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Research Article

Efficacy of green tea extract on *Staphylococcus aureus* and *Klebsiella pneumoniae* of raw chevon

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Abstract

The efficacy of green tea extract with different strengths on selective microorganisms of raw chevon was investigated in this study. The experiment was conducted using completely randomized design (CRD) six treatments (T₀=0 ml of green tea; T₁=1 ml of green tea; T₂=2 ml of green tea; T₃=3 ml of green tea; T₄=4 ml of green tea and T₅=5 ml of green tea) having three replications. Drop plate method was used for analyzing antimicrobial activity in this experiment. The outcome illustrated a declining microbial population in samples while incorporating green tea with colony-forming units steadily reducing from T₀ to T₅. There was a significant effect (p<0.0001) of green tea on the number of *E. coli* and *Staphylococcus epidermidis*. The minimum and maximum population of TVC of *E. coli*, in the treated sample were 6×10⁵ CFU/ml in T₅ and 45×10⁵ CFU/ml in T₀ respectively while for *Staphylococcus epidermidis*, it was 4×10⁵ CFU/ml in T₅ and 10×10⁵ CFU/ml in T₀ respectively. Green tea extracts showed equal growth suppression and extremely significant differences (p<0.0001) in their antimicrobial activity against both *E. coli* and *Staphylococcus epidermidis*. Given the results, it is possible to draw the conclusion that adding green tea extract at a level of 5 ml to raw chevon will diminish its microbial population. For *Staphylococcus aureus* and *Klebsiella pneumoniae* were significant reduction (p<0.0001) of the microbes among samples treated with green tea extracts.

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Introduction

Meat is perhaps one of the most nutrient-dense foods on which a notable section of the world's population relies as a dietary source, providing important amino acids, minerals, and essential vitamins (Akhter et al., 2022 and 2009; Akter et al., 2022 and 2009; Alam et al., 2011; Ateba and Setona, 2011). Chevon, which is preferred because it has less fat and cholesterol than other regularly consumed meats, is generally in higher demand worldwide (Gipson, 1998). Being a potential competitor of beef and sheep meat (Singh et al., 2014), red meat chevon is universally acceptable and unaffected by culture, tradition, social norms, and economic conditions (Verma et al., 2014). Furthermore, because chevon meat tends to be lean and nutritious, it may appeal to consumers who are health conscious (Casey et al., 2003; Dhanda et al., 2003) which could be reasoned as customers' purchasing decisions are usually based on the meat quality which is a combination of chemical, microbial and sensorial attributes (Madruga et al., 2009).

However, due to its considerably nutritious nature, meat is renowned to serve an inhabitable environment for pathogenic, nonpathogenic as well as spoilage organisms to grow (Steinkraus, 1994) which can also pave their way through animal skin, hide and feet, fecal material and the hands, clothing, and equipment of slaughter men (Adetunde et al., 2011). *E. coli*, *S. aureus*, *Salmonella spp.*, *Campylobacter jejuni*, and *Listeria monocytogenes* are to name a few pathogenic microbes which may reside in raw meat, putting human health at risk upon consumption (Mead et al., 1999). Enzyme, microbial action, and fat oxidation by microorganisms usually lead to the alteration of visual, textural, and organoleptic properties of meat when metabolites are being released (Johnson and McGowan, 1998). Very recently, plant and herbal extract has utilized for increasing shelf life and quality such as Centella leaf (*Centella asiatica*) extracts (Akter et al., 2022), telakucha (*Coccinia cordifolia*) leaves extract (Bithi et al., 2020), long coriander leaf (*Eryngium foetidum*) extract (Boby et al., 2021), lemon extract (Disha et al., 2020), ginger extract (Hossain et al., 2021), pomegranate (*Punica granatum*) extract (Jahan et al., 2018), carrot extract (Khatun et al., 2022), bottle gourd leaf (*Lagenaria siceraria*) extract (Saba et al., 2018), tulsi (*Ocimum sanctum*) leaf extract (Siddiqua et al., 2018), synthetic antioxidants (Azad et al., 2021), mustard seed extract (Das et al., 2022) might be due to these plant extracts contains natural antioxidant that reduced microbial load.

Therefore, a thorough microbial check and hygiene practices have been advocated with the goals of ensuring that consumers receive disease-free meat, checking the spread of disease among meat consumers, and identifying the source of disease through proper examination of animals prior to slaughter (Ramasastry et al. 1999; Dhanze et al., 2012). For this reason, and in response to rising consumer demand for chemical-free additives, the utilization of natural preservatives technology has been popularized since many vegetal compounds like tea contain antioxidant and antimicrobial qualities (Bañón et al., 2007).

L. monocytogenes, *E. coli* O157:H7, *Salmonella Typhimurium*, and *Campylobacter jejuni*, are among the major foodborne pathogens that green tea extract (GTE) has illustrated inhibitory capabilities against. The list also contains *S. aureus*, *Staphylococcus epidermidis*, *Salmonella Enteritidis*, *Shigella flexneri*, *Shigella dysenteriae*, and *Vibrio cholera* (An et al., 2004; Gadang et al., 2008; Hamilton-Miller, 1995; Toda et al., 1991). While high (100 °C/60 min) or pasteurization temperatures (121 °C/15 min) are usually unable to create an impact on the efficiency of GTE (Diker and Hascelik, 1994; Oh et al., 1999), polyphenols of green tea, when irradiated could have amplified anti-microbial characteristics against *S. aureus*, *S. epidermidis*, *E. coli* and *S. mutans* (An et al., 2004). In broth culture tests, it was shown by Over et al., (2009) that GTE alone (20 or 40 mg/mL) or in conjunction with tartaric acid (37.5 mM) decreased *Salmonella*, *Listeria*, and *E. coli* by at least 3.5 log CFU/mL.

With the intention to enrich the knowledge base, this study focuses on comprehending the potential of green tea as a preservative for meat and its bacteriostatic effect on meat spoilage organisms, particularly specific pathogens. Therefore, this study was conducted to-

- To investigate the effect of green tea extract as an antimicrobial agent in raw chevon.
- The efficacy of various strengths of green tea on *E. coli* and *Staphylococcus epidermidis*.

Materials and Methods

Collection of raw materials

Chevon weighing 100 gm after 6hr post-mortem was purchased from Santidham More, Khulna. Green tea was purchased from the super shop called Safe 'n' Save in Khulna City.

Bacteriological media & Chemicals

Media used for bacteriological analysis, detection, count and identification of *Staphylococcus epidermidis* and *E. coli* in chevon were Mannitol Salt Agar (Hi-Media India) and Eosin Methylene Blue Agar (Hi-Media India).

Experimental Design

The study was conducted using a completely randomized design (CRD) having six (6) treatments ($T_0=0$ ml of green tea; $T_1=1$ ml of green tea; $T_2=2$ ml of green tea; $T_3=3$ ml of green tea; $T_4=4$ ml of green tea and $T_5=5$ ml of green tea) with three (3) replications. Six different meat mixes were prepared; the 1st meat mix (control) was prepared with 1gm chevon and 10 ml normal saline. The second mix was prepared with 1ml green tea, the third mix was prepared with 2 ml green tea, the fourth mix was prepared with 3 ml green tea, the fifth mix was prepared with replacement 4 ml green tea and the sixth mix was prepared with replacement 5 ml green tea. All measurements were made in duplicate.

Preparation of Sample for Bacteriological Studies

The chevon was washed in normal saline (0.9% NaCl). Ten grams of ground dry green tea was added to 100 ml of distilled water and heated at 30-40°C for 45 min with a magnetic stirrer. Then the mixture was filtrated with a Wattman filtration paper (No.42) and the filtered solution with soluble solid content was applied as green tea extract (GTE) in the experiment.

1 gm meat sample was added with 1000 μ l (1ml) saline and grinded properly. After grinding 9 ml saline was added. On the other hand, 1 gm meat sample was soaked in 1ml green tea extract for 30min then 10000 μ l (10ml) saline was added and grinded properly. Following grinding, meat (chevon) was assigned into six treatments.

Monitoring parameter: Total viable count

1 g of meat sample for each treatment was weighed and put in T_0 , T_1 , T_2 , T_3 , T_4 , T_5 . T_0 was left as the fresh sample while T_1 , T_2 , T_3 , T_4 , T_5 was subjected to surface treatment using 1ml, 2ml, 3ml, 4ml, & 5ml of the green tea respectively. All samples were stored at room temperature (25°C) and were analyzed immediately 30 minutes after treatment.

Sample plating (by using the drop plate method)

A series of dilutions of the sample were prepared. Then two sterilized Petri dishes were taken and agar medium was poured into them and allowed to dry. Each agar plate was divided into four quadrants by marking over the lid with a glass marking marker each quadrant was reserved for one dilution in the series. Then the plates were arranged, the sample was drawn by the pipette from the highest dilution first (10^{-7}), the segment of the plate marked 10^{-7} was brought nearer, the lid was lifted on one side and a drop of the suspension was delivered from the pipette aseptically on the marked segment. The electronic pipette was programmed to pick up 10 μ l and volume. The first dilution tube was vortexed for approximately 6 s and 10 μ l was picked up using the electronic pipette. Eighty microliters were dispensed in 8 evenly spaced 10 μ l drops onto the designated quadrant of the Petri plate. The tip and the remaining sample were discarded. The sample was vortexed again for approximately 6 s and 10 μ l was picked up using the electronic pipette. Eighty microliters were dispensed in eight evenly spaced 10 μ l drops onto the designated quadrant of the duplicate plate. In that way, suspensions of bacteria of different dilutions were separated by plates on marked areas of the Petri dish. After the drops on the agar dried, the Petri plates were inverted and incubated.

Observation

The petri plates were incubated at 37°C for 18–72 hrs. While the incubation period was over the microorganisms were grown in the petri plates and it was counted the CFU/ml via visual method.

Enumeration of Total Viable Count of Bacteria (TVC), *E. coli* count (ECC) and *Staphylococcus epidermidis* count (SEC)

TVC, SEC, and ECC in the samples were enumerated following the methods of Viable Count of Bacteria by Drop Technique with suitable modifications whenever necessary. Viable bacteria might be counted by diluting samples, plating the dilutions on the solid medium, and counting the colonies that arise. Results were usually expressed as colony-forming units (CFU). The number of CFU per gram of the original sample was calculated by using the formula:

$$\text{Number of CFU ml}^{-1} \text{ (or g}^{-1}\text{)} = N \times 10^n \times 10$$

Where,

N = no. of colonies on the plate at the selected dilution.

n = no. of dilution.

Total Viable Count (TVC)

For evaluating Total Viable Count (TVC), the drop plate technique was followed using 10^{-1} to 10^{-7} dilutions. Briefly, 10 μ l from each dilution were drop plated on solidified plates of agar and incubated at 37 ± 2 °C for 18 to 72 hrs. The plates containing between 5-50 colonies in consecutive dilutions were selected to calculate the results.

The assumption was that each viable bacterial cell was separate from all others and will develop into a single discrete colony (CFU). Thus, the number of colonies should be given the number of bacteria that could grow under the incubation conditions employed. A wide series of dilutions (e.g., 10^{-4} to 10^{-10}) was normally plated because the exact number of bacteria was usually unknown. Greater accuracy was achieved by plating.

***E. coli* count (ECC)**

E. coli was isolated and enumerated using EMB agar (Hi Media, Mumbai, India). Briefly, 10 μ l of 10^{-1} to 10^{-7} dilution were drop plated on dried plates of EMB agar and incubated at 37°C for 18-24 hrs. The presumptive colonies were determined by counting the number of small purple colonies. The colonies were confirmed by streaking 2-3 colonies onto EMB (Levine) agar (Eosin methylene blue agar) and colonies of *E. coli* species produces small black dot purple colonies with a metallic green sheen on EMB agar. The average number of colonies was recorded in the sample.

***Staphylococcus epidermidis* count (SEC)**

Mannitol salt agar was used for the isolation and enumeration of *Staphylococcus epidermidis*. Briefly, 10 μ l of 10^{-1} to 10^{-7} dilution were drop plated on dried plates of mannitol salt agar (Hi-Media Mumbai, India) and incubated at 37°C for 18-24 hrs. The presumptive colonies were determined by pink colonies and by streaking 2-3 colonies on MSA (Mannitol salt agar) for a better view. Average numbers of colonies were recorded for the sample.

Statistical analysis

Microsoft Excel was employed for data entry and the data were analyzed using the GLM procedure of SAS version 9.1, (SAS Institute, Inc.1996). Effects of green tea in chicken meat were tested by analysis of variance and when differences were detected, DMRT (Duncan's Multiple Range Test) was used to compare the treatment means, with significance considered at $P < 0.0001$.

Results and Discussion

Bacteria count with varying dilution factor of raw chevon meat

The data was collected from the drop plate count of *E. coli* and *S. epidermidis* cultures on Petri dishes. The effects of green tea (catechins) on the microbial population of raw chevon are shown in figure-1.

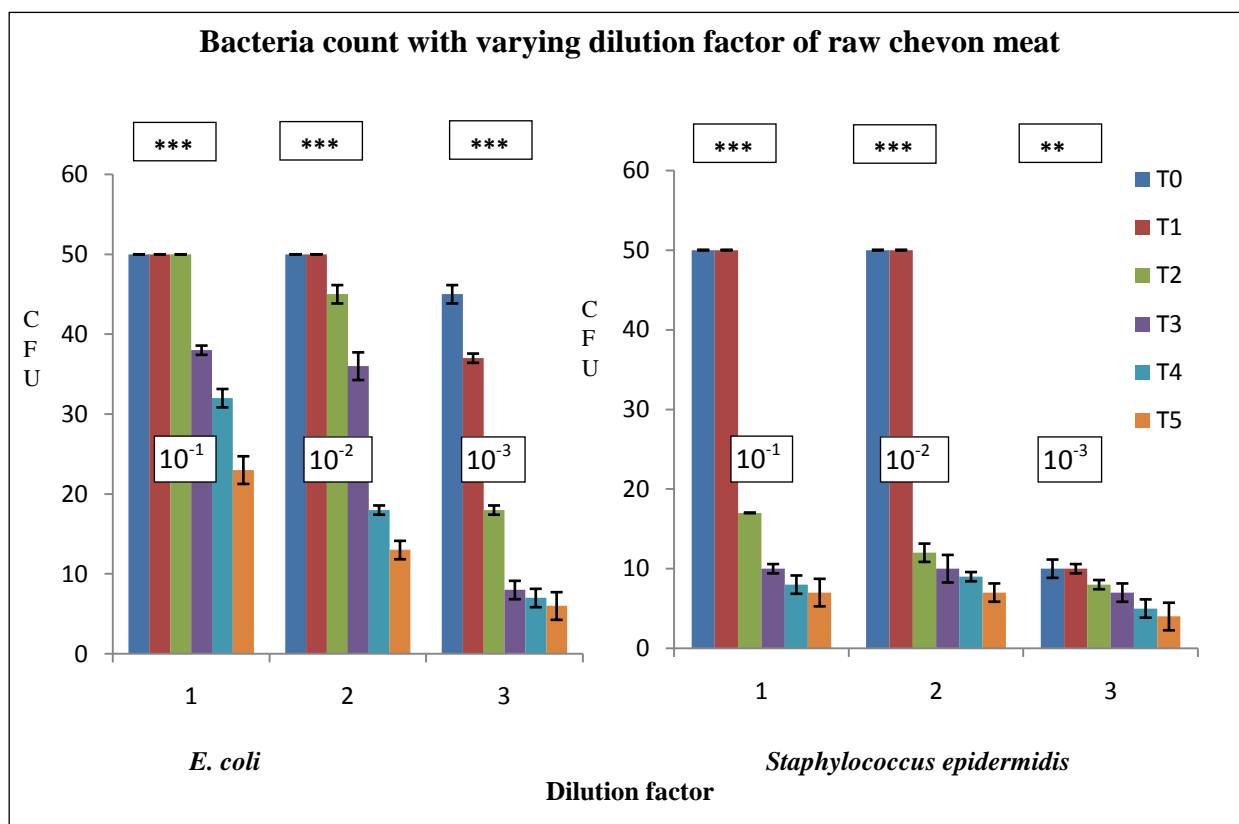


Figure-1. Bacteria count with varying dilution factor of raw chevon meat.

For *E. coli*, colony forming unit (CFU) in 10⁻¹ dilution factor were noted down 50, 50, 50, 38, 32 and 23 again, colony forming units (CFU) in 10⁻² dilution factor were noted down 50, 50, 45, 36, 18 and 13 while colony forming unit (CFU) in 10⁻³ dilution factors were noted down 45, 37, 18, 8.00, 7.00 and 6.00 in T₀, T₁, T₂, T₃, T₄ and T₅, respectively. The colony-forming unit gradually decreased from T₀ to T₅. The lowest colony formation (CFU) was found in T₅ having in 10⁻³ dilution factor. It was found that colony forming unit (CFU)/ml decreased with the addition of green tea into raw chevon meat. There was a highly significant difference (p<0.0001) in colony forming unit of *E. coli* in raw chevon meat among the treatments.

For *S. epidermidis*, colony forming unit (CFU) in 10⁻¹ dilution factor were noted down 50.00, 50.00, 17.00, 10.00, 8.00 and 7.00 in T₀, T₁, T₂, T₃, T₄ and T₅, respectively. Again, colony forming unit (CFU) in 10⁻² dilution factor were noted down 50.00, 50.00, 12.00, 10.00, 9.00 and 7.00 in T₀, T₁, T₂, T₃, T₄ and T₅, respectively. While colony forming unit (CFU) in 10⁻³ dilution factors were noted down 10.00, 10.00, 8.00, 7.00, 5.00 and 4.00 in T₀, T₁, T₂, T₃, T₄ and T₅, respectively. Colony formation (CFU) gradually decreased from T₀ to T₅. The lowest colony forming unit (CFU) was found in T₅ having in 10⁻³ dilution factor. It was found that colony forming unit (CFU) /ml decreased with the addition of green tea into raw chevon meat. There were highly significant differences (p<0.0001) in the colony forming unit (CFU) of *S. epidermidis* in raw chevon meat among the treatments.

A similar result was obtained by Carl, (1975) who reported the microbial profile of the control and the treated chevon during storage at ambient temperature. The SPC levels were well within the acceptable limit in the case of treated samples. Studies have shown that GT catechins effectively inhibit the growth of several strains of pathogens like *E. coli*, *Staphylococcus* etc. Similar findings were reported by Toda et al. (1989).

The effect of green tea on the microbial population of raw chevon

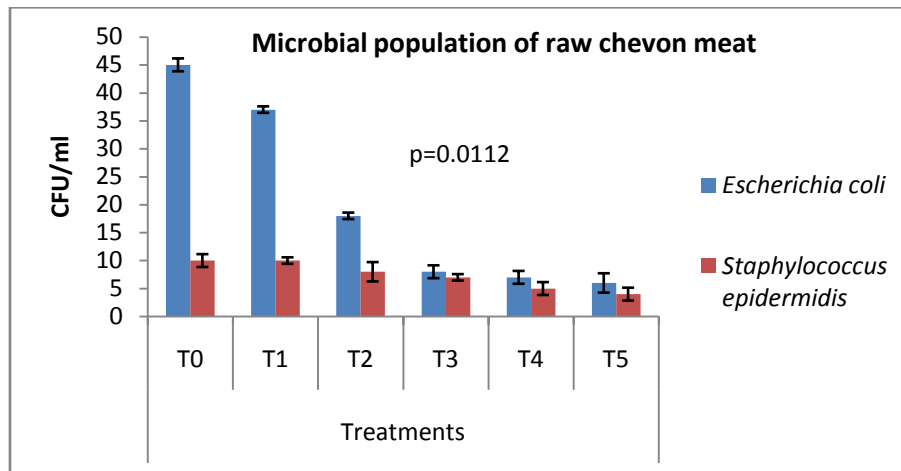


Figure 2. The effect of green tea on the microbial population of raw chevon meat.

The highest initial mean value of *E. coli* was 45 in T₀ while the lowest value was 6 in T₅. It was found that the average colony forming unit (CFU)/ml decreased with the addition of green tea to raw chevon meat. There was a significant effect ($p < 0.0001$) of green tea on the number of *E. coli*.

The highest initial mean value of *Staphylococcus epidermidis* was 10 in T₀ while the lowest value was 4 in T₅. It was found that the average colony forming unit (CFU) /ml decreased with the addition of green tea to raw chevon meat. There was a significant effect ($p < 0.01$) of green tea on the number of *S. epidermidis*.

It is shown in the figure-2 has a germicidal effect on the growth of the spoilage microorganism surface treatment on raw chevon meat. Colony-forming units in fresh meat treated with green tea were lower than the control. A similar result was obtained by Carl (1975) who reported the microbial profile of the control and the treated chevon during storage at ambient temperature. The SPC levels were well within the acceptable limit in the case of treated samples.

Total viable count (TVC) of bacteria in raw chevon treated with green tea

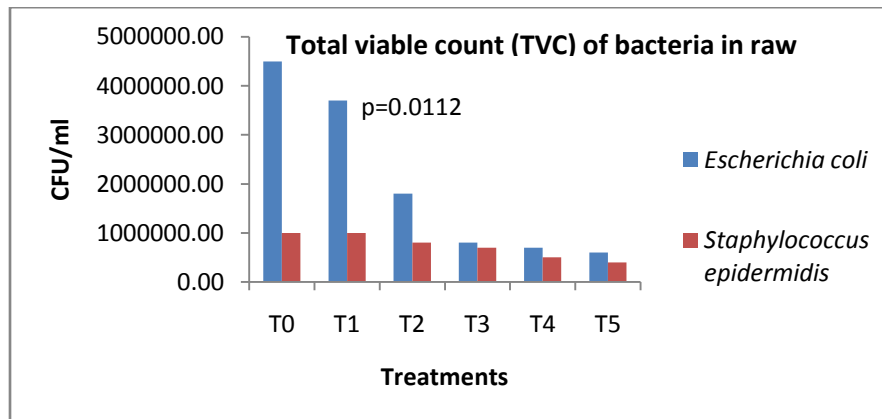


Figure 3. Total viable count (TVC) of bacteria in raw chevon treated with green tea.

The maximum range of TVC for *E. coli* in raw chevon meat was 45×10^5 CFU/ml in T₀, while the minimum range of TVC in raw chevon meat was 6×10^5 CFU/ml in T₅. The variation of TVC in raw chevon meat of different treatments was significant ($p < 0.0001$). (figure-3)

For *S. epidermidis*, the colony forming units of average bacteria per ml of raw chevon, the maximum range was 10×10^5 CFU/ml in T₀ while the minimum range of TVC in raw chevon meat was 4×10^5 CFU/ml in T₅. The variation of TVC in raw chevon meat of different treatments was significant ($p < 0.0001$). (figure-3). A similar result was reported by Carl (1975) showing the microbial profile of the control and the treated chevon during storage at ambient temperature. The SPC levels were well within the acceptable limit in the case of treated samples.

Antimicrobial activity of green tea against *Klebsiella pneumonia* and *Staphylococcus aureus*

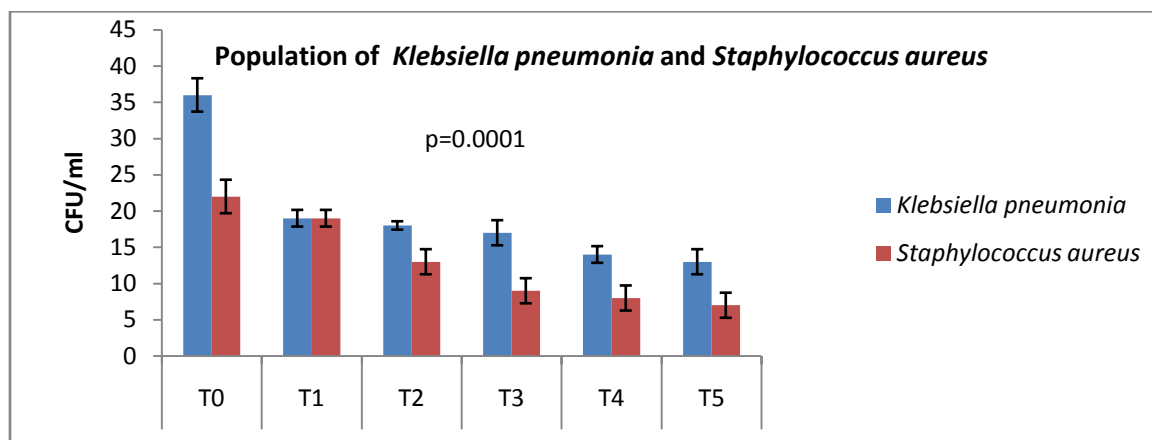


Figure 4. Antimicrobial activity of green tea against *Klebsiella pneumonia* and *Staphylococcus aureus*.

For *Klebsiella pneumonia*, the highest initial mean value of the colony-forming units was 36 in T₀ while the lowest value was 13 in T₅. It was found that the average colony forming unit /ml decreased with the addition of green tea to raw chevon meat. There was a significant reduction ($p < 0.0001$) of the microbial counts among samples with green tea extracts for 30 minutes.

For *S. aureus*, the highest initial mean value was 22 in T₀ while the lowest value was 7 in T₅. It was found that the average colony forming unit /ml decreased with the addition of green tea to raw chevon meat. There was a significant reduction ($p < 0.0001$) of the microbial counts among samples with green tea extracts for 30 minutes. A similar result was obtained by Carl, (1975) who reported the microbial profile of the control and the treated chevon during storage at ambient temperature. The SPC levels were well within the acceptable limit in the case of treated samples.

However, green tea showed the greatest inhibition against the standard cultures. Studies have shown that, GT catechins effectively inhibit the growth of several strains of pathogens like *E. coli*, *Staphylococcus*, *Campylobacter*, etc. Similar findings were reported by Toda et al. (1989). In the present study, the phenolic component of green tea is found to exert a profound inhibitory activity against spoilage organisms including certain pathogens in treated chevon and thus help in extending the shelf life.

Conclusion

The experiment was conducted to find out the effect of adding varying amounts of green tea on the microbial population (*E. coli* and *Staphylococcus epidermidis*) of raw Chevon meat. According to the results T₀ had the highest microbial count, but adding more GTE to the raw chevon progressively reduced its microbial load. There was a highly significant difference in colony forming unit among the treatments and GTE also showed significant antimicrobial activity against all the spoilage organisms found in the sample. Thus, it can be concluded that the addition of 5 ml green tea resulted in a progressive effect in the microbial population of raw chevon meat making it more acceptable.

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Conflicts of Interest

The authors declare that there are no potential conflicts of interest.

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