SINAI Journal of Applied Sciences (ISSN: 2314-6079) Vol. (4) Is. (3), Dec. 2015 221-228

Sinai Journal of Applied Sciences

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

Marwa M. El-Shreef[1], M.R.E. Shahein[1], A.M. Abou El-Nour[2] and M.M.K. Metwally[1]

1. Dept. Food and Dairy Sc. and Techno., Fac. Environ. Agri. Sc., Suez Canal Univ., El-Arish, Egypt

2. Dept. Dairy, Fac. Agri., Suez Canal Univ., Ismailia, Egypt

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 pathoginic 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 parasitucus* at 200-250 µg/ml. It has been reported that application of cinnamon revealed potent antimicrobial effects against *Clostridium perfringens*, *Bacteroïdes fragilis* and *Bifidobacterium bifidus* **(Senhaji *et al.*, 2007).**

cinnamon is a good detoxifying herb and acts as a pain reliever. Various terpenoides 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 cerivisiae* and *Serratia*.

The bark oil is anti-fungal and anti-bacterial 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_3OH-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.

Organism	Type of culture	Gram Type
Staphylococcus aureus	ATCC[a] 29213	Gram-positive
Staphylococcus epidermidis	ATCC 12228	Gram-positive
Escherichia coli	ATCC 25922	Gram-negative
Salmonella typhimurium	ATCC 14028	Gram-negative

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 (10^6 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 DISCISSION

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 **(Singh *et al.*, 2005).**

A possible explanation for these observations may lie in the differences in the outer layers of Gram-negative and Gram positive bacteria. The resistance of Gram-negative bacteria towards antibacterial substances is related to the hydrophilic surface of their outer membrane which is rich in lipopolysaccharide molecules, presenting a barrier to the penetration of numerous antibiotic molecules and is also associated with the enzymes in the periplasmic space, which are capable of breaking down the molecules introduced from outside. Gram-positive bacteria do not have such an outer membrane and cell wall structure.

Finally, the antibacterial activity is closely related to the concentration of phenolic compounds and thus to the antioxidant capacity of the extracts 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).**

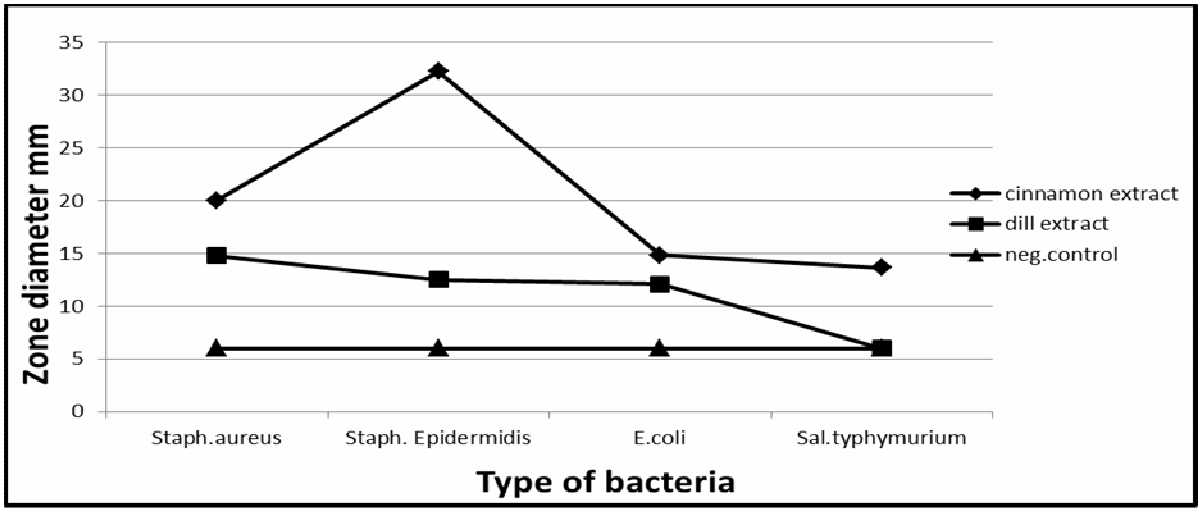

Fig. (1): Inhibition zone of cinnamon and dill extracts against Tested bacteria on Mueller-Hinton agar plates.

Table (1): Minimum inhibitory concentration of various Concentrations of extracts of Cinnamon on the growth of bacterial pathogens.

cinnamon extract concentration (mg/well)	IZD of Pathogenic Bacteria			
	Staphylococcus aureus	*Staphylococcus epidermidis*	*Escherichia coli*	*Salmonella typhimurium*
0.5	6.0	6.0	6.0	6.0
1.0	8	15.4	6.0	6.0
3.0	11.8	20.1	6.0	6.0
5.0	17.8	21.9	6.0	6.0

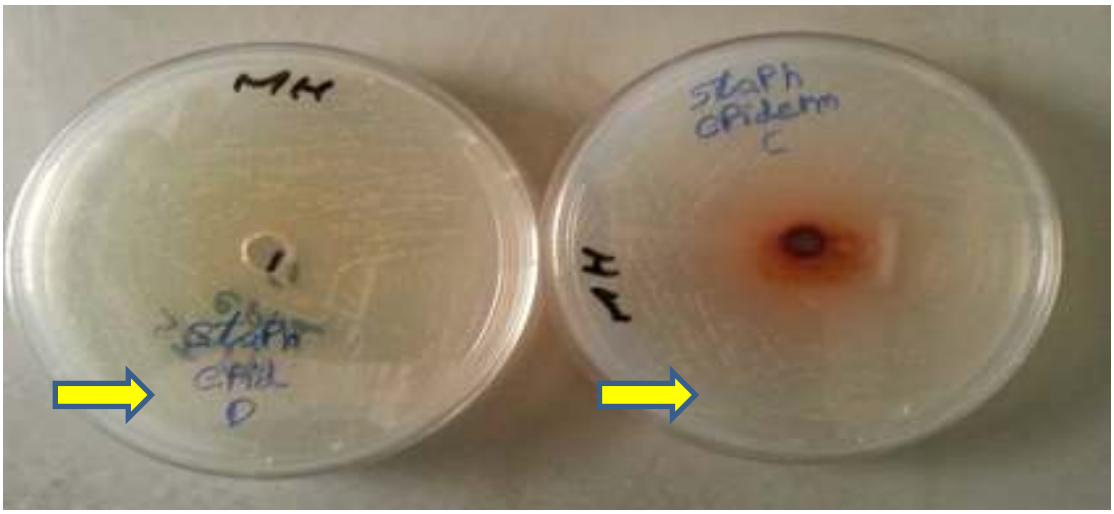

Fig (2): Inhibition zone of cinnamon and dill extracts against *Staphylococcus epidermidis* bacteria on Mueller-Hinton agar plates.

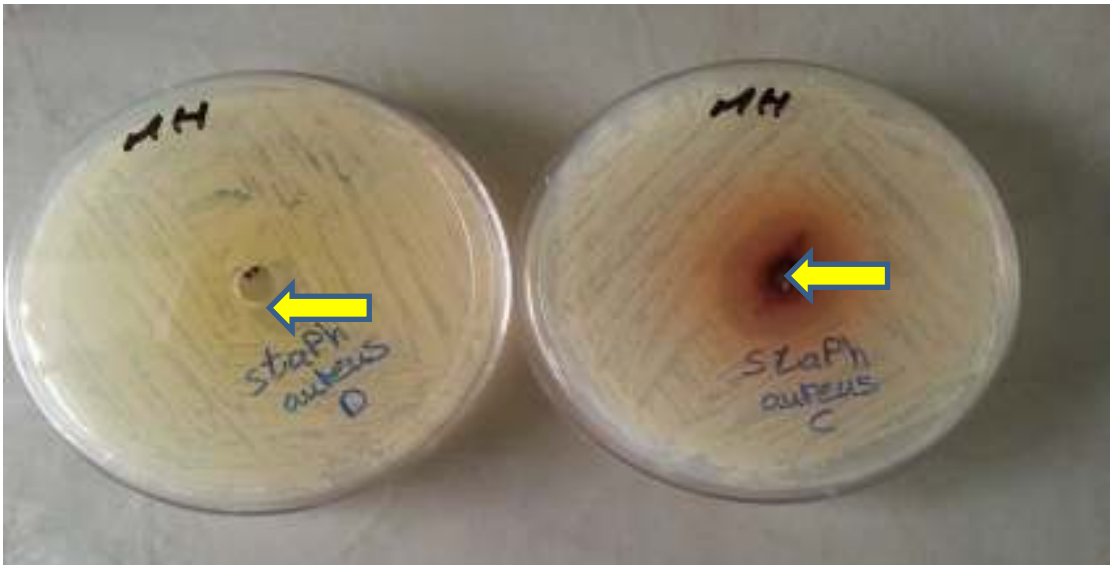

Fig. (3): Inhibition zone of cinnamon and dill extracts against *Staphylococcus aureus* bacteria on Mueller-Hinton agar plates.

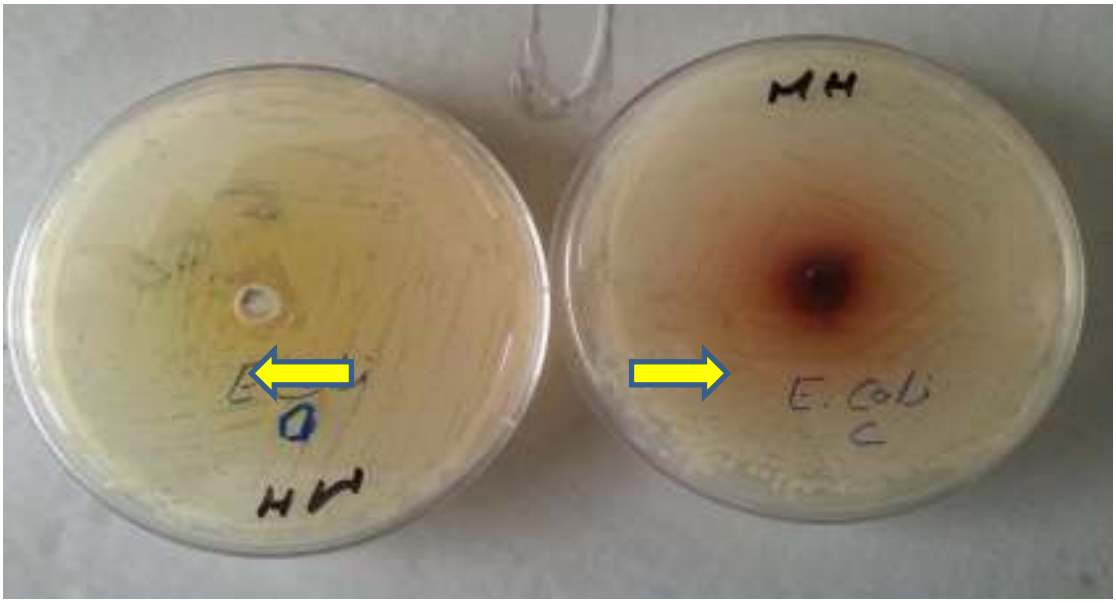

Fig (4): Inhibition zone of cinnamon and dill extracts against *E. coli* bacteria on Mueller-Hinton agar plates.

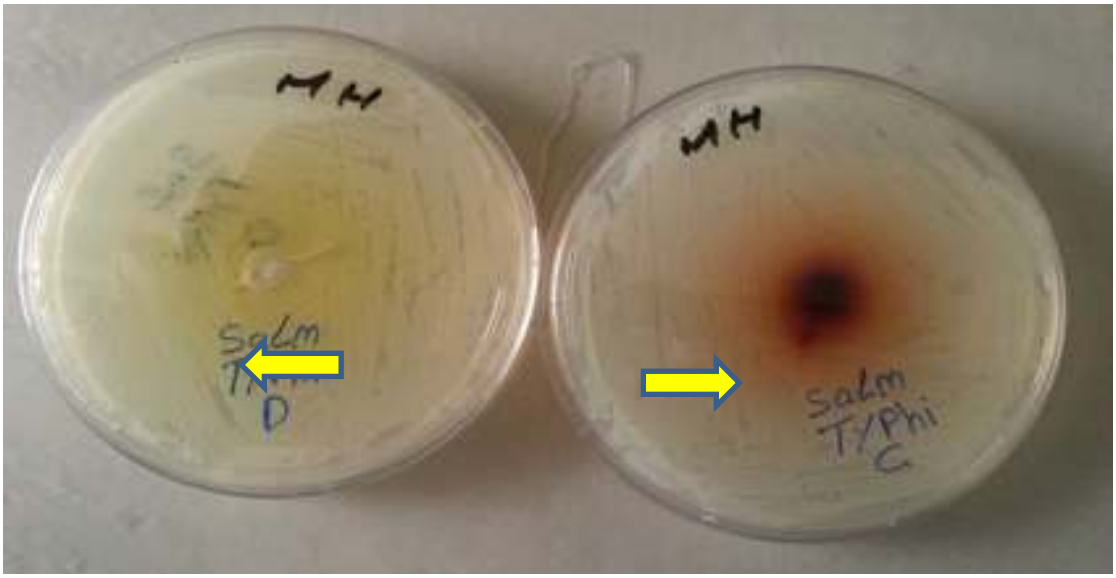

Fig (5): Inhibition zone of cinnamon and dill extracts against *Salmonella typhimurium* bacteria on Mueller-Hinton agar plates.

REFERENCES

Gonzalez, N.J.; K. Adhikari and M.F. Sancho-Madriz (2011). Sensory characteristics of peach-flavored yogurt drinks containing prebiotics and synbiotics. Food Science and Technology, 44: 158-163.

Goni, P.; P. Lopez; C. Sanchez; R. Gomez-Lus; R. Becerril and C. Nerin (2009). Antimicrobial activity in the vapour phase of a combination of cinnamon and clove essential oils. Food Chemistry, 116: 982–989.

Senhaji, O.; M. Faid and I. Kalalou (2007). Inactivation of Escherichia coli O157:H7 by essential Oil from *Cinnamomum zeylanicum*. The Brazilian Journal of Infectious Diseases, 11(2):234-236.

Peter, K.V. (2001). Handbook of herbs and spices. First published 2001 Woodhead Publishing Limited CRC Press ISBN-0 8493-1217-5.

Gupta, C.; A.P. Garg; R.C. Uniyal and A. Kumari (2008). Comparative analysis of the antimicrobial activity of cinnamon oil and cinnamon extract on some food-borne microbes. African Journal of Microbiology Research, 2 (9): 247-251.

Shan, B.; Y. Cai; J.D. Brooks and H. Corke (2007). The *in vitro* antibacterial activity of dietary spice and medicinal herb extracts. International Journal of Food Microbiology, 117: 112–119.

Ponce, A.G.; R. Fritz; C.D. Valle and S.I. Roura (2003). Antimicrobial activity of essential oils on the native microflora of organic Swiss chard. Lebensm.-Wiss. u.-Technol, 36 : 679–684.

Babu, A.J.; A.R. Sundari, J. Indumathi; R.V.N. Srujan and M. Sravanthi (2011). Study on the antimicrobial activity and minimum inhibitory concentration of essential oils of spices. Veterinary World, 4 (7): 311-316.

Singh, G.; S. Maurya; M.P. deLampasona and C. Catalan (2005). Chemical constituents, antimicrobial investigations, and antioxidative potentials of *Anethum graveolens* L. essential oil and acetone Extract: Part 52. Journal of Food Science, 70 (4): 208 – 215.

El-Baroty, G.S.; H.H. Abd El-Baky; R.S. Farag and M.A. Saleh (2010). Characterization of antioxidant and antimicrobial compounds of cinnamon and ginger essential oils. African Journal of Biochemistry Research, 4(6): 167-174.

الملخص العربى

تقييم النشاط المضاد للميكروبات للمستخلص الميثانولي لقلف القرفة وبذور الشبت على بعض البكتريا الممرضة الملوثة للأغذية

مروة محمد الشريف[١] - مجدي رمضان شاهين[١] - عاطف أبو النور[٢] - ممدوح مصطفى كمال[١]

١- قسم علوم وتكنولوجيا الأغذية والألبان - كلية العلوم الزراعية البيئية بالعريش- جامعة قناة السويس - مصر
٢- قسم الألبان - كلية الزراعة بالإسماعيلية - جامعة قناة السويس - مصر

يهدف هذا البحث الى دراسة الفاعلية التثبيطية للمستخلصات الكحولية لنباتي القرفة (اللحاء) والشبت (البذور) باستخدام كحول الميثانول ضد بعض انواع البكتريا المرضية باستعمال طريقة الإنتشار حول الحفر يمكن القول أن التأثير المضاد للميكروبات لمستخلص القرفة ربما يعود إلى مادة ال cinnamaldehyde وأن بكتريا *Staphylococcus epidermidis* وجدت أنها أكثر حساسية للمستخلص مع مساحة منع من الظهور تبلغ ٣٢,٢٥مم يتبعها *Staphylococcus aureus* مع مساحة منع من الظهور تبلغ ٢٠,٠٣ مم ويتبعها *Escherichia coli*, *Salmonella typhimurium* وجد انها حساسة قليلا للمستخلص الكحولي للقرفة مع مساحة منع من الظهور تبلغ ١٤,٨٥ مم لبكتريا ال *Escherichia coli* ومساحة منع من الظهور تبلغ مساحتها ١٣,٦٥ مم لبكتريا ال *Salmonella typhimurium*. أن مستخلص الشبت أقل تأثيراً على البكتريا المرضية عند مقارنته بمستخلص القرفة. أوضحت النتائج المتحصل عليها أن المستخلصات الكحولية لكلا من نباتي القرفة والشبت اعلى في تأثيرها على البكتريا الموجبة لجرام عن البكتريا السالبة لجرام.

الكلمات الإسترشادية: قلف القرفة، المستخلص الميثانولي، وبذور الشبت، البكتريا الممرضة الملوثة.

المحكمون:
١- أ.د. حسن عبد الفتاح أستاذ الميكروبيولوجى المتفرغ - كلية الزراعة - جامعة الزقازيق.
٢- أ.د. أميـــرة الخولى أستاذ بقسم علوم وتكنولوجيا الأغذية والألبان - كلية الزراعة بالإسماعيلية - جامعة قناة السويس.