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Phytochemical and Antibacterial Studies of Chicory (*Cichorium intybus* L.) - A Multipurpose Medicinal Plant

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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 root extracts of chicory against pathogenic bacteria like gram positive (*Bacillus subtilis, Staphylococcus aureus* and *Micrococcus luteus*) and gram negative (*Escherichia coli* and S*almonella typhi*) bacteria by *in vitro* agar well diffusion method. The hexane and ethyl acetate root extracts of chicory showed pronounced inhibition than chloroform, petroleum ether and water extracts. Root extracts showed more inhibitory action on *Bacillus subtilis, Staphylococcus aureus* and S*almonella typhi* than *Micrococcus luteus* and *Escherichia coli*.

Key words: *Cichorium intybus* L. · antibacterial activity · root extracts · zone of inhibition · volatile compounds

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, antiinflammatory, 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, world wide 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 root against the important human pathogenic bacteria so far. In the

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present study we reported the antibacterial activity of *Cichorium intybus* L. against pathogenic bacteria. The study confirms that both aqueous as well as organic solvent root extracts possess strong antibacterial properties against various pathogens such as gram positive (*Bacillus subtilis*, *Staphylococcus aureus* and *Micrococcus luteus*) and gram negative (*Escherichia coli* and S*almonella typhi*) bacteria.

MATERIALS AND METHODS

Preparation of root extracts: Apparently healthy plant roots were collected, washed thoroughly in tap water and dried in room temperature for 15 days. The dried 25 g root was powdered and soaked separately in 100 ml petroleum ether, hexane, chloroform, ethyl acetate and water by keeping it in a shaker for 3 days. The extracts were filtered through cheesecloth and the extracts were reduced to 10% of its original volume. The organic solvent filtrates were concentrated in vacuum using a rotary evaporator, while aqueous extract was dried using water bath.

Phytochemical screening of root extracts: The phytochemical components of the chicory roots were screened by using the methods of Brindha *et al*. [18] and Harbone [19]. The components analysed were alkaloids, volatile oils, fatty acids, emodins, flavonoids, triterpenoids, antharacene glycosides, tannins, phenolics and saponins.

Separation of the compounds: The compounds present in chicory root extracts were qualitatively analyzed by using thin layer chromatography which is commercially available; TLC aluminum sheets with silica gel $60F_{254}$ were used. The isolation and separation of monoterpenes and sesquiterpenes was done by using the procedure of Brindha *et al*. [18] and Janusz Malarz [20].

Inoculums: The test microorganisms, gram positive (*Bacillus subtilis, Staphylococcus aureus* and *Micrococcus luteus*) and gram negative (*Escherichia coli* and S*almonella typhi*) bacteria were obtained from culture repository of Best Biotech culture collection, Bangalore, India. The organisms were inoculated into NB (Nutrient Broth) medium, (0.5% Peptone, 0.5% Sodium Chloride, 0.15% Yeast extract; pH 7.4) and incubated at 37°C for overnight. The bacterial cells were harvested by centrifuging at 5000g for 15 min. The pellet formed was washed twice with PBS (Phosphate Buffer Saline), (10 mM Sodium Chloride, pH 7.4) and the cells were counted by hemocytometer. The bacterial cells were diluted to approximately 10^5 CFU ml^{-1} before use [21].

Determination of antibacterial activity: The antibacterial activity of the root extracts 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 plant extracts. Control wells containing neat solvents (negative control) or standard antibiotic solution (positive control) viz., chloromphenicol (50 and 100 μg ml^{-1}) were also run parallel in the same plate [23]. The plates were incubated at 37°C for 18 h 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 drug chloromphenicol.

Statistical analysis: The resultant clear zones around the discs were measured in mm. The antibacterial activity of chicory root extracts was indicated by clear zones of growth inhibition. Data of three independent experiments represented by five replicates from each experiment were subjected to statistical analysis (Mean±SE), according to New Duncan's Multiple Range Test [24].

RESULTS

Phytochemical screening of root extracts: The preliminary phytochemical screening of the root extracts using different solvents was reported (Table 1). All the

Table 1: Preliminary phytochemical analysis of chicory root extracted with different solvents

S. No.	Name of the compound	PE	C	H	EA	W
1	Alkaloids	-	-	-	+	+
2	Volatile oils	+	+	+	+	-
3	Fatty acids	+	+	+	+	-
4	Emodins	-	-	-	-	-
5	Flavonoids	-	-	-	-	+
6	Triterpenoids	-	-	+	-	-
7	Antharacene glycosides	-	-	-	-	-
8	Tannins	-	-	-	-	+
9	Phenolics	-	-	-	-	-
10	Saponins	+	+	-	+	+

+ Present, -Absent, PE-Petroleum ether, C-Chloroform, H-Hexane, EA-Ethyl acetate, W-Water

Table 2: Antibacterial activity of root extracts of chicory at 50 µl concentration

	Zone of inhibition in mm (Mean±SD)				
	Gram(+)			Gram(-)	
Solvent	*B. subtilis*	*S. aureus*	*M. luteus*	*E. coli*	*S. typhi*
Petroleum ether	6.8±0.17^{cd}	5.8±0.21^{c}	4.2±0.13^{d}	4.7±0.41^{d}	3.5±0.31^{e}
Chloroform	4.1±0.25^{ef}	3.6±0.23^{d}	2.6±0.28^{e}	3.0±0.28^{e}	5.4±0.32^{c}
Hexane	12.3±0.21^{a}	9.8±0.03^{a}	8.0±0.41^{a}	9.6±0.32^{a}	7.5±0.44^{a}
Ethyl acetate	10.5±0.28^{b}	2.0±0.27^{e}	6.8±0.30^{b}	6.3±0.41^{bc}	3.0±0.25^{ef}
Water	4.8±0.25^{e}	2.8±0.23^{de}	3.3±0.23^{de}	1.9±0.35^{ef}	4.5±0.33^{d}
chloromphenicol	8.4±0.23^{c}	9.6±0.23^{ab}	6.0±0.14^{bc}	7.5±0.29^{b}	7.3±0.30^{ab}

The negative control wells were exposed with the neat solvent and the positive control was chloromphenicol (50 µg ml^{-1}). Each value represents the mean±standard error (SE) of five replicates per treatment in three repeated experiments. Means within a row followed by the same letters are not significant at p = 0.05 according to DMRT

Table 3: Antibacterial activity of root extracts of chicory at 100 µl Concentration

	Zone of inhibition in mm (Mean±SD)				
	Gram(+)			Gram(-)	
Solvent	*B. subtilis*	*S. aureus*	*M. luteus*	*E. coli*	*S. typhi*
Petroleum ether	14.3±0.39^{c}	10.10±0.16^{c}	5.8±0.33^{d}	8.0±0.33^{cd}	5.7±0.32^{de}
Chloroform	10.5±0.21^{d}	8.20±0.22^{cd}	4.8±0.32^{de}	3.2±0.30^{f}	14.0±0.31^{b}
Hexane	18.2±0.47^{a}	18.23±0.18^{a}	15.2±0.04^{a}	14.5±0.22^{a}	19.2±0.43^{a}
Ethyl acetate	15.0±0.17^{bc}	3.60±0.20^{e}	13.6±0.29^{ab}	10.1±0.26^{c}	10.7±0.30^{c}
Water	8.0±0.27^{e}	6.70±0.28^{d}	10.3±0.56^{c}	5.6±0.23^{e}	5.8±0.28^{d}
chloromphenicol	16.0±0.18^{b}	17.30±0.37^{ab}	13.0±0.33^{b}	13.5±0.37^{ab}	14.0±0.23^{b}

The negative control wells were exposed with the neat solvent and the positive control was chloromphenicol (100 µg ml^{-1}). Each value represents the mean±standard error (SE) of five replicates per treatment in three repeated experiments. Means within a row followed by the same letters are not significant at p = 0.05 according to DMRT

four organic solvents such as petroleum ether, chloroform hexane and ethyl acetate showed positive result for the presence of volatile oils and fatty acids which were absent in the water extract. The ethyl acetate and water extracts showed the presence of alkaloids. Only in the hexane extract presence of triterpenes was observed. In the water extract, flavonoids, tannins and saponins were present which were absent in the organic solvent extracts.

Antibacterial activity of different solvent extracts: We used both polar as well as non-polar solvents for the extraction of active components from the roots of chicory plant. The antibacterial activity of the chicory roots was assessed using the agar well diffusion method by measuring the diameter of growth inhibition zones with 50 and 100 µl of different solvent extracts (Table 2 and 3). The results showed that all the five solvent extracts possesses antibacterial activity against the tested gram positive (*Bacillus subtilis, Staphylococcus aureus* and *Micrococcus luteus*) and gram negative (*Escherichia coli* and *Salmonella typhi*) bacteria. At 50 µl concentration, the hexane extract showed pronounced inhibition against all the tested organisms, the maximum inhibition was observed on *B. subtilis* (12.3±0.21 mm), *S. aureus* (9.8±0.03 mm) and *E. coli* (9.6±0.32 mm) and moderate inhibition was observed on *M. luteus* (8.0±0.41 mm) and *S. typhi* (7.5±0.44 mm). The other solvents extracts did not actively inhibit the growth of the bacteria at 50 µl concentration except ethyl acetate against *B. subtilis* (10.5±0.21 mm) (Table 2). The growth of *B. subtilis* was inhibited by all the root extracts at 100 µl concentration and maximum inhibition was observed with hexane extract as 18.2±0.47 mm zone of inhibition, which was higher than the zone of inhibition caused by the standard drug chloromphenicol (16.0±0.18 mm). Similarly, hexane root extract produced maximum inhibition (18.0±0.18 mm) to the growth of *S. aureus* than chloromphenicol (17.3±0.37 mm) whereas other extracts showed less

inhibitory activity than chloromphenicol. The hexane (15.2±0.04 mm) and ethyl acetate (13.6±0.29 mm) extracts were effective against *M. luteus* and the water extract (10.3±0.56 mm) showed less inhibition than the standard drug whereas the other two extracts showed very minimum inhibition on the growth of *M. luteus*. The hexane (14.5±0.22 mm) and ethyl acetate (10.1±0.26 mm) root extracts showed maximum inhibition and petroleum ether extract (8.0±0.33 mm) showed moderate inhibition and the water (5.6±0.23 mm) and chloroform (3.2±0.30 mm) extracts showed less inhibition on *E. coli*. *S. typhi* was inhibited by all the root extracts and the maximum inhibition was observed with hexane (19.2±0.43 mm), chloroform (14.0±0.31 mm) and ethyl acetate (10.7±0.30 mm) whereas petroleum ether (5.7±0.32 mm) and water (5.8±0.28 mm) extracts showed least inhibition at 100 µl concentration (Table 3). We found that both aqueous as well as organic extracts of the roots were successful in inhibiting the bacteria in a dose dependent manner. Besides the 50 µl concentration of root extracts, the 100 µl concentration of root extracts were found to possess maximum inhibition (Table 2 and 3).

DISCUSSION

We used both the aqueous and organic solvents for the extraction of active components from roots of the chicory plant. The result of the study reveals that the non-polar and polar solvent extracts were active against the strains of the bacteria that are common cause of infections. Chicory shows significant antibacterial activity that may be due to the presence of many potent compounds such as inulin, bitter sesquiterpene lactones, coumarins, flavonoids etc. The biologically active compounds are screened by dissolving the crude powder on various compound specific solvents confirmed by the TLC (data not shown). The antibacterial activity was expressed at varying degrees which was being both strain and dose dependent. The various crude extracts of chicory showed significant activity against all the bacteria tested. Similar to our results, the biological activity of *Mentha piperita* against the pathogenic bacteria was reported [25]. We used five different solvent extracts of chicory roots which showed activity against all bacterial species tested at all the dosages. The root extracts of chicory exhibited antibacterial activity on hexane, ethyl acetate, petroleum ether, chloroform and aqueous extracts against the bacteria tested at 50 µl-100 µl concentrations by following the method of Valsaraj *et al*. [23]. We observed maximum activity at 100 µl concentration against the tested gram positive (*Bacillus subtilis*, *Staphylococcus aureus* and *Micrococcus luteus*) and gram negative (*Escherichia coli* and *Salmonella typhi*) bacteria. The present report indicates that increased lipophillic compounds are extracted using the petroleum ether, chloroform and hexane increased the suspended higher compounds in the above solvents as stated [26]. Chicory plant was active against gram positive and gram negative bacteria, yeast and filamentous fungi [17]. The antibacterial activity of water, ethanol and ethyl acetate extracts of chicory was reported against *Agrobacterium radiobacter*, *Erwinia carotovora*, *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* [27]. The present work shows that the compounds from chicory possess potent antimicrobial activity and suggesting that the chicory root extracts contains the effective active constituents responsible for eliminating the bacterial pathogens. Finally, it can be concluded that the active chemical compounds 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.

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