Study antibacterial effects of cinnamon extract on

Staphylococcus aureus and Escherichia coli

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Abstract

Cinnamon (Cinnamomum verum, synonym C. zeylanicum) is a small evergreen tree, 10-15 meters (32.8-49.2 feet) tall, belonging to the family Lauraceae, native to Sri Lanka and South India. For this research 95% ethanol was used as solvent for extraction of cinnamon. Nine experimental tubes were selected and 0.5 ml of Dimethyl sulfoxide 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. We were designed 4 disks per culture plate for each dilution that one of them was control disk. For this research Staphylococcus aureus (PTCC1431) and Escherichia coli (PTCC1399) were used. In staphylococcus aureus cultures there were inhibition zones around disks with cinnamon extract. This inhibition zones were on 1/2, 1/4, 1/8 Dilutions. Also in Escherichia coli cultures there were inhibition zones on 1/2, 1/4, 1/8 dilutions.

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Introduction

The growing concern about food safety has recently led to the development of natural antimicrobials to control food borne pathogens and spoilage bacteria (Snyder 1997). Spices are one of the most commonly used natural antimicrobial agents in foods and have been used traditionally for thousands of years by many cultures for preserving foods and as food additives to enhance aroma and flavor. The antimicrobial properties of some spices and their components have been documented (Shelef 1983, Hirasa and Takeanasa 1998, Nevas et al. 2004, Kim et al. 1994). Cinnamon (Cinnamomum verum, synonym C. zeylanicum) is a small evergreen tree, 10-15 meters (32.8-49.2 feet) tall, belonging to the family Lauraceae, native to Sri Lanka and South India (Dragland et al. 2003). The flowers, which are arranged in panicles, have a greenish colour and have a distinct odour. The fruit is a purple one-centimeter berry containing a single seed. Its flavour is due to an aromatic essential oil which makes up 0.5 to 1% of its composition (Dragland et al. 2003, Friedman et al. 2002). In medicine it acts like other volatile oils and once had a reputation as a cure for colds. It has also been used to treat diarrhoea and other problems of the digestive system. Cinnamon is high in antioxidant activity. The essential oil of Cinnamon also has antimicrobial properties, which aid in the preservation of certain foods (Jakhhetia et al. 2010). "Cinnamon" has been reported to have remarkable pharmacological effects in the treatment of type II diabetes. Cinnamon has traditionally been used to treat toothache and fight bad breath and its regular use is believed to stave off common cold and aid digestion. The Cinnamon is having essential oils, resinous compounds, Cinnamic acid, Cinnamaldehyde and Cinnamate. Essential oil such as trans-cinnamaldehyde, caryophyllene oxide, L-borneol, L-bornyl acetate, eugenol, b-caryophyllene, E-nerolidol, and cinnamyl acetate was reported by Tung et al. Some other constituents are Terpinolene, α-Terpineol, α-Cubebene, and α-Thujene. (Jakhhetia et al. 2010) Several articles have been written about the effects of cinnamon for example In Al-Kassie (2010) research, effect of thyme and cinnamon on the microbial balance in gastro intestinal tract on broiler chicks has been studied. Also Mahfuzul Hoque et al. (2008) Ethanol, aqueous extracts, and essential oils of Cloves (Syzygium aromaticum), and Cinnamon (Cinnamomum cassia) were analyzed for determination of antibacterial activity against 21 food borne pathogens. According to these researches we decided to design this research to investigate the antibacterial effects of cinnamon extract on two pathogen bacteria include staphylococcus aureus and Escherichia coli.

Materials and methods

Extraction

Cinnamon (Cinnamomum verum) were purchased from the local market of Azerbaijan Shargi and Tabriz city then identified from the Department of botany and Agricultural, Islamic Azad University, Tabriz branch, Iran. After preparation were crushed using a ball mill and powder was obtained. In this study, 95% ethanol was used as solvent for extraction. After mixing the solvent and cinnamon (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.

Disc diffusion method

The antimicrobial activity of the C. verum extract 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. 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 bacteria individually. For this research Staphylococcus aureus (PTCC1431) and Escherichia coli (PTCC1399) were used.

**Results**

In staphylococcus aureus cultures there were inhibition zones around disks with cinnamon extract. This inhibition zones were on 1/2, 1/4, 1/8 Dilutions. Also in Escherichi a coli cultures there were inhibition zones on 1/2, 1/4, 1/8 dilutions. Results are given in table 2.

**Table 1.** Serial dilution of extract by DMSO.

<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>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

**Table 2.** Results of disk diffusion by cinnamon extract on Staph.aureus and E.coli.

<table>
<thead>
<tr>
<th>Num</th>
<th>Staphylococcus aureus</th>
<th>Escherichia coli</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dilution</td>
<td>Inhibition zone mean ± St(cm)</td>
</tr>
<tr>
<td>1</td>
<td>1/2</td>
<td>1.8±0.685</td>
</tr>
<tr>
<td>2</td>
<td>1/4</td>
<td>1.1±0.510</td>
</tr>
<tr>
<td>3</td>
<td>1/8</td>
<td>0.9±0.085</td>
</tr>
<tr>
<td>4</td>
<td>1/16</td>
<td>Neg</td>
</tr>
<tr>
<td>5</td>
<td>1/32</td>
<td>Neg</td>
</tr>
<tr>
<td>6</td>
<td>1/64</td>
<td>Neg</td>
</tr>
<tr>
<td>7</td>
<td>1/128</td>
<td>Neg</td>
</tr>
<tr>
<td>8</td>
<td>1/256</td>
<td>Neg</td>
</tr>
<tr>
<td>9</td>
<td>1/512</td>
<td>Neg</td>
</tr>
<tr>
<td>10</td>
<td>Control</td>
<td>Neg</td>
</tr>
</tbody>
</table>

*Neg: Negative.

**Discussion**

Ethanol (ethyl alcohol, grain alcohol) is a clear, colorless liquid with a characteristic, agreeable odor (Mohaghegi 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).

Escherichia coli is a Gram negative rod (bacillus) in the family Enterobacteriaceae (Quinn et al.1994, Naderinasab et al. 1997, Tabatabayi and Firouzi 2011). 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 (Naderinasab 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, Tadjbakhsh 1997, Tabatabayi and Firouzi 2011). 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 (Farrell et al. 2002, Kloos and Lambe 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 and Lambe 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 and Nahmias 1972, Tadjbakhsh 1997, Tabatabayi and Firouzi 2011). 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 (Klein and Finland 1963), ampicillin (Klein and Finland 1963), and tetracycline (Lowy 1998).

In Sana Mukhtar and Ifra Ghori (2012) research, spices such as garlic turmeric and cinnamon have been used as antimicrobial agents in their raw form for the treatment of wounds and injuries and joint pains etc. The Mukhtar and Ghori (2012) study was conducted to investigate the antibacterial activity of garlic, cinnamon and turmeric. Different concentrations of extracts were prepared by using two solvents water and ethanol. The antibacterial activity was tested against Bacillus subtilis (DSM 3256) and E.coli (ATCC25922) at different concentration of extracts of spices by using disc diffusion method. The cinnamon ethanolic extracts are equally effective against both Gram negative and Gram positive bacteria (Mukhtar and Ghori 2012). Study of Mukhtar and Ghori shows antibacterial effects of cinnamon on E.coli so their studies are similar to our results.

In other research conducted by Mahfuzul Hoque et al. (2008) Ethanol, aqueous extracts, and essential oils of Cloves (Syzygium aromaticum), and Cinnamon (Cinnamonum cassia) were analyzed for determination of antibacterial activity against 21 food borne pathogens: Listeria monocytogenes (5 strains), Staphylococcus aureus (4 strains), Escherichia coli O157: H7 (6 strains), Salmonella Enteritidis (4 strains), Vibrio parahaemolyticus and Bacillus cereus and 5 food spoilage bacteria: Pseudomonas aeroginosa, P. putida, Alcaligenes faealis, and Aeromonas hydrophila (2 strains). In their research for cinnamon, the ethanol extract was active only against Staphylococcus aureus strains except S. aureus JCM 2874 and V. parahaemolyticus with zones of inhibition ranging from 10.0 to 11.4 mm. The EOs from clove or cinnamon have antimicrobial properties and shown to inhibit all test organisms. The EOs of clove and cinnamon showed maximum inhibition for A. hydrophila (32.0mm) and B. cereus IFO 3457 (46.5 mm), respectively, with zones of inhibition larger than those observed against the antibiotic, gentamycin. (Mahfuzul et al. 2008).

In a research conducted by Thakare (2004) pharmacological screening of some medical plants as antimicrobial and feed additives have been studied.
In Thakare study Ethanol extracts of different medicinal plants including *Curcuma longa* (Turmeric), *Zingiber officinale* (Ginger), *Piper nigrum* (Black Pepper), *Cinnamomum cassia* (Cinnamon), *Thymus vulgaris* (Thyme), *Laurus nobilis* (Bay leaf), and *Syzygium aromaticum* (Clove) were tested using the disc diffusion method for their antimicrobial activity against the common poultry pathogens *E. coli*, *S. typhimurium*, *E. faecium*, and *E. faecalis*. Thakare results suggest that cinnamon and thyme have antibacterial activity *in vitro* (Thakare 2004).

![Fig. 2. E.coli culture, inhibition zone differences of cinnamon disks in various dilutions.](image)

In Mohamed Al-Kassie (2010) research, effect of thyme and cinnamon on the microbial balance in gastro intestinal tract on broiler chicks has been studied. This study was conducted to determine the effect of the addition of different percent levels of thyme and cinnamon, added to a standard diet, on the gastro intestinal tract. Total bacteria count, *coli form* bacteria, *lactobacilli* bacteria and fungal counts were determined in different region crop, jejunum and large intestine. The data showed that the additive thyme and cinnamon has statistical effect the decrease in the total bacteria count, *coli form* in group, jejunum and large intestine compared with the control. Different levels of thyme and cinnamon used as antimicrobial balance in gastro intestinal tract for broiler chicks (Al-kassie 2011).

**Acknowledgment**

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**References**


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