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* (1 year old) were collected from Hindustan Uniliver Pvt. Ltd., Etah Kasganj Road, Etah and its nearby areas.

2.2 Extraction of active principles:

A thimble was prepared by using 0.5 mm Wattman filter paper. About 25 gm of powdered material (root and leaf of different stages) was uniformly packed in a thimble and run in soxhlet extractor. The extraction was carried out in methanol, distilled water, chloroform, petroleum ether and acetone (Merck).The thimble was placed in an extraction chamber that was suspended above the flask containing the solvent and below a condenser. The flask was heated at the boiling temperature and the solvent get evaporated and moved into the condenser where it was converted into liquid that trickled into the extraction chamber containing the plant material. The extraction chamber was designed so that when the solvent surrounding the sample exceeded a certain level it overflowed and trickled back down into the boiling flask. At the end of the extraction process, the flask containing the extract was removed and solvent was evaporated by using rotary evaporator. Then extract was kept in refrigerator at 4ºC to determine antibacterial properties. The extraction was carried three times to get the desired amount of crude extract (3 replicates).

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. Both the stains are gram negative, which are resistant to most of the synthetic antibiotics. The bacterial strains 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 DMSO (Dimethyl sulphoxide). 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. On the other hand inocula were prepared by emulsifying some colonies from the pure culture in nutrient broth (7 µl/ml broth). These innocula were kept at 37ºC for overnight in incubator. The inoculum was adjusted to McFarland 0.5 turbidity standard by adjusting the OD of the solution and inoculum to 0.08 – 0.1 at 625 nm. The plates

were inoculated with the bacterial cell culture of concentration 10^8 CFU/ml. Sterile discs loaded with extracts were placed on inoculated surface of nutrient 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:

The data of antibacterial activity of *Cichorium intybus* L. was 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. There is no comparison between root and leaf extract and also between solvent forever comparison was made among different concentration at the same solvent.

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.* 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 *C. intybus.* It is also clear that methanol and acetone were the best extractive solvents for the antibacterial activity against the pathogens used. This is in accordance with the results reported by [14] in *C. intybus.* Most of antibacterial agents in plants are soluble in methanol [15]. This may be attributed to the presence of soluble phenolic and polyphenolic compounds [16]. The low activity of the water extract against most bacterial strains investigated in this study is in agreement with previous reports which show that aqueous extract of plants generally have little or no antibacterial activities [17 – 18].

According to [19] it may be possibly because some active substances are present in water extract but in low concentration. Active substances are soluble in organic fractions and therefore not present in water extract.

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 [20] 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 sensitivity 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 ($p < 0.05$). Means are significantly different for all the solvent extracts.

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 ($p < 0.05$). Means are significantly different for petroleum ether, aqueous, chloroform extracts and highly different for acetone extract.

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

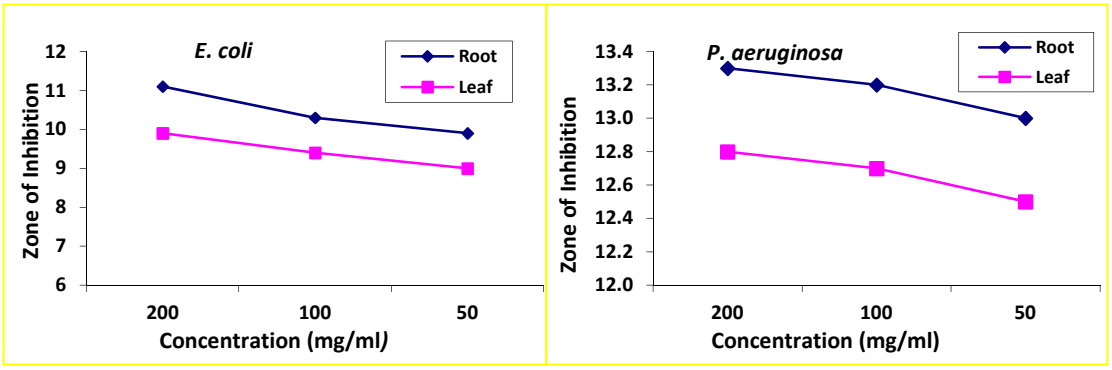
The different concentration of all the test extracts does not have significant effect at 0.05 level of significance ($p < 0.05$). Means are not significantly different for all the solvent extracts.

Table 4. Zone of inhibition of different leaf fractions of *Cichorium intybus* against *P. aeruginosa*.

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

±: Standard Deviation

The different concentration of all the test extracts does not have significant effect at 0.05 level of significance ($p < 0.05$).). Means are not significantly different for all the solvent extracts.



Ia Ib

Fig. 1a-b: Comparative antibacterial activity of root and leaf extracts against *E. coli* and *P. aeruginosa,* respectively

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REFERENCES

1. Ebna RUB, Madunagu BE, Ekpe ED, Otung IN. Microbiological exploitation of cardiac glycoside and alkaloids from *Garcinia kola, Borreria ocymoides, Kola nitida* and *Citrus aurantifolia.* J Appl Biotechnol. 1991; 71:398-401.
2. Williams VL. The Witwater Strand Multritrade. Veld and Flora. 1996; 82:12-14.
3. Pamplona–Roger GD. Encyclopedia of Medicinal Plants. Vol. 1 and 2, 2nd Ed. Education and Health Library. The European Union U.K; 1999.
4. Manna A, Abalaka ME. Preliminary screening of the various extracts of *Physalis angulata* for antimicrobial activities. Spectrum Journal. 2000; 7:119-125.
5. Shariff ZU. Modern Herbal Therapy for Common Ailments. Nature Pharmacy Series (Volume 1), Spectrum Books Limited, Ibadan, Nigeria in Association with Safari Books (Export) Limited, United Kingdom; 2001.
6. Kroschwitz JI, Howe-Grant M. Kirk-Othmer encyclopedia of chemical Technology. 1992; 2: 893.
7. Newman DJ, Cragg GM, Snader KM. The influence of natural products upon drug discovery. Nat Prod Res. 2000; 17: 215-234.
8. Varotto S, Lucchin M, Parrin P. Immature embryos culture in Italian red Chicory (*Cichorium intybus*). Plant Cell Tiss Org Cult. 2000; 62: 75-77.
9. Duke JA. Medicinal Plants of the Bible. (Illustrated by Peggy K. Duke) Out of print Trado Medic Books Buffalo and NY; 1983.
10. Angelina QA, Dolores J. Antitumor activity of Pyrimidine derivatives of sesquiterpene lactones. J Pharm Pharmaceut Sci. 1999; 3: 108-112.
11. Bischoff TA, Nguyen-Dinh P, Arefi AG, Laurantos M, Kelley CJ, Karchesy Y. Antimalarial activity of Lactucin and Lactucopicrin: sesquiterpene lactones isolated from *Cichorium intybus*. J Ethnopharmacol. 2004; 95: 455-457.

12. Farrukh A, Iqbal A. Broadspectrum antibacterial and antifungal properties of certain traditionally used Indian medicinal plants. World J Microbiol Biotechnol. 2003; 19: 653-657.

13. Mukherjee PK, Giri SN, Pal M, Saha BP. Antibacterial efficiency of *Nelumbo nucifera* (Nymphaceae) rhizome extract. Indian drugs. 1996; 32:274–276.

14. Deans SG, Baratta MT. Antimicrobial and Antioxidant properties of some essential oils. Flau Fragrance. 1998; 13: 235-244.

15. Chandrasekaran N, Venkatesalu V. Antibacterial and antifungal activity of *Syzygium jabolanum* beets. J Ether Pharmacol. 2009; 91: 105 – 108.

16. Kowalski R, Ketzia B. Antibacterial activity of *Silphium perftidum* extracts. Pharm Biol. 2007; 45: 495 – 500.

17. Aliero AA, Griersm DS, Afolayan AJ. Antifungal activity of *Solanum pseudocapsicum*. Res J Bot. 2006; 1: 129 – 133.

18. Aiyegose OA, Akinpelr DA, Afolayan AJ, Okoh AI. Antibacterial activities of crude stem bark extracts of *D. benthamianus*. J Biol Sci. 2008; 8(2): 356 – 436.

19. DeBoer HJ, Kool A, Broberg A, Mziraf WR, Hedberg I, Leurfors JJ. Antifungal and antibacterial activity of some herbal remedies from Tanzania. J Etharophormacol. 2005; 96: 461 – 469.

20. Ahmad S, Sharma R, Mahajan S, Agnihotri RK, Gupta A. Antibacterial evaluation and preliminary phytochemical analysis of the leaf extract of *Holoptelea integrifolia.* J Pharm Res. 2012; 5(7): 3823-3825.