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The Effect of Myrtus Communis Extracts on the Pathogenic *Salmonella SPP*. Isolated from Milk and Soft Cheese in Baghdad City

Moutaz A.W. Abdul Mounam[1*]

Department of Veterinary Public Health, College of Veterinary Medicine, University of Baghdad, Iraq[1]

Corresponding Author: 1*

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ABSTRACT

Milk and cheese contamination with harmful bacteria is more common particularly those made from raw or inadequately pasteurized milk, have been implicated in the transfer of many foodborne diseases to humans. Pathogens enter raw milk from a variety of sources, salmonella is the most prevalent pathogenic bacterium that causes salmonellosis in people and animals. salmonella serovars produce the illness, which can show as anything from mild food poisoning to severe enteric fever. In recent years, extensive research into the antibacterial effects of essential oils and extracts has been conducted, revealing their potency and potential to inhibit the growth of a wide range of hazardous microbes. Fifty (50) samples of raw cow milk and soft white cheese randomly collected from different local market and bulk farm between (February 2021 - May 15, 2022), from the different Area of Baghdad city. Milk and cheese are significant sources of salmonella, particularly for those who prefer raw milk. the prevalence of salmonella was (20% of 50) among different types of samples. We conclude that *Myrtus communis* essential oil possesses antimicrobial properties against the three tested strains: *salmonella typhimurium, salmonella enteritidis and salmonella infantis*. Raw milk and soft white cheese act as a wide source of contamination with pathogen, and we consider *Myrtus communis* oil act as antibacterial action.

1. Introduction

Milk is regarded as a high-quality, nutrient-dense nourishment for humans [15]. The composition and cleanliness provided during milk production and storage are largely responsible for this outstanding quality. However, if suitable sanitary conditions are not followed, germs can infect the milk, causing it to deteriorate quickly.

Milk and products of milk, particularly those derived from fresh or insufficiently sterilized milk, have been linked to the transmission of a variety of foodborne illnesses to humans. Raw milk and its derivatives are regarded additional sources of human non-typhoidal salmonellosis, in addition to chicken items [29].

Pathogens enter raw milk from a variety of sources, including infected bovine excrement, contaminated skin, diseased udders, Airborn illness, [26]. The microbiological quality of several dairy products made from raw milk, such as soft cheese, kariesh cheese, and ice cream, increased the salinity and/or acidity of the manufactured dairy products [27]. Another hurdle in underdeveloped nations is the rudimentary manufacturing process carried out under poor or unregulated sanitary conditions.

Milk contamination with harmful bacteria is more common small livestock keepers in Iraq, particularly in rural areas, tend to raise dairy animals for the milk production and products of dairy.

salmonella is most prevalent pathogenic bacterium that causes salmonellosis in people and animals. salmonella serovars produce the illness, it can manifest itself in a variety of ways, ranging from minor food poisoning to acute enteric fever [17]. Animals infected with salmonelosis incur enormous economic losses as a result of increased mortality and morbidity, and these diseased animals may infect humans through direct or indirect contact. [21]. contamination by salmonella of the nutrient and environment occur mostly over the feces of infected people and animals [22]. salmonella is primarily found in the gastro-intestinal system [23].

Despite scientific advances and the creation of synthetic medications, medicinal plants have been the only source of pain control for millennia and are still commonly utilized today [24]. Numerous studies on the antibacterial properties of essential extractions and oils have been undertaken recently, indicating their potency and capacity to stop the growth of a variety of dangerous pathogens [25].

Myrtus communis is a fragrant evergreen shrub in the Mytaseae family with many stems and branches [16]. Numerous studies have demonstrated the anti-parasitic and anti-infective benefits of this plant's extract, which contains terpinolene, cineol, linalool, terpineol, linalyl acetate, tannins, and flavonoids.

The high rates of infection have persisted despite the use of antibiotics because microorganisms can spread antibiotic resistance from one generation to the next and even from one microbial species to another by developing an antibiotic-resistant gene. Until recently, several studies on myrtle leaf and stem extract have antibacterial effects against harmful microorganisms. such as *staph. aureus*, *Lactobacillus plantarume*, *Bacillus cereus*, *Listeria monocytogenes*, *pseudomonas aeruginosa*, Klebsiella, and Shigella, were conducted with promising results [28].

2. Methodology

- Sample collection: Fifty (50) samples of raw cow milk and soft white cheese were collected randomly from different markets and bulk farms and, small independent dairy farms that provide milk and cheese, local grocers were chosen at random from (February 2021 until May 15, 2022), from the different area of Baghdad city at various points. At the point of sale, the samples were taken in two separate batches and stored in clean plastic containers. They were labeled, and then transported to the laboratory in an ice box in order to do a quick evaluation for the conventional salmonella diagnosis.
- Myrtle (Myrtus communis) leaves were collected in January and dried for two weeks in the shade in a dry, ventilated area before being extracted for essential oil. Hydro-distillation in a Clevenger device was used to produce the essential oil. [20], In a 2-Liter flask, a plant mass of 100 g is submerged in distilled water. The whole thing is then cooked for 3 hours at 100 degrees Celsius. The oil was collected, dehydrated with sodium sulfate anhydrous (Na2SO4), and kept in dark bottles in the refrigerator at (4 C°) to protect it from light and heat [18].
- - Traditional salmonella isolation and identification: Standardized portions of 25 mL of milk and 25

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g of cheese were incubated for 24 hours at 37°C in buffered peptone water in 225 mL of 0.1 percent sterile water. 9 milliliters of (Rappaport Vassilliadis) (RV) broth were combined with one milliliter of the homogenates, and the mixture was then kept at 45°C overnight. The surface of XLD agar was infected with a loop-filling of the improved broth, which was then incubated for one day at 37°C [19]. Three to five pinkish to red colonies with or without a black core from Salmonella colonies that were common on XLD were selected, streaked onto nutritional agar, and then examined. After an (18-24) hour incubation period at 37 C, they were maintained on a slant.

- Congo red agar method: The samples were spread onto congo red agars plates and aerobically incubated for (24-48) hrs at 37°C. The presence of black colonies with a dry crystalline consistency was considered a sign of slime formation. Strong biofilm producers were identified in isolates that produced extremely dark colored colonies. Moderate biofilm producers were defined as bacteria that formed black colonies, whereas weak biofilm producers were defined as bacteria that formed virtually black colonies [30]. Isolates creating red colonies were reflected as non-biofilm producers [31].

3. Results and Discussion

3.1 Isolated Salmonella species:

Milk and cheese are significant causes of salmonella, particularly for those who prefer raw milk. salmonella infections can be caused by a variety of circumstances, including unhygienic farm conditions, handler, and fresh milk and product of milk consumption [1]. In the current study as presented in the table 1, the occurrence of salmonella, was (20% of 50) among different types of samples. The results of the study are in agreement with that of other studies reported by, [9], salmonella was found in 3 (12%) market milk samples. [10], also establish high occurrence of salmonella in dairy products.

Table 1: Occurrence of salmonella at different types of samples (raw milk and soft cheese).

Type of sample		Number	Positive N. (%)
Milk	Market milk	17	3(6)
	Farm milk	8	2(4)
Cheese	Soft white cheese	15	1(2)
	Farm cheese	10	4(8)
Total	50		10(20%)

The predominance of salmonella as 8% (n.4/10) in the farm cheese as shown in table1, followed by 6% (n.3/17) in the market milk samples while 4% (n.2/8) and 2 % (n.1/15) was detected in farm milk and soft white cheese subsequently. At the farm bulk milk salmonella was not founded as mentioned by [11]. This discrepancy could be attributed to differences in milking hygiene and sanitation methods. Very low prevalence of salmonella in soft white cheese this result is nearly in agreement with [12]. Our study on farm cheese samples revealed that sanitary standards during cheese manufacturing and handling were insufficient. In addition, the majority of these cheeses were marketed in their natural state, with no covering. As a result, the risk of *salmonella spp*. contamination was high. the predominance of salmonella

was (2%) (n.1/15), In case of white cheese as shown in (table 1). Our findings matched those of [13], however [14] found a reduced occurrence of salmonella in white soft cheese. Soft cheese has a high moisture and pH content, especially on the surface, which is assisted throughout the ripening process, making it more susceptible to microbial development than hard or semi hard cheeses [2]. Salmonella could be spread through the use of tainted milk, inappropriate heat treatment, improper manufacturing practices, or polluted brine. *salmonella typhimurium*, *salmonella enteritidis* and *salmonella infantis* were detected in different milk and cheeses samples as shown in figures 1,2,3 subsequently. These were the most common salmonellosis serotypes that cause gastroenteritis in humans in most regions of the world, which is usually straightforward and requires no treatment. However, it can be severe in young and elderly people, as well as individuals with impaired immunity [3]. *salmonella typhimurium*, *salmonella enteritidis*, and *salmonella infantis* were found in farm cheese in this research, with *salmonella enteritidis* being the most prevalent. *salmonella typhimurium* was exposed to acidic medium for 4 hours at pH 5.5. As a consequence of this, acid adaptation is a risky survival strategy for salmonella species in fermented dairy products and likely in other acidic meals as well. [4]. *s. typhimurium* had high rate of prevalence in our study and these result in compatible with other study on sheep animal mentioned that *s. typhimurium* had more prevalence [34].

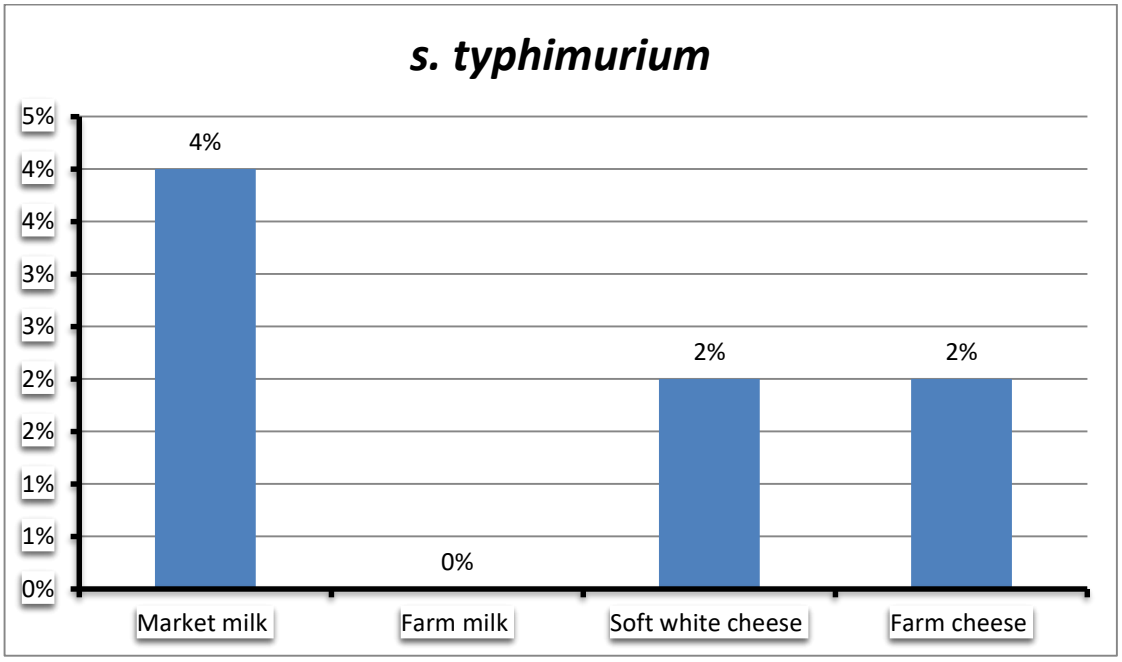

Figure (1): Occurrence of *sallmonella typhimurium* in different types of raw milk and soft cheese samples.

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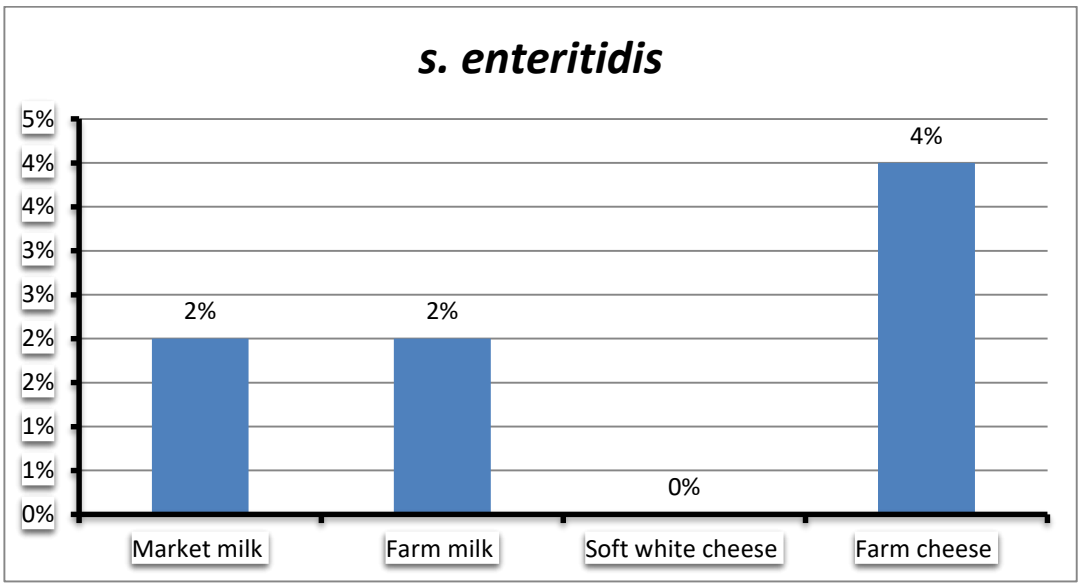

Figure (2): Occurrence of *sallmonella enteritidis* in different types of raw milk soft and cheese samples.

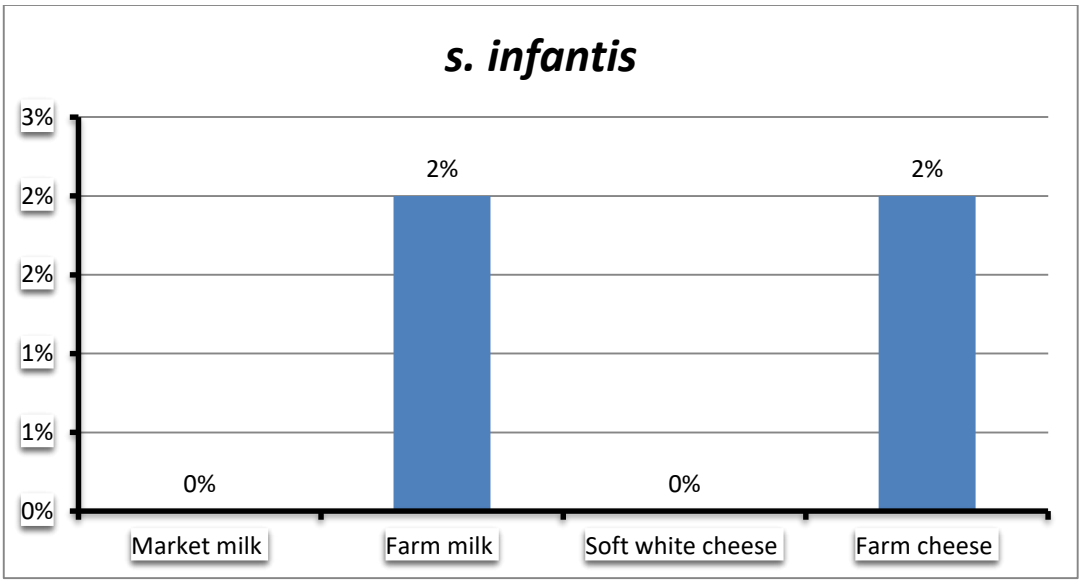

Figure (3): Prevalence of *salmonella infantis* in different types of raw milk and soft cheese samples.

3.2 Susceptibility of isolated salmonella species to medical plant:

The sizes of inhibition zones determined around the discs are used to express the results of the antibacterial activity investigation of our extract (Table 2). Based on the results (Table2), we may establish that the vital oil of *M. communis* has antibacterial action against the three tested strains: *salmonella typhimurium*, *salmonella enteritidis* and *salmonella infantis*. *salmonella typhimurium* has the broadest inhibition zone (18 mm), followed by *salmonella enteritidis*, which has a 15 mm inhibition zone, and *salmonella Infantis*, which has an 11 mm inhibition zone.

Table (2): *Myrtus communis* oil activity against bacterial isolates.

Bacteria	Diameter of inhibition zone (mm)
s. typhimurium	18

s. enteritidis	15
s. infantis	11

[5] reported that, after four different treatments, the myrtle leaves oil induced a significant reduction in *salmonella typhimurium* population compared to the controls ($p \leq 0.05$). Table (3) displays that MIC of *Myrtus communis* oil, the concentration of 0.66 was sufficient to inhibit the growth of *salmonella Infantis* followed by *salmonella enteritidis* that was inhibited by minimum concentration of 1.32. On the other hand, *salmonella typhimurium* was inhibited at minimum concentration of 2.64.

Table (3): The essential oil of *Myrtus communis* at its minimum inhibitory concentration (MIC).

Concentration mg/ml								
Isolates	**1/100 6.6**	**1/250 2.64**	**1/500 1.32**	**1/1000 0.66**	**1/2000 0.33**	**1/3000 0.22**	**1/5000 0.13**	**Control**
s. typhimurium	-	-	+	+	+	+	+	+
s. enteritidis	-	-	-	+	+	+	+	+
s. infantis	-	-	-	-	+	+	+	+

-: inhibition; + grawth

The first biolite devoid of bacterium is used to calculate the minimum bactericidal concentration. The minimal bactericidal concentration provided in (Table 4) appears to be based on this criterion. 2.64 mg/mL for *salmonella typhimurium*. For *salmonella enteritidis*, the minimum bactericidal concentration is 1.32 mg/mL, whereas the minimum bactericidal concentration for *salmonella infantis* is 0.66 mg/mL.

Table (4): The minimum bactericidal concentration (MBC) essential oil of *Myrtus communis*.

Tested strains	**1/100 6.6**	**1/250 2.64**	**1/500 1.32**	**1/1000 0.66**
s. typhimurium	-	-	+	+
s. enteritidis	-	-	-	+
s. infantis	-	-	-	-

The elemental composition of *Myrtus communis* particularly Monoterpenes such as eucalyptol (1,8-cineole) and a-pinene are primarily responsible for variances in microorganism sensitivity [7]. Antimicrobial characteristics are widely recognized for these chemicals, which belong to the ether and hydrocarbon groups. Indeed, Eucalyptol is a natural saturated monoterpene with mucolytic, antibacterial, and spasmolytic characteristics that has been used to treat sinusitis and bronchitis symptoms. It should also be noted that the antibacterial activity of the essential oil of *M. communis* is most likely responsible for the synergistic action amongst all components [8]. Furthermore, distinct chemotypes of myrtle essential oils have been proven to have significantly higher inhibitory effect against Gram-positive bacteria and Gram-negative bacteria [6].

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Congo red agar method approach was used to biofilms identification. A number of recurrent infections are resistant to traditional antibiotic treatment have been linked to microbial biofilms production. The antibiotic resistant features in nosocomial infections also disseminated by increase mutation rates and enabling the exchange of antibiotic resistance genes [32].

According to the findings of this paper, the adhesive characteristics were put to use in the investigation of the pathogenic properties of microorganisms. Previous research has revealed that one of the most essential variables in the formation of biofilm architectonics, which is characterized by a rise in optical density and multiple drug resistance, is a high adhesive potential. This potential is one of the most critical aspects [33]. Detected biofilm formation in five positive isolates corresponding to 50 % of salmonella isolates from different types of samples, the result of biofilm production property shows; 2(20%) strong biofilm production, 1(10%) appeared as moderate and 2(20%) showed weak reaction as shown in (table 5).

Table 5: Biofilm production of salmonella isolates.

Methods	Biofilm producer NO. (%).				Non-Biofilm producer NO. (%).	Total
	Strong	Moderate	Weak	Total		
Congo red agar method	NO. 2 (20%)	NO. 1 (10%)	NO. 2 (20%)	NO. 5 (50%)	NO. 5 (50%)	NO. 10 (100%)

4. Conclusion

Raw milk and soft white cheese act as a wide source of contamination with pathogens and we may consider *Myrtus communis* oil act as antibacterial action.

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