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The Activity of Eucalyptus Leaves Extract against some Bacteria isolated from Cattle Minced Meat in Diyala Province

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Abstract:

The Meat and Food Manufacturing are susceptible to contamination and many other foodborne diseases that can arise in food manufacturing areas due to poor personal hygiene and sanitation practices, leading to the growing of multiple pathogenic agents. Beef can be a foodborne pathogens due to unsanitary conditions during animal slaughter and meat processing. In recent decades, scientific strategies have been developed to reduce meat contamination and its effects on human health.

Forty meat samples were randomly collected from several butcher shops in Diyala province, minced, and prepared for the experiment to know the antibacterial activity of Eucalyptus leaves. Leaves were collected from the garden of Diyala University, cleaned and ground. Eucalyptus leaves extract was prepared against some bacteria isolated from minced meat. The aqueous extract of eucalyptus leaves was prepared at different concentrations (100%, 85%, 70%, 55%, and 40%) and used as an antibacterial against Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Klebsiella spp.*) investigated the antibacterial activity of Eucalyptus leaves extract toward some bacteria isolated from minced meat, Forty meat samples collected randomly from Many meat shops Diyala province, water extract (100%, 85%, 70%, 55% and 40%) prepared from Eucalyptus leaves and used as antibacterial activity against gram-positive bacteria (*Staphylococcus aureus*) and gram-negative bacteria (*Klebsiella spp*.), Eucalyptus leaves were collected from the garden of Diyala University, the leaves was cleaned and ground. 25 grams of Eucalyptus leaves were put in 150 ml deionized water, placed on a hot stirrer plate at 90°C for 1 hour, and left for 5 hours, then purified using Whatman No. 1 filter paper. Isolated bacteria were identified based on colony description, gram stain, coagulase test, and catalase test—the antimicrobial activity of the extract against isolates carried out by the good diffusion method.

Results of this study revealed the prevalence of contamination of meat with *Staphylococcus aureus* spp and Klebsiella spp (65, 52.5) % respectively. Eucalyptus leaves extract displayed great activity against *Staphylococcus aureus and Klebsiella spp.* The highest antibacterial

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activity was (33, 20 mm) against *Staphylococcus aureus* and *Klebsiella spp*. respectively, while the lowest activity against these bacteria was (21, 10) mm respectively.

Key word: Eucalyptus, *Klebsiella spp., stap. Aureus*

Introduction:

The greatest consumed foods, meat, and meat products are vital sources of a range of B-complex vitamins, as well as proteins, amino acids, and minerals in humans, Therefore, the body needs animal protein for growth, vitality restoration, and repair of body cells (1,2). Because of their biological characteristics and chemical content, meats are greatly fresh foods providing a better source of nutrients for humans growing (3). Also, it is found that meat contains a high amount of iron, which is one of the micronutrients that humans lack (4).

Local markets in Iraq usually contain various kinds of food including meat and meat products from several countries regardless of whether or not this food is valid for consumption by humans (5). The inappropriate storage, transport, and retail marketing of these food products can increase the opportunity of contamination with various biological, chemical, and physical contaminants, this threat of food contamination may lead to serious health complications, particularly by the imported food (6).

Meat spoilage can occur due to changes in the number of microorganisms on the fresh meat surfaces when stored in cold conditions due to microbial growth. The range of bacterial counts in meat reach about 10^2-10^5 cfu/g, however, approximately 10% can initiate growing (7). Escherichia coli O157:H7 and Staphylococcus aureus are the most common pathogen that contaminates meat, while meat may be contaminated with E. coli O157:H7 by direct contact with sewage or contaminated skin and equipment during animal slaughtering. The carcass surface may become contaminated with S. aureus from numerous sources such as equipment, cattle hides, and infected workers (8).

Recently most major problem to public health is the increased resistance of pathogens to antibiotics because of a decrease in the effect of antibiotic treatment which increases morbidity and mortality. The most important bacteria resistant to the antibiotic are gram-positive bacteria, including vancomycin-resistant enterococci and methicillin-resistant *Staphylococcus aureus* (9). Antibiotic resistance of Gram-

negative bacteria has been reported in *Escherichia coli*, *Klebsiella pneumonia*, actinobacteria, and *Pseudomonas aeruginosa* (10,11). Various antibiotics used frequently have become less effective against these microorganisms. For these reasons, there is a need to find other antimicrobic agents to get rid of antimicrobial-resistant problems (12).

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Staphylococcus aureus is a gram-positive bacterium with a spherical shape non-spore-forming and it is one of the most important reasons of staphylococcal food poisoning, a type of gastroenteritis (13). These bacteria produce *staphylococcal* enterotoxin which causes staphylococcal food poisoning. The symptoms of staphylococcal food position appear after three to six hours of ingestion, including diarrhea, nausea, vomiting, abdominal pain, and headache. It also causes nonfood poisons such as skin irritation, respiratory contagion, mastitis, and wound sepsis (14).

Klebsiella spp is a gram-negative bacterium, rod-shaped, non-motile, facultative anaerobic bacteria, encapsulated, lactose fermenting, normally established in intestine flora and feces. Infection in causes pain, headache, fever, swelling, and pus from a wound, causing severe infection in the liver, bladder, brain, lung, eye, and blood (15, 16).

Eucalyptus trees grow naturally to heights 60 to 80m, Eucalyptus contain bioactive compounds including flavonoid, carotenoid, polyphenols, phytosterol, Phytoestrogen, glucosinolates, coenzyme Q, carnitine, choline, and taurine (17). Aqueous extracts of dried eucalyptus leaves are commonly used as an anti-inflammatory, analgesic, anti-bacterial, antiseptic, antifungal, antiradical activity, antioxidative, antiparasitic, anti-rheumatism, anti-urinary tract infection and treatment of respirational infections symptoms like cold, flu, and sinus blocking (17). Besides, it works as an antibacterial (17), antifungal (18), analgesic and anti-inflammatory effects (19), and anti-oxidative action (20).

Materials and Methods:

Minced Meat Samples:

A total of 40 samples of locally minced meat were collected randomly from various areas in Diyala city, meat samples were saved in safe sterilized plastic polyethylene bags and transported in ice buckets to the public health laboratory for microbiological analysis bags and preserved in a protected freeze case, thereafter they were transferred directly to the public health laboratory under hygienic condition then rapidly examined without interruption.

All isolates were identified based on colony description, gram stain, and biochemical tests. Whole 25 g of meat mixture specimens were crushed and weighed under sanitary conditions, mixed with 225 mL of sterile saline solution in a stomacher bag, and homogenized (14). To create decimal dilutions, 1.0 mL of the primary suspension, (10^{-1}) was added to 9 mL of 0.1% peptone water, taking into consideration no direct contact between the tip of the pipette and the diluent, and then mixed cautiously by a vortex mixer for 5–10 s (21). When a ten-fold sequential dilution, 0.1 mL of diluted homogenate was spread-plated in duplicates

on mannitol salt agar (MSA) and then incubated at 35 °C for 24 h. Typical colonies of Staphylococcus spp. were isolated from similar morphologies plates and cultivated separately on MSA previously smearing in Nutrient Agar Slant for further confirmation (5).

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(12, 13), concluded that purification and identification colonies were distinguished depending on morphological appearance and biochemical tests. All strains were negative on methyl red tests, indol, urease tests, and Voges Proskauer (VP). On the contrary, all isolated microbial strains showed positive results in both catalase and motility tests. In the current study, total testers who seemed no ferment colonies on MacConkey agar and showed (dark purple at first 10 seconds) in the Oxidase test affirm *Pseudomonas* bacteria.

According to (22), Coliforms were grown on MacConkey through incubation with aerobic environments at 37°C for 24 – 48 h. Colonies were noticed by colony counter (Stuart, UK) and counted (pink colony), Colonies were identified using selective media EMB (eosin methylene blue agar).

Preparation and Extraction of *Eucalyptus* leaves extract:

Eucalyptus leaves collected from the garden of Diyala University, kept in a plastic page container, and transported to the workroom (20). The leaves were cleaned by washed with tap water and deionized water individually. The leaves were left to dehydrate at the workroom temperature for a week and after drying the leaves were crushed in electrical grinding as in Figure (1). After that, 25 grams of leaves of Eucalyptus, were placed in a beaker size 500 ml comprising 150 ml deionized water, placed on a hot plate stirrer at 90℃ for one hour, and left to rest for 5 hours. The extract was purified through No.1 Whatman filter paper (20).

Preparing different concentrations of *Eucalyptus* leaves extract:

According to (2), the steps are:

(a) Steam distillation for removing essential oil from Eucalyptus leaves then dehydrating and grinding the leaves to obtain a desiccated leaves powder.

(b) Powder extraction using the first solvent (ethanol aqueous solution at a concentration of less than or equal to 30 %).

(c) Purifying the solvent to get an extraction residue.

(d) Heating the extraction residue between 20° C. to 100° C. for about 10 minutes to 25 hours using a second solvent (ethanol aqueous solution 50 to 80%).

(e) Filtering the second solvent to obtain the required Eucalyptus extract.

Bacterial Suspensions Preparation for Antimicrobial Sensitivity:

An antimicrobial sensitivity test was executed with the isolated bacteria. They were a subculture in nutrient media. Firstly, after overnight incubation of culture, bacterial swabs were taken from 5-6 colonies, diluted in brain-heart infusion broth, and incubated under anaerobic conditions. For 1-2 h at 35°C extend the concentricity of 1.5 × 108 CFU/ml.

The colonies were diluted by adding saline water to the last concentration of 1.5 × 106 CFU/ml. 0. 2 ml was added from

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standardized suspension to 20 ml of Hinton agar at 40 C°. The test was done worked in 3 replicates and plate incubated 24 hr, 37°C aerobically. Inhibition zone diameters were measured in mm for evaluation of the antibacterial action (23).

Figure (1) preparation of Eucalyptus leaves extract.

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Results:

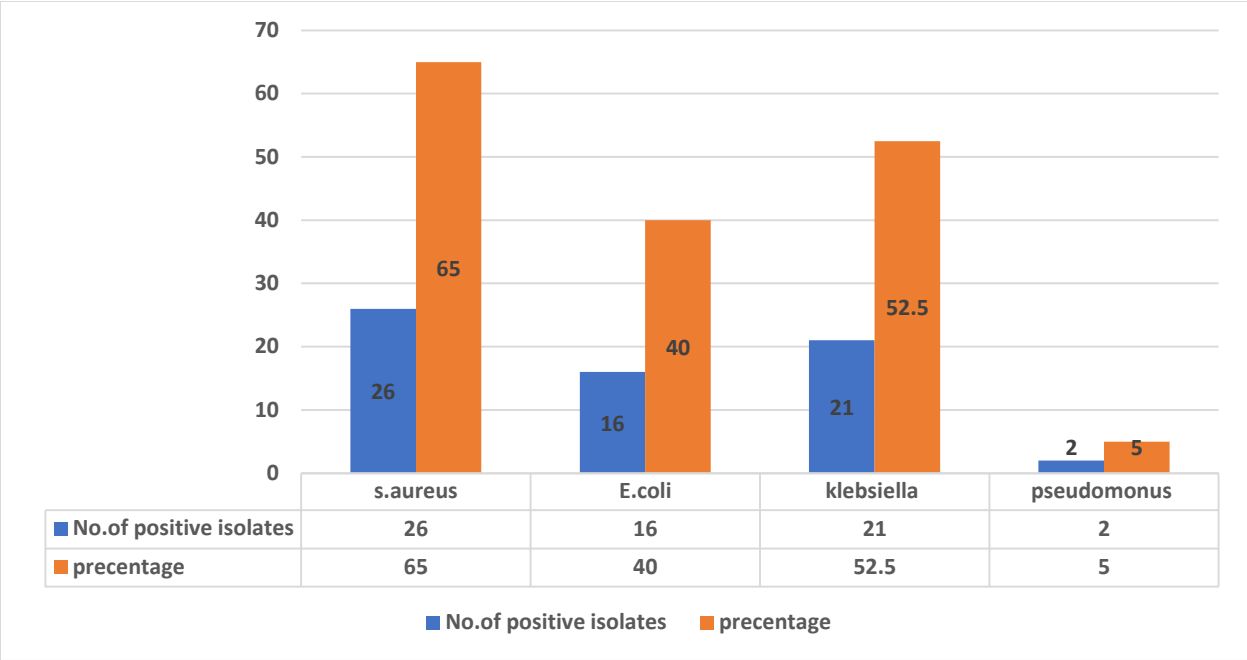

Figure (2) displays bacterial spread isolated from minced meat.

Table (1): Staphylococcus aureus and Klebsiella spp inhibition zone by *Eucalyptus* leaves extract.

Concentrationof *Eucalyptus* leaves extract	**Inhibition zone Dimeter against bacteria**(mm)	
	Staph aureus	Klebsiella
100 %	33	20
85 %	31	17
70 %	28	15
55 %	24	12
40 %	21	10

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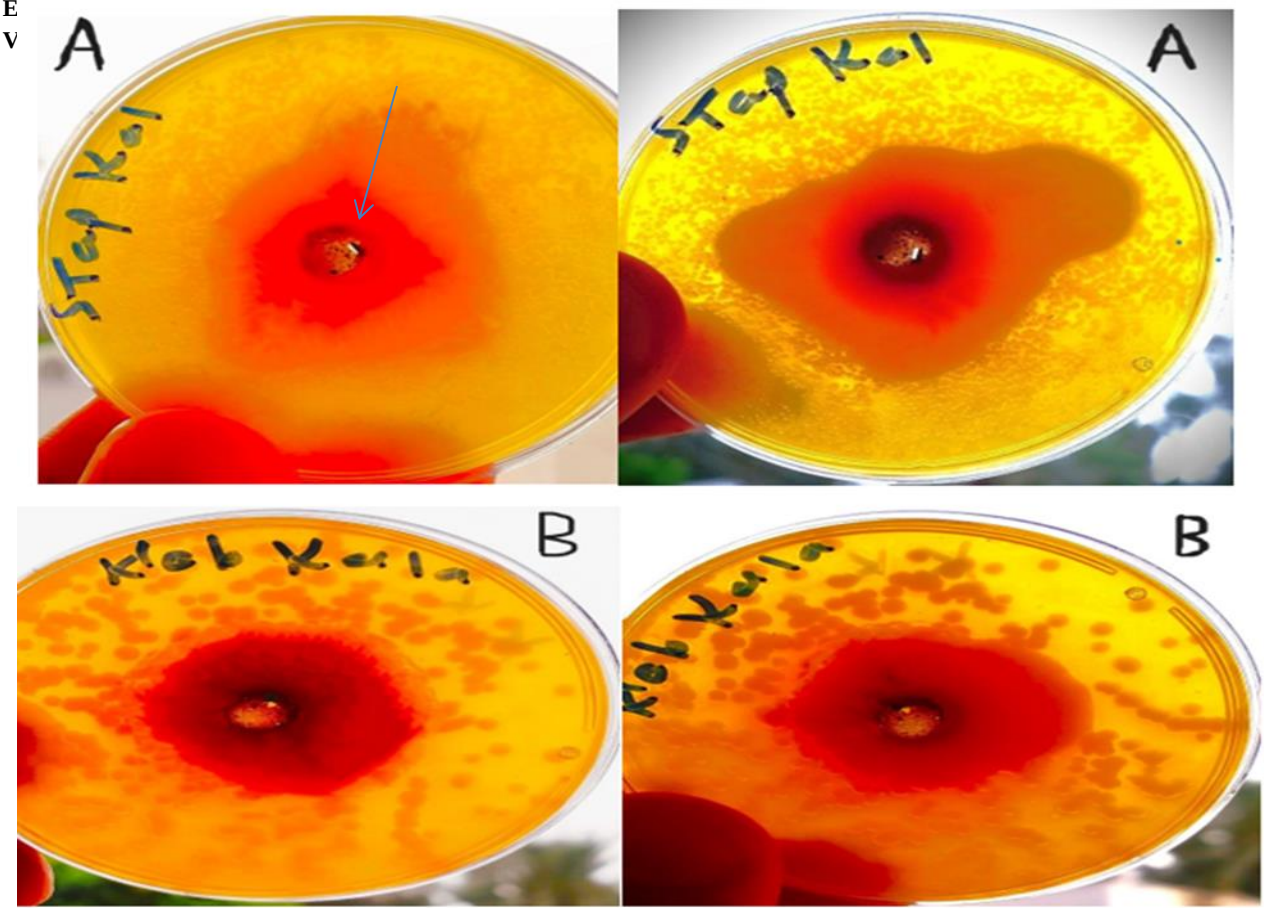

Figure (3): Antibacterial effect against Staphylococcus aureus(A) and Klebsiella(B) by Eucalyptus leaves extract.

Discussion:

It is known that minced beef meat is an excellent medium for microorganism growth. Generally, microorganisms derive thoroughly across on meat surface and start to replicate, unsanitary conditions during meat processing as mixing, grinding, packaging, storage, and display, contaminants may enter meat or meat products, resulting in reduced product value and the emergence of potentially health risks. (24,3,10). Besides, variation in results may be due to poor sanitation at the processing plant, climate, difference in sample sites, quantity of samples, or analytical methods used. Also, a high bacteria count indicates the short probable shelf life of meat and increases the probability in bad time meat spoilage (8).

(ACMSF) Advisory Committee on the Microbiological Safety of Food mentioned that cold water meat washes provide no perspective for effective microbial load reduction (1). The high (TVBC) Total

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Viable Bacterial Count values described in the current study might be related to varied influences, including insufficient refrigeration of stewed meat, and poor time and/or temperature through cooking or treat handling of meat products. Besides, the marketing presentation of uncovered meat products for sale irregularly at cooling is inappropriate for storing, on account of mutable and poor electricity supply. The inadequacy and fluctuation of electric supply have been reported by (7, 25) as well as the great factor that contributes to the spoilage of meat products spoilage and elevated total bacteria count.

The microbiological quality of meat related to the physiological status of the animals at slaughter, sanitary conditions and contamination levels of the slaughterhouse, instability of temperature, and other storage and distribution conditions are important factors that determine the microbiological quality of meat. (7). In addition, preparation and unsanitary conditions when handling these products increase the exposed area of meat and ultimately distribute pathogens all over the product, thus increasing the microbial load of the final product (21).

Nevertheless, the Misuse of antibiotics, prophylactically or therapeutically against bacterial infections in livestock and the consequential deposit, have been responsible for the emergence of bacterial-resistant isolates and may be considered key to health problems (19). Many microorganisms have developed resistance to different antibiotics (26, 19).

Our result found incidence of S. aureus in raw meat was 65% this result was in disagreement with many researchers and revealed the presence of S. aureus in raw meat and meat products from marketing stores with a diffusion below 1% in Asia (23), up to about 12% in Europe (6).

The presence of flavonoids, tannin, phytosterol, glycosides, steroids, volatile oils, carotenoid, saponin, polyphenols, Phytoestrogen, glucosinolates, coenzyme Q, carnitine, choline, and taurine in the Eucalyptus leaves causes the inhibition of this organism (2). The tannin presence in the leaves of Eucalyptus has greater antimicrobial activity because can bind proteins and prevent the protein manufacture of pathogens (27). The difference in the rates of inhibition of Gram-negative bacteria (Klebsiella) and Gram-positive bacteria (Staphylococcus aureus) is due to the difference in the components of the cell wall.

As the previous results showed, in this study it was found that there is an antibacterial effect in the aqueous and ethanolic extract of Eucalyptus camaldulensis leaves. As well as the antibacterial effect of the extracts was conditional on the nature of the microorganism. Thus, the sensitivity of the gram-positive bacterium Staphylococcus aureus is more compared to gram-negative bacteria E. coli and the inhibitory properties of Eucalyptus camaldulensis leaves extracts at minimal concentrations. Gram-negative bacteria are lower sensitive than gram-positive bacteria to the Eucalyptus volatile oils according to variances in the bacterial

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cells composition, this is because there is extra mucous- peptide is present in the cell wall conformation of Gram-positive bacteria, whereas Gram-negative bacteria contain a thin layer and greatest of their cell structure is lip-polysaccharides and lipoprotein. Therefore, Gram-negative bacteria are further rigid and renitent (9).

The prior studies demonstrate that the extract of alcoholic and aqueous concentrations has a reserve influence on *Staphylococcus aureus* and *Penicillium digitatum* growth (28). Nevertheless, alcoholic and aqueous extracts of *Eucalyptus* grasses have growth inhibition properties on *E. coli* growth in 60 and 80 mg/ml concentrations, and no antimicrobial effect was detected at 20 and 40 concentrations.

Antibacterial effects of five types of eucalyptus essential oils were examined by (29), the results show that at 5 mg/ml concentration of five types of *Eucalyptus* grasses volatile oils have been shown to have significant antibacterial effects contra gram-negative bacteria, gram-positive bacteria, and molds. As well, the alcoholic extract was extra effective and had a superior preventive effect compared with the aqueous extract. This phenomenon may be due to the extraction of more active substances from eucalyptus trees through ethanol extraction.

(20) Were pointed out that the antimicrobial compounds in the vegetal excerpt interact with the phospholipid bilayer membrane, affecting bacterial cell membrane permeability and releasing intracellular constituents. In various types of research, the mechanism of cell wall action has been in consideration, they have reported that cell wall and cell membrane influenced and altered their penetrability causes releasing of intracellular matters, which can be related to poor membrane functions such as nutrient uptake or enzyme efficiency and electron conveyance.

Conclusions:

We concluded that the high frequency of isolation for S.aureus, E. coli, Klebsiella, and Pseudomonas spp from minced meat indicated that storage and slaughtering conditions were not sufficient to ensure consumer safety. Also, the antibacterial effect of the leaves extract of Eucalyptus against Staph aureus is stronger than against Klebsiella. More extensive studies of the active ingredient of the plant for the accurate mode of action will aid the advancement of modern drugs.

Recommendations:

1. 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
2. Further studies are necessary to investigate the quality and validity of imported meat, particularly meat imported from unknown sources

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3. Investigate alcoholic extract against many Gram-positive and Gram-negative bacteria

References:

1. Olmedilla-Alonso, B., Jiménez-Colmenero, F., & Sánchez-Muniz, F. J. (2013). Development and assessment of healthy properties of meat and meat products designed as functional foods. *Meat Science*, *95*(4), 919-930.
2. Pereira, P. M. D. C. C., & Vicente, A. F. D. R. B. (2013). Meat nutritional composition and nutritive role in the human diet. *Meat Science*, *93*(3), 586-592.
3. Lika, E. (2021). Sustainable Rural Development in Albania Through Agriculture and Livestock: Challenges in the European Union Perspective. *J. Agron. Technol. Eng. Manag*, *4*(2), 577-582.
4. Shubham, K., Anukiruthika, T., Dutta, S., Kashyap, A. V., Moses, J. A., & Anandharamakrishnan, C. (2020). Iron deficiency anemia: A comprehensive review on iron absorption, bioavailability, and emerging food fortification approaches. *Trends in Food Science & Technology*, *99*, 58-75.
5. Jarullah B. A. (2015). Isolation and identification of bacterial contamination of raw meat in Thi-Qar province markets. J. of Thi-qar Sci. 5(3): 13-6.
6. Haleem A. M.; Al-bakri S. A. and Al-Hiyaly S. A. (2013). Determination of microbial content in poultry meat in local Iraqi markets. J. of Microbiol. Res. 3(6): 205-7.
7. Nychas, G. J., Dillon, V., & Board, R. G. (1988). Glucose is the key substrate in the microbiological changes occurring in meat and certain meat products. *Biotechnology and applied biochemistry*, *10*(3), 203-231.
8. Jay, J. M., Loessner, M. J., & Golden, D. A. (2008). *Modern food microbiology*. Springer Science & Business Media.
9. Lim, S. K., Nam, H. M., Park, H. J., Lee, H. S., Choi, M. J., Jung, S. C., ... & Wee, S. H. (2010). Prevalence and characterization of methicillin-resistant Staphylococcus aureus in raw meat in Korea. *Journal of microbiology and biotechnology*, *20*(4), 775-778.
10. Kimera, Z. I., Mgaya, F. X., Misinzo, G., Mshana, S. E., Moremi, N., & Matee, M. I. (2021). Multidrug-resistant, including extended-spectrum beta-lactamase-producing and quinolone-resistant, Escherichia coli isolated from poultry and domestic pigs in Dar es Salaam, Tanzania. *Antibiotics*, *10*(4), 406.
11. Abdulameer, S. J. (2024). Isolation, Characterization, and Antimicrobial Sensitivity of Enterobacter Aerogenes in Locally Produced Cheese from

Diyala Journal for Veterinary sciences
Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
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Diyala Province. *Diyala Journal for Veterinary Sciences*, *2*(1), 46-53.

12. Mancuso, G., Midiri, A., Gerace, E., and Biondo, C. (2021). Bacterial antibiotic resistance: The most critical pathogens. Pathogens, 10(10), 1310.
13. Monteville, T. J., and Matthews, K. R. (2008). Food microbiology: An introduction. Washington, 428.
14. Norman GM, Gravani RB (2006). Principles of Food Sanitation. 5th Edition, Springer, USA.
15. Jasim, S. T., and Farhan, A. S. (2020). Article Review: Klebsiella Pneumonia: Epidemiology, Virulence Factors and Treatment. flora, 14(2), 5-10.
16. Ali, A. T., & Minnat, T. R. (2024). Isolation and Identification of Klebsiella pneumoniae causing Urinary Tract Infection and Histopathological Changes of the Bladder and Kidney in Slaughtered Cattle. *Diyala Journal for Veterinary Sciences*, *2*(3), 84-97.
17. Babayi, H., Kolo, I., Okogun, J. I., and Ijah, U. J. J. (2004). The antimicrobial activities of methanolic extracts of Eucalyptus camalctulensis and Terminalia catappa against some pathogenic microorganisms.
18. Silva, J., Abebe, W., Sousa, S. M., Duarte, V. G., Machado, M. I. L., and Matos, F. J. A. (2003). Analgesic and anti-inflammatory effects of essential oils of Eucalyptus. Journal of Ethnopharmacology, 89(2-3), 277-283.
19. Cimanga, K., Kambu, K., Tona, L., Apers, S., De Bruyne, T., Hermans, N., and Vlietinck, A. J. (2002). Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo. Journal of Ethnopharmacology, 79(2), 213-220.
20. Su, Y. C., Ho, C. L., Eugene, I., Wang, C., and Chang, S. T. (2006). Antifungal Activities and Chemical Compositions of Essential Oils from Leaves of Four Eucalypts, 21(1), 49-61.
21. Siramon, P., and Ohtani, Y. (2007). Antioxidative and antiradical activities of Eucalyptus camaldulensis leaves oils from Thailand. Journal of Wood Science, 53, 498-504.
22. Gholami, D., Emruzi, Z., Noori, A. R., & Aminzadeh, S. (2019). Advances in bacterial identification and characterization: methods and applications. *Microbiology, Metabolites and Biotechnology*, *2*(2), 119-136.
23. Isenberg, H. D. (2004). Clinical microbiology procedures handbook. *(No Title)*.
24. Siriken, B. (2004). The microbiological quality of ground beef in Aydin and Afyon Provinces, Turkey. *Revue Méd Vét*, *155*(12), 632-6.
25. Wang, T., Lin, J., Chen, Z., Megharaj, M., and Naidu, R. (2014). Green synthesized iron nanoparticles by

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Open Access Journal Published by College of Veterinary Medicine
University of Diyala, Iraq
P-ISSN: 2410-8863
E-ISSN:2958-6178
Vol. 2, No. 4, December 2024

green tea and Eucalyptus leaves extracts used for removal of nitrate in aqueous solution. Journal of Cleaner Production, 83, 413-419.

26. Mohamed,M.W(2019). Synergistic Effects of Magnesium Oxide Nanoparticles and Nisin against Staphylococcus aureus and coliforms isolated from Raw Milk of Cows and Ewes in Diyala province (MSc Thesis College of Veterinary Medicine, Baghdad University.
27. Başkaya R, Karaca T, Sevinç İ, Çakmak Ö, Yıldız A, Yörük M (2004). İstanbul'da satışa sunulan hazır kıymaların histolojik, mikrobiyolojik ve serolojik kalitesi. *YYÜ Vet Fak Derg*, 15 (1-2), 41–46.
28. Kotzekidou, P., Giannakidis, P., & Boulamatsis, A. (2008). Antimicrobial activity of some plant extracts and essential oils against foodborne pathogens in vitro and on the fate of inoculated pathogens in chocolate. *LWT-Food Science and Technology*, *41*(1), 119-127.
29. Oyedeji, A. O., Ekundayo, O., Olawore, O. N., Adeniyi, B. A., & Koenig, W. A. (1999). Antimicrobial activity of the essential oils of five Eucalyptus species growing in Nigeria. *Fitoterapia*, *70*(5), 526-528.