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Comparison of Antibacterial Effect of *Cichorium Intybus* L. with Vancomycin, Ceftriaxone, Ciprofloxacin and Penicillin (*In Vitro*)

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Abstract

Background and aim: Although antibiotics are used in the treatment of infectious diseases nevertheless, there are many problems such as adverse drug reactions and resistance to antibiotics. Plants, which may have less adverse reactions, can be suitable substitute for chemical drugs. *Cichorium intybus* L.which is one of the herbs that can be easily found in many areas of Iran, has antibacterial effect and can be used in the treatment of infectious diseases. The present study was planned for comparison of antibacterial effect of *Cichorium intybus* L. with vancomycin, ceftriaxone, ciprofloxacin and penicillin.

Methods: In the present experimental study *Streptococcus pyogen*, *Staphylococcus aureus* and *Enterococcus* were cultured on blood-agar medium. Alcoholic extract of *Cichorium intybus* L. (AECI) was added to culture media along with antibiotics (vancomycin, ceftriaxone, ciprofloxacin and penicillin) discs. Zone of inhibition of samples were measured and the data was analyzed by using Chi square and Fisher´s exact tests.

Results: AECI had no antibacterial effect on the respective microorganisms, whereas Vancomycin had antibacterial effect on *Staphylococcus aureus* and *Streptococcus pyogen* but no effect on *Enterococcus*. Ceftriaxone had antibacterial effect on *Enterococcus* and *Streptococcus pyogen* with no effect on *Staphylococcus aureus*. Ciprofloxacin had antibacterial effect on *Enterococcus* and *Staphylococcus aureus* and Penicillin had only effect on *Streptococcus pyogen*.

Conclusion: AECI had no antibacterial effect on *Streptococcus pyogen*, *Staphylococcus aureus* and *Enterococcus*. We conclude that other extracts of *Cichorium intybus* L. such as aqueous or ethyl acetate may have antibacterial effect on gram positive bacteria which requiring more studies to prove.

Keywords: Ceftriaxone; *Cichorium intybus* L; Ciprofloxacin; Penicillin; Vancomycin

Introduction

Incorrect use of antibiotics causes increase of resistance to microorganisms. This not only increase mortality rate but also induce economical damages [1]. Side effects and adverse drug reactions are the biggest problems in the treatment of diseases and are the fourth factor of death in the USA [2,3]. Therefore, actions must be taken to reduce this problem, for example, to develop new drugs, either from synthetic or natural sources [4]. *Cichorium intybus* L. (Compositae family) is a widespread weed with antibacterial effect. Its habitants are roadsides, railroads and waste grounds, flowering period lasts from June to October. Leaves of the plant contain salts such as sulphates and phosphates of sodium, magnesium and potassium as well as potassium nitrate. It also contains a bitter glycoside named cichorine [5,6]. In traditional medicine, all parts of the plant specially root and leaves are used as diuretic, laxative, antibilious, antipyretic, blood purification and strengthen of the stomach. It is also used as an appetizer as well as in the treatment of hepatic failure, jaundice, intermittent fever and mild states of chronic skin diseases [5].

Penicillins are bacteriocidal antibiotics and are active against gram positive and gram negative microorganisms. Ceftriaxone belongs to the third generation of cephalosporins with increasing effect on gram negative bacteria. Ciprofloxacin is a fluoroquinolone and is mainly used in the treatment of Enterobacter and other gram negative bacillus infectious. Vancomycin is effective against gram positive bacteria in particular Staphylococcus [7]. The objective of present study was comparison of antibacterial effect of alcoholic extract of *Cichorium intybus* L. (AECI) with vancomycin, ceftriaxone, ciprofloxacin and penicillin against gram positive bacteria *in vitro*.

Materials and methods

All chemicals used, were of analytical grade. The bacterial species of *Staphylococcus aureus*, *Streptococcus pyogen* and *Enterococcus* were purchased from Pasteur Institute, Tehran, Iran and were cultured on blood agar medium. Leaves of *Cichorium intybus* L was collected locally, authenticated by botanist and a voucher specimen preserved at Birjand University of Medical Sciences (BUMS). Then leaves was dried in shade and pulverized to fine particles. The obtained powder was macerated in ethanol 96% for duration of 72 h by shaking the mixture every 12 h. The final extract was passed through No. 1 whatman filter paper and the obtained filtrate was concentrated under vacuum on a rotary evaporator and stored for further use. Three concentrations of the extract containing 10%, 15% and 20% were prepared in sterile distilled water and ethanol with the proportion of nine to one respectively.

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The antibacterial activity of samples was determined by well and disc diffusion methods by some modifications [8,9]. A suspension of 0.5×10^8 microorganism per mL of three bacteria including *Streptococcus pyogen*, *Staphylococcus aureus* and *Enterococcus* were prepared. The obtained suspensions with the turbidity of 0.5 McFarland were spread on the plates containing blood agar. Then seven wells with the diameter of 6 mm were made on the plate with the distance of at least 2 cm from each other and 1.5 cm from the edge of culture medium. On each plate one well for negative control and for each concentration of AECI, two wells were used. The experiment was carried out 15 times for each microorganism. The discs of four reference antibiotics including vancomycin, ceftriaxone, ciprofloxacin and penicillin purchased from Padtan, Tehran Teb Company were also placed on the cultured plates. In this case for each microorganism, it was performed six times. All the plates were incubated at 37°C for 24 h and antibacterial activity was evaluated by measuring the diameter of the inhibition zone in mm. According to the report of NCCLS the zone of inhibition (ZOI) was categorized in to sensitive, semi-sensitive and resistant [10]. Data was collected and analyzed with Chi square and Fisher´s exact test by using SPSS software.

Results

ZOI of AECI and antibiotics and their sensitivity to microorganisms have been presented in Table 1. In this experimental study, the AECI with three concentrations (10, 15 and 20%) had no effect on *Streptococcus pyogen*, *Staphylococcus aureus* and *Enterococcus*. As, it was shown in Table 2, Vancomycin had antibacterial effect on *Staphylococcus aureus* and *Streptococcus pyogen* but no effect on *Enterococcus*. Ceftriaxone had antibacterial effect on *Enterococcus* and *Streptococcus pyogen* with no effect on *Staphylococcus aureus*. Ciprofloxacin had antibacterial effect on *Enterococcus* and *Staphylococcus aureus* and Penicillin had only effect on *Streptococcus pyogen*.

Discussion

The AECI in the concentrations of 10%, 15% and 20% did not exhibited any effect on gram positive bacteria including *Streptococcus pyogen*, *Staphylococcus aureus* and *Enterococcus* (five strains for each bacterium). Mosadegh et al. have been indicated that *Cichorium intybus* L. had partly antiobacterial effect on *Escherichia coli* and *Pseudomonas aeruginosa* [11]. According to this study *Cichorium intybus* L. had antibacterial effect on gram negative bacteria whereas our research was on gram positive bacteria. The results obtained from Petrovic et al. displayed that alcoholic, aqueous and ethyl acetate extracts of *Cichorium intybus* L. had antibacterial effect and in case of ethyl acetate extract this effect was prominent [12]. They had also showen that aqueous extract had antibacterial effect on *Pseudomonas aeruginosa*. In our study, alcoholic extract of plant was used and may be by using other extracts such as aqueous or ethyl acetate, the same results have been obtained which requiring more studies.

Shirazi et al. [13] studied the antibacterial effect of ten herbal extracts including *Glycyrrhiza glabra* L., *Salvia officinalis* L., *Myrtus*

Antibiotic	Concentration	Sensitive (mm)	Semi-sensitive (mm)	Resistant (mm)	Statistical test and significancy	Particulars
ciprofloxacin	5 µg	≥ 21	16-20	≤ 15	X2=83.5, p<0.001	-
Ceftriaxone	30 µg	≥ 21	14-20	≤ 13	fisher´s exact test=101.1, p<0.001	-
Penicillin	10 unit	≥ 29	--	≤ 28	fisher´s exact test=10.09, p<0.003	When testing staphylococci
	10 unit	≥ 15	--	≤ 14	fisher´s exact test=10.09, p<0.003	When testing enterococci
	10 unit	≥ 28	20-27	≤ 19	fisher´s exact test=10.09, p<0.003	When testing streptococci (not s. pneumoniae)
vancomycin	30 µg	≥ 17	15-16	≤ 14	X2=61.07, p<0.001	When testing enterococci
	30 µg	≥ 12	10-11	≤ 9	X2=61.07, p<0.001	When testing other positive organisms

Table 1: Zone of inhibition (mm) according to type of sensitivity.

Bacterium type		Antibiotics	Sensitive (no. of strain)	Semi-sensitive (no. of strain)	Resistant (no. of strain)	Total
Streptococcus		Vancomycin	3	0	2	5
pyogen		Ceftriaxone	0	1	4	5
		Ciprofloxacin	0	1	4	5
		Penicillin	0	1	4	5
		AECI (10, 15 and 20%)	0	0	15	15
		Ethanol 96%	0	0	15	15
aureus	***Staphylococcus***	Vancomycin	5	0	0	5
		Ceftriaxone	0	0	5	5
		Ciprofloxacin	0	2	3	5
		Penicillin	0	0	5	5
		AECI (10, 15 and 20%)	0	0	15	15
		Ethanol 96%	0	0	15	15
Enterococcus		Vancomycin	0	0	5	5
		Ceftriaxone	5	0	0	5
		Ciprofloxacin	5	0	0	5
		Penicillin	0	0	5	5
	AECI (10, 15 and 20%)		0	0	15		15
	Ethanol 96%		0	0	15		15

Table 2: Comparison of *in vitro* antibacterial effect of alcoholic extract of *Cichorium intybus* L. (AECI) with ceftriaxone, ciprofloxacin, penicillin and vancomycin.

communis L., *Achillea millefolium* L., *Cichorium intybus* L., *Citrus bigaradia L.*, *Artemisia absinthium* L., *Peganum harmala* L., *Heracleum persicum* Desf., and *Melia ozedarach* L. on *Helicobacter pylori* by disc diffusion method. The results of the research indicated that extracts of *Glycyrrhiza glabra* L., *Salvia officinalis* L., *Myrtus communis* L., *Artemisia absinthium* L. and *Melia ozedarach* L. showed inhibitory effect on the growth of *Helicobacter pylori* whereas *Cichorium intybus* L. did not show remarkable effect [13]. Antiviral effects of 20 medicinal plants which are traditionally used against infectious diseases were evaluated by zyaei et al. [14]. They revealed that *Aristolochia*, *Terminalia chebula* Retz and *Cichorium intybus* indicated antiviral effect on adenoviruses. The root of *Cichorium intybus* also inhibited replication of Herpes type one [14]. In a clinical trial on children who used toothpastes containing extracts of *Salvia officinalis* L., *Cichorium intybus* L. and *Salvadora persica* L., the microbial plaque and gingivitis decreased significantly compared to children who used normal toothpastes [15].

According the study of Aqil and Ahmad [16], the extract of *Cichorium intybus* L. had mild antibacterial effect against *Escherichia coli* and *Staphylococcus aureus*. They also showed that there is a synergistic antibacterial effect between the respective medicinal plants with tetracycline, chloramphenicol and ciprofloxacin. This evidence displays that *Cichorium intybus* L. has synergistic effect with some antibiotics which is needed more research on this issue.

It was concluded that AECI had no antibacterial effect on gram positive bacteria including *Streptococcus pyogen*, *Staphylococcus aureus* and *Enterococcus*. Other extracts of *Cichorium intybus* L. such as aqueous or ethyl acetate or higher concentration of AECI may have antibacterial effect on gram positive bacteria which requiring more studies to prove.

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