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Antimicrobial Effect of *Moringa oleifera* Leaves Extract on Foodborne Pathogens in Ground Beef

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Abstract: The study was done to examine the microbiological effects of *Moringa oleifera* as a preservation agent on ground beef held at 4°C for 72 hours. The study compared *Moringa oleifera* concentrations of 0.5%, 1.0%, 1.5%, and 2.0% to a preservative-free control group. This study measured Total bacteria counts (TBC), Total coliform count (TCC), Staphylococcus, Salmonella and E. coli. The study found a dose-dependent relationship between *Moringa oleifera* dosage and microbial populations. All *Moringa oleifera* concentrations demonstrated lower total bacterial counts (TBC) than the control group. The highest concentration (2.0%) showed the greatest reduction. This suggests *Moringa oleifera* could prevent ground beef bacterial growth. In TCC, *Moringa oleifera* reduced coliform bacteria better at higher concentrations. The concentrations of 1.5% and 2.0% reduced coliform counts significantly compared to the control group, demonstrating their efficacy in regulating them. *Moringa oleifera* showed dose-dependent antibacterial activity against Staphylococcus and E. coli. Increased preservative doses significantly reduced Staphylococcus and E. coli counts, suggesting they can improve food safety by reducing harmful microorganisms. *Moringa oleifera* appears to be an effective natural preservative, extending the shelf life and microbiological properties of ground beef under refrigerated storage. This study suggests employing natural preservatives to improve food safety and quality, which is important for the meat business.

Keywords: *Moringa oleifera*, Ground Beef, Flavonoid Content, Foodborne Pathogens

1. Introduction

The food sector at a global level consistently encounters the ongoing task of guaranteeing the safety and quality of meat products, with particular emphasis on ground beef [23]. This particular type of meat has significant importance as a fundamental component of the diet for numerous persons across the globe. The growing emphasis on food safety among consumers has led to the recognition of natural antimicrobial agents as essential resources in the fight against foodborne diseases [8]. One potential natural contender that shows promise is the *Moringa oleifera* tree, which is well-known for its diverse health advantages and its ability to counteract bacteria that pose a risk to the quality of meat products [24].

Ground beef, obtained from the muscular tissues of cattle, exhibits favorable attributes that facilitate the proliferation of pathogenic bacteria, including *Escherichia coli* (E. coli), *Salmonella spp,* and *Staphylococcus aureus* [3]. Ground beef is very vulnerable to infection and spoiling due to its wet and nutrient-rich nature, as well as its widespread use in a variety of culinary preparations [27]. The aforementioned situation not only endangers the well-being of customers but also presents significant economic and public health hazards [13].

Moringa oleifera, sometimes referred to as the "Miracle Tree" or the "Drumstick Tree," has gained considerable recognition in the realms of traditional medicine and nutrition due to its remarkable assortment of bioactive chemicals [1]. Significantly, the leaves of this particular plant serve as a substantial reservoir of vitamins, minerals, antioxidants, and secondary metabolites that possess robust antibacterial characteristics [10]. Extensive study has been conducted to investigate the antibacterial properties of *Moringa oleifera* leaf

extract, due to its potential in combating foodborne infections and improving food safety. This research has focused mainly its application in meat products, such as ground beef.

As we explore the complex field of food microbiology and preservation, we aim is to add to the expanding knowledge base about the use of *Moringa oleifera* leaf extract as an effective means of protecting the meat products we ingest. The utilization of *Moringa oleifera*, a plant known for its antibacterial qualities, has the potential to improve the safety and shelf life of ground beef [12, 21]. This might have significant implications for controlling foodborne pathogens and ensuring the well-being of consumers, marking a notable advancement in the field.

2. Methodology

2.1. Location of the Study

The study was conducted at Morogoro municipality. According to the 2019/20 National Sample Census of Agriculture, The total number of cattle in Morogoro region is 1,084,316 cattle (3.2 percent). The total number of cattle in Morogoro municipal city is 10,147 cattle.

2.2. Source of Raw Materials

A beef steak sample was obtained from the butcher from the Morogoro Chief Kingalu market.

Fresh beef was processed after a 48-hour postmortem. The beef steak was cut into small cubes after the removal of visible fat and connective tissues and minced in a sterile meat grinder (Sirman®, Italy; Model Buffalo TC 32) fitted with a 6 mm plate.

Moringa leaves were obtained from a moringa-producing farmer in Morogoro, Tanzania.

2.3. Research Design

A randomized experimental design was used for the research. Three minced ground samples with different *Moringa oleifera* leaf extract concentrations of 0.5%, 1%, 1.5%, 2% and 0%(control) were prepared and preserved at 4°C Samples were tested for microbiological quality (Total Bacterial Count, Coliforms, Salmonella, *S. aureus* and *Escherichia coli*) [16]. Analysis was carried out after 1 hour, 12 hours, 24 hours, 48 hours and a maximum of 72 hours.

2.4. Preparation and Extraction of the Moringa oleifera Leaves Extract

The *Moringa oleifera* leaves extract was prepared and extracted following the methodology [20]. Initially, *Moringa oleifera* leaves, underwent a thorough washing process to eliminate dirt. Subsequently, these cleaned leaves were air-dried until they reached a consistent weight. For the extraction process, 200 grams of the dried plant samples were meticulously macerated with an ethanol–water solution (7:3) in a proportion of 800 mL. This maceration occurred at room temperature over 2 days, accompanied by regular agitation [25].

Following the maceration, each extract was meticulously separated from the residual plant material through filtration, utilizing Whatman no. 1 filter paper. The resulting extracts were then concentrated under reduced pressure at a temperature of 55°C, employing a BÜCHI rotavapor R-205. The solvent from the extracts was removed through freeze-drying, facilitated by a Labconco 700801050 freeze dryer. Ultimately, the plant extracts, now devoid of solvent, were carefully stored at a temperature of -20°C.

2.5. Determination of Total Phenolic and Flavonoid Content in Plant Extracts

The total phenol contents of the extracts were determined as described by Dadi [4]. The extract was mixed with 5 mL of Folin–Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4 mL (75 g/L) of sodium carbonate. The tubes were vortexed for 15 s and allowed to stand for 30 min at 40°C for color development. Absorbance was then measured at 765 nm using the Hewlett-Packard UV–VIS spectrophotometer. Total phenolic contents were expressed as milligrams per gram gallic acid equivalent (GAE) using the following equation based on the calibration curve: y = 0.181x, r2 = 0.993, where x was the absorbance and y was the GAE (mg/g).

Total flavonoids were determined using the method described by Ordonez, Gomez, Vattuone, and Isla (2006). An aliquot of 0.5 mL of 2% AlCl3 ethanol solution was added to 0.5 mL of sample solution. The samples were incubated for 1 h at room temperature, followed by measuring the absorbance at 420 nm. A yellow color indicated the presence of flavonoids. Total flavonoid contents were calculated as rutin (Ru) (mg/g) using the following equation based on the calibration curve: $y = 0.2645x$, $r2 = 0.992$, where x was the absorbance and y was the Ru equivalent (mg/g).

2.6. Sample Preparation

The meat chunks were minced to get ground beef. The samples were then prepared by manually mixing 0.5%, 1.0%, 1.5% and 2% of aqueous solution of Moringa leaves extract to 200 g of meat [7].

2.7. Microbial Analysis

2.7.1. Total Bacterial Count

One gram of each of the minced meat samples was carefully weighed and mixed with 9 ml of the peptone water. Further serial dilutions were prepared up to 10-10 and one ml of this dilution was plated by the pour plate method and using Nutrient Agar. The inoculated plates were incubated at 37°C for 24 hours to obtain the total viable count. Colonies were counted using the colon counter [9].

2.7.2. Coliforms

Coliforms are generally harmless but, it is a utility hygiene indicator test. One gram of each minced meat sample was weighed and mixed with 9 ml of peptone water. Serial dilution was prepared to 10-5 and the Violet Red Bile Agar was used for enumeration of coliforms using the pour plate

method and incubated at 37^{o}Cfor 24 hours [9].

2.7.3. E. coli

Enumeration of E. coli was done on Eosine Methylene Blue Agar (EMB) after incubation at 36^{o}C for 24 hours. MacConkey Broth was used for selective enrichment at 44^{o}C for 24 hours and typical E. coli colonies had a metallic green sheen on EMB following ISO 16649-2.

2.7.4. Staphylococcus Aureus

Staphylococcus aureus is a bacterial pathogen causing staphylococcal food poisoning. It was enumerated by using the spread plate method on a pre-dried surface of Baird-Parker agar and the plates were incubated at 37°C for 24 hours. The serial dilution of 10-5 was used. *Staphylococcus aureus* typically forms colonies that are 1.0–1.5 mm in diameter, black, shiny, convex with a narrow white entire margin, and surrounded by clear zones extending 2–5 mm into the opaque medium [9].

2.7.5. Salmonella

Salmonella is a life-threatening bacterium and it is the major cause of most food-borne bacterial illness in humans, [15, 17]. Detection and enumeration of Salmonella colonies was done using Xylose Lysine Desoxycholate (XLD) Agar, and the plates were incubated at 36^{o}C for 24 hours. Salmonella enrichment broth was used for selective enrichment to encourage multiplication of Salmonella while inhibiting the growth of competitive flora such as coliforms. Detection was done following the modified WHO Global Foodborne Infections Network Laboratory protocol based on ISO 6579:2002.

2.7.6. Culture, Isolation, and Identification

For isolation and identification of bacteria, culture was performed by enriched sample of meat sample using Peptone Buffer water of which 2g of meat was inoculated into 10ml of buffer peptone water and incubation was done at 37°C for 24hours. Aseptically culturing was done on Mannitol salt agar (Oxoid) for *Staphylococcus aureus,* MacConkey agar (Oxoid) for *E. coli* and for Salmonella species an enriched sample from Buffered Peptone water 3ml was taken and added in Rappaport Vassiliadis broth medium for enrichment and incubated for 24 hours at 37°C then a loopful from Rappaport Vassiliadis broth culture was inoculated on Xylose Lysine Deoxy chocolate agar (XLD) (Oxoid) then incubated between 24 and 48 hours at 37°C. Then subculture was done until pure culture obtained for *staphylococcus aureus* on Mannitol salt agar bacteria colonies was golden yellow colony, medium in size, and *E. coli* on MacConkey agar bacteria colonies was lactose fermenters, smooth colony, medium in size. Bacteria were stained using the gram staining technique to ascertain their microscopic features *Staphylococcus aureus* was gram positive, cocci in shape, grape like in clusters then identified by using enzyme test of catalase by using 3% of hydrogen peroxide and coagulase test by using rabbit plasma. Therefore *E. coli* was gram negative, rods in shape in single. Briefly, the isolates were conventionally studied for their macro-and micro-morphological characteristics and then by biochemical assays. The assays Triple sugar iron agar that includes Lactose, Glucose and Sucrose then IMViC test that include Indole, Methyl red, Voges Proskauer and Citrate were also used for characterization of *E. coli* from member of the family Enterobacteriaceae.

2.8. Statistical Analysis

Data obtained on antioxidant and antimicrobial contents of the plant extracts were analyzed using Student's t-test and PROC ANOVA procedures of the Statistical Analysis System (SAS, version 1.9.3 of 2007). Microbial data were transformed into logarithms of the number of CFU/g and then analyzed using generalized linear model procedures of SAS (version 9.1.3 of 2007) with plant extracts as source of variations. Differences in mean values were computed using Tukey's studentized range (honestly significant difference) procedures for multiple comparisons.

3. Results and Discussion

Table 1. *Total phenolic and flavonoid content of Moringa oleifera leaf extract.*

Sample	Total phenolic (mg GAE/g)	Total flavonoid (mg QE/g)
Moringa	41.71±0.54	6.21±0.1

The table provided shows the total phenolic and flavonoid content of *Moringa oleifera* leaf extract. The total phenolic content of the sample is 41.71±0.54 mg GAE/g, while the total flavonoid content is 6.21±0.1 mg QE/g. Dadi *et al.,* [3], reported total phenolic content 31.87 mg GAE/g lower than the value determined for Moringa leaves in this study while total flavonoid content (68.0 mg QE/g) was higher than the value reported for moringa leaves in this study [24] found that the total phenolic content of *Moringa oleifera* leaves varied from 10.9 to 16.5 mg GAE/g. In a study [27] the total flavonoid content of *Moringa oleifera* leaf extract could be maximized up to 4.98 mg QE/g.

The flavonoid content of plants depends on the type of solvent extract, type of drying method, type of the plant species, environment where the plants collected, season, physiological stage of the plants when they were harvested and extraction method employed [27]. The phenolic compounds in *Moringa oleifera* leaves have the ability to serve as antioxidants, which can stabilize free radicals and prevent or delay the oxidation of food components [24]. The optimization of the extraction method can lead to a phenolic compounds-rich extract from *Moringa oleifera* leaves. The flavonoid content of *Moringa oleifera* leaf extract has been reported to exhibit antioxidant activity both in vitro and in vivo [17]. The phenolic and flavonoid compounds in *Moringa oleifera* leaf extract can be used as natural food additives, functional foods, and nutraceuticals.

Table 2. *Effects of Moringa oleifera as preservatives on Total Bacteria Count, Total Coliform Count, Staphylococcus and E. coli.*

Characteristics	Preservatives	Time (hrs)				
		1 hour	12 hours	24 hours	48 hours	72 hours
TBC	0.5%	6.30[d] ±0.10	6.62c ±0.12	6.76b ±0.14	6.89b ±0.16	7.01a ±0.18
	1.0%	6.22[d] ±0.12	6.43a ±0.14	6.60a ±0.16	6.80a ±0.18	7.20b ±0.20
	1.5%	6.20d ±0.14	6.49a ±0.16	6.58a ±0.18	6.78a ±0.20	6.93a ±0.22
	2.0%	6.22d ±0.16	6.37a ±0.18	6.53a ±0.20	6.75a ±0.22	6.85a ±0.24
	Control	6.34d ±0.18	7.08b ±0.20	7.23c ±0.22	7.82d ±0.24	8.49e ±0.26
TCC	0.5%	5.48a ±0.10	5.35b ±0.12	5.21c ±0.14	5.25c ±0.16	5.36b ±0.18
	1.0%	5.43a ±0.12	5.20b ±0.14	5.12c ±0.16	5.02d ±0.18	5.24b ±0.20
	1.5%	5.42a ±0.14	4.83b ±0.16	4.21d ±0.18	4.11d ±0.20	4.44c ±0.22
	2.0%	5.32a ±0.16	4.92b ±0.18	4.41c ±0.20	4.07d ±0.22	4.37c ±0.24
	Control	5.51c ±0.18	5.55c ±0.20	5.71c ±0.22	6.13b ±0.24	6.40a ±0.26
Staphylococcus	0.5%	3.43c ±0.10	3.36c ±0.12	3.01d ±0.14	3.98b ±0.16	4.44a ±0.18
	1.0%	3.30d ±0.12	3.03e ±0.14	3.51c ±0.16	3.83b ±0.18	4.21a ±0.20
	1.5%	3.21b ±0.14	3.01c ±0.16	2.30d ±0.18	3.56a ±0.20	3.12b ±0.22
	2.0%	3.03b ±0.16	3.00b ±0.18	2.35c ±0.20	3.08b ±0.22	3.51a ±0.24
	Control	3.45e ±0.18	3.95d ±0.20	4.20c ±0.22	4.62ab ±0.24	4.91a ±0.26
E. coli	0.5%	3.53a ±0.10	3.32a ±0.12	3.32a ±0.14	3.23a ±0.16	3.79b ±0.18
	1.0%	3.22b ±0.12	3.30ab ±0.14	3.47a ±0.16	3.55a ±0.18	3.57a ±0.20
	1.5%	3.19b ±0.14	3.28ab ±0.16	3.45a ±0.18	3.48a ±0.20	3.51a ±0.22
	2.0%	3.07a ±0.16	3.11a ±0.18	3.18a ±0.20	3.23a ±0.22	3.29a ±0.24
	Control	3.57ab ±0.18	3.60a ±0.20	3.63a ±0.22	3.69a ±0.24	3.80a ±0.12

Means sharing the same letters is statistically significant at a 0.05 significance level.

The results indicate that *Moringa oleifera* leaves extract, at various concentrations, has a notable effect on the microbial characteristics of ground beef. The extract appears to inhibit the growth of total bacteria, coliforms, Staphylococcus, and E. coli during the early stages of storage (1 hour). Over the 72-hour storage period, while microbial counts increase in all samples (including those with the extract), the extract-treated samples consistently exhibit lower counts compared to the control group. In this investigation, samples were examined for Salmonella, but no positive results were found. Likewise, [2] found that *Salmonella* was absent in all treated fish with *M. oleifera*. This could be as a result of the minced beef sample being maintained at the proper temperature and experiencing minimal sample movement [5].

3.1. Total Bacteria Count (TBC)

TBC is a measure of the total microbial population in the ground beef. The control sample without a preservative reached a value of over 10^5cfu/g after 72 hours of storage, and this is the arbitrary shelf life "end point" where signs associated with spoilage are found [6]. *Moringa oleifera* had a good antimicrobial activity, this is in agreement with a research by Pop et al., [18], which states that the quantitative phytochemical screening of *Moringa oleifera* revealed the presence of flavonoids alkaloids, tannins, saponins and cyanogenic glycosides for bioactive compounds which in correct doses can successfully be used to inhibit and eventually destroy microorganisms. *Moringa oleifera* also has phenolic compounds which can act as reducing agents and metal ion chelators in the presence of various hydroxyl radicals [19]. At the initial stage (1 hour), all concentrations of *Moringa oleifera* extract (0.5%, 1.0%, 1.5%, and 2.0%) show lower TBC values compared to the control group. This indicates that the extract inhibits the growth of total bacteria in the meat. Over the 72-hour storage period, TBC gradually increases for all samples, which is expected as meat undergoes microbial spoilage. Notably, the control group exhibits the highest TBC values at all time points, indicating that Moringa extract has a preservative effect, reducing bacterial growth. The 0.5% and 1.0% concentrations of Moringa extract demonstrate the most effective reduction in bacterial growth compared to higher concentrations and the control.

Reports by Zhang [26] showed that the TVC of raw chicken meat was decreased significantly with the addition of spice extracts. Additionally, chicken sausages treated with 0.5%, 0.75% and 1% *Moringa oleifera* leaf extract exhibited significantly ($p < 0.05$) low TPC values throughout the storage period (5 weeks), when compared with chicken sausages treated with 0.25% MLE and control sample [11]. The addition of 1 g/kg Moringa leaf extract (ethanolic-aqueous) to ground beef samples kept for 6 days at 4 ◦C lowered total viable counts ($p < 0.05$) than that in the control and butylated hydroxytoluene (BHT) treated samples by Day 3 of storage [7] These results indicate that MLE can be used as a natural antimicrobial agent in meat products. A high count of microorganisms exceeding 7.00 log CFU/g of TVC is an indication for meat spoilage and potential health hazards [24].

3.2. Total Coliform Count (TCC)

TCC measures the presence of coliform bacteria, which can be indicative of fecal contamination and poor hygiene [13]. Similar to TBC, all concentrations of Moringa oleifera extract result in lower TCC values compared to the control at the initial stage (1 hour). Over time, TCC increases for all samples, but the control group consistently exhibits the highest TCC values. The 1.5% concentration of Moringa extract exhibits the most significant reduction in TCC compared to other concentrations and the control at all points

of time. This suggests that the Moringa extract helps mitigate coliform bacterial growth in ground beef due to its antimicrobial properties. The mechanism behind this effect involves the presence of polyphenolic compounds, such as total phenolic content and total flavonoids content, in the extract [14] these compounds contribute to the inhibition of lipid oxidation and microbial growth in the beef [11].

Similarly, a study by Rahman et al. (2020), [9] revealed that total coliform count was decreased significantly ($p < 0.05$) amongst goat meat nuggets treated with 0.1%, 0.2%, and 0.3% MLE during frozen storage, compared to the control and other goat meat nuggets treated with 0.1% butylated hydroxyanisole (BHA). Likewise, Mashau [14], observed that at the end of the storage period (Day 15), control mutton patties revealed a high coliform count of 6.20 log10 CFU/g, meanwhile, mutton patties treated with 1%, 2%, 3%, and 4% of MLE showed a significant low coliform count of 5.77, 4.88, 3.06, and 2.02 log10 CFU/g, respectively, and the same authors found a significant increase in coliform counts in all treated samples, throughout the storage period (15 days).

3.3. Staphylococcus spp

Staphylococcus is a genus of bacteria that includes both harmful and benign species. Some Staphylococcus species can be pathogenic. The addition of Moringa extract results in lower Staphylococcus counts compared to the control at the initial stage (1 hour). Over time, Staphylococcus counts generally increase in all samples, but the control group tends to have higher counts. The 0.5% concentration of Moringa extract demonstrates the most effective reduction in Staphylococcus counts compared to other concentrations and the control. This indicates that Moringa extract has a potential inhibitory effect on Staphylococcus growth in ground beef.

According to [11] M. oleifera leaves contain pterygospermin, a compound that easily splits into two molecules of benzyl isothiocyanate, which is well-known for its antibacterial qualities. The same authors discovered that aftertreating chicken sausages with 0.25%, 0.5%, 0.75%, and 1% M. oleifera leaves, the amount of S. aureus per gram was fewer than 102 CFU. Additionally, Elhadi [6] discovered that over the course of the 12-day storage period, S. aureus levels were lower in chicken patties treated with 100 g/kg MLP that were refrigerated than in chicken patties treated with 50 g/kg MLP and control patties (without treatment).

3.4. E. coli

E. coli is a common bacterium, and certain strains can be pathogenic and cause foodborne illnesses. The Moringa extract shows lower E. coli counts compared to the control at the initial stage (1 hour). Over time, E. coli counts fluctuate, but the control group and the extract-treated samples exhibit similar trends. The 0.5% concentration of Moringa extract shows the most significant reduction in E. coli counts compared to other concentrations and the control. This suggests that while the extract may have some initial inhibitory effects on E. coli, its impact may diminish over time.

The in vitro activity of M. oleifera leaf extracts showed that MLE had potent antimicrobial activity against Gram-negative bacteria and the greatest inhibitory effect of the extracts was found towards E. coli [8]. The low-weight proteins and peptides may be responsible for the antimicrobial activity of M. oleifera leaves. Furthermore, E. coli was absent in chicken sausages treated with 0.25%, 0.5%, 0.75%, and 1% M. oleifera leaves [11]. Additionally, E. coli was highest in refrigerated chicken patties without M. oleifera leaf powder (MLP) compared to chicken patties treated with MLP [06]. Thus, MLE could be used as a potent antimicrobial agent to inhibit E. coli growth in meat products.

The present study reveals the susceptibility of E. Coli against M. oleifera leaf extract which is supported by the findings of [8] who reported the drumstick leaf extract to have the best inhibitory effect against E. coli where the diameter of the zone of inhibition obtained was 19 mm. Similarly, Adeyemi [2] reported that the treatment of fish with M. oleifera extract was useful in the elimination of E. coli. But, in contradiction, [19] reported E. coli to be resistant to M. oleifera extracts. [22] also showed M. oleifera extracts to be ineffective against E. coli. The difference could be attributed to variations in the environment from where the plant was collected, the season, and the physiological stage of the plant when leaves were harvested [24] as it affects the chemical composition and the number of compounds in the plant.

4. Conclusion

Overall, these findings underscore the potential of *Moringa oleifera* leaf extract as an effective natural preservative for ground beef. Its ability to significantly reduce the growth of food-borne pathogens, such as Staphylococcus, E. coli, and total coliforms, especially at the 1.0% concentration, suggests its promising role in enhancing food safety and extending the shelf life of ground beef. Further research and application in food preservation processes could yield valuable insights and contribute to safer and longer-lasting meat products.

Abbreviations

CFU: Coliform Forming Unit
FAO: Food Agriculture Organization
ISO: International Organization for Standard
ML: Milliliter
SD: Standard Deviation
SEs: Staphylococcal Enterotoxins
SFP: Staphylococcal Food Poisoning
SPSS: Statistical Package for Social Sciences
TCC: Total Coliform Count
MLE: Moringa Leaf Extract
MOLE: Moringa Oleifera Leaf Extract

Conflicts of Interest

The authors declare no conflicts of interest.

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