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Evaluating the activity of the moringa plant oil extract in preserving some foods

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Abstract:

This study was conducted to evaluate the oil extract of Moringa oleifera seeds and assess its effectiveness against selected food-contaminating bacteria. The effectiveness of the oil extract in inhibiting bacteria was tested at concentrations of 50, 100, 200, and 300 mg/ml) against (Pseudomonas spp., Escherichia coli, and Bacillus spp. bacterial isolates. The results indicated that the concentration of 50 mg/ml exhibited the lowest inhibitory activity, whereas 300 mg/ml showed the highest antibacterial effect. However, the concentration of 200 mg/ml demonstrated moderate inhibitory activity, with inhibition zones measuring 14, 12, and 8 mm, respectively), and (was therefore selected for application in food preservation tests involving cheese, milk, and ground meat. The effectiveness of the oil extract at a concentration of 200 mg/ml was tested in reducing the total number of aerobic bacteria in cheese, local yoghurt, and minced meat samples during storage periods of (1, 3, 5, 7, and 12 days) at (6 ± 1°C). In comparison with untreated control samples, the results showed that the oil extract at 200 mg/ml effectively reduced total aerobic bacterial counts in accordance with the Iraqi Central Organization for Standardization and Quality Control specifications for soft minced meat and yoghurt intended for human consumption.

Keywords: *Moringa oil, inhibition, bacteria, contaminating food.*

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1. Introduction

Contaminated food is a major cause of foodborne diseases worldwide and represents a serious public health concern (Ali et al, 2015). Approximately one in ten people globally suffer from illness each year due to the consumption of contaminated food (Nova et al, 2020). Protecting food from microbial and chemical deterioration is therefore essential in the food industry. Pathogenic bacteria transmitted through food, such as Escherichia coli, Staphylococcus aureus, Salmonella typhi, Shigella spp., and Pseudomonas aeruginosa not only cause foodborne illness but also contribute significantly to food spoilage (Nadeem, et al, 2016). Chemically synthesized preservatives including butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butyl hydroquinone (TBHQ), and propyl gallate (PG), are used in the food industry to prevent

microbial growth and oxidative deterioration (Chen et al., 2019).). However, increasing concerns have been raised regarding the potential health risks and toxicity associated with synthetic preservatives. (Cui et al, 2018). In recent years, natural alternatives such as essential oils have gained attention due to their antimicrobial properties and potential role in food preservation (Sharma, Mendiratta, Agarwal, Kumar, & Soni, 2017). Moringa oil extracted from Moringa oleifera, is considered a promising edible oil due to its safety, high efficiency, and rich bioactive composition (Zhao, Li, Lan, Wu, & Chen, 2019), the oil is safe, high-efficiency, and non-polluting. It is an antibacterial agent, non-toxic and natural and has abundant and complex chemical compounds (Chen et al., 2019; Zhao & Zhang, 2014). The chemical components of extracted moringa oil vary to varying degrees, as it contains different proportions of palmitic acid, stearic acid, and oleic acid as the main components of the oil. (Lee, Kim, Park, & Lee, 2017). The microbiological activity of moringa oil has been studied by many researchers. They reported that they possess high antibacterial activity. It also possesses non-specific and antibacterial function, and 750 mg/kg moringa oil can resist and eliminate the failure (Gupta et al., 2012). However limited studies have investigated its antimicrobial mechanism at the molecular level due to the complexity of its chemical composition. Therefore, further research is required to better understand the antibacterial mechanism of moringa oil. (Food and Drug Administration, 2017).

2-Meterail and Methods

2-1 Preparing moringa seed samples

Moringa seeds were obtained from local markets identified microscopically according to FAO guidelines (1988). The seeds were ground using an electric grinder to obtain a homogeneous powde*r*) and (stored in sterile glass containers until extraction 2-2 Preparation of the oil extract of moringa seeds

To prepare the oil extract of moringa seeds, use the method described by Desmukh and Borle (1975) with some modifications: It was carried out by placing 40 grams of moringa seed powder in a thimble, which was done in a Soxhlet continuous tracking device, and using 200 milliliters of hexane at a temperature of 70°C. The extraction process continued for 8 hours. The solvent was evaporated using Rotary evaporator device under vacuum pressure at a temperature of 45°C, and stored in the refrigerator until use.

2-3 The effectiveness of Moringa seed oil extract on some food contamination bacteria:

The effectiveness of the 0.1 ml of moringa oil extract in concentrations (50, 100, 200, 300) mg/ ml in nutrient agar well were tested by using well diffusion method against some food contamination isolates. Testing using a sterile smear (sterile swab) of bacterial suspension containing. 1.5×10^8 cells/ ml, and three replicates were made for each treatment. Left the dishes for half an hour in the cold condition at a temperature 6°C (Crespo *et al*., 1990). Then the plates were incubated at a temperature of 37°C for a period of (24 + 2) hour, and estimate the diameter of the inhibition zone around each well.

2-4 Selection of the Optimal Concentration for Food Preservation

Samples of cheese, local yoghurt and ground meat were purchased from local markets. The effect of the oil extract of moringa seeds was studied according to the method mentioned in : As follows Andrews (1992). The oil extract was added at concentration 200 mg/ml to 10 g of each food sample after thorough mixing, Both in private after mixing well, the ground meat was stored in the cold condition at a temperature of 6 ºC in a periods (1, 3, 5, 7 and 12) days and with three replicates for each treatment. The ground meat samples not treated with the oil extract were adopted as control, and after the end of each period of storage, the following was done.

- 90 ml of sterile 0.85% physiological saline was added to a container mixer containing 10 grams of cheese, yoghurt and minced meat separately, and mix for two minutes to obtain On dilution 10 – 1.
- Decimal dilutions were made from 10 - 2 to 10 - 8 by transferring 1 ml of the mixture
- Dilute 9 ml of sterile physiological saline solution at a concentration of 0.85% and shake each Dilute well (25-30) times within several seconds.
- Transfer 1 ml of each dilution to a petri dish.
- Pour 20 ml of agar medium into sterile dish.
- After the incubation period, the bacterial colonies in the dishes were counted for three dilutions and a sample the rate is for three replicates for each treatment and according to the following equation:

Number of bacterial cells/ml = number of colonies in the dish x inverse of dilution x 10.

Statistical Analysis

The obtained experimental data were analyzed statistically using SPSS software (version 26.0, IBM Corp., Armonk, NY, USA). Descriptive statistical methods were applied, and the results were expressed as mean ± standard deviation (SD) based on replicate measurements of the tested bacterial isolates. The inhibition zone diameters were calculated as mean values from the recorded measurements. In addition, the reduction percentage in bacterial counts during storage periods was calculated by comparing treated samples with untreated control samples according to the following equation: Reduction (%) = [(Control − Treatment) / Control] × 100. This statistical approach was used to evaluate the antimicrobial effectiveness of Moringa oleifera seed oil extract at different concentrations and its efficiency in reducing bacterial growth in food samples during storage.

3. Result and Discussion:

3-1 The effectiveness of Moringa seed oil extract in experimental microbiology

The results of tab. (1) showed significant differences in inhibition zone diameters among the tested concentrations of the oil extract (50, 100, 200, 300) mg/ ml, At the lowest tested concentration of 50 mg/ml, the oil produced modest inhibition zones (7, 8, and 7 mm for Pseudomonas spp., E. coli, and Bacillus spp., respectively), indicating limited antibacterial potency. The relatively smaller diameters at this concentration suggest that only a fraction of the bioactive molecules could interact with bacterial membranes, insufficient to cause strong disruption, At the highest concentration of 300 mg/ml, inhibition zones expanded substantially (18, 20, and 10 mm, respectively), demonstrating the enhanced antimicrobial efficacy associated with increased availability of active lipid constituents. This stronger effect likely results from the cumulative interaction of unsaturated fatty acids, flavonoids, and other bioactive molecules with bacterial membranes, leading to destabilization, leakage of intracellular components, and impairment of metabolic functions.

The 200 mg/ml concentration produced moderate yet consistent inhibition (14, 12, and 8 mm, respectively), which was considered optimal for practical application in food preservation. This concentration balances antimicrobial efficacy with potential considerations for food taste, texture, and cost, making it suitable for maintaining safety without compromising sensory quality. tab. (1) and figure (1). These results show that the effectiveness of this extract, at a concentration of 200 mg/ml, can, we believe, be applied in inhibiting. The growth of contaminated microorganisms that cause spoilage of cheese, local yogurt, and minced meat, and thus their use increases the period storage this product.

Table 1: The effectiveness of the oil extract of moringa seeds in experimental microorganisms

Sample Ex conce. mg/ml / Bacteria Isolate.	**300**	**200**	**100**	**50**
	Inhibition Zone mm			
PS.	18	14	11	7
E. coli	20	12	10	8
Bacillus	10	8	7	7

The variation in inhibition zones among the tested bacterial species reflects structural and physiological differences in their cell envelopes. Gram-negative bacteria, including E. coli and Pseudomonas spp., possess an outer membrane enriched with lipopolysaccharides that can partially restrict the penetration of hydrophobic bioactive compounds. In contrast, Gram-positive Bacillus spp., despite having a thick peptidoglycan layer, remain susceptible to membrane-disrupting molecules. These structural and compositional differences in the bacterial cell walls account for the observed variability in inhibition diameters, highlighting the selective interactions of the bioactive components of Moringa oleifera oil with bacterial membranes.

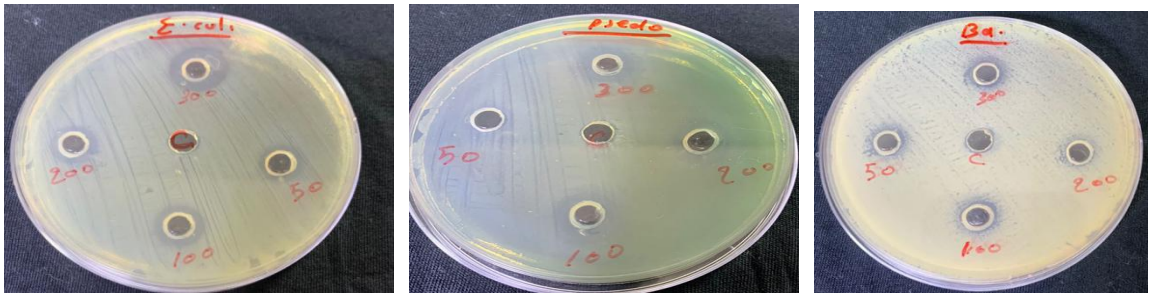
The current findings are consistent with previous research demonstrating significant antibacterial effects of Moringa oleifera seed oil against both Gram-positive and Gram-negative bacteria. The observed inhibition zones are within the ranges reported in similar studies, reinforcing the reproducibility of the oil's antimicrobial potential. The combined presence of unsaturated fatty acids, phenolic compounds, flavonoids, and glucosinolates likely contributes synergistically to bacterial membrane destabilization, metabolic disruption, and growth inhibition, providing a mechanistic explanation for the observed antimicrobial effects.

The statistical evaluation of inhibition zone diameters demonstrated a concentration-dependent antibacterial activity of Moringa oleifera seed oil extract against the tested bacterial isolates.

The mean inhibition zones increased progressively with increasing extract concentration. At the lowest concentration (50 mg/ml), the mean inhibition diameter was approximately 7.33 mm, indicating weak antibacterial activity. Increasing the concentration to 100 mg/ml resulted in a moderate increase in the inhibition zone with an average value of 9.33 mm.

At the concentration of 200 mg/ml, the average inhibition zone reached 11.33 mm, reflecting a moderate yet consistent antibacterial effect. The highest antibacterial activity was observed at 300 mg/ml, where the mean inhibition diameter reached approximately 16 mm. These results confirm that increasing the concentration of the oil extract enhances the antibacterial efficiency due to the greater availability of active bio-compounds capable of interacting with bacterial cell membranes. The sensitivity of bacterial isolates to the oil extract varied among the tested species. Escherichia coli exhibited the highest susceptibility with the largest inhibition zone (20 mm) at the highest concentration tested. Pseudomonas spp. showed moderate sensitivity, while Bacillus spp. exhibited comparatively lower inhibition diameters. These variations may be attributed to

structural differences in bacterial cell walls and membrane permeability, which influence the interaction between antimicrobial compounds and bacteria cells.



(A) **(B)** **(C)**

Figure 1: The inhibitory effectiveness of moringa oil extract on food contamination bacteria. (A) *E. coli*, (B) Pseudomonus spp. and (C) Bacillus spp.

3.2 Testing the optimal concentration of moringa seed oil extract in preserving Cheese, local yoghurt

The effectiveness of the oil extract of moringa seeds at a concentration of 20% was tested in an experiment to study the effectiveness of the moringa oil extract in reducing the total number of aerobic bacteria contaminating local cheese and local yoghurt. The results obtained tab. (2, 3) showed a clear decrease in the number of cells forming aerobic bacterial colonies in local cheese and during periods storage (1, 3, 5, 7) days at alone at a temperature of 4 degrees Celsius, will compared to the cheese models not treated with the oil extract as a control. It is noted from tab. (2) that the concentration of the extract oily 20% (200) mg/ ml showed the least decrease in the numbers of total cells forming bacterial colonies aerobic contaminants of chees and local yoghurt and the total numbers of cells forming bacterial colonies were estimated aerobic cells/ mm to make the product acceptable, Fig. (2).

Table 2: Effectiveness of Moringa seed oil extract in experimental microbiology in a chees sample.

Treatment	**Preserve. Day**	**Staphylococcus spp.**	***E. coli***	**MPN Index Per/ gm**	**0.01 ml**	**0.1 ml**	**1 ml**	**Result**
oil extract 200 mg/ml	1	4	3	≤0.02	0	0	0	Aid
	3	16	11	≤0.02	0	0	0	Acid
	5	31	19	0.06	0	0	1	Acid
	7	44	31	≤0.06	0	1	2	Acid
Control	1	10	4	≤0.02	0	0	0	Acid
	3	42	33	0.06	1	1	1	Acid
	5	96	74	0.17	1	2	3	Acid & Gas

	7	169	129	P ≥ 0.17	2	2	3	Acid & Gas

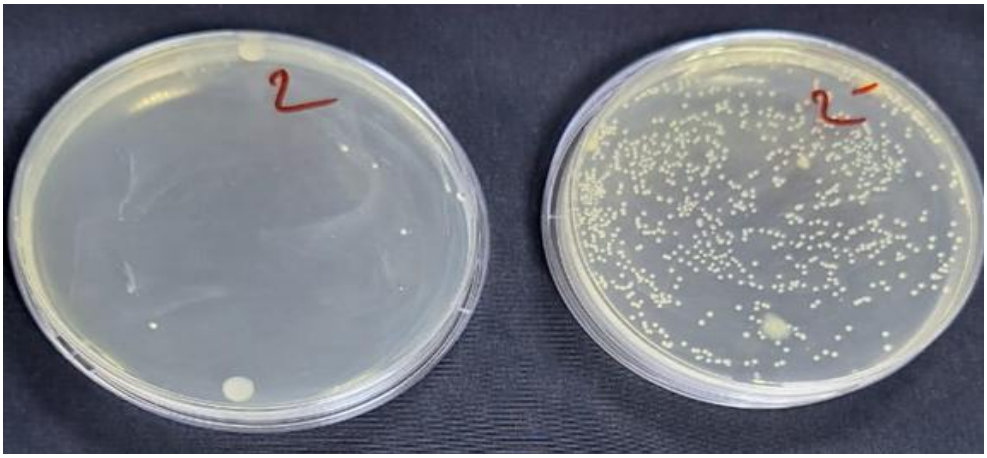
The results obtained from cheese samples demonstrated a noticeable reduction in the total aerobic bacterial counts in samples treated with Moringa oleifera oil extract compared with untreated control samples during storage. The bacterial counts in treated samples increased gradually from 4 cells/ml on the first day to 44 cells/ml after seven days of storage. In contrast, the control samples exhibited significantly higher bacterial growth, increasing from 10 cells/ml on the first day to 169 cells/ml after seven days. The calculated mean bacterial count in treated samples was 23.75 cells/ml compared with 79.25 cells/ml in the control samples. This indicates that the oil extract reduced bacterial growth by approximately 65% during the storage period, demonstrating its potential effectiveness as a natural preservative for cheese.

Table 3: Effectiveness of Moringa seed oil extract in experimental microbiology in a local yoghurt sample.

Treatment	**Preserve. Day**	**Staphylococcus spp.**	***E. coli***	**MPN Index Per/ gm**	**0.01 ml**	**0.1 ml**	**1 ml**	**Result**
oil extract 200 mg/ml	1	0	0	≤0.02	0	0	0	Aid
	3	4	0	≤0.02	0	0	1	Acid
	5	5	0	0.06	0	0	1	Acid
	7	11	0	≤0.06	1	1	1	Acid
Control	1	0	0	≤0.02	0	0	0	Acid
	3	13	2	0.06	1	1	1	Acid
	5	24	6	0.17	1	2	3	Acid & Gas
	7	39	9	P ≥ 0.17	2	2	3	Acid & Gas

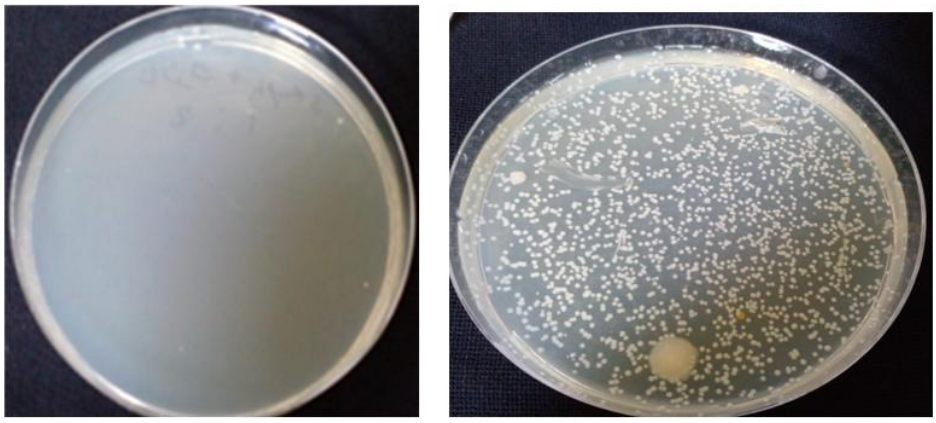
n local yoghurt samples, treatment with Moringa oleifera oil extract also resulted in a clear inhibitory effect on bacterial growth during refrigerated storage. The bacterial counts in treated samples remained very low during the early storage period and increased slowly over time, reaching 11 cells/ml after seven days. In comparison, untreated control samples showed a more pronounced bacterial growth, increasing from 0 cells/ml at day one to 39 cells/ml after seven days.

The mean bacterial count recorded in treated yoghurt samples was approximately 5 cells/ml, while the control samples recorded a mean value of about 19 cells/ml. This represents a bacterial reduction ranging from approximately 70% to 75%, confirming the antimicrobial effectiveness of moringa oil extract in fermented dairy products.



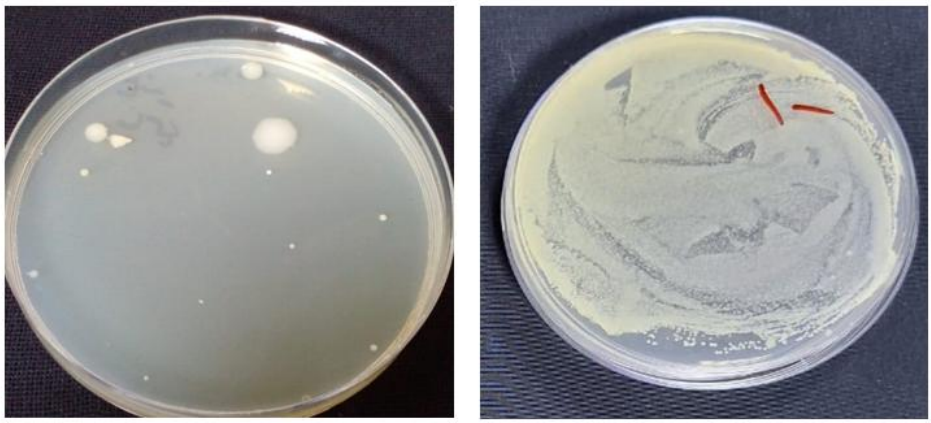
(A) **(B)**

Figure 2: the antibacterial activity of the oil extract in cheese sample (A) total aerobic bacteria of treatment sample (B) total aerobic bacteria of control sample.



(A) **(B)**

Figure 3: Antibacterial activity of moringa oil extract in yoghurt local sample (A) total aerobic bacteria of treatment sample (B) total aerobic bacteria of control sample.



(A) **(B)**

Figure 4: Pictures of the bacterial inhibitory activity of the oil extract of ground meat sample. (A) total aerobic bacteria of treatment sample (B) total aerobic bacteria of control sample.

3.3 Activity of moringa seed oil extract to ground meat preservative.

An extract of moringa oil Extract in 200 mg/ ml concentration was used in an experiment. Study of the conclusion in eliminating the total count of contaminating bacteria causing spoilage the ground meat. the results obtained tab. (4) have a clear quality in the number of cells and colonies of bacteria in whole meat and they were not preserved during storage periods (1, 3, 5, 7 and 12) days, at a temperature of 4°C, compared to the (control ground meat not treated with oil extract), were noted in tab. (4) that the concentration of the extract oily 200 mg/ml showed the higher decreasing in the total numbers cells forming aerobic bacterial colonies contaminants the ground meat, and the mean of the total numbers of cells forming bacterial colonies were determined aerobic acids(3, 14, 88, 19.5×10^2, and 287×10^3) cells/ ml during a storage period of (1, 3, 5, 7 and 12) days, treated samples exhibited markedly lower bacterial growth compared with control samples.

Table 4: Effectiveness of Moringa seed oil extract in experimental microbiology in a ground meat sample

Treatments	**Preserve. day**	***Total count***	***E. coli***	**Staphylococcus spp.**	**Salmonellae Spp.**
Treatment with oil extract	1	3	0	3	0
	3	14	3	10	0
	7	88	13	69	0
	9	$19.5x10^2$	39	121	0
	12	$287x10^3$	87	$197x10^2$	1
control Without presence	1	$35x10^4$	160	$12x10^2$	0
	3	$97x\ 10^5$	42	$93x10^2$	0
	7	$173x\ 10^6$	96	$24.6x10^3$	1
	9	$25.1x10^7$	$13.9x10^2$	$39.3x10^3$	3
	12	$49.2x10^9$	$213x10^2$	$46.9x10^5$	15

The application of Moringa oleifera seed oil extract in ground meat samples showed a significant inhibitory effect on bacterial growth throughout the storage period. The total bacterial counts in treated samples increased gradually from 3 cells/ml on the first day to 2.87×10^5 cells/ml after twelve days of storage. However, the control samples exhibited a dramatic increase in bacterial load, reaching 4.92×10^{10} cells/ml at the end of the storage period.

This difference indicates that the oil extract effectively slowed bacterial multiplication and reduced microbial growth by several logarithmic units. The reduction reached approximately five logarithmic cycles compared with untreated samples, highlighting the strong preservative potential of moringa seed oil in meat products.

The current study confirmed the multiple pathway inhibitory mechanism of MO against *L. monocytogenes*. Firstly, the increase of conductivity, surface charge, and the loss of intracellular proteins verified that MO could cause irreversible damage to cell structure. In addition, MO significantly inhibited the viability of *L. monocytogenes* through the action on the activities of key enzymes (AKP and *β*-galactosidase), cell metabolism activity, ATP content and respiration metabolism. t is reported that *M. oleifera* seeds contain 19–47% oil, 10–52% proteins, and 2.5–

20% glucosinolates. Moringa oil, which has more than 70% unsaturated fatty acids and is rich in oleic acid, has been used as a high-quality vegetable oil and in traditional medicine for the treatment of arthritis, rheumatism, and hypertension (Fernandes *et al*., 2015, Gopalakrishnan *et al.*, 2016). Moringa proteins, which have anti-microbial potential, can be made into food, beverage, and animal feed (Baptista *et al*., 2017, Medeiros *et al*., 2018). Moringa glucosinolates are a group of glucosinolats comprising a benzene ring with two rhamnose moieties attached. There are four kinds of glucosinolates in *M. oleifera* which are unique and important functional components of *M. oleifera*, the main glucosinolate in *M. oleifera* seeds is GLC (4-α-rhamnopyranosyloxy-benzyl glucosinolate). Moringa glucosinolates have anti-inflammatory, anticancer, and hypoglycemic potential, and can be used in phytopharmaceutical, nutraceutical and healthy food products (Jaja-Chimedza *et al*., 2020).

Overall, the statistical analysis of the obtained data confirms the strong antibacterial activity of Moringa oleifera seed oil extract against food contaminating bacteria. The antimicrobial effect was concentration dependent, with the most suitable practical concentration being 200 mg/ml. Furthermore, the application of the extract in different food products significantly reduced bacterial growth during refrigerated storage, indicating its potential use as a natural food preservative.

4. Conclusions

Among the tested concentrations, the extract at 300 mg/ml produced the largest inhibition zones against the tested bacterial isolates, while the concentration of 200 mg/ml showed moderate but consistent inhibitory activity. Based on these observations, the concentration of 200 mg/ml was considered suitable for practical application in food preservation experiments

Application of the oil extract at a concentration of 200 mg/ml in different food samples resulted in a noticeable reduction in total aerobic bacterial counts during refrigerated storage. The treated cheese and local yoghurt samples showed lower bacterial growth compared with untreated control samples throughout the storage period.

Similarly, the addition of moringa seed oil extract to ground meat significantly limited the increase of total bacterial counts during storage at low temperature. The treated samples maintained substantially lower microbial loads than the control samples, indicating an inhibitory effect on bacterial multiplication.

Overall, the findings indicate that Moringa oleifera seed oil extract can contribute to the reduction of microbial contamination in certain food products during storage. Therefore, the extract may represent a promising natural alternative for improving microbiological quality and extending the storage stability of some refrigerated foods.

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