Investigate the antimicrobial effect of chicory leaf extract with different solvents on *Staphylococcus aureus* and *Escherichia coli*

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Abstract

Recent studies have found some of the important constituents in chicory such as caffeic acid derivatives, fructooligosaccharides, flavonoids, inulin, and polyph-enol. *Cichorium intybus* L. is a widespread weed with antibacterial effect. In other reports on the antimicrobial activity of *C. intybus*, the crude aqueous and organic seed extracts were found to be active against four pathogenic microorganisms, namely, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Candida albicans*, and root extracts had pronounced effects on *Bacillus subtilis*, *S. aureus*, *Salmonella typhi*, *Micrococcus luteus*, and *E. coli*. The main goal of our research is to investigate the antimicrobial effect of chicory extract with different solvents on *Staphylococcus aureus* and *Escherichia coli*. In our research the antimicrobial activity of the *C. intybus* leaves extract and its different fractions was determined by the disc diffusion method. In this study, 67% ethanol, 67% acetone and 67% hexane were used for extraction. Three Blank discs placed in each extract dilution tube and after 30 minutes, disks were removed from the tubes and placed in an incubator. For control group we used Blank discs with solvents that they were dried in same method. We were designed 4 disks per culture plate for each dilution that one of them was control disk. *Staph*- aureus and *E-Coli* antibiograms with different solvents had no significant difference between groups and no inhibition zone observed around different extract disks and control disks on Mueller-Hinton agar culture. In our research we couldn't find any chicory leaves extract antibacterial effect against *S.aureus* and *E.coli*.

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Introduction

*Cichorium intybus* L is a perennial herb of 1.0 to 1.8 m height with a deep dandelion type root and bright blue flowers. Chicory is one of the earliest known and most widely used raw materials for manufacturing of coffee substitutes (Pazola 1987). The name of the plant is derived from Greek and Latin. *Cichorium* means field and *intybus* is partly derived from the Greek “to cut”, because of the leaves, and partly from the Latin tubus to indicate the hollow stem (EMA 2013). *Cichorium intybus* L is a member of the family Asteraceae. It is an as an important medicinal herb has been used Ayurveda, Unani and Siddha system of medicine for diseases of hepatobiliary system and renal system (Zaman et al. 2013, Zargari 1996).

Recent studies have found some of the important constituents in chicory such as caffeic acid derivatives, fructooligosaccharides, flavonoids, inulin, and polyphenol *Cichorium intybus* L. (Compositae family) is a widespread weed with antibacterial effect (Zaman et al. 2013, Tabrizi et al 2014). Historically, chicory was grown by the ancient Egyptians as a medicinal plant, coffee substitute, and vegetable crop and was occasionally used for animal forage. In the 1970s, it was discovered that the root of *C. intybus* contained up to 40% inulin, which has a negligible impact on blood sugar and thus is suitable for diabetics (Judsoniene et al. 2008, Zargari 1996). The experimental plant Chicory (*Cichorium intybus* L) grows as a wild plant on roadsides in its native Europe, and in North America and Australia, where it has become naturalized. It is variously used as a tonic and appetite stimulant, and as a treatment for gallstones, gastro-enteritis, sinus problems cuts and bruises. Chicory is well known for its toxicity to internal parasites (Zubair et al. 2012). The antibacterial activity of the organic acid-rich extract of fresh red chicory (*C. intybus* var. sylvestre) was tested against periodontopathic bacteria including *Streptococcus mutans*, *Actinomyces naeslundii*, and *Prevotella intermedia*. The compounds identified from the active extract include oxalic acid, succinic acid, quinic acid, and shikimic acid. All of the organic acids were found to decrease biofilm formation and adhesion of bacteria to the cells, with different levels of efficacy. These compounds also induced biofilm disruption and detachment of dead cells for the cultured substratum (Gazzani et al. 2000). In other reports on the antimicrobial activity of *C. intybus*, the crude aqueous and organic seed extracts were found to be active against four pathogenic microorganisms, namely, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Candida albicans*, and root extracts had pronounced effects on *Bacillus subtillis*, *S. aureus*, *Salmonella typhi*, *Micrococcus luteus*, and *E. coli* (Nandagopal et al. 2007, Shaikh et al 2012). The leaf extract of *C. intybus* also showed a moderate activity against multidrug resistant *S. typhi* (Rani et al. 2004). Guananolides rich root extracts of *C. intybus* have shown antifungal properties against anthropophilic fungi Trichophyton tonsurans, *T. rubrum*, and *T. violaceum* (Mares et al. 2005). A sesquiterpenoid phytoalexin cichoralexin isolated from chicory exhibited potent antifungal activity against *Pseudomonas cichorii* (Monde et al. 1990). Also some scientists have told the antimicrobial properties of other parts of this plant, for example in a research conducted by Koner et al. (2011) antibacterial effects of different solvent extracts of chicory roots have been studied. In Koner et al. research *Staphylococcus aureus*, *Bacillus subtillis*, *Pseudomonas fluorescens*, *Rhizobium leguminosarum*, *Escherichia coli* and *Vibrio cholera* were selected and after research they were found antibacterial effects of chicory root (Koner et al. 2011). There was not many reports about chicory leaf so we decided to investigate the antibacterial effects of chicory leaves extract. The main goal of our research is to investigate the antimicrobial effect of chicory extract with different solvents on *Staphylococcus aureus* and *Escherichia coli*.

Material and methods

Extraction

Leaves of the selected medicinal plant *Cichorium intybus* were purchased from the local market of Arasbaran and identified from the Department of botany and Agricultural, Islamic Azad University, Tabriz branch, Iran. After preparation of the leaves, they were dried by ambient temperature. Then the
leaves were crushed using a ball mill and Leaf powder was obtained. In this study, 67% ethanol (solvent I), 67% acetone (solvent II) and 67% hexane (solvent III) were used for extraction (table 1-1). After mixing the solvent and chicory leaves (18gr leaf powder and 100 ml solvent), it made 48 hours in the darkroom, and then extracted with rotary device. Extract was filtered by filter paper (125 mm, produced by Whatman Company) after extraction.

The antimicrobial activity of the C. intybus leaves extract and its different fractions was determined by the disc diffusion method. For this research nine experimental tubes were selected and 0.5 ml of Dimethyl sulfoxide (DMSO-Merck©) was poured for all tubes. 0.5 ml of extract was added to the first tube, and then by 0.5 ml transferring, serial dilutions were prepared. At the end 0.5 ml was poured out from last tube. This method used for each extract individually (67% ethanol, 67% acetone and 67% hexane). After all, three Blank discs (6.4mm diameter, produced by Padtanteb Company) placed in each tube and after half an hour, disks were removed from the tubes and placed in an incubator with 37±2 degrees Celsius for 20 minutes, for drying. After drying, disks were ready to use. For control group we were used Blank discs with solvents that they were dried in same method.

For Antibiogram method we were used Mueller-Hinton agar culture that produced by Merck Company. 24 hour BHI-broth culture was used for this research. For Mueller-Hinton agar culture, we were used McFarland half. We were designed 4 disks per culture plate for each dilution that one of them was control disk (dried extract-free solvent). Cultures were placed in an incubator at 37 degrees Celsius for 18 hours. We were used these levels for each solvents and each bacteria individually. For this research Staphylococcus aureus (PTCC1431) and Escherichia coli (PTCC1399) were used.

**Results**

In this research by all different solvents, there was no antibiotic effect from chicory extracts (table1-3). Staphylococcus aureus and Escherichia coli antibiograms with different solvents had no significant difference between groups and no inhibition zone observed around different extract disks and control disks on Mueller-Hinton agar culture.

<table>
<thead>
<tr>
<th>Tube number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilution</td>
<td>1/2</td>
<td>1/4</td>
<td>1/8</td>
<td>1/16</td>
<td>1/32</td>
<td>1/64</td>
<td>1/128</td>
<td>1/256</td>
<td>1/512</td>
</tr>
<tr>
<td>ml</td>
<td>0.5</td>
<td>0.5</td>
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</table>

**Discussion**

In this research we used Hexane, Ethanol and Acetone solvents. Ethanol (ethyl alcohol, grain alcohol) is a clear, colorless liquid with a characteristic, agreeable odor (Mohagheghi et al. 2011, Reichard 2011). In dilute aqueous solution, it has a somewhat sweet flavor, but in more concentrated solutions it has a burning taste (Reichard 2011) Ethanol, CH₃CH₂OH, is an alcohol, a group of chemical compounds whose molecules contain a hydroxyl group, –OH, bonded to a carbon atom (Reichard 2011). The selection of hexane as the solvent of choice for extraction of oils, other nonpolar constituents from plant foods, or removal of undesirable constituents from plant foods is one of the most common practices in the food industry (Reichard 2011, Liauw et al. 2008, Sepidar et al. 2009). The solvent extraction process generally involves solubilization and partitioning in hexane, separation of the extract, and solvent volatilization and removal to recover the extracted constituents (Liauw et al. 2008, Sotillo et al. 1994, Mohagheghi 2011). Acetone is the organic compound with the formula (CH₃)₂CO. It is a colorless, volatile, flammable liquid, and is the simplest ketone (Mohagheghi et al. 2011, Reichard 2011).
In this research by all different solvents, there was no antibiotic effect on chicory extracts. Staphylococcus aureus and Escherichia coli antibiograms with different solvents had no significant difference between groups and no inhibition zone observed around different extract disks and control disks on Mueller-Hinton agar culture. Escherichia coli is a Gram negative rod (bacillus) in the family Enterobacteriaceae (Reichard 2011, Quinn et al. 1994, Naderianasab et al. 1997, Tadjbakhsh 1997). Few microorganisms are as versatile as Escherichia coli. An important member of the normal intestinal microflora of humans and other mammals, it can be a highly versatile, and frequently deadly, pathogen.

Several different E. coli strains cause diverse intestinal and extraintestinal diseases by means of virulence factors that affect a wide range of cellular processes (Naderianasab et al. 1997, Kaper et al. 2004). Escherichia coli typically colonizes the gastrointestinal tract of some mammals and human infants within a few hours after birth. Usually, E. coli and its host coexist in good health and with mutual benefit for decades. These commensal E. coli strains rarely cause disease except in immunocompromised hosts or where the normal gastrointestinal barriers are breached (Kaper et al. 2004, Tabatabayi et al. 2011, Tadjbakhsh 1997).

Table 1-3. Antibiogram results by different solvent extracts.

<table>
<thead>
<tr>
<th>Staphylococcus aureus</th>
<th>Escherichia coli</th>
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<tbody>
<tr>
<td>Solvent I extract</td>
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<td>Solvent I extract</td>
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<td>1/4 Negative 1/4</td>
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<td>1/8 Negative 1/8</td>
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<tr>
<td>1/16 Negative 1/16</td>
<td>Negative 1/16</td>
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<tr>
<td>1/32 Negative 1/32</td>
<td>Negative 1/32</td>
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<tr>
<td>1/64 Negative 1/64</td>
<td>Negative 1/64</td>
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<tr>
<td>1/128 Negative 1/128</td>
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<td>1/256 Negative 1/256</td>
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Staphylococci are Gram-positive bacteria, with diameters of 0.5 – 1.5 μm and characterised by individual cocci, which divide in more than one plane to form grape-like clusters (Reichard 2011, Farrell et al. 2002, Kloos et al. 1991). To date, there are 32 species and eight sub-species in the genus Staphylococcus, many of which preferentially colonise the human body (Quinn et al. 1994, Kloos et al. 1991). S. aureus is considered to be a major pathogen that colonises and infects both hospitalised patients with decreased immunity, and healthy immunocompetent people and animals in the community. This bacterium is found naturally on the skin and in the nasopharynx of the some animals and human body. It can cause local infections of the skin, nose, urethra, vagina and gastrointestinal tract, most of which are minor and not life-threatening (Shulman et al. 1972, Tabatabayi et al. 2011, Tadjbakhsh 1997).

The excessive use of antibiotics has led to the emergence of multiple drug resistant S. aureus strains (Lowy 1998). Penicillin was introduced for treating S. aureus infections in the 1940s, and
effectively decreased morbidity and mortality. However, by the late 1940s, resistance due to the presence of penicillinase emerged (Eickhoff 1972). The staphylococci are very capable of evolving resistance to the commonly used antimicrobial agents, such as, erythromycin (Tabrizi et al. 2014), ampicillin (Klein and Finland 1963), and tetracycline (Lowy 1998).

Cichorium intybus is a medicinal and culinary herb which is used in traditional system of medicine since many years, even though it has many medicinal uses but still it is necessary to scientifically validate with experimental and clinical study(Ghaderi et al. 2012). Some scientists have told the antimicrobial properties of this plant, for example in a research conducted by Koner et al. (2011) antibacterial effects of different solvent extracts of chicory roots have been studied. In Koner et al. research Staphylococcus aureus, Bacillus subtilis, Pseudomonas fluorescens, Rhizobium leguminosarum, Escherichia coli and Vibrio cholera were selected and after research they were found antibacterial effects of chicory root (Koner et al. 2011). Also in research that designed by Ghaderi et al. (2012) Comparison of Antibacterial Effect of Cichorium Intybus L. with Vancomycin, Ceftriaxone, Ciprofloxacin and Penicillin (In Vitro) have been studied. In Ghaderi et al. (2012) research, it was concluded that Alcoholic extract of Cichorium intybus L. had no antibacterial effect on gram positive bacteria including Streptococcus pyogen, Staphylococcus aureus and Enterococcus (Ghaderi et al. 2012). In other research conducted by Zubair et al. (2012) they found out the methanolic extract and ethylacetate fraction of seeds exhibited good antioxidant activity. The various fractions of C. intybus showed moderate activity as antibacterial agent while antifungal activity of C. intybus extract/fractions was very low against A. flavus and A. niger while mild against R. solani. A compaeison among the results leads them to the conclusion that constituents of this plant extract may serve as a source of drugs useful in the chemotherapy of some infections caused by bacteria and also as an antioxidant agent (Zubair et al. 2012). According to these researches we were decided to investigate the antimicrobial effect of chicory extract with different solvents on Staphylococcus aureus and Escherichia coli but in our research there was no significant difference between groups, which mean chicory leaf extract had no antibacterial effect on Staphylococcus aureus and Escherichia coli.

Conclusion

In our research we couldn’t find any antibacterial effect from chicory leaves extract against S.aureus and E.coli.

Acknowledgments

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