

# Microbiological quality of locally and imported minced meat at Diyala province and study antibacterial activity of Rosemary extract against some foodborne pathogens

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## Abstract:

**background:** Beef can act as a route for illnesses transmitted by food due to insufficient hygiene standards at the slaughter of livestock. Minced meat becomes more vulnerable to deteriorating versus whole slices due to the breakdown of the muscle tissue throughout preparation, the release of moisture, and a greater surface area exposed to microbial growth. Several kinds of bacteria that contribute to the decay of minced meat and meat products populate the outer layers of the flesh and invade its core during all various stages. peoples consume medicinal plants, which play an important role in the alleviation of sickness in individuals. A major challenge with modern antibiotic therapy is drug resistance and its related negative effects. Rosemary extracts are being utilized for their antibacterial properties. Its characteristics owing to its chemical components.

## Aims:

To investigate contaminated locally and imported minced meat, all samples submitted to laboratory investigation to assess bacterial load, isolate and identify the contaminated bacteria, according to their cultural morphology and biochemical properties, then determine antibacterial action of rosemary extract with different concentration against some bacteria isolated from minced meat.

**Results:** the microbial load of Total bacterial, coliform bacterial and staphylococcus were (4.93), (4.56) and (4.01) log cfu/gm respectively in the imported minced meat while the bacterial load at local meat were (4.01) (3.69) (3.38) log cfu/gm respectively. The percentage of bacterial contamination in imported meat was (100%) (80%) (72%) with Total, klebsiella and staphylococcus respectively, while the contamination percentage of locally meat with Total, klebsiella and staphylococcus respectively was (100 %) (48%) (20%). The antibacterial effect of the Rosemary alcoholic extract against *Klebsiella* the finding were ( 44 ) ( 20 ) mm at concentration ( 100% ) (80%) respectively while the antibacterial effect of the aqueous extract against *Klebsiella* were ( 22), ( 20 ) at concentration ( 100% ) (80%) respectively .There were no inhibition effect of alcoholic extract at concentration (60% ) (40% )mm against (klebsiella ) also the watery extract at concentration (40% ) not appeared any inhibition activity against (klebsiella ) . while the result of antibacterial activity of alcoholic extract against *Staph. aureus* was (40) (20) mm at concentration (100) (80) respectively while the antibacterial effect of the aqueous extract against *Staph aureus* was (24), (18) mm at concentration (100%) (80%) respectively. There was no inhibition effect of alcoholic and watery extract at concentration

(60%,40%) against this bacterium.

**Conclusions:** The high bacterial for *S. aureus*, *coliform*, total aerobic from minced meat indicated that storage and slaughtering condition not sufficient to ensure consumer safety. Also Conclude Antibacterial effect of Rosemary extract increase with increase its concentration.

**Keyword:** Minced meat, coliform, Rosemary, antibacterial



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## Introduction:

local markets in Iraq usually contains various kinds of food including meat and meat products from several countries regardless of whether or not this food is valid for consumption by human (Jarallah, *et al.*,2014).The inappropriate storage, transport and retail marketing of these food products can increase the opportunity of contamination withvarious biological, chemical and physical contaminants, this threat of food contamination may lead to serious health complications, particularlyby the imported food (Haleem,*et al.*,2013).

Beef can act as a route for illnesses transmitted by food due to insufficient hygiene standards at the slaughter of livestock (Hernandez *et al.*, 2007). Meat frequently exhibits a shortened shelf life owing to its perishable features. The increasing fat, protein, and humidity levels in meat makes it more susceptible to bacterial degradation, discoloration, lipid oxidation (resulting in off-odors), and overall nutritional decline (Zhang *et al.*, 2021). Minced meat becomes more vulnerable to deteriorating versus whole slices due to the breakdown of the muscle tissue throughout preparation, the release of moisture, and a greater surface area exposed to microbial growth. Several kinds of bacteria that contribute to the decay of minced meat and meat products populate the outer layers of the flesh and invade its core during all various stages (Jay *et al.*, 2005).

Internationally, native peoples consume medicinal plants, which play an important role in the alleviation of sickness in individuals as well as animals) Zainab *et al.*, 2013). A major challenge with modern antibiotic therapy is drug resistance and its related negative effects. As a result, following a study into the adverse consequences of plants, humans are using them within numerous sectors (Sales *et al.*,2015). During the discovery of penicillin in the 1940s and subsequent use in medical therapy, novel antibiotics were developed daily for combating infections (Mobaiyen *et al.*, 2016). The widespread utilization of these antimicrobial medications has caused in elevated drug resistance for different antibiotics in a large number of bacterial groups (Jafari-Sales and Bolouri, (2018).

The increasing popularity of herbal as low-risk, economical alternative treatments for curing bacteria-related illnesses, in opposition to synthetic antibiotics, has been a contributory factor (Jafari-Sales, and Hossein-Nezhad, (2019). Besides this, these medicinal plants acquire more popularity among persons (Sales, (2014). The important role of natural substances in the pharmaceutical industry continues to rise, both in their immediate use as therapeutic agents and as raw materials for drug manufacturing or

as examples for the generation of novel physiologically chemically active substances (Tajehmiri *et al.*, 2014).

In the past few decades, there has been a growing demand in herbal preparations owing to their natural source and improving health aspects. These ingredients display anticancer and anti-inflammatory actions, minimize the dangers of myocardial infarction, diabetes, atherosclerosis, also, lower blood cholesterol and blood pressure, and improve immune function (Mahboubi and Haghi, (2008). Rosemary extracts as well as their essential oils are widely used for their antioxidant, antifungal, and antibacterial capabilities. The properties of this compound are attributed to its chemical elements, particularly rosmanol, carnosol, carnosic acid, ursolic acid, rosmariquinone, caffeic acid, and rosmaridiphenol (Kloy *et al.*, 2020).

### **Materials and Methods:**

#### **Watery Extract rosemary:**

Employing a grinder and a mortar, 10 g of the various leaves (rosemary) were crushed, and they were subsequently immersed in 100 ml of distilled water for the whole night. After centrifuging the substances for 10 minutes at 5000 RPM, the supernatants were utilized to make a liquid extract. The sample (extract) was produced from the combined supernatants (Hamasadek *et al.*, 2023).

#### **Alcoholic Extraction rosemary:**

Freshly *Rosmarinus officinalis* leaves have been collected and dried in a location with shade. The dried leaves were subsequently crushed into a fine powder. Fifty grams of leaves were measured and placed to a sterile flask. In the next stage, 250 milliliters of ethanol (98%) were utilized to dissolve the herb's ingredients. A flask of alcohol and herb particles had been shaking for two days at 40°C to allow the solvent to demonstrate its effects. Then, the solvent was eliminated using rotating. Finally, the Rosemary extracts were kept in sterilized dishes in a refrigerator, secured from light by aluminum coverings (Golshani and Sharifzadeh. (2014).



**Figure 1:** Rosemary plant

### Microbiological analysis:

A total of 40 imported minced beef samples weighing 100grams per each were purchased from local supermarkets in Diyala. The samples were taken and transferred directly to the food hygiene laboratory using an ice box under complete aseptic conditions without undue delay. 25 g of product were taken from each sample and placed in a homogenization bag together with 225 ml of sterile 0.1% peptone water. These samples were homogenized for two minutes. Subsequent decimal dilutions were performed in test tubes with 9 ml of the same diluent. All inoculations were carried out in duplicate. Plates with between 30 and 300 colonies (pour-plate technique) were counted, and mean counts were calculated. Isolation and identification of *klebsiella* and *Staph aureus* isolates Between four and six colonies were taken for later identification. Samples were cultured on different media, all the samples were appeared as golden yellow colonies in color on the mannitol salt agar that were first identified as *S. aureus*, then confirmed with Gram staining also biochemical tests, coagulase and catalase activity assays. Moreover, the other samples appear on MacConkey agar opaque, pink in color, mucoid colonies and were positive for Simmon citrate test also demonstrated urease action, which is characteristic as *Klebsiella* (Mohamed *et al.*, 2021).

**Antimicrobial susceptibility of the extract:** Antimicrobial sensitivity test was carried out with the bacterial isolated. They were a subculture in Nutrient Agar. In order to accomplish a dose of  $1.5 \times 10^8$  CFU/ml, five to six colonies from a culture grown overnight was initially diluted in brain heart infusion broth and maintained in anaerobic circumstances for one to two hours at 35°C. A saline solution was then utilized to dilute the colonies till the last concentration was  $1.5 \times 10^6$  CFU/ml. the agar well diffusion method. The bacterial culture was infused in LB broth media at 37 °C for 3 hours, and turbidity was adjusted to a 0.5 value McFarland's index in phosphate-buffered saline. Wells measuring 6 mm in width were created in the LB agar via a sterile syringe cap, and a lawn culture was introduced on the agar with a sterile cotton swab from a diluted culture .Numerous concentrations of herb extract (100%, 80%, 60%, 40%) were inserted into each well, and the plates had been incubated for one day at 37 °C. width of the microbial inhibitory zone surrounding each well was measured and recorded in millimeters (Mohamed *et al.*, 2021).

### Result:

#### Enumeration bacteria:

Total aerobic count (TAC) is utilized as an index to calculate the life span of meat, as an elevated level of this bacterial group may contribute to quick product degradation. The mean values of total bacterial, staph aureus and coliform count (log cfu/gm) in the both locally and imported samples collected of Diyala province are shown in (Table 1 and fig 2).

**Table 1:** The mean values of Bacterial count (log cfu/gm) in Minced meat

Minced raw meat origin	<i>S.aureus</i> log cfu/gm	coliform log cfu/gm	Total viable bacteria log cfu/gm
Local	3.38	3.69	4.01
imported	4.01	4.56	4.93
T test p value	0.000	0.000	0.000

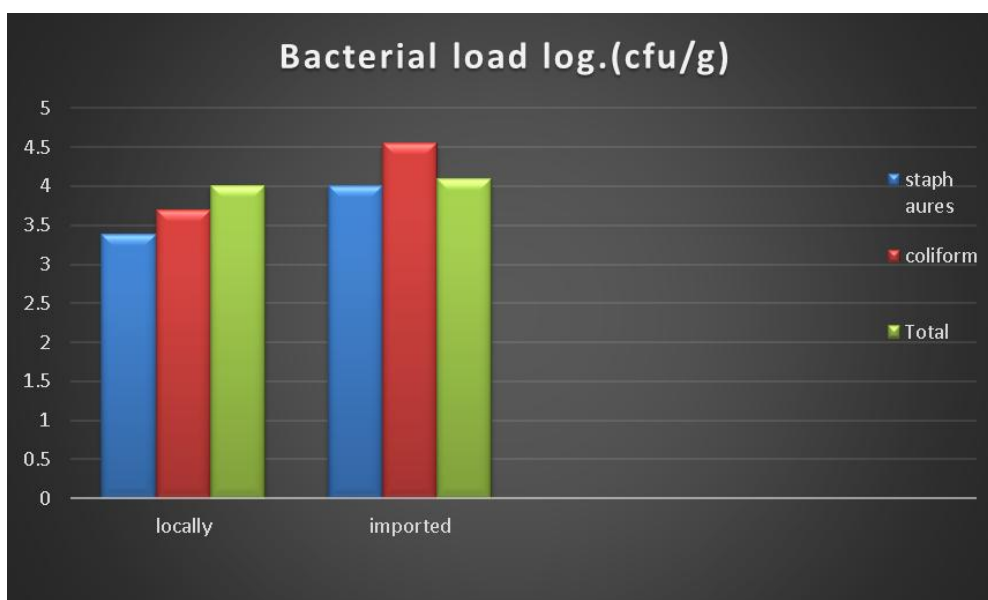


Figure 2: Bacterial load log. (cfu/g) at locally and imported minced meat

### Isolation bacteria:

results showed that the prevalence of *S. aureus* 5 out of 25 (20%), 18 out of 25 (72%) in locally and imported minced meat respectively, *Klebsiella* contamination was 12 out of 25 (48%), 20 out of 25 (80%) in locally and imported meat, while contamination of locally and imported minced meat with Total viable bacteria was 25 out of 25 (100%), 25 out of 25 (100%) respectively. No substantial variances were discovered among the groups at  $P < 0.05$ , as illustrated in (Table 2, Figure 3).

Table 2: percentage bacterial contamination in different source minced meat:

		Bacterial contamination		
		Staph aureus	Klebsiella	Total bacteria
Meat source	Locally	5 (20%)	12 (48%)	25(100%)
	Imported	18(72%)	20 (80%)	25 (100%)
Total		23 out of 50 (46 %)	32 out of 50 (64 %)	50 out of 50(100%)
Chi-Square: 0.068				

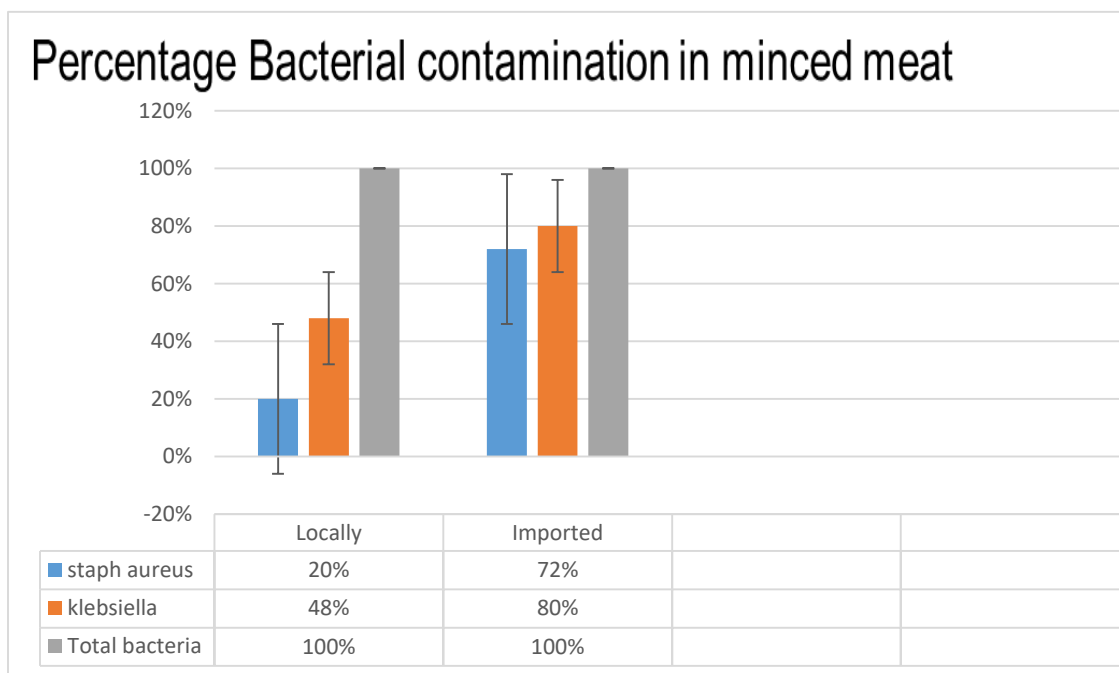


Figure 3: Percentage bacterial contamination in minced meat.

### Antibacterial effect of Rose marry extract against bacteria isolated from Minced meat:

The antimicrobial activity of Rose marry extract with different concentration (100%,80%,60%,40%) against Staphylococcus aureus and Klebsiella isolated from the locally and imported samples by well diffusion assay according to instructions of (NCCLS) are shown in Figure 4.



Figure 4: Antibiotics Sensitivity test

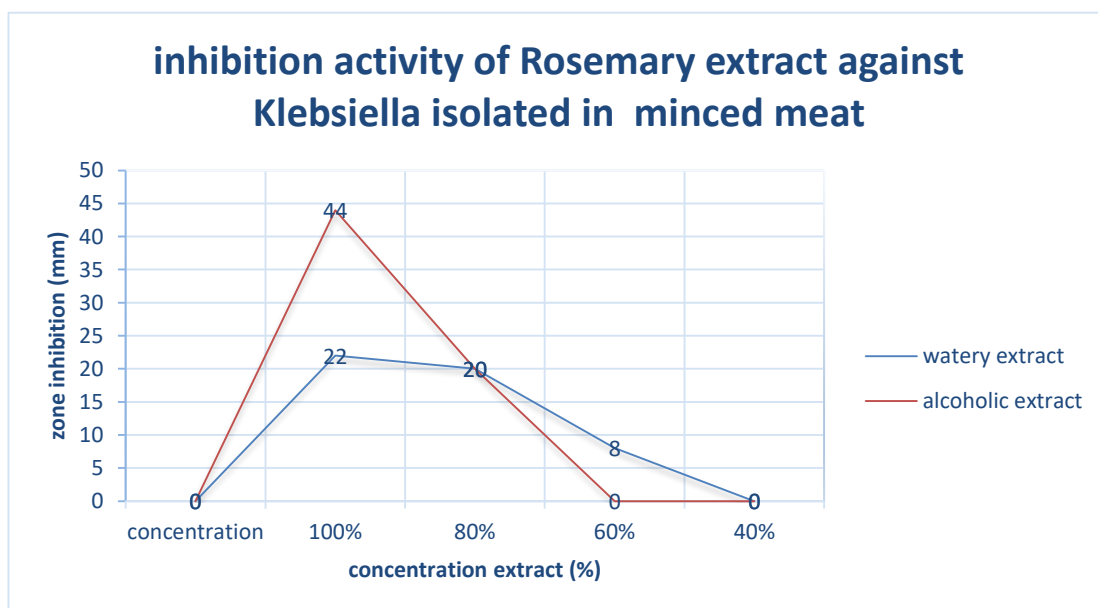


Figure 5: Antibacterial activity of Rosemary extract

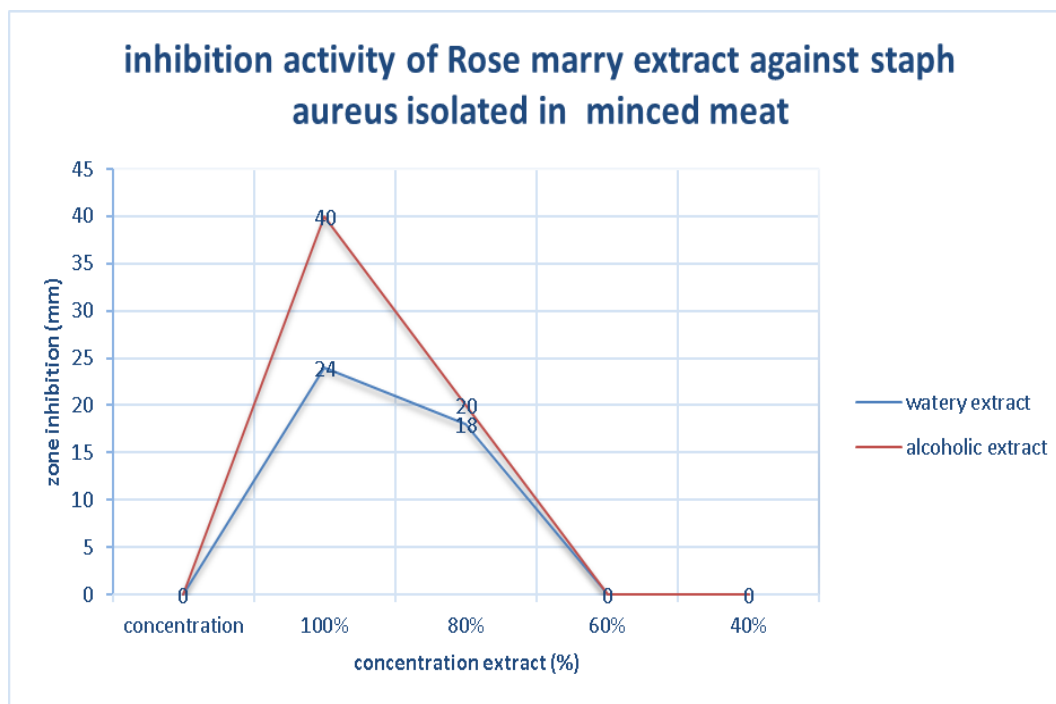


Figure 6: Antibacterial activity of Rosemary extract

### Discussion:

The principal threat to global public health is food contamination caused by foodborne and opportunistic pathogenic microbes, which are the main contributors to foodborne illnesses and fatalities (Tropea, 2022). Coliform bacteria, which develop in animal guts and fecal matter versus muscle tissue, demonstrate that bacterial passage from the gastrointestinal tract to the finished meat product has taken place when detected in wrapped meat (Kassem, Issmat. *et al.*, 2020). This is critical as coliforms involve an extensive range of bacteria, notably pathogenic species such as *Enterobacter* spp. and *E. coli* O157:H7 (Patel *et al.*, 2014).

Most consumers prefer meat from freshly slaughtered animals, which is therefore less contaminated. In contrast, imported meat exceeded the maximum permissible limits for bacterial contamination. If the meat's origin is unknown and the health procedures followed in its production do not adhere to international health standards, this will result in spoiled goods, even if transportation, storage, handling, and display are within the required standards (Khalil, 2020). Throughout history, medicinal plants have played an essential role in healthcare due to their natural bioactive compounds, which exhibit antimicrobial, anti-inflammatory, and immunomodulatory properties, with the emergence of antibiotic-resistant bacteria, researchers have turned to plant based compounds as alternative antimicrobial agents capable of inhibiting bacterial growth and resistance mechanisms (Angelini, 2024).

Because medicinal plants are often used to treat illnesses, scientists have been encouraged to research plants that have pharmacological value and can be used therapeutically to regulate human health (Hina *et al.*, 2020). Antibacterial properties have been identified for several medicinal herbs (Anand *et al.*, 2019). Herbs and spices, which include rosemary (*Rosmarinus officinalis*), serve as an important source of natural antioxidants for usage in the meat manufacturing sector. It can also be employed as a flavoring in different forms of fresh or dried leaves, essential oil, or extracts (including oleoresin).

Rosemary extracts and their essential oils are being utilized for their antioxidant, antifungal, and antibacterial properties (Djenane *et al.*, 2002). Its characteristics can be attributed to its chemical components, notably rosmanol, carnosol, carnosic acid, ursolic acid, rosmariquinone, caffeic acid, and rosmaridiphenol (Kaur, 2023).

The introduction of extracts of oils into chicken meat had been documented to reduced Salmonella numbers without negatively impacting quality through keep at 4°C and 18°C. Stojanović-Radić *et al.*, (2018) assessed the antibacterial and antioxidant properties of rosemary by-products resulting from essential oil distillation, showing that they hinder the spread of Bacillus strains (*B. subtilis*, *B. licheniformis*, *B. cereus*) and *Listeria monocytogenes*, they also noticed an improvement in the antioxidant properties of rosemary (Jafari-Sales *et al.*, 2019). The inhibiting effect of rosemary results from the actions of rosmarinic acid, rosmaridiphenol, carnosol, epirosmanol, carnosic acid, rosmanol, and isorosmanol. They interface with the cell membrane, leading to alterations to genetic material and nutrition, impacting electron transport, the leaching of cellular constituents, and variations in fatty acid formation. additionally, it created an interactions with the protein membrane that led to the loss of both membrane functions and structural (Walid ,*et al.*, 2022).

The bactericidal abilities of rosemary have been successfully established in many nutritional investigations, involving beef meatballs (Fernández-López *et al.*, 2005), cooked beef, and pig sausage. Burt *et al.* (2004) confirmed the antibacterial property of rosemary essential oil against *E. coli*, *Bacillus cereus*, and *S. aureus*. Camo *et al.* (2008) similarly reported the inhibiting impact of rosemary extract used in lamb meat stored in an altered condition on the development of psychrotrophic bacteria, as opposed to control meat. This identical effect was seen by Quattara *et al.* (2001).

**Conclusions:** The high bacterial for *S. aureus*, *coliform*, *total* from minced meat indicated that storage and slaughtering condition not sufficient to ensure consumer safety. Also Conclude Antibacterial effect of Rose marry extract increase with increase its concentration.

**Recommendations:** The meat processing must be monitored by public health authorities (Ministries of Health and Agriculture) from the initial steps of slaughtering in slaughterhouses till marketing and consumption by consumers. In addition Further studies are necessary to investigate the quality and validity of imported meat, particularly meat imported from unknown sources, finally, the further research is needed to assess synergistic effects between Rosemary and Standard

antibiotics

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