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Antimicrobial Effect of Cinnamon Bark Hot and Cold Watery Extract against Extraintestinal Pathogenic Escherichia Coli (ExPEC) and Staphylococcus Aureus

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Abstract
The aim of this work is to study the antimicrobial effect of hot and cold watery extract of cinnamon bark against the Extraintestinal pathogenic *Escherichia coli* (ExPEC) and *Staphylococcus aureus*.Where prepared the hot watery extract by powdering the cinnamon bark and boiled 100 gm for 30 min with 200 ml of distill water then centrifuged at 5000 pm\s for 8 min, also prepared the cold watery extract in the same method except the boiling step. And tested the cinnamon watery extract activity against ExPEC and *S. aureus* and measured the inhibition zones by well diffusion method.Where the results show that both ExPEC and *S. aureus* not affected with the concentration 100, 150 mg\mL for hot and cold watery extract, but they affected with high concentration as 300, 450 mg\mL, where the inhibition zones with cold watery extract was 2, 3 mm for ExPEC and 2, 3.5 mm for *S. aureus*. And it's was 4, 8 mm for ExPEC and 8, 10 mm for *S. aureus* at the same concentrations above but with hot watery extract.Hot and cold watery extract of cinnamon bark contained glycosides, phenolic compounds, alkaloids, saponins, and tannins, these compounds are well known to be as antimicrobial growth inhibitors, the presence of similar antimicrobial compounds in both hot and cold watery extracts of cinnamon bark could be explained to dealing with the same solvent in both extracts. The only is the amount of active compounds; hence heat may increases the dissolved active compounds, and that's explains the relative increase of inhibition for hot watery extract than the cold watery extract. And *S. aureus* is more affected than *E. coli* as showed in the results.
Keywords: Cinnamon bark, Well diffusion method, Essential oils (EOs), Cold and hot watery extract, Staphylococcus aureus, Extraintestinal Eschaerchia coli.

1. Introduction

Essentials oils (EOs) obtained from plant material have been used for centuries as antimicrobial agents. The increase of bacterial resistance to antibiotics and the efforts to develop natural preservatives in food manufacturing has increased interest in possible applications of EOs. Since the 1990s a fair number of trials have been carried out with EOs in food. Most studies use EOs as antimicrobial agents incorporated into the food. However the use of these agents as active antimicrobial compounds in packaging materials has been less developed and only some works study this application [1].

Staphylococcus aureus is found in the nostrils as well as on the skin and hair of warm-blooded animals, and up to 30–50% of human population are carriers[2] It has been isolated from several foods including meat and meat products, chicken, milk and dairy products, fermented food items, salads, vegetables, fish products, etc.[3-6]. Staphylococcal food poisoning is among the most common causes of reported food-borne diseases[7-10], requiring hospital attention by up to 19.5% of the affected individuals[11] Most strains are capable of producing one or more heat stable enterotoxins[12,13] which are the cause of the gastrointestinal symptoms observed during intoxications[5]. *S. aureus* is also widely disseminated in nosocomial infections, where it poses a threat due to its acquired resistance to most common antimicrobials. Methicillin-resistant *S. aureus* strains are of particular concern [14]. Extraintestinal pathogenic *Escherichia coli* (ExPEC) cause a wide spectrum of illnesses including cystitis, pyelonephritis, bacteremia, prostatitis and other infections which occur outside the human intestine. The most common type of infection due to ExPEC is urinary tract infection (UTI); 70–95% of UTIs are caused by ExPEC [15, 16]. It is estimated that 11% of women aged of 18 years are affected by UTIs annually, resulting in over 1 billion dollars of direct and indirect costs per year [17-19].

In the last ten years many studies have been published about the development of active packaging materials, most of them focused on food applications [20-24]. This is an area of great interest for both industry and academia, as the introduction of protective agents in the packaging materials can be used to protect the food without direct addition of new chemicals. The current trend of having more natural and ecologically produced foodstuffs, while simultaneously requiring longer shelf life, is a challenge the food industry has to face. One key point in this research is the selection of the active agents, i.e. the protective substances to be incorporated into the packaging materials [25]. Natural extracts, such as essential oils (EO) and their constituents, are categorized as flavorings in Europe (European Decision 2002/113/EC of January 23rd 2002, notified under document number C (2002) 88). In addition, essential oils and their constituents are categorized as GRAS (Generally Recognized as Safe) by the US Food and Drug Administration. For this reason, essential oils (EOs) have been often proposed and used as antimicrobial, antifungal and antioxidant agents, in general with good results [26-30].

Matan et al. have reported Antimicrobial activity of Cinnamon bark. The volatile gas phase of combinations of Cinnamon oil and clove oil showed good potential to inhibit growth of spoilage fungi, yeast and bacteria normally found on IMF (Intermediate Moisture Foods) when combined with a modified atmosphere comprising a high concentration of CO_2 (40%) and low concentration of O_2 (<0.05%). *A. flavus*, which is known to produce toxins, was found to be the most resistant microorganism [31].

2. Experimental work

2.1 Microorganisms and culturing

Strains of Extraintestinal *Escherichia coli* and *Staphylococcus aureus* were provided by the Department of Biology laboratories from University of Mustansiriya (Iraq).

2.2 Preparation of Cinnamon-derived Extracts

Extracts for antimicrobial screening were prepared from cinnamon bark that was obtained from the local market and triturate as a powder. 100 grams of cinnamon powder was boiled in 200 mL of distilled water for 30 min. The crude extract was centrifuged at 5,000 pm\s for 8 min and filtered. The filtrate was used for further antimicrobial screening. The cold-water extracts were prepared as described above, except the boiling step.

2.3 Antimicrobial activity of Cinnamon extracts

From the prepared stock solution that has the concentration 500 mg\mL prepared the concentrations 450, 300, 150, and 100 mg\mL from each watery extract (hot and cold) then mixed in an exact volume from the stock solution with distilled water as in the equation $C_1 \cdot V_1 = C_2 \cdot V_2$ where $C_1 \cdot V_1$ is the stock solution concentration and volume, and $C_2 \cdot V_2$ is the concentration and volume of the solution that we hope to prepare. Prepared Petri dish that have nutrient agar with 3 holes that used to put the cinnamon watery extract as 1 ml from each concentration in each hole then was made a series dilutions for *S. aureus* and *E. coli* then used the concentration 10^{-2} from each other. Then cultured by continuous streaking method and incubated for 24 and 48 h at 37 c°.

3. Results

The results of the experiment showed as in (Table 1) and through the inhibition zones that were measured by well diffusion method that hot watery extract was the most efficient from cold watery extract through its effect on the growth of ExPEC and *S. aureus* and they not affected with low concentration as 100, 150 mg\mL of cold and hot watery extract, but they affected with high concentration as 300, 450 mg\mL where the inhibition zones was 2, 3 mm for ExPEC and 2, 3.5 mm for *S. aureus* respectively for cold watery extract.

And 4, 8 mm for ExPEC and 8, 10 for *S. aureus* respectively at the same concentration above but with hot watery extract.

It is thus clear that the hot watery extract is better than cold watery extract, can be inferred that the high concentrations are the best in the practical application for better results, where did not affected the low concentrations 100 and 150 mg\mL in the growth of *S. aureus* and *E. coli* from the hot and cold watery extract.

Table 1. Shows the inhibition zones of *E.coli* and *S. aureus* by well diffusion method.

Concentration (mg\mL)	*E.coli* Inhibition (mm)		*S. aureus* Inhibition (mm)	
	Cold watery extract	Hot watery extract	Cold watery extract	Hot watery extract
100	-	-	-	-
150	-	-	-	-
300	2	4	2	8
450	3	8	3.5	10

4. Discussion

Chemical analysis of raw extract of cinnamon bark that was used in this study showed that they contain the active compounds as a secondary metabolism Table 2 [32].

Table 2. Show the active compounds that contained in *Cinnamomum zeylanicum.*

Active compound	Hot watery extract	Cold watery extract
Glycosides	+ve	+ve
Phenolic compounds	+ve	+ve
Alkaloids	+ve	+ve
Terpins	-ve	-ve
Resins	-ve	-ve
Saponins	+ve	+ve
Tanins	+ve	+ve
Flavones	-ve	-ve
Coumarins	-ve	-ve
Volatile oils	-ve	-ve

-ve is negative, +ve is positive

Inhibitory effect restored to the nature of the material contained in cinnamon. As the presence of compounds alkaloids, tannins, volatile oils, saponins, terpins flavones and coumarins which is one of the antibacterial was its effect in inhibiting the growth of bacteria, alkaloids characterized by its ability to break into the bacterial cell and interfering with DNA, while working tannins on the inhibition of enzymes and transport proteins in the cell membrane [33]. Either saponins is working to reduce the proportion of sugar within the bacteria that lead to bacterial cell death as well as for to glycosides which have a similar effect, but has a lesser effect [34]. The phenols as that these compounds have the ability to formation a complex with sulfhydryl group leading to damage the cell wall [35].

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