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Article:

Impact of cinnamon oil and its nanoemulsion on *Staphylococcus aureus* isolated from luncheon samples sold in Sohag city

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Abstract

This study was conducted to determine the antibacterial effect of cinnamon essential oil (CEO) and its nanoemulsion (CNE) on *Staphylococcus aureus* isolated from luncheon samples sold in Sohag city. Forty luncheon samples were randomly collected from different supermarkets in Sohag city. The samples were examined bacteriology for detection of *Staph. aureus.* The results revealed that the incidence of *Staph. aureus* in luncheon samples was 10% (4/40). The minimum inhibitory concentration (MIC) of the prepared CEO and its nanoemulsion CNE were detected in-vitro against the isolated *Staph. aureus* strains by using agar well diffusion method, in which, the results revealed that the MIC were 0.39% for CEO and 0.78% for CNE. The different concentrations of CEO and CNE have a great effect against *Staph. aureus* growth and the most effective one at the MIC (0.39%) was CEO with 11.67±0.66 mm.

Keywords: luncheon, *Staphylococcus aureus,* cinnamon essential oil, nanoemulsion.

Introduction

Egyptian beef luncheon is considered as one of the ready-to-eat meat products and highly demanded due to its high biological value, reasonable price, agreeable taste, and easy serving. However, meat products are considered to be risky to human health [1].

Staphylococcus aureus food poisoning is related to improper handling and processing of food, food containing raw ingredients or those from unsafe origin and food storage under the required conditions which allows the *Staph. aureus* growth and enterotoxins production [2]. *Staph. aureus* causing both animal and human illness such as toxic shock syndrome, Staphylococcal Food Poisoning (SFP) and mastitis [3]. SFP is associated with ingestion of food containing heat stable enterotoxins with symptoms including nausea, abdominal cramps, vomiting and diarrhea which may be accompanied with blood [4].

Plant essential oils (EOs) including cinnamon, clove, oregano, and ginger have strong antibacterial and antifungal properties and are natural and environmentally safe, have recently received increased attention [5]. They have been researched for their possible use as a natural ingredient in food preservation in addition to their known antioxidant action [6]. These oils are used to improve the flavor, color, and aroma of foods and comprise a crucial component of the human diet [7]. More specifically, cinnamon consists mainly of cinnamaldehyde, reported as an active volatile ingredient that contributes to the strong cinnamon-like flavor and antimicrobial activities [8].

Nanotechnology, by the year 2000, was universally recognized as a landmark innovation, and named the sixth truly revolutionary technology introduced in the modern world and become an emerging technology for food preservation [9]. Nanotechnology refers to the technology dealing with the fabrication and application of nanomaterials with sizes from 1–100 nm; the physical and chemical properties of a substance will vary with this size, while its water solubility, stability, bioavailability and physiological activities can be enhanced substantially with

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minimum side effects [10]**.** This study aimed to detect the presence of *Staph. aureus* in luncheon samples and investigate the effect of cinnamon oil and its nanoemulsion on the isolated strains of *Staph. Aureus*

Materials and Methods

Isolation and identification of *Staph. aureus*

1- Collection and preparation of samples according to ISO.

A total of 40 luncheon samples were collected based on their availability from the supermarket located in Sohag city, Egypt. An adequate quantity of each product was purchased and placed aseptically into a clean, dry and sterile polythene bag. All collected samples were transported to the laboratory in ice boxes to be prepared and examined as soon as possible. Twenty-five grams of the examined samples recommended cuts samples, were transferred to a septic homogenizer (made by DAI HAN scientific) and 225 ml of 0.1% sterile peptone water were aseptically added. Each sample was then homogenized in the homogenizer at 2000 r.p.m for 1-2 minutes to provide a homogenate [11].

2- Isolation of *Staph. Aureus*

Aseptically 10g of each prepared sample was suspended in 100 ml of Trypticase Soya broth (Hi Media) with 70 mg of NaCl /ml and incubated at 35□C for 18 - 24 hrs for enrichment [12] then inoculated onto Baird Parker agar Medium (HiMedia, India), and incubated at 37 ºC for 24 hrs [13]**.**

3- Enumeration of *Staph. aureus* was done using plate method technique according to **A.O.A.C.** [14].

4- Identification of *Staph. aureus*

Staph. aureus colonies were identified by morphological examination and using biochemical tests as catalase, coagulase and anaerobic fermentation of mannitol according to **Lancette and Bennett, Collee *et al.,*** and **Kateete *et al.*** [15,16 and 17]**.** The isolated strains were preserved at -20 C until use.

5-The antibacterial effect of cinnamon oil and its nanoemulsion on *Staph. aureus*–strains isolated from the luncheon samples

The isolated strains were prepared according to **Isenberg.** [18]. The CEO was serially diluted by two-fold serial dilutions to achieve 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, 0.78% and 0.39% concentrations. While the CNE was prepared according to **Ghosh *et al.*** [19] to achieve 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, 0.78% and 0.39% concentrations.

6- Determination of the minimum inhibitory concentration (MIC) of cinnamon essential oil and its nanoemulsions

MIC was conducted by using the agar well diffusion method. The isolated strains were cultured on Müller-Hinton agar (Hi media) and the prepared CEO and CNE with different concentrations were added into the wells on the agar plates, then incubated at 37°C for 24hr [20].

7- Characterization of the prepared nanoemulsion

Achieved by measuring particle size and polydispersity index (PDI) according to **Baboota *et al.*** [21], Fourier-transform infrared spectroscopy (FTIR) spectral analysis according to **Gurpreet and Singh.** [22] and Morphological study by using Transmission Electron Microscope according to **Shakeel *et al.*** [23]**.**

Results

The results are presented in **Table 1.** showed that the incidence of *Staph. aureus* in examined luncheon samples was 10% (4/40). The level of contamination ranged from 12 x 10^2 to 31 x 10^3 CFU/g with an average count of 11.3 x10^3 CFU/g **Table 2.** and the percentage of contamination was 100% obtained in 10^2 <10^3 CFU/g and 10^3 < 10^4 CFU/g in 50% of positive samples for each, while the lower percentage of contamination was zero in 10^4 < 10^7CFU/g. **Table 3.**

The minimum inhibitory concentration (MIC) of cinnamon essential oil (CEO) was 0.39%, while average zone of inhibition was 11.67± 0.66 mm and the MIC of CNE was 0.78%, while average zone of inhibition was 2.67 ± 2.66 mm. **Table 4.**

Discussion

The Incident of *Staph. aureus* isolated from the examined luncheon samples was 10% (4/40**) Table 1**. Nearly similar results were reported by **Eid *et al.*** [24] who revealed that 12 % of luncheon samples were contaminated by *Staph. aureus*. Higher results were reported by **Abdelfattah *et al.*** [25] who detect *Staph. aureus* in luncheon samples with percentage of 20%. While opposite results were obtained by **Mansour *et al.*** [26] who mentioned that all examined luncheon samples were free from *Staph. aureus*.

In the present study, the mean counts of *Staph. aureus* in the examined samples were 11.3 x10^3 CFU/g **Table 2,3**. Lower results were achieved by **Eltanani and Arab**. [27] who found that the mean count of *Staph. aureus* in luncheon

samples was 0.3 $x10^2 \pm 0.12 \times 10^2$ CFU/g. The examined luncheon samples are above the acceptable limits of **Egyptian Standard**. [28] which stipulated that *Staph. aureus* must be absent in luncheon. The microbial load of meat products depends on several factors, such as the initial physiological status of the animal, contamination at slaughterhouses, the equipment used for the meat manipulation, and the temperature of storage conditions [29]**.** The MIC of CEO was 0.39%, while average zone of inhibition was 11.67± 0.66 mm and the MIC of CNE was 0.78%, while average zone of inhibition was 2.67 ± 2.66 mm **Table 4**. Lower MIC results were mentioned by **Kaskatepe *et al.*** [30] who reveal that the MIC was 0.09%. Higher MIC results were recorded by **El Atki *et al.*** [31] who mentioned that the MIC was 4.88%.

Table 1 Incidence of Staph. aureus in the examined sample

samples	No of examined samples	positive samples	
		No	**%**
luncheon samples	40	4	**10**

Table2: Statistical analytical results of Staph. aureus count in the examined samples

Type of samples	Positive *Staph. aureus*		Count of positive samples/ ml or g			No. of samples above E.S.	
	No./ 40	**%**	**Min.**	**Max.**	**Average**	**No.**	**%**
Luncheon	4	10	12×10^2	31×10^3	11.3×10^3	4	**10**

E.S. = Egyptian Standard (2005)

Table 3: Frequency distribution of the Staph. aureus based on their Staph. aureus counts / ml

Luncheon samples	Count/ml									
	$10^2<10^3$		$10^3<10^4$		$10^4<10^5$		$10^5<10^6$		**$10^6<10^7$**	
	No	%	No	%	No	%	No	%	No	**%**
4	2	50	2	50	0	0	0	0	0	**0**

Table 4: MIC of cinnamon oil and its nanoemulsion by mm (mean± SE) through three time replicate by agar well diffusion method

Conc.%	Average zone of inhibition by mm (mean± SE)	
	CEO	**CNE**
100	31±4.04	**30.33±3.38**
50	33.33±0.33	**28±4**
25	30±2.08	**22±2**
12.5	25.67±2.60	**14±0.57**
6.25	23±2.88	**11.5±0.67**
3.125	17.67±0.66	**9.67±0.72**
1.56	15.33±1.45	**8±0.57**
0.78	13±1.0	**2.67±2.66**
0.39	11.67±0.66	**NZ**

NZ: no zone

In this study, the PDI was 0.234 with average droplet size 352.8 nm for the prepared CNE **Table 5.** lower results were obtained by **Ghosh *et al.*** [32] with particle size ranged (254-96 nm) and PDI (0.25-0.21), and **Paudel *et al.*** [33] where the mean droplet size of CNEs 5% (2.5% Tween 80) with an ultra-sonication time of 10 min 9.6±0.3 nm and a pretty PDI value 0.1. While higher results were achieved by **Pimple *et al.*** [34] with PDI 0.382.

Fig 1. showed FTIR of CEO and its nanoemulsions and also mentioned in several previous studies as **Moustafa *et al.*** [35]**.**

In the present study, the morphology and size of CNE fabricated by Nano precipitation were determined by Transmission Electron Microscope (TEM) showed that separate spherical shape particles with ranged size 16.9-19 nm, without any aggregation and perfect stability. Higher results were obtained by **Haider *et al.*** [36] with values of 289.09 nm **Picture 1.**

Table 5: Physical properties of formulated cinnamon oil nanoemulsion

Type of nanomaterial	Average droplet size (nm)	Polydispersity index (PDI)
CNE	352.8	**0.234**

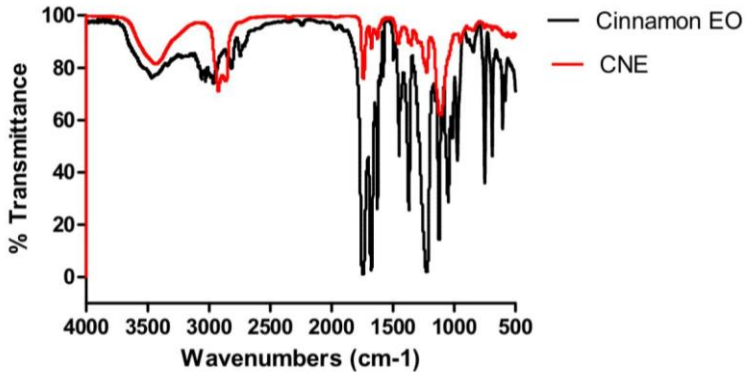
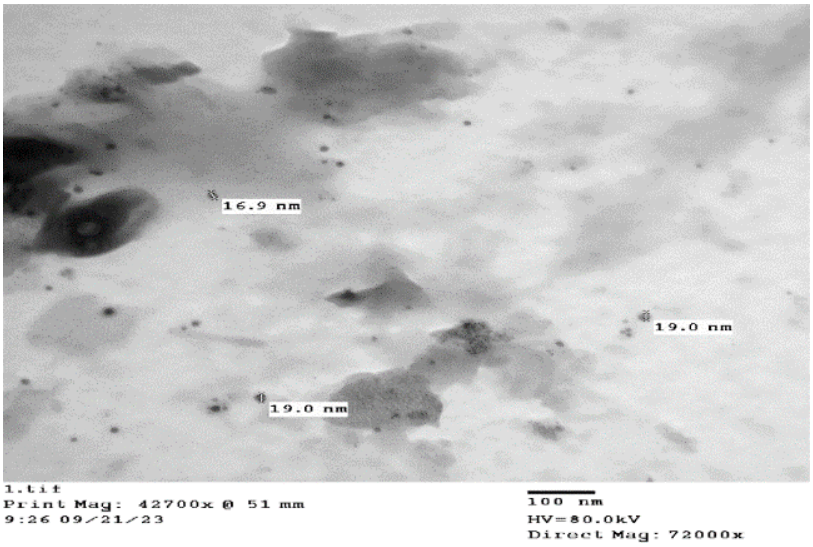


Figure 1. FTIR of cinnamon essential oil and cinnamon nanoemulsion



Picture 1. Morphological study of cinnamon nanoemulsion (CNE) by using Transmission Electron Microscope

Conclusion

The examined luncheon samples were highly contaminated with high number of *Staph. aureus* which renders them unfit for human consumption and indicates impersonal hygiene and unsanitary conditions during processing and handling. Natural preservatives like (CEO) can be used as promising alternative to chemical preservatives against *Staph. aureus*. Indeed, CNE can easily be formulated with existing food ingredients and technologies and has unique characterization, high biosafety, rapid onset of action and long-term stability. They exhibited inhibitory activity against *Staph. aureus*.

Authors' contribution

The work was equally distributed between authors. All authors have read and approved the final version of the manuscript. There is no need for ethical approve because the samples weren`t animals but meat products.

Conflict of interest

There is no conflict of interest.

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