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A STUDY ON OXYTETRACYCLINE RESIDUE IN MILK AND KAREISH CHEESE WITH REGARD TO THE ANTIMICROBIAL ACTIVITY OF SOME ESSENTIAL OILS

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ABSTRACT

The presence of antibiotic residues in milk and kareish cheese poses potential risks to human health, attributed to the improper use of antibiotics in veterinary practices. To assess oxytetracycline residues by double-beam UV spectrophotometer, a total of 90 random samples were collected from markets in Assiut City, Egypt. These samples included raw and UHT milk, and kareish cheese. The results showed that oxytetracycline residues were detected in 100% of the analyzed milk samples and in 96.67% of kareish cheese. The means of oxytetracycline residue in raw milk, UHT milk, kareish cheese were 38.68 ± 2.15, 35.15 ± 3.05 and 40.98 ± 3.54 μg/kg, respectively. Also, the results showed that all analyzed milk were below the codex alimentarius maximum residual limit for oxytetracycline. Thermal treatments (boiling and freezing) of raw milk were followed by a determination of the content of oxytetracycline, and the results revealed that thermal treatment was effective in lowering such content. The reduction percentage of OTC residue after boiling and freezing of milk ranged from 8.32 to 68.28% and 2.58 to 81.63%, respectively, the antibacterial activities of some essential oils were studied against selected reference pathogenic bacteria (*Escherichia coli* O157, *Staphylococcus aureus*, and *Salmonella typhimurium*). The obtained results indicated that the used oils had high antibacterial activity. So, this research suggests that essential oils should be used as feed additives for livestock as they are effective antibacterial agents and safer than antibiotics.

Keywords: Oxytetracycline, Milk, Kareish cheese, Thermal, Essential oils.

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INTRODUCTION

Potential adverse health effects are occasionally associated with milk and kareish cheese, particularly due to antibiotic residues. Drug residue may exist in the food due to the use of drugs in food-producing animals to prevent diseases and improve the feed conversion rate. (Li *et al.,* 2020).

The existence of drug residue in food occurs due to uncommitment to the withdrawal period for each medication, the use of excess doses, and contamination of feed with treated animal waste. (Leibovici *et al.,* 2016).

Oxytetracycline (OTC) is one of the tetracycline antibiotic groups, produced by *Streptomyces rimosus* (Li *et al.,* 2015). OTC is widely used as a broad-spectrum antibiotic (Mevius *et al.,* 1986). It is the preferred drug in veterinary medicine due to its potent antimicrobial activity, affordable cost, and ease of use by farmers (Nonga *et al.,* 2009; Katakweba *et al.,* 2012)

Exceeding antibiotic residue levels in food has adverse effects on health, including the development of antibiotic resistance, disruptions to normal gut microflora, sensitive reactions, carcinogenicity, mutagenicity, nephropathy, hepatotoxicity, and bone marrow toxicity (Mitchell *et al.,* 1998; Kabrite *et al.,* 2019). Moreover, Aalipour *et al.,* 2015 indicated that the OTC pollution poses a risk of teratogenicity. The long-term consumption of milk polluted with OTC has been linked to teeth pigmentation among infants and children (Alanazi *et al.,* 2021).

Heat treatment of raw milk is applied in dairy manufacturing and at home to enhance safety and prolong shelf life (László *et al.,* 2018). Boiling temperature can reduce OTC residues in milk, although complete degradation of the antibiotic may not be achieved (Fathy *et al.,* 2019).

Due to overuse and misuse of antibiotics in dairy farms leading to antibiotic-resistant bacteria (ARB), ARB are becoming a major global health concern. Every year, millions of people contract antibiotic-resistant bacteria, resulting in over 20,000 deaths worldwide (Cheng *et al.,* 2020). This raises concerns about both human health and the future use of antibiotics. The use of antibiotics in animal feeds has been strictly prohibited by numerous nations so organizations make an effort to reduce the hazards associated with ARB. The goal of the livestock and poultry industries is to reduce antibiotic use without compromising animal growth performance (Zhang *et al.,* 2021a).

Finding an alternative derived from plants with excellent antibacterial activity to replace the role of antibiotics in animal feeds seems to be a possible approach to solve this challenge (Freitas *et al.,* 2020). Essential oils (EOs) and their bioactive components have greater appeal as they are matched with current opinions on the future of livestock and poultry industries (Zhai *et al.,* 2018; zhang *et al.,* 2021b).

Natural additives such as essential oils (EOs) and herbal extracts have recently replaced chemical antibacterial agents for their medicinal effects, low toxicity, and cost-effectiveness (Aminzare *et al.,* 2017). EOs consist of many bioactive substances and have wide pharmacological properties, including antibacterial, antifungal, and antioxidant activities (El Hachlafi *et al.,* 2023). They may be used as anti-inflammatory agents (Chamkhi *et al.,* 2022) and as therapeutics for cancer and diabetes (Bakrim *et al.,* 2022). So, it is considered

a potential replacement for antibiotic treatment (Oliva *et al.*, 2018).
These oils had hydrophobic characteristics, allowing them to break down the cell membrane and mitochondria of bacteria, leading to disruptions in cell structure (Sikkema *et al.,* 1994).

More research on the potential use of EOs in animal feed as an antibiotic alternative. Furthermore, it is difficult for bacteria to build resistance against EOs due to the many action sites that EOs have on pathogenic bacteria (Lin *et al.,* 2018).

The study aims to estimate the concentration of OTC residue in milk (raw and UHT) and kareish cheese by the spectrophotometric method. Furthermore, determines the effect of both thermal treatments (boiling and freezing) of raw milk. Finally, examine the antibacterial activity of some EOs (thyme, black seed, and cinnamon oils) against selected pathogenic bacteria (*E. coli, Staph. aureus, and Salmonella typhimurium*) so Thus, the goal of this review is to provide an overview of the viability of using EOs as in vitro antibiotic replacements in animal feed.

MATERIALS AND METHODS

1- Determination of OTC residue in milk and Kareish cheese:

1. Samples collection:
A total of 90 random samples of raw and UHT milk, kareish cheese (30 each) were collected from different localities in Assiut city, Egypt, in the period from March to December 2023. The collected samples were transferred to the laboratory of the Food Safety Center, Faculty of Veterinary Medicine, Assiut University, Egypt, as soon as possible for examination.

2. Samples extraction:

2.1. Milk and kareish cheese samples: milk (raw and UHT) and kareish cheese samples were made using techniques described by Samanidou and Nisyriou (2008). Five grams of each sample was combined with 2.5 ml of 0.1 M succinic acid (pH 4). 10 ml of Mcllvaine-EDTA (0.1 M sodium EDTA, 0.1 M citric acid and 0.2 M di sodium hydrogen phosphate Na_2HPO_4) was added to the mixture. Then the mixture was sonicated for ten minutes before being frozen for fifteen minutes. The mixture was centrifuged for 15 minutes at 4000 rpm and 10°C. The supernatant was filtered with Whatman filter paper, and kept at 4°C until analysis.

3. Calibration curve determination:
According to the procedure done by Merey *et al.* (2017) and Kaluka Tshibamba *et al.* (2023). The stock solution (1000 μg/ml) was prepared by dissolving, in a 100 ml volumetric flask, 0.1 g of OTC dihydrate powder (Sigma, Aldrich) in 20 ml of 0.1 N HCl. Then it was completed to the volume with the same solvent. Then a working solution (100 μg/ml) was prepared by dissolving 10 ml of stock solution in a 100 ml volumetric flask with 100 ml of 0.1 N HCl. From this working solution 0.25, 1, 2, 3, and 4 ml were placed into 5 separate 10 ml volumetric flasks. Then they were completed to volume with 0.1 N HCl to obtain calibration standard concentrations of 2.5, 10, 20, 30, and 40 μg/ml of OTC. A blank solution was prepared by using only 0.1 N HCl.

4. The limit of detection (LOD) and limit of quantitation (LOQ):
They were calculated by the following equations: $LOD = 3.3\ \sigma/S$ and $LOQ = 10\ \sigma/S$ (Shabir, 2003). Both calculations were based on the standard deviation of the y-intercepts of regression analysis (σ) and the slope (S).

5. Sample analysis:
The samples were analyzed according to Malgwi *et al.* (2023). Pipette 0.5 ml of

standard working concentration (40 µg/ml) into a 10 ml test tube. Then, 2 ml of each extracted sample and 2.5 ml of 0.1 N HCl were added and vortexed. A blank test tube was made using only 0.1 N HCl. The absorption was measured at 327 nm against blank by a UV-VIS double-beam spectrophotometer (Jenway).

6. Calculation of OTC residue in the examined samples:

The residue concentration (µg/ml) was estimated by the linear curve equation (Figure 1) according to Beer Lambert's law, and then it was converted to µg/kg.

7. Estimation of human health risk based on hazard quotient (HQ) for OTC residue:

HQ = estimated daily intake (EDI) / acceptable daily intake (ADI) Where

ADI was 30 µg/kg, according to Australian Pesticides and Veterinary Medicines Authority (2021).

EDI = (Concentration of OTC residue in µg/mL × Daily Intake of each type of sample in kg/person) / Adult Body Weight (kg). Where:

- Daily intake (kg/person) of milk was 0.05897 kg**,** and Kareish cheese was 0.01894 kg, according to the Food and Agriculture Organization (2013).

- Body weight was 70 kg.

2. Effect of thermal treatment of raw milk on the level of OTC residue:

Raw milk samples were subjected to (boiling and freezing) to compare the effect of these treatments on the concentration of OTC with the original sample. The boiling was done for 5 minutes (Almashhadany, 2021). The freezing was done at -20°C for 24h. The OTC concentration was quantified by the previous spectrophotometric method

3. Antimicrobial activity of some EOs against selected pathogenic bacteria.

The antimicrobial activity of some EOs against selected pathogenic bacteria was determined by the agar well diffusion method. Then, the minimum inhibitory concentration (MIC) of the EOs and OTC against pathogenic strains was measured using the resazurin-based 96-well plate microdilution method.

3.1. Essential oils:

EOs include thyme, black seed, and cinnamon oil. These oils were obtained as reference oils from the National Research Centre, Cairo, Egypt.

3.2. Pathogenic microorganisms:

Microorganisms include *Echerichia coli* O157 (ATCC25922), *Staphylococcus aureus* (ATCC6538), and *Salmonella typhimurium* (ATCC14028). These strains were obtained as reference microorganisms from the Animal Health Research Institute in Cairo, Egypt.

3.3. Preparation of tested pathogenic strains:

Tested pathogenic strains were prepared according to the method described by the Clinical and Laboratory Standards Institute (2006), the turbidity was adjusted to approximately 1×10^8 CFU/ml by comparing it to a McFarland barium sulfate standard 0.5%.

3.4. Determination of Zone of Inhibition

The antibacterial activity of used EOs was determined in duplicate by the Agar well diffusion method according to Joshi *et al*., (2009), Firstly, 20 ml of sterilized Mueller Hinton agar medium was poured into the sterile Petri dishes and left to set. The freshly prepared bacterial inoculum (1×10^8 CFU/ml) for each strain was swabbed onto individual Petri dishes by using sterilized cotton swabs. The wells were drilled into the agar (with a diameter of 6 mm) using a sterile borer. Each well was sterilely injected with 30 -100 µl of essential oil (1000 mg/ml), and the petri dishes were left for 30 min to facilitate diffusion of the oil in the medium prior to incubation. Then the plates were incubated at 37°C for 24 h. Antimicrobial effectiveness was determined by measuring the diameter of the zone of inhibition (DIZ) against the examined bacteria.

3.5. Determination of MIC of used EOs against selected pathogenic strains:

According to methods of Elshikh *et al.* (2016), the plates were prepared in triplicate by pipetting 200 ul of essential oil solution (1000 mg/ml) into the first well of column of the 96 well plates. Then 100 ul of Muller Hinton broth (MHB) was added to all other wells. Two-fold serial dilutions were performed from well 1 to well 10 using a multichannel pipette such that each well had 100 ul of essential oil solution in serially descending concentrations. After 10 ul of each bacterial suspension (1×10^8 cfu /ml) were added to each well (1 to 11) then mix well. Finally, the plates were incubated at 37 °C for 24 h. Then, 20 ul of resazurin indicator solution (0.015%) was added in each well, further incubation for 2-4 h for the observation of color change. Each plate had a set of controls: a column with all solutions except the essential oils (positive control) and a column with all solutions except for the bacterial solution (negative control). The MIC value was achieved when there was no colony growth in the well of the plate as color of reszaurin had not changed.

Statistical analysis:

The statistical program GraphPad Prism 5 (version 5.01) was used for data analysis. Then descriptive statistics of ANOVA were performed to measure the mean ± standard error (SE). Differences between concentrations were assessed by Tukey's multiple comparison test ($P < 0.05$).

RESULTS

1 -Determination of OTC residue in raw, UHT milk and Kareish cheese:

The detection of OTC residue (Table 1) postulated that the residue was found in 100% of the analyzed milk samples (raw and UHT). It was detected in 96.67% of kareish cheese, while 3.33% of these samples (kareish cheese) were free from such residue.

The means of OTC residue in the analyzed raw milk, UHT milk, kareish cheese and samples were 38.68 ± 2.15, 35.15 ± 3.05 and 40.98 ± 3.54 µg/kg, respectively, with minimum values of 21.75, 5.87and 7.06 µg/kg, respectively, while, the maximum values were 71.68, 72.82 and 85.03 µg/kg, respectively. Also, the residue in all milk samples was below the Maximum Residual Limit (MRL) set by the Codex Alimentarius Commission (CAC,2021). But there is no available MRL for OTC in kareish cheese. The statistical analysis revealed that the different types of samples did not differ significantly as p-value > 0.05 (Table 2).

Risk assessment utilizes the HQ to represent the ratio of OTC exposure concentration and level. The risk to human health is considered not significant and has no harmful effects when HQ ≤ 1. If it increases above this level (HQ >1), that indicates a potential risk to human health (Moudgil *et al.*, 2019).

Moreover, Table 3 denotes the EDI and HQ of OTC residue based on the consumption of analyzed samples and a standardized body weight of 70 kg, in which the EDI values associated with raw milk, UHT milk, and kareish cheese were 0.0326, 0.0296 and 0.0111 µg/kg, respectively, and HQ values were 0.0011, 0.0010 and 0.0004, respectively. The HQ values of all examined samples in this study were below the risk level.

2-Effect of thermal treatment on OTC levels in raw milk:

The contents of OTC residue after (boiling and freezing) the raw milk samples were tabulated in Table 4. Thermal treatment was applied to samples with a higher level of OTC residues. The boiling process could reduce the content of OTC from 42.59±3.11 to 26.01±1.82 µg/kg in raw milk samples, with a reduction % ranging from 8.32 to 68.28%. While the freezing process decreases such content to 26.57±1.85 µg/kg, the reduction ranged from 2.58% to 81.63%. There are significant differences (p-value < 0.05) observed when comparing pretreated raw milk to both boiled and frozen milk samples.

3- Antibacterial activity of some EOs against selected pathogenic bacteria:

3.1. Determination of Zone of Inhibition

The result shown in Table 5 and Figs. 2, 3, and 4 found that thyme oil showed antibacterial activity against *E. coli*, *S. aureus*, and *S. typhimurium* with diameter inhibition zone (DIZ) of 47.5, 54, and 55 mm, respectively.

While the DIZ of black seed oil is shown in Table 5 and Figs. 5, 6, and 7 for both *E. coli* and *S. typhimurium* was 10.5 mm and 51.5 mm for *S. aureus*.

The result of the antibacterial activity of cinnamon oil against *E. coli*, *S. aureus*, and *S. typhimurium* was 54.5, 57, and 51.5 mm, respectively, as shown in Table (5) and Figs. (5, 6, and 7).

3.2. Determination of the MIC of used EOs:

The results of the MIC of EOs against pathogenic strains presented in Table 6 and Fig. 8 indicated that thyme oil has lower MIC values of 2 mg/ml for all bacterial strains.

Moreover, the MIC of black seed oil against *E. coli*, *S. aureus*, and *S. typhimurium* was 62.5, 4, and 31.3 mg/ml, respectively.

The MIC of cinnamon oil against both *E. coli* and *S. typhimurium* was 4 mg/ml, and against *S. aureus* it was 2 mg/ml.

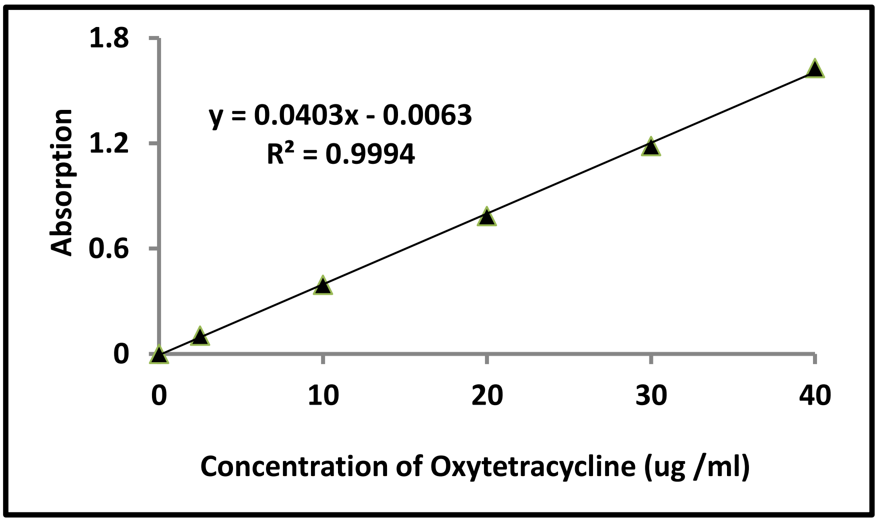

Fig. 1: Calibration curve and equation of the linear curve of Oxytetracycline
X = concentration
0.0403 = slope of the calibration graph
0.0063=Y-intercept

Fig. 2: Thyme EO's effect on *E. coli* growth.

Fig. 3: Thyme EO's effect on *S. aureus* growth.

Fig. 4: Thyme EO's effect on *S. typhimurium* growth.

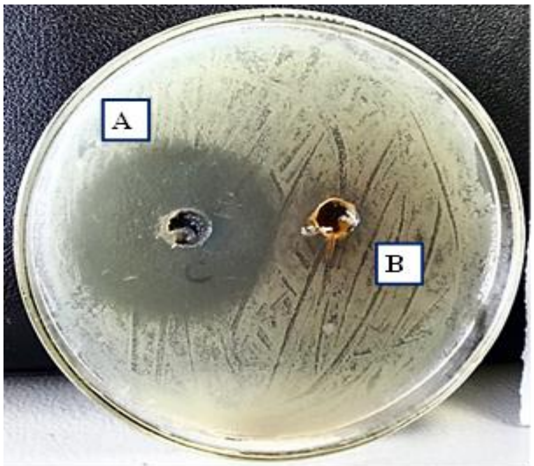

Fig. 5: Cinnamon (A) and black seed (B) EO's effect on *E. coli* growth.

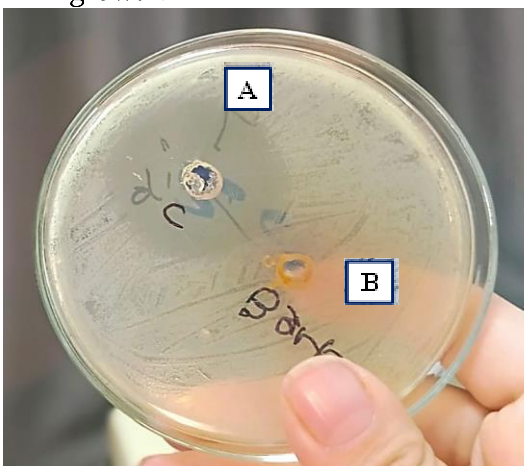

Fig. 6: Cinnamon (A) and black seed (B) EO's effect on S. aureus growth.

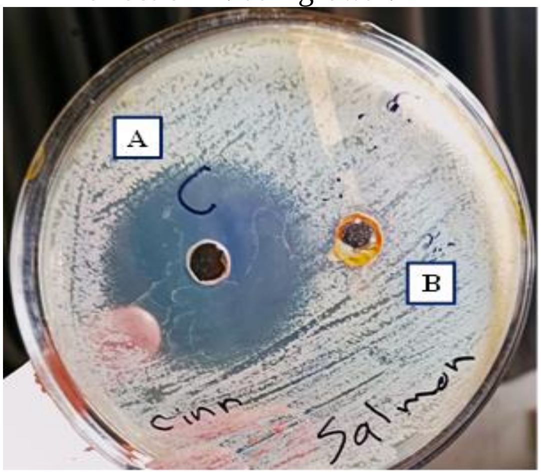

Fig. 7: Cinnamon (A) and black seed (B) EO's effect on *S. typhimurium* growth.

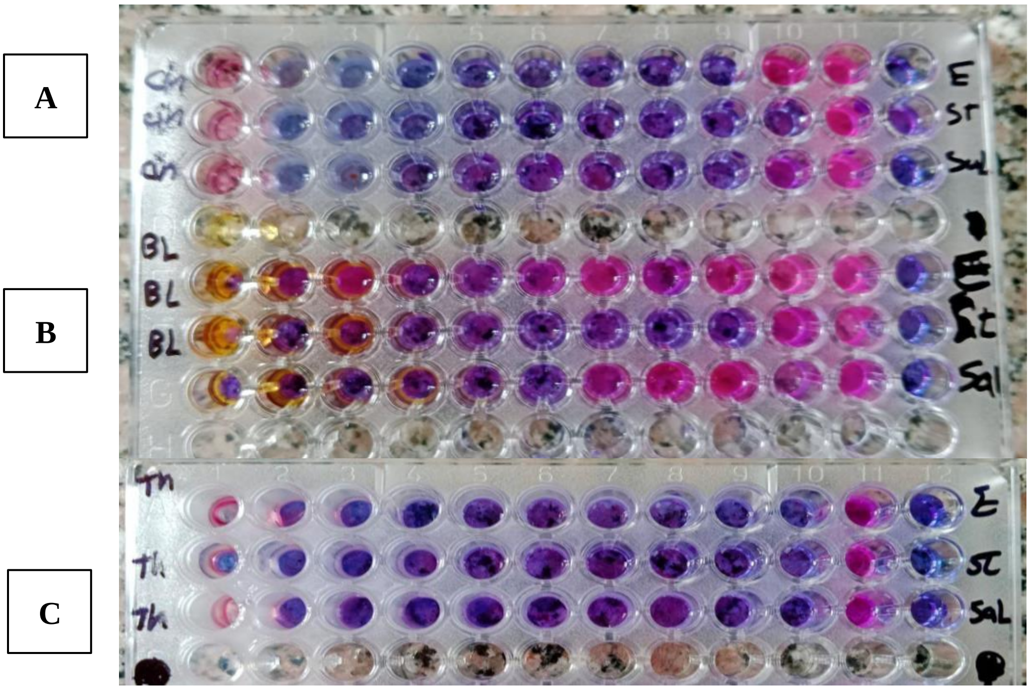

Fig. 8: MIC determination of cinnamon (A), black seed (B), and thyme (C) EOs on *E. coli* (E), *S. aureus* (ST), and *S. typhimurium* (Sal) by the resazurin microdilution plate technique.

Table 1: Oxytetracycline residue in the analyzed samples

Sample type	No. of samples	undetectable levels		detectable levels*	
		No.	%	No.	%
Raw milk	30	nil	nil	30	100
UHT milk	30	nil	nil	30	100
Kareish cheese	30	1	3.33	29	96.67

* The detectable levels of OTC residue were detected with a detection limit of 1.4 µg/ml and a quantification limit of 4.4 µg/ml.

Table 2: The content of oxytetracycline residue in the analyzed samples

Sample type	The content of the residue (µg/kg)			Samples above the Maximum residual limit (MRL)**	
	Min.	Max.	Mean ± SE	No.	%
Raw milk (n=30)	21.75	71.68	38.68 ± 2.15*	Nil	nil
UHT milk (n=30)	5.87	72.82	35.15 ± 3.05*	Nil	nil
Kareish cheese (n=30)	7.06	85.03	40.98 ± 3.54*	N.A"	N.A"

Data are presented as Mean±SE
*Mean no significant difference (p-value > 0.05)
"N.A means not available MRL.
** Mean Codex Alimentarius Commission (2021) stated that the maximum residual limit (MRL) of oxytetracycline in milk should be < 100 µg/kg.

Table 3: Human health risk based on estimated daily intake of oxytetracycline (OTC) residue through the analyzed samples consumption

Sample type	Mean of OTC residue (µg/kg)	Daily Intake (kg/person) *	Body weight (kg)	Estimated daily intake (µg/kg) (EDI)	Acceptable daily intake (µg/kg) (ADI) **	Hazard Quotient
Raw milk	38.68	0.05897	70	0.0326	30	0.0011
UHT milk	35.15	0.05897	70	0.0296	30	0.0010
Kareish cheese	40.98	0.01894	70	0.0111	30	0.0004

*Daily Intake in Egypt (kg/person) of milk was 0.05897 kg, and Kareish cheese was 0.01894 kg according to Food and Agriculture Organization (2013).

** Acceptable daily intake (ADI) was 30µg/kg according to the Australian Pesticides and Veterinary Medicines Authority (APVM) (2021).

Table 4: Effect of thermal treatments of raw milk on oxytetracycline (OTC) levels

Thermal treatment	OTC level (µg/kg)			Reduction%
	Min.	Max.	Mean ± SE	
Pretreatment	27.41	71.68	42.59 ± 3.11	
Boiling	15.80	42.25	26.01 ± 1.82*	8.32-68.28
Freezing	13.17	40.26	26.57 ± 1.85*	2.58-81.63

Data are presented as Mean±SE

* mean a significant difference (P-value < 0.05)

Table 5: Diameter of inhibition zone (DIZ) of tested essential oil against selected pathogenic bacteria

Strain of pathogenic bacteria	DIZ of essential oil (mm)		
	Thyme	Black seed	Cinnamon
E. coli (ATCC25922)	47.5	10.5	54
S. aureus (ATCC6538)	55	51.5	57
S. typhimurium (ATCC14028)	54	10.5	51.5

Table 6: Minimum inhibitory concentration (MIC) of the tested essential oils against selected pathogenic bacteria

Strain of pathogenic bacteria	MIC (mg/ml)		
	Thyme oil	Black seed oil	Cinnamon oil
E. coli (ATCC25922)	2	62.5	4
S. aureus (ATCC6538)	2	4	2
S. typhimurium (ATCC14028)	2	31.3	4

DISCUSSION

1- Determination of OTC residue in raw, UHT milk and Kareish cheese:

The detection of OTC residue in milk was found in 100%, these results may be due to the intramammary infusion of antibiotics for treating mastitis and may be due to the use of oxytetracycline in the treatment of various bacterial diseases in dairy farms. A high percentage of kareish cheese is due to the transfer of antibiotic residue that exists in milk to the cheese during the manufacture of cheese as revealed by Cabizza *et al.* (2017).

The same results were obtained by Beltrán *et al.* (2013), who revealed that OTC residue was detected in all examined sheep and goat milk samples. However, the lower detection limit of residue in milk samples (30%) was noticed by El-Makarem *et al.* (2020), who found that the detection rate of OTC in examined raw milk samples was 30%.

The residue in all milk samples was below the Maximum Residual Limit (MRL) set by the Codex Alimentarius Commission (CAC,2021). But there is no available MRL for OTC in kareish cheese. The statistical analysis revealed that the different types of samples did not differ significantly as p-value > 0.05.

The results showed that the OTC residues in all examined milk (raw and UHT) and samples were compatible with the MRL stated by (CAC,2021). These observations were consistent with those indicated by Malgwi *et al.* (2023), who found that all examined raw milk samples were below the MRL. El-Makarem *et al.* (2020) noted that the residue was greater than the MRL in 15% and 10% of the examined cow and buffalo milk samples, respectively.
Moreover, The HQ values of all examined samples in this study were below the risk level, and that is considered safe for human consumption. The same findings were achieved by Al-Shaalan *et al.* (2022) and Rahman *et al.* (2021), who observed insignificant adverse impacts on consumer health associated with the intake of the investigated milk samples as HQ values were lower than the toxicological standard value.

2- Effect of thermal treatment on OTC levels in raw milk:

High temperatures can disrupt covalent bonds and destabilize ring structures in antibiotics, explaining the efficiency of boiling in the deactivation and degradation of antibiotics. Moreover, raising the temperature from 60°C to 100°C considerably lowers the duration of action antibiotic residues in milk (Kurjogi *et al.*, 2019). Pasteurization of milk does not achieve full inactivation and degradation of drug residues (Botsoglou & Fletouris, 2001).

There are significant differences (p-value < 0.05) observed when comparing pretreated raw milk to both boiled and frozen milk samples.

This study indicated that thermal treatments (boiling and freezing) of raw milk samples were effective in lowering OTC residues detected in these samples, and from comparing the results of both thermal treatments, it was clear that boiling was more significant. This was similar to that studied by Almashhadany (2020), who demonstrated that the boiling of milk samples significantly eliminated antibiotic residues in these samples.

3. Antibacterial activity of some EOs against selected pathogenic bacteria:

3.1. Determination of Zone of Inhibition

The use of EOs is growing to combat multidrug-resistant pandemic pathogens (Mulyaningsih *et al.*, 2010).

These results were higher than that obtained by Mahmood *et al.* (2023), who observed that (DIZ) of thyme EO ranged from 8 mm to 20mm.

While the DIZ of black seed oil in this result is nearly similar to that obtained by Abraham *et al.* (2019), who noticed that the zone of inhibition of Nigella. sativa for *S. aureus* was 8.35±0.35–18.35±0.53 mm and for *E. coli* was 5.43±0.15–11.33±0.85 mm.

The results of the antibacterial activity of cinnamon oil against *E. coli*, *S. aureus*, and *S. typhimurium* were similar to those achieved by Lalami *et al.* (2019). The high activity of cinnamon EO was confirmed by Singh *et al.* (2007), who found inhibition diameters of 56.7 ± 0.1mm for *S. aureus* and 35.1 ± 0.3mm for *E. coli*. Also, Unlu *et al.* (2010) found inhibition diameters higher than 40mm for *S. aureus* and 26mm for *E. coli*.

3.2. Determination of the MIC of used EOs:

The results of the MIC of EOs against pathogenic strains indicated that thyme oil has lower MIC values of 2 mg/ml for all bacterial strains. These results agreed with Gonçalves *et al.* (2017), who noted that thyme EO had the strongest antimicrobial activity against *S. aureus* (MIC varied between 0.125 and 0.6 mg/ml). Some previous studies demonstrated that thyme EO administration inhibited the activity of S. *aureus* and *E. coli* (Yazdi *et al.*, 2013 and Elhofy *et al.*, 2019).

Moreover, the results of MIC of black seed oil against *E. coli*, *S. aureus*, and *S. typhimurium* were agreed with by Abraham *et al.* (2019), who found that the MIC of N. sativa was 1.28 mg/ml. in harmony, the result of the MIC of black seed EO against *E. coli* disagreed with the same author of 32 mg/ml.

The MIC of cinnamon oil in this study agreed with Reyad (2023), who detected that the cinnamon MIC was 2.5 mg/ml for *S. aureus*. Cinnamon EO exhibited potent antibacterial activity against foodborne spoilage and pathogenic bacteria, including *E. coli* and *Staphylococcus spp*. (Zhang *et al.*, 2016; Intorasoot *et al.*, 2017 and Al-Garadi *et al.*, 2023).

In vitro investigations in this work revealed that thyme and cinnamon oils had the highest antimicrobial activity, followed by black seed oil. Similar finding was obtained by Valdivieso-Ugarte *et al.* (2021), who revealed that thyme and cinnamon oils had a better antimicrobial effect against *E. coli and S. typhi*.

The strongest antibacterial activity of EOs is due to chemical compounds found in them such as thymol, cinnamaldehyde, camphor, eugenol, and carvacrol that change the structure and integrity of membranes, inhibit ATPases, and prevent the formation of cell walls (Bajpai *et al.*, 2012; Mohamed *et al.;* 2013; Shreaz *et al.*, 2016 and Salehi *et al.*, 2018).

The present study found that the most susceptible pathogen to the EOs was *S. aureus*. This result was consistent with those of Evangelista-Martínez *et al.* (2018).

CONCLUSION

OTC residue was detected in all analyzed milk samples and in the majority of Kareish cheese. Thermal treatments of raw milk were effective in reducing OTC residue. Moreover, EOs can be used in feed additives of livestock as an alternative to antibiotics in the dairy field.

Conflicts of interest

All authors have approved the submission, and none declares any conflict of interest in the work performed or in the submission of the manuscript.

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دراسة عن متبقيات الأوكسي تتراسيكلين في الألبان والجبن القريش والتأثير المضاد للنشاط الميكروبي لبعض الزيوت العطرية

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إن وجود بقايا المضادات الحيوية في الألبان وجبن القريش يشكل مخاطر محتملة على صحة الإنسان، ويعزى ذلك إلى الاستخدام غير السليم للمضادات الحيوية في الممارسات البيطرية. لتقييم بقايا الأوكسي تتراسيكلين بواسطة مقياس الطيف الضوئي للأشعة فوق البنفسجية مزدوج الشعاع، تم جمع ٩٠ عينة عشوائية من الأسواق في مدينه أسيوط، مصر. وشملت هذه العينات اللبن الخام والمعقم، وجبن القريش (٣٠ لكل منها). أظهرت النتائج وجود بقايا الأوكسي تتراسيكلين في ١٠٠% من عينات اللبن التي تم تحليلها و٩٦,٦٧% من عينات الجبن القريش وكانت متوسط بقايا الأوكسي تتراسيكلين في اللبن الخام واللبن المعقم وجبن القريش ٣٨,٦٨ ± ٢,١٥، ٣٥,١٥ ± ٣,٠٥و٤٠,٩٨ ± ٣,٥٤، ميكروغرام / كجم على التوالي. كما أظهرت النتائج أن جميع عينات اللبن التي تم تحليلها كانت أقل من الحد الأقصى المتبقي للأوكسي تتراسيكلين لدى الدستور الغذائي. تم تطبيق المعاملات الحرارية (الغلي والتجميد) على اللبن الخام وتلاها تحديد محتوى الأوكسي تتراسيكلين، وسجلت النتائج أن كلا من المعاملات الحرارية كانت فعالة في خفض هذا المحتوى. نسبة الخفض في بقايا الـ الأوكسي تتراسيكلين بعد غلي وتجميد اللبن تراوحت من ٨,٣٢ إلى ٦٨,٢٨% ومن ٢,٥٨ إلى ٨١,٦٣% على التوالي. وتمت دراسة الأنشطة المضادة للبكتيريا لبعض الزيوت العطرية ضد البكتيريا المسببة للأمراض المرجعية المختارة (الميكروب الايشيرشى القولونيةO157 ، المكور العنقود الذهبي وسالمونيلا تيفيموريوم). أشارت النتائج المتحصل عليها إلى أن الزيوت المستعملة لها نشاط مضاد للجراثيم عالي ولذلك يقترح هذا البحث باستخدام الزيوت العطرية في إضافات الأعلاف للماشية الحية باعتبارها أكثر أمانًا من المضادات الحيوية ومضاد بكتيرى فعال.