EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF METHANOLIC EXTRACT OF CINNAMON BARK AND DILL SEEDS AGAINST SOME FOOD-BORNE PATHOGENIC BACTERIA

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ABSTRACT

This study was carried out to evaluate the susceptibility of some food-borne pathogens bacteria (two Gram-positive including Staphylococcus aureus ATCC 29213, Staphylococcus epidermidis ATCC 12228 and two Gram-negative including Escherichia coli ATCC 25922, Salmonella typhimurium ATCC 14028) to cinnamon and dill methanolic extracts by using an agar well diffusion method. The antimicrobial activity of cinnamon and dill extracts against the four microbial species was assessed by the presence or absence of inhibition zone. Also, the minimum inhibitory concentration (MIC) of cinnamon extracts on these four pathogenic bacteria at different concentrations were determined. Staph. epidermidis and Staph. aureus were found to be extremely sensitive to cinnamon extract with an inhibition zone diameter (IZD) of (32.25mm) and (20.03 mm) respectively, followed by E. coli and Salmonella typhimurium were found to be partially sensitive to the test extract with an IZD of 14.85 and 13.65 mm, respectively. Dill extract showed less effective when compared with cinnamon extract.

Key words: Cinnamon, dill methanolic extracts, pathogenic bacteria, antibacterial effect, minimum inhibitory concentration (MIC).

INTRODUCTION

In recent years, many consumers have developed an interest in learning more about nutrition and food. Consumers want food that is inherently healthy, yet easy to prepare and consume, especially with women and men working an average of 7 and 8 h per day, respectively (Gonzalez et al., 2011).

Numerous food products require protection against microbial spoilage during their shelf life. The growing demand of consumers for safe and natural products, without chemical preservatives, has resulted in thorough investigations from food authorities and researchers to assess the feasibility of mild preservation techniques and to improve the microbial quality and safety of products, while maintaining their good nutritional and organoleptic properties.

Essential oils (EOs) are volatile oily liquids obtained from different plant parts and widely used as food flavors.

In spite of having been long recognized for their antibacterial, antifungal, insecticidal, antiviral, and antioxidant properties. The recent interest in alternative natural substances has led to a new scientific awareness of these substances
(Goni et al., 2009). *Cinnamomum zeylanicum Blume* is one of the world’s oldest spices that has been used as a natural preservative in food, beverage and cosmetic industries.

Its oil has been reported to inhibit the growth and subsequent toxin production from *Aspergillus parasiticus* at 200-250 µg/ml. It has been reported that application of cinnamon revealed potent antimicrobial effects against *Clostridium perfringens*, *Bacteroides fragilis* and *Bifidobacterium bifidus* (Senhaji et al., 2007).

Cinnamon is a good detoxifying herb and acts as a pain reliever. Various terpenoids found in essential oil are believed to account for cinnamon’s medicinal effects. Important among these compounds are eugenol and cinnamaldehyde. The essential oil also shows antimicrobial activity against *Pseudomonas, Aspergillus parasiticus, Staphylococcus aureus, Candida* and *Saccharomyces cerevisiae* and *Serratia*.

The bark oil is anti-fungal and antibacterial agents (Peter 2001). Cinnamon oil exhibited a broad spectrum of antagonistic activity, as compared to its extract, by inhibiting both bacteria and fungi.

The oil was found to be very effective with a lowest minimum inhibitory concentration (MIC) of 1.25% (v/v) against *Bacillus sp.*, *Listeria monocytogenes, E. coli* and *Klebsiella sp.* Amongst the fungi, *Rhizomucor sp.* (Gupta et al., 2008).

The objective of this study was to examine the effect of cinnamon bark and dill seeds methanolic extracts on some foodborne organisms.

### MATERIALS AND METHODS

#### Materials:

1. **Plant materials:**

   Cinnamon Bark and dill seeds were purchased from market of herbal medicines in El-Arish, North Sinai Egypt. Then they processed immediately and extracted.

2. **Bacterial strains:**

   A total of four foodborne pathogens bacteria (two Gram-positive and two Gram-negative) were kindly provided by the Department of Microbiology, Naval Medical Research Unit3 (NAMRU-3) Cairo, Egypt as follows:

   - *Methyl alcohol pure (methanol) CH$_3$OH-99.9%.*
   - Phosphate Buffered Salin (PBS) pH 7-7.2 from Department of Microbiology, Naval Medical Research Unit3 (NAMRU-3) Cairo, Egypt.
   - MillexOr 0.22 µm Filter unit Carrigtwohill, Co. Cork, Ireland.
   - Mcfarland Turbidity Standard No 0.5 Approximate Formula per 100 ml purified Water consist of Sulfuric acid 0.18 ml 99.5 ml and Barium chloride, 0.048 ml 0.5 ml from Department of Microbiology, Naval Medical Research Unit3 (NAMRU-3) Cairo, Egypt.
   - Mcfarland Equivalence Turbidity Standard Comparison Card.
   - Muller Hinton agar (MHA) from Department of Microbiology, Naval Medical Research Unit3 (NAMRU-3) Cairo, Egypt.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Type of culture</th>
<th>Gram Type</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>ATCC® 29213</td>
<td>Gram-positive</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>ATCC 12228</td>
<td>Gram-positive</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>ATCC 25922</td>
<td>Gram-negative</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em></td>
<td>ATCC 14028</td>
<td>Gram-negative</td>
</tr>
</tbody>
</table>
2. Methods of analysis:

Antimicrobial tests of plant extracts were determined according to (Goni et al., 2009). Working cultures which were prepared by transferring a loop of cells from the stock cultures to Mueller-Hinton agar, then they incubated at 37°C for 24 h. Preparation of methanolic extracts was described according to (Shan et al., 2007).

An agar-well diffusion method was employed for determination of antibacterial activities according to (Goni et al., 2009) and well of 6 mm punched in Mueller-Hinton agar plates. The sensitivity of the individual extract was recorded by the diameter of the inhibition zone according to (Ponce et al, 2003), (Babu et al., 2001).

The minimum inhibitory concentration (MIC) was defined as the lowest essential oil concentration resulting in the lack of visible microorganism growth according to (Goni et al., 2009).

And the MIC was defined as the lowest concentration that completely inhibited the growth for 24 h. The MIC for the extracts was determined by the agar well diffusion method as described by (Gupta et al., 2008).

A four-fold serial dilutions of the cinnamon and dill extracts to achieve a decreasing concentrations range of 5 to 0.5 mg/well. a decreasing each dilution was added aseptically into the wells in Mueller Hinton agar plates that had been inoculated with standardized inoculums (10⁶ CFU/ml) of the tested bacteria. The agar plates were incubated at 37°C for 24 h. All experiments were performed in duplicate. Zone of inhibition was considered as the MIC.

RESULTS AND DISCUSSION

Antimicrobial activity of the tested methanolic extracts on selected bacterial strains

The antimicrobial activity of cinnamon and dill extracts against the four microbial species (Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli and Salmonella typhimurium) was assessed by the presence or absence of inhibition zone.

The diameter of the inhibition zone is given in Fig (1). cinnamon extract was found to be highly effective against almost all of the food-borne microbes in this study and these results were in accordance with these founded by (Gupta et al., 2008). Staph. epidermidis and S. aureus were found to be extremely sensitive to cinnamon extract with an inhibition zone diameter (IZD) of (32.25mm) and (20.03 mm) respectively, as shown in Figs. (2,3), followed by E. coli and Salmonella typhimurium which were found to be partially sensitive to the test extract with an IZD of 14.85and 13.65 mm, respectively, as showed in Figs. (4,5).

The antimicrobial activity of cinnamon extract may be attributed to cinnamaldehyde. The antimicrobial compound of cinnamon Cinnamaldehyde exhibits its antibacterial activity due to its lipophilicity of terpenoids and phenyl propanoids, which can penetrate the membrane and reach the inner part of the cell and impair bacterial enzyme system and these data are in agreement with these obtained by (Babu et al., 2001).

Using agar well diffusion, dill extract showed less effective when compared with cinnamon extract. The extract found to be highly effect on Staphylococcus aureus and Staphylococcus epidermidis with an inhibition zone diameter (14.8) and (12.5) mm respectively, as showed in Figs. (2,3) but was less effective on Escherichia coli with an inhibition zone diameter (12.1) mm as showed in Fig. (4) On the other hand, dill extract found to be ineffective on Salmonella typhimurium which explained that dill extract has a less effective on Negative strains bacteria.
The higher activity of extract can be explained on the basis of the chemical structure of their major constituents such as dill Apiole which have aromatic nucleus containing polar functional group that is known to form hydrogen bonds with active sites of the target enzyme. Carvone, the major component of dill seed oil, has already been reported as having ability to inhibit the growth of bacteria these data are in agreement with that obtained by (Shan et al., 2007).

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of cinnamon extract on various pathogenic bacteria strains at different concentrations are given in Table (1).

Among the bacterial pathogens tested, the results showed that Staphylococcus epidermidis and Staphylococcus aureus were found to be most sensitive with a higher minimum inhibitory concentrations of cinnamon methanolic extract of 5 mg/well to produce IZD of 21.9 and 17.8 respectively, except the lower concentration of cinnamon (0.5 mg/well) has no effect on the tested bacteria.

At concentration of (1mg/well), minimum inhibitory concentration occur for positive bacterial strains Staphylococcus epidermidis and Staphylococcus aureus to be (15.4) and (8) mm inhibition zone diameter respectively. There was a little inhibition effect of different concentrations of cinnamon extracts on the two type of negative strains pathogens bacteria (El-Baroty et al., 2010).

![Fig. (1): Inhibition zone of cinnamon and dill extracts against Tested bacteria on Mueller-Hinton agar plates.](image-url)
Table (1): Minimum inhibitory concentration of various concentrations of extracts of Cinnamon on the growth of bacterial pathogens.

<table>
<thead>
<tr>
<th>Cinnamon extract concentration (mg/well)</th>
<th>Staphylococcus aureus</th>
<th>Staphylococcus epidermidis</th>
<th>Escherichia coli</th>
<th>Salmonella typhimurium</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td>1.0</td>
<td>8</td>
<td>15.4</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td>3.0</td>
<td>11.8</td>
<td>20.1</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td>5.0</td>
<td>17.8</td>
<td>21.9</td>
<td>6.0</td>
<td>6.0</td>
</tr>
</tbody>
</table>

Fig (2): Inhibition zone of cinnamon and dill extracts against *Staphylococcus epidermidis* bacteria on Mueller-Hinton agar plates.

Fig. (3): Inhibition zone of cinnamon and dill extracts against *Staphylococcus aureus* bacteria on Mueller-Hinton agar plates.
Fig (4): Inhibition zone of cinnamon and dill extracts against *E. coli* bacteria on Mueller-Hinton agar plates.

Fig (5): Inhibition zone of cinnamon and dill extracts against *Salmonella typhimurium* bacteria on Mueller-Hinton agar plates.
REFERENCES


المنشور العربي

تقييم النشاط المضاد للميكروبات المستخلص الميثانولي للفقرة وبذور البترويا المرضية الملوثة للأغذية

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يهدف هذا البحث إلى دراسة الفاعلية التحيطية للمستخلصات الكحولية لنباتي القرفة (اللحماء) والشيث (البذور) باستخدام كحول الميثانول ضد بعض أنواع البكتيريا المرضية باستخدام طريقة الانتشار حول الحفر يمكن القول أن التأثير المضاد Staphylococcus cinnamaldehyde للميكروبات المستخلص القرفة رزمة يعود إلى مادة الوجدة أنها أحد جوانبها أكثر حساسية للمستخلص مع مساحة منع من الظهور تبلغ 12.85 مم وتعبعها Escherichia coli, Salmonella. وجد أنها حساسة قليلا للمستخلص الكحولي للقرفة مع مساحة منع من الظهور تبلغ 14.85 مم لبيكتريا typhimurium Salmonella typhimurium Escherichia coli. ومساحة منع من الظهور تبلغ 13.15 مم لبيكتريا الأن مستخلص البترويا أقل تأثيراً على البكتيريا المرضية عند مقارنتها بمستخلص القرفة. أوضحت النتائج المحصل عليها أن المستخلصات الكحولية تكلا من نباتي القرفة والشيث أطول في تأثيرها على البكتيريا الموجبة لجرام عن البكتيريا السالبة.

الكلمات الإشرافية: قلق الشرطة، المستخلص الميثانولي، بذور البترويا، البكتيريا المرضية الملوثة.

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