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Effect of *Moringa oliefera* Aqueous Extract on Food Poisoning Pathogens Isolated from Dairy Products

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Abstract

The high nutritional value of milk and dairy products renders them susceptible for bacterial contamination easily under the absence of hygienic practices and regulations. Therefore, a total of 100 random samples of milk and milk products (25 of each: raw milk, kareish cheese, processed cheese and ice cream) were collected from both Sharkia and Dakahlia Provinces, Egypt, for microbiological examinations. Staphylococcu*S. aureus* and *Pseudomonas aeruginosa* counts were performed using HiCrome™ Staph Selective Agar (Himedia, Mumbai) and *Pseudomonas* Agar Base (Himedia, Mumbai) with glycerol and CetriNix Supplement (Himedia, Mumbai) as a selective media. The obtained results revealed that the mean *S. aureus* counts in the examined raw milk, kariesh cheese, processed cheese and ice cream were $3.08x10^5 \pm 1.73x10^5$, $3.20x10^5 \pm 2.80x10^5$, $5.78x10^4 \pm 4.24x10^4$ and $6.60x10^5 \pm 2.93x10^5$ CFU/g, respectively; the mean *Pseudomonas aeruginosa* counts in the examined raw milk, kariesh cheese, processed cheese and ice cream were $2.02x10^6 \pm 1.24x10^6$, $1.85x10^4 \pm 8.80x10^4$, $2.26x10^5 \pm 1.43x10^5$ and $7.68x10^5 \pm 4.22x10^5$ CFU/g, respectively According to the Egyptian Organization for Standardization and Quality (EOS) all samples examined in this study exceeded the permissible limits of *S. aureus* counts reported in Egyptian Standards. The examined samples were not accepted for *S. aureus*, *Pseudomonas aeruginosa* count. In conclusion, the examined products revealed unsatisfactory hygienic measures. Therefore, strict hygienic practices should be adopted during processing of dairy products to improve the bacteriological quality of such products. *Moringa oliefera* 0.05% aqueous extract showed antibacterial effect against *S. aureus* by 56.7% and 83.3% reduction percentage in examined yogurt at day one and day three, respectively, but no effect against *Pseudomonas aeruginosa*.

KEYWORDS
Moringa oliefera, Food Poisoning, Milk products, raw milk

INTRODUCTION

All the people all over the world take up milk and or dairy products that are highly nutritious and rich of all nutrients (Dehkordi *et al*., 2013; Rozenberg *et al*., 2016). The most consumed dairy product is cheese (Ranadheera *et al*., 2019), that is produced in several ways and types (Fox *et al*., 2017; Tarakçı and Deveci, 2019). One of the most popular type of cheese in Egypt is kariesh cheese which is light acidic cheese made of skimmed milk (Fayed *et al*., 2014; Mohammed *et al*., 2021), while the most popular frozen milk is ice cream for all children, adult and even ill people suffered from inflamed mouth and throat (EL-Prince and Hussien, 2000). Keeping raw milk in chilling environments for few days (De Jonghe *et al*., 2011) allow the growth of psychrotrophic microorganisms (Xin *et al*., 2017), also the spoilage of chilled cheese may be due to contamination of *Pseudomonas* spp. (Ibrahim *et al*., 2022), where it can grow easily at low temperature as it is classified as psychro¬trophic pathogen (Nowak *et al*., 2012). Moreover, *Pseudomonas* can grow in rough environmental conditions with minimum requirements (Bamaiyi, 2013). *P. aeruginosa* is incriminated in food poisoning and as a causative agent of severe clinical cases (CDC, 2015; Rehm, 2008). Poor sanitary practice, improper handling, low quality materials and weak regulations are the main causes of foodborne diseases in developing countries (Farzanh *et al*., 2012; WHO, 2015). On the other hand, food handlers are incriminated obviously in Staphylococcal food poisoning through poor hygienic practices that contaminate food with *S. aureus* during handling and preparation of food (Viktoria *et al*., 2001; Todd *et al*., 2008). It was proofed that 100 ng of *S. aureus* enterotoxins or 10^6 *S. aureus* CFU/ gm can contaminate food causing Staphylococcal food poisoning (Balaban and Rasooly 2000), so it is very important to evaluate the microbial content of milk and dairy products (Momtaz *et al*., 2012), particularly, ready to eat food that will not be heat treated further, making it under great concern (Baumgartner *et al*., 2014). Recently, there is a great attention to use natural additives in food manufacturing as antibacterial agents (Abou-taleb & Kawai, 2008; Puvača *et al*., 2020); these agents were used as natural preservatives by ancient Egyptian then in China and India (Tajkarimi *et al*., 2010). *Moringa oliefera* has been examined under many research for several years as a medicinal plant with antibacterial effect (Ghebremichael *et al*., 2005; Suarez *et al*., 2005; Vieira *et al*., 2010). Thus, this present study was planned to evaluate *S. aureus* and *Pseudomonas aeruginosa* counts in raw milk, Kareish cheese processed cheese, and ice cream, marketed in small outlets, vendors and markets in both Sharkia and Dakahlia Provinces, Egypt and to gain better insight into the effect of aqueous plant extract (*Moringa oliefera*) on these two pathogens.

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MATERIALS AND METHODS

Collection of samples

A total of 100 random samples of milk and milk products as raw milk, kareish cheese, processed cheese and ice cream (25 of each) were collected from different supermarkets, small outlets, vendors and markets in Sharkia and Dakahlia Provinces, Egypt. All samples were transported to the Milk Hygiene Laboratory, Faculty of Veterinary Medicine, Zagazig University in an ice box in their containers as purchased to the public with no delay for conventional bacteriological analysis.

This study was done under the ethics of Zagazig University guidelines.

Preparation of ten folds serial dilution

This step was done according to APHA (1992) and APHA (2004).

Determination of S. aureus count

0.1 ml from each previously prepared serial dilution was spread on the surface of pre-poured Petri dishes with HiCrome™ Staph Selective Agar (Himedia, Mumbai) (M1913) (APHA, 2004; APHA, 2015). The inoculated and control plates were inverted after 10 min to allow the agar to absorb the inoculum and incubated at 35-37°C for 24-48 hours, Staphylococcus will grow as green colonies which were recorded, then *Staphylococcus aureus* count was calculated according to FDA (2001).

Determination of Pseudomonas aeruginosa count

0.1 ml from each previously prepared serial dilution was spread on the surface of *Pseudomonas* Agar Base (Himedia, Mumbai) (M085) with glycerol and CetriNix Supplement (Himedia, Mumbai) (FD029) (APHA, 2015). After evenly spreading, the plates were incubated to obtain presumptive colonies after the plates had been incubated at 34-38°C and examined inoculated plates after 24 hours and 48 hours. The presence of blue-green fluorescence under UV lamp is considered as presumptive evidence of *Pseudomonas aeruginosa* then all developed presumptive colonies were counted (ISO, 2004).

Moringa oliefera treatment:

Extracts preparation

Aqueous extract was prepared according to (Shah *et al.*, 2015).

Preparation of yogurt

At first we heated buffaloes' milk to 85°C for 15 min then it was rapidly cooled at 43°C. Heat treated milk was portioned into 2 portions, where the first one was the control one, the second was treated with 0.5% of aqueous extract of *Moringa oliefera*, then the two portions were inoculated with 2% starter culture and an ideal confirmed isolated strain of *S. aureus* and were incubated at 43°C for 3 h. The same was done to examine *Moringa oliefera* aqueous extract against *P. aeruginosa* (El-Gammal el al., 2017).

Determination of antibacterial activity of aqueous extract of Moringa oliefera Reduction test of S. aureus and P. aeruginosa

Serial dilutions 0.1 mL of different dilutions were spreaded onto pre-poured plates of *Pseudomonas* Agar Base (Himedia, Mumbai) (M085) with glycerol and CetriNix Supplement (Himedia, Mumbai) (FD029) (APHA, 2015) and HiCrome™ Staph Selective Agar (Himedia, Mumbai) (M1913) plates which were incubated at 37°C for 24hrs. Bacterial count was obtained from the control and examined portions yogurt at day 1 and day 3 to determine the antibacterial effect of plant extract on bacteria count was performed as mentioned by APHA (2004).

Statistical analysis

Means ± standard error (S.E) represented bacterial counts which were analyzed by SPSS and One-Way Analysis of Variance (ANOVA) at a 95% level of confidence showing the significant differences between examined sam¬ples using the Duncan Multiple Range test where $p<0.05$ as significant.

RESULTS

S. aureus count

The *S. aureus* mean counts in the examined raw milk, kariesh cheese, processed cheese and ice cream were $3.08x10^5\pm1.73x10^5$, $3.20x10^5\pm2.80x10^5$, $5.78x10^4\pm4.24x10^4$ and $6.60x10^5\pm2.93x10^5$ CFU/g, respectively; with minimum counts of $1.0x10^3$, $3.8x10^2$, $5.0x10^2$ and $1.5x10^2$CFU/g, respectively and maximum counts of $1.80x10^6$, $2.0x10^6$, $1.80x10^6$ and $2.0x10^6$ CFU/g, respectively (Table 1).

Table 1. *S. aureus* count (CFU/g) in raw milk and some dairy products (N=25, of each).

Type of Samples	*S. aureus* count/g		
	Min.	Max.	Mean ±S.E
Raw milk (n=25)	$1.0x10^3$	$1.80x10^6$	$3.08x10^5\pm1.73x10^5$
Kariesh cheese (n=25)	$3.8x10^2$	$2.0x10^6$	$3.20x10^5\pm2.80x10^5$
Processed cheese (n=25)	$5.0x10^2$	$1.80x10^6$	$5.78x10^4\pm2.24x10^4$
Ice cream (n=25)	$1.5x10^2$	$2.0x10^6$	$6.60x10^5\pm2.93x10^5$

S.E.: Standard error of mean; N: Number of examined samples; CFU/g: Colony Forming Unit per gram.

Pseudomonas aeruginosa count

The *Pseudomonas aeruginosa* mean counts in the examined raw milk, kariesh cheese, processed cheese and ice cream were $2.02x10^6\pm1.24x10^6$, $1.85x10^4\pm8.80x10^4$, $2.26x10^5\pm1.43x10^5$ and $7.68x10^5\pm4.22x10^5$ CFU/g, respectively; with minimum counts of $2.3x10^3$, $1.2x10^3$, $2.3x10^3$ and $2.5x10^3$CFU/g, respectively and maximum counts of $1.50x10^7$, $4.40x10^5$, $6.0x10^5$ and $4.0x10^6$ CFU/g, respectively (Table 2).

Moringa oliefera treatment (Reduction test)

Mean count of *S. aureus* (CFU/g) in control samples was $1.53X10^7\pm2.08X10^7$ CFU/g, while it was $6.61X10^6\pm9.04X10^6$ and $2.54X10^4\pm3.48X10^6$ CFU/g af¬ter treatment by *Moringa oliefera* 0.05% aqueous extract at day 1 and day 3, respectively, a significant reduction % was found between control and treated samples as 56.7% and 83.3% at day one and day three, respectively, while *Moringa oliefera* aqueous extract shows no effect on *P. aeruginosa* count or reduction.

Table 2. Total *Pseudomonas aeruginosa* count (CFU/g) in raw milk and dairy products (N=25, of each).

Type of Samples	*Pseudomonas aeruginosa* count/gm.		
	Min.	Max.	Mean ±S.E
Raw milk (n=25)	$2.30x10^3$	$1.50x10^7$	$2.02x10^6±1.24x10^6$
Kariesh cheese (n=25)	$1.20x10^3$	$4.40x10^5$	$1.85x10^4±8.80x10^4$
Processed cheese (n=25)	$2.30x10^3$	$6.0x10^5$	$2.26x10^5±1.43x10^5$
Ice cream (n=25)	$2.50x10^3$	$4.0x10^6$	$7.68x10^5±4.22x10^5$

S.E.: Standard error of mean; N: Number of examined samples; CFU/g: Colony Forming Unit per gram.

Table 3. Reduction test using Moringa olifera aquas extract treatment against Staph. aureus and reduction percentage at days 1 and 3 in yogurt samples.

	S. aureus			
	Min.	Max.	Reduction%	Mean ±S.D
Control	$4.80 x10^5$	$3.0x10^7$	0	$1.53x10^7±2.08x10^7$
Day1	$2.10 x10^5$	$1.30x10^7$	56.70%	$6.61x10^6±9.04x10^6$
Day3	$8.00 x10^4$	$5.00x10^6$	83.30%	$2.54x10^4±3.48x10^6$

DISCUSSION

The Egyptian Standards (ES, 2005) for raw milk classified samples that exceed 10^2 for *S. aureus* as unaccepted samples while, soft cheeses (like Kareish and processed) and ice cream must be free from pathogenic organisms and their toxins or render those samples as unsatisfactory items, where count of *S. aureus* acts as a hygienic and sanitary indication factor (Morshdy *et al*., 2023). Therefore, all raw milk, cheese and ice cream samples in our study exceeded the permissible counts limits of *S. aureus* mentioned in Egyptian Standards (ES, 2005), where the *S. aureus* mean counts in the examined raw milk, kariesh cheese, processed cheese and ice cream were $3.08x10^5±1.73x10^5$, $3.20x10^5±2.80x10^5$, $5.78x10^4±4.24x10^4$ and $6.60x10^5±2.93x10^5$ CFU/g, respectively, (Table 1). These results were nearly in accordance with Baumgartner *et al*. (2014) that examined soft cheese in Switzerland for *S. aureus* and found that about 49% of soft cheese ranged between 10^2 and 10^5 CFU/g. Our results in this study were higher than those mentioned by AL-Tahiri (2005) who declared that *S. aureus* counts in fresh milk and farmer produced cheese in Jordan were $3x10^2$ and $5x10^3$ (CFU)/g, respectively. On the other results these results were lower than results obtained by Al-Ashmawy *et al*. (2016) who found 3.49, 2.93 and 3.40, $\log_{10}$ (CFU)/g among tested raw milk, Kareish cheese and ice cream samples, respectively in a study was carried in Egypt. Different results were detected by Salem *et al*. (2016) who found the mean *S. aureus* counts $1.26x10^9±9.7x10^8$ and 7.80 x 102±$3.2x10^2$ in Kareish cheese samples collected from street vendors and supermarkets, respectively in Egypt. When a contaminated surface is in a direct contact with milk like milking equipment or milker,s hands that result in unaccepted contaminated milk with high viable counts (AL-Tahiri, 2005), moreover, using this contaminated milk in production of traditional dairy products allows presence of foodborne pathogens like *S. aureus* in this products (Kadariya *et al*., 2014). *Pseudomonas* is a psychrophilic bacterium that is obviously detected in raw milk and cold stored products as it can grow easily below 8°C in the absence of good hygienic practices. (von Neubeck *et al*., 2015; Li *et al*., 2018; Hahne *et al*., 2019; Skeie *et al*., 2019). Our study showed *P. aeruginosa* mean count in the examined samples were $2.02x10^6±1.24x10^6$, $1.85x10^4±8.80x10^4$, $2.26x10^5±1.43x10^5$ and $7.68x10^5±4.22x10^5$ CFU/g in raw milk, kariesh cheese, processed cheese and ice cream samples, respectively (table 2). Atia *et al*. (2022) reported lower results of *P. aeruginosa* mean count in examined samples of raw milk, kariesh cheese and Ice cream collected from Egypt as 1.9± 2.1, 6.3±1.1 and 1.0±1.6 $\log_{10}$ CFU/ml, respectively, also Lower results of *P. aeruginosa* mean count were reported by B€ohnlein *et al*. (2021) as value of 3.9±1.3 log cfu mL^{-1} in raw milk in Northern Germany. El-Leboudy *et al*. (2015) examined Damietta and kariesh cheese for *P. aeruginosa* count and mean counts were $9.02x10^4±2.87x10^4$ and $2.43x10^5±9.32x10^4$ for Damietta and kariesh cheese, respectively with higher results than those in our studies. There are many sources of *P. aeruginosa* in dairy products such as fecal contamination, unhygienic practices or even unchlorinated water used in cleaning or steps of production (Bartram *et al*., 2003; El-Leboudy *et al*. 2015). The most predominant psychrotrophic pathogen in raw milk is *Pseudomonas aeruginosa* (Ibrahim *et al*., 2022). *Pseudomonas aeruginosa* was proofed to have a great public health hazard, mainly for immune compromised persons or who are suffering from Cystic fibrosis as it can invade several tissue cells causing severe diseases like septicemia and meningitis (De Victorica and Galvān, 2001). *Moringa oliefera* is a medicinal plant; its aqueous extract contains some antibacterial and antioxidant components like gallic acid (El-Gammal *et al*., 2017). In our study, aqueous extract 0.5% of *Moringa oliefera* reduced *S. aureus* count by 56.7% and 83.3% reduction percentage at day one and day three, respectively in examined produced yogurt and rendered its main count at day three less than the health risk limit which is105 CFU/g (Baumgartner *et al*., 2014), where it was $2.54X10^4±3.48X10^6$ after three days of treatment with *Moringa oliefera* aqueous extract 0.5% (The mean count was at first$1.53X10^7±2.08X10^7$), but *Pseudomonas aeruginosa* didn't change or reduced. El-Gammal el al. (2017) illustrated that *Moringa oliefera* aqueous extract has antimicrobial effect against Gram positive pathogens as *S. aureus*, but not against Gram negative pathogens in an agreement with a study was performed by Vieira *et al*. (2010). Future studies may be directed to show more antimicrobial effect of *Moringa oliefera*.

CONCLUSION

Results achieved in this study represent health risks due to high level of food poisoning pathogens in raw milk and some dairy products those got contaminated easily by improper hygienic practices and, or fecal contamination, our trial to reduce that risk using 0.5% of *Moringa oliefera* aqueous extract against examined pathogens in manufacture of functional yogurt was nearly satisfactory allowing the interested researchers to perform more better further.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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