Research Article

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Evaluation of Anti-Bacterial Activity of *Silybum Marianum* Against *Proteus* Spp. in Minced Meat

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Abstract

This study was conducted to investigate the antibacterial activity of *Silybum marianum* (silymarin) against *Proteus* spp. bacteria isolated from minced meat. Two concentrations (1000 µg/ml and 2000 µg/ml) of *Silybum marianum* were assay against *proteus* spp., by inoculating minced meat for (60 and 120 min): stored at refrigerator temperature for 24, 72, and 120 hours, respectively; the bacterial counting was done on selective media. The results showed that *proteus* spp. were resistant to low concentrations of silymarin, while the inhibitory concentration of silymarin against *Proteus* strains was ≥ 2000 µg/ml. It was concluded that silymarin is a more beneficial antibacterial in the 2000 µg/ml concentration for the elimination of *Proteus* spp. pathogenic bacteria in minced meat than in the low concentration.

Keywards: *Proteus*, Silymarin, Antimicrobial.

تقييم كفاءة مستخلص السيليبوم (*Silybum marianum*) كمضاد بكتيري لبكتريا نوع المتقلبة والموجودة في اللحم المفروم

الخلاصة

أجريت هذه الدراسة للتحقق من النشاط المضاد للبكتيريا لمستخلص *Silybum marianum* (silymarin) ضد بكتيريا نوع المتقلبة والمعزولة من اللحم المفروم. تم اختبار تركيزين من مستخلص *Silybum marianum* (1000 ميكروغرام / مل و2000 ميكروغرام / مل) ضد بكتيريا المتقلبة، عن طريق نقع اللحم المفروم لمدة (60 و120 دقيقة): تخزينها في درجة حرارة الثلاجة لمدة 24 و72 و120 ساعة، على التوالي؛ تم العد البكتيري على وسط انتقائي. أظهرت النتائج أن بكتيريا نوع المتقلبة. كانت مقاومة للتركيزات المنخفضة من سيليمارين ، بينما كان التركيز المثبط للسيليمارين ضد سلالات المتقلبة كانت ≤ 2000 ميكروغرام / مل. نستنتج إلى أن سيليمارين هو مضاد للجراثيم أكثر فائدة في تركيز 2000 ميكروغرام / مل للتخلص من بكتيريا المتقلبة والمسببة للأمراض في اللحم المفروم من التركيز المنخفض.

Introduction

Meat is a nutritious source of protein for humans and contains numerous compounds such as phosphate, vitamins, fat, water, protein, and iron. The majority of the meat has a lot of water, which encourages microorganism to proliferation (1). Meat contaminated by a variety of factors, including the environment, human handling, manipulation, and/or the animal itself (2).

The *Enterobacteriaceae* family is the most difficult to eradicate from meat and meat products around the world. In all cases of food poisoning linked to meat products, *E. coli, Salmonella, Proteus, and Klebsiella* are the most common bacteria (3 and 4). Many food poisoning outbreaks have been linked to *Proteus* group organisms, and with the increased incidence of *Proteus* spp. related food borne diseases, there is

an urgent need for control and/or prophylactic for food poisoning outbreaks linked to meat products. To ensure food safety and protect public health from microbial contamination of food, it is critical to investigate such agents in foods and eliminate them (5).

Enteric bacterial pathogens (*Proteus* spp.) cause food-borne diseases, and the major problem with these bacteria is antimicrobial resistance. Silymarin is a standardized extract from the milk thistle that is clinically used as a hepatoprotective agent (6). However, research on its antibacterial effectiveness against intestinal bacterial infections is limited (7). *Silybum marianum* extract has antifungal properties, inhibiting dermatophyte fungi growth more than saprophyte fungi (8). *Silybum marianum* was shown to possess antimicrobial substances such as terpenoids, flavonoids, and tannins, according to (9). The *Silybum marianum* seed extract was found to be efficient against all bacterial species tested, and showed antibacterial activity against the Gram-positive *Bacillus subtilis* and *Staphylococcus epidermidis* (10). However, this research was conducted to assay the antibacterial effectiveness against enteric bacterial infections is limited (11,12,13). Therefore the present study aimed to investigation the effect of Silymarin extract against *Proteus* that contaminates minced beef.

Materials and Methods

Proteus spp. Isolated bacteria from minced meat were grown in nutrient broth at 37 °C for 24 hours to induce artificial contamination of meat in order to test the effect of silymarin on our isolated bacteria.

Local minced meat was purchased from a local market in Baghdad province,Iraq for about 250 grams per sample, put in a poly ethylene sac and tokened to the lab by using the cool box to be free from *Proteus* spp (14).

The minced meat was inoculated the culture mixture as (15). And the contaminated samples were kept at room temperature for 20 min. for attachment and absorption (16).

The silymarin powder made by Meda (Hamburg, Germany) was purchased from pharmacy. Stock solution of silymarin was prepared at a concentration of 1000 µg/mL and 2000 µg/mL in distal water. These concentrations were chosen according to a study by (17). They found that silymarin is not a beneficial drug for treatment of gram-negative enteric bacterial pathogens at lesser concentration.

Treatment inoculation meat:

Pure Silymarin is purchased from a pharmacy as a capsule and dissolved in distilled water. Each capsule contains 140 mg of pure silymarin, which is dissolved in 140 ml to obtain 1000µg/ml. Another concentration is dissolved in 70 ml to obtain 2000µg/ml. Each sample was separated into two categories, each separated by turn into three samples: one inoculum with a Silymarin concentration of 1000µg /ml, a second part inoculum with 2000 µg /ml, and the last one inoculum with distil water as a control for 60 minutes, while the other categories were treated with the same concentration but a difference in time for 120 minutes. After the inoculum, we take that sample and butt in lockable containers and transfer them to refrigerators at 4 °C and compare the bacterial load three times after 24 h, 72 h, and 120 h, respectively.

Serial decimal dilutions to count bacterial load after treatment

A tenfold decimal serial dilution was prepared using sterile test tubes with nine ml of peptone water in each. The number of cells per ml was determined by the surface spreading of a (0.1) decimal dilution of *proteus* spp. suspension on the *proteus* selective agar plate (19).

Then incubated it for 24 hrs at 37 °C. The microbial load log titers of the counted infected test samples were calculated by the equation:

(No. of counted colony x 10^x)

X is the inverted dilution.

Statistical analysis

The data obtained were expressed as Mean and Standard Error (Mean±S.E) and a two-way analysis of variance (ANOVA) was used in the statistical analysis for comparison between the different groups. In addition, post hoc test was used when appropriate to find the least significant differences (LSD) between different means groups (Statistical significance was defined as a $P \leq 0.05$). The Statistical Package for the Social Sciences (SPSS) version 24 packages were used for all statistical analyses (20).

Result and discussion

The Results showed in Table (1 and 2) that treatment of meat with Silymarin for 60 and 120 minutes respectively showed a significant decrease at ($p \leq 0.05$) in bacterial count in all concentration treatments through meat preservative at refrigerator temperature of (4) °C after 24 hours compared after 72 hours and 120 hours, respectively. While the results showed a significant increase in the bacterial load in meat inoculated with Silymarin concentrations of 1000 µl compared to inoculated with 2000 µl after meat preservation in refrigerator at (4) °C, there was also a significant increase at ($p \leq 0.05$) in the bacterial load in meat inoculated with distal water compared to inoculated with 2000µl after preservation meat in refrigerator temperature (4) °C. Results obtained from Tables 1 and 2 show that Silymarin has less inhibition effect on proteus spp. bacteria in minced meat in low concentrations of Silymarin and that inhibition increases with increasing Silymarin concentration compared with distil water at the same time. noticed that two concentrations of Silymarin in the test don't prevent the full growth of *proteus* bacteria in minced meat and the growth increases with time progress, which means that maybe *proteus* spp. is resistant to low concentrations of Silymarin, while the *proteus* spp. was inhibited by a high concentration of Silymarin. And that agreed with (13) who revealed that silymarin showed significant activity against Enterobacteriaceae. While (21) and (10) confirm that gram-negative bacteria showed resistance to silymarin. Also, (11) showed that gram-negative bacteria were not inhibited by the silymarin concentrations included in their study.

Table 1 effect Silymarin on the bacterial count ($X10^{6-}$) at 60 minute inoculum (+ve) of meat (Mean±S.E.)

Con. Time	1000µg/ml	2000µg/ml	D.w
24 h.	25.0 ± 2.88 C a	9.66 ± 1.2 C b	27.66 ± 1.45 C a
72 h.	139.6 ± 43.01 B a	85.33 ± 7.51 B b	145.6 ± 59.5 B a
120 h.	300.0 ± 20.20 A a	280.6 ± 12.45 A b	294.6 ± 10.45 A ab
LSD	14.2		

The upper or lower case letter refers to significant differences at ($p \leq 0.05$)

Table 2 effect Silymarin on the bacterial count ($X10^{6-}$) at 120 minute inoculum (+ve) of meat (Mean±S.E.)

Con. Time	1000µg/ml	2000µg/ml	D.w
24 h.	54.6 ± 0.88 C b	19 ± 1.15 C c	71.6 ± 3.17 B a
72 h.	160.6 ± 52.5 B a	66.3 ± 5.8 B c	129.6 ± 28.0 B b
120 h.	301.3 ± 12.4 A a	239.6 ± 39.9 A b	242.0 ± 9.81 A b
LSD	16.3		

The upper or lower case letter refers to significant differences at ($p \leq 0.05$)

This study suggests that *proteus* spp. have an additional layer to the outer membrane and that may resistant to silymarin in low concentrations, which agrees with (22) that showed gram negative bacteria have an additional layer based on phospholipids, proteins, and lipopolysaccharides that forms an impermeable barrier to most hydrophobic molecules, making it more resistant than gram positive bacteria.as well as, The *Proteus* species possess an extracytoplasmic outer membrane that contains a lipid bilayer, lipoproteins, polysaccharides, and lipopolysaccharides, various components of the membrane of the proteus species (23).

Another probability The high concentration of silymarin was more effective than the low concentration in inhibiting the growth of bacteria, which suggests that it may have largely belonged to the flavonoids found in the seeds of milk thistle, which are composed of a high proportion of silymarin that has lower melting in water. Therefore, it is not diffused in meat when using distal water as a melted liquid, which agrees with (9).

Conclusion:

The Silymarin extract was effective against *proteus* spp. and have a decreased the bacterial count of treated minced meat.

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Conflict of Interest:

The authors didn't have any conflicts with anyone.

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