mg/ml)	Zone of inhibition (mm)
Leaf	Methanol	200	9.9 ± 0.08
		100	9.4 ± 0.04
		50	9 ± 0.81
	Aqueous	200	7 ± 0.47
		100	—
		50	—
	Chloroform	200	7.2 ± 0.08
		100	7 ± 0.47
		50	—
	Petroleum ether	200	9.2 ± 0.08
		100	9 ± 0.81
		50	8.5 ± 0.08
	Acetone	200	9.8 ± 0.08
		100	9 ± 0.47
		50	8.6 ± 0.08
Cephotaxime		30 mcg	19.5

±: Standard Deviation

—: no activity

The different concentration of aqueous, chloroform and petroleum ether extract have significant effect, while acetone extract has highly significant effect at 0.05 level of significance ($p < 0.05$).

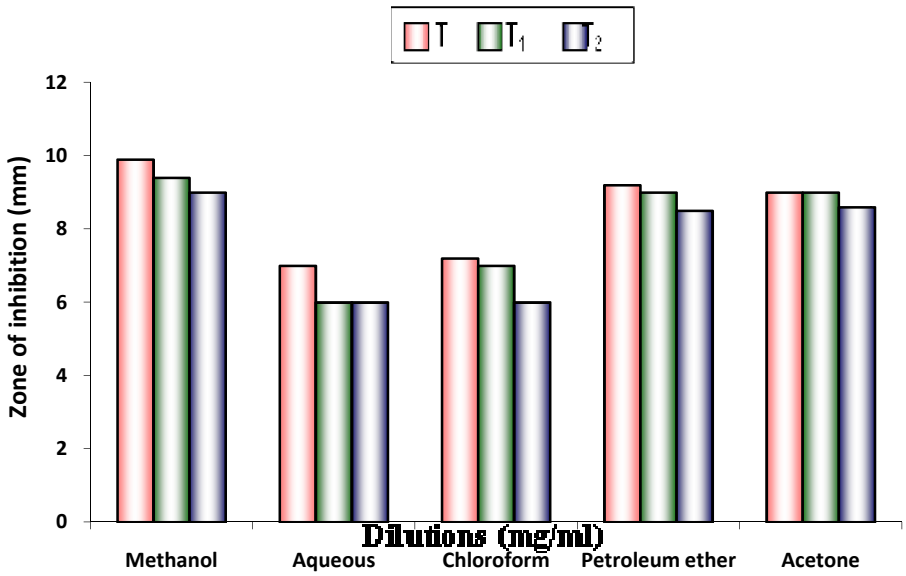

Fig. 2: Graphical representation of zone of inhibition of different leaf fractions of *Cichorium intybus* against *E. coli*.

Table 3. Zone of inhibition of different root fractions of *Cichorium intybus* against *P. aeruginosa*.

Plant part	solvent	Concentration (mg/ml)	Zone of Inhibition (mm)
Root	Methanol	200	13.3 ± 0.08
		100	13.2 ± 0.08
		50	13± 0.81
	Aqueous	200	9.3 ± 0.12
		100	8.9 ± 0.08
		50	8.6 ± 0.08
	Chloroform	200	9 ± 0.47
		100	8.5 ± 0.08
		50	8.2 ± 0.04
	Petroleum ether	200	10.5 ± 0.24
		100	10.2 ± 0.20
		50	10.1 ± 0.04
	Acetone	200	11.4 ± 0.08
		100	11.2 ± 0.04
		50	11.1 ± 0.04
Cephotaxime		30 mcg	22. l

±: Standard Deviation

The different concentration of all the test extracts does not have significant effect at 0.05 level of significance ($p < 0.05$)

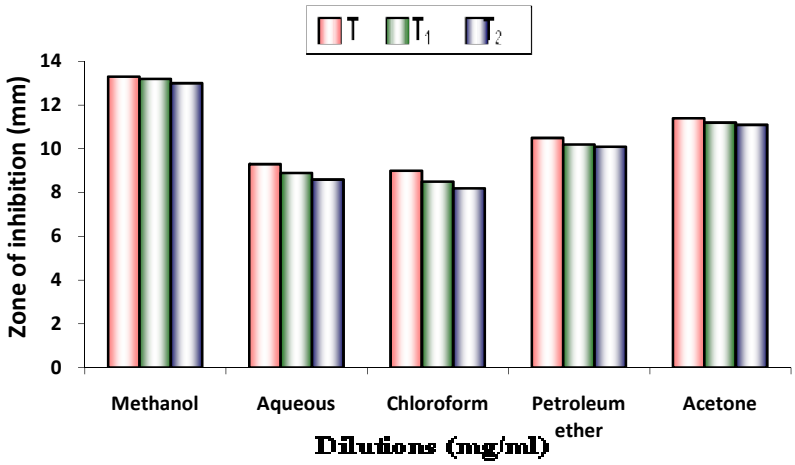

Fig. 3: Graphical representation of zone of inhibition of different root fractions of *Cichorium intybus* against *P. aeruginosa.*

318 **Table 4. Zone of inhibition of different leaf fractions of *Cichorium intybus* against *P. aeruginosa*.**
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Plant part	solvent	Concentration (mg/ml)	Zone of inhibition (mm)
Leaf	Methanol	200	12.8 ± 0.12
		100	12.7 ± 0.08
		50	12.5 ± 0.04
	Aqueous	200	8.8 ± 0.08
		100	8.4 ± 0.08
		50	8.1 ± 0.04
	Chloroform	200	8.5 ± 0.04
		100	8.1 ± 0.08
		50	7.7 ± 0.08
	Petroleum ether	200	10.0 ± 0.12
		100	9.5 ± 0.12
		50	9.1 ± 0.04
	Acetone	200	10.9 ± 0.08
		100	10.4 ± 0.08
		50	10.1 ± 0.04
Cephotaxime		30 mcg	20. l

320 ±: Standard Deviation
321 The different concentration of all the test extracts does not have significant effect at 0.05 level of
322 significance ($p < 0.05$).

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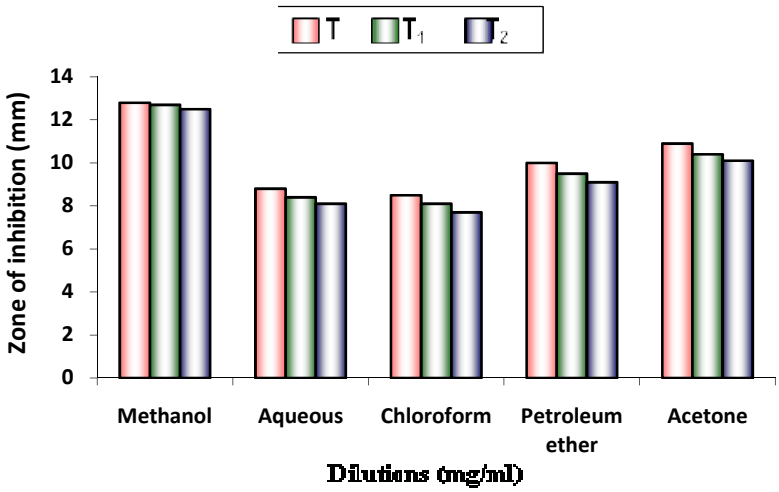

Fig. 4: Graphical representation of zone of inhibition of different leaf fractions of *Cichorium intybus* against *P. aeruginosa.*

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