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## Original Article

# *Solanum Lycopersicum* Phenotypes Juice Food Preservative Potentials: Its Antimicrobial Investigation

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**ABSTRACT**

Consumers' increasing awareness of the health implication of synthetic food preservatives alongside the perceived benefits of natural food additives calls for the search for natural food preservatives as an alternative. This study employed total microbial counts, antimicrobial susceptibility test and sensory evaluation methods to investigate meat preservative potential of lyophilized *Solanum lycopersicum* L. (Tomato) phenotypes' juice organic solvent extracts in comparison to other preservative methods including salting and boiling. The findings revealed significant ($P< 0.05$) decrease in the total microbial load alongside 24-hour microbial growth lag in meat treated with the Tomato juice in comparison to untreated (control) and salted meat; but increase as compared to boiled meat. Relative to other tomato phenotypes extracts, the ethyl acetate extract of ripe round-undulated Tomato (YRR) and ethanolic extract of ripe elongated Tomato (HER) exhibited the highest inhibitory potential against *Staphylococcus aureus* (16.2 ± 0.40 mm), and *Staphylococcus aureus* (12.0 ± 0.25 mm) and *Bacillus subtilis* (1.5 ± 0.20 mm) respectively. Averagely, very good sensory qualities (colour, odour and general acceptability) were recorded within 24 hours for the Tomato juice treated meat as well as boiled meat. A time-dependent decrease in the overall sensory qualities was observed for all the preservative treatments. Our results have highlighted the preservative potential of lyophilized *S. lycopersicum* juice. Most importantly, it offers comparably better preservation potential than salting method.

## INTRODUCTION

Food spoilage is a colossal loss in terms of human endeavour, but the reverse is associated with microbes. Unpalatable for human consumption but palatable for microbial consumption: such is the case of spoiled foods. The deterioration of sensory quality of foods has a linkage with food spoilage, which is a metabolic process that causes foods to be undesirable for human consumption [1,2]. Foods sensory qualities alterations are triggered by metabolic activities of a variety of microbes, including bacteria and fungi that utilize food for their growth and other physiological functions [3-5]. The multiplication of these microbes in foods and food products presents adverse effects on the shelf-life span, textural characteristics, and overall quality [6]. Consequently, the consumer choice for such food is disfavoured; thereby leading to significant commercial loss [7]. In uncoupling sensory qualities alteration from economic loss of food, the prevention of microbial growth in foods via sustainable food preservation methods is of utmost importance for the current globalized food production [8].

Food preservation is known as a process of treating and handling foods to prevent loss of quality, maintaining edibility properties and nutritive value of food through inhibition of the growth of bacteria,

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fungi, and other food spoilage microorganisms, as well as slowing down the oxidation of fats that leads to rancidity of oil [6,9,10]. Several preservation techniques including heat treatments, application of food additives or synthetic preservatives, salting, acidification, canning and other preservation methods have been widely adopted in various food industries to prevent the growth of spoilage and pathogenic microorganisms in foods [5-14]. Increasing awareness of the negative health implications of synthetic food preservatives alongside with benefits of natural food additives, by consumers, calls for searching better alternative means of preserving foods that involve the use of natural products most especially of plant origin [15, 16]. Plants with antimicrobial properties are gaining importance as potential treatments to extend product shelf life and reduce risk of disease-causing microorganisms growth in contaminated food substance as several studies related to plant antimicrobials have demonstrated the efficacy of compounds extracted from plants in food applications [17-19].

Tomato (*S. lycopersicum*), one of the most widely consumed vegetables in the world, belongs to the leafy vegetables with several types and varieties [20]. Several studies have reported remarkably antimicrobial activities of extracts of different parts of the plant on human pathogens that of medical importance, including microbes implicated in oral infection [21,22]. However, its potentials and applications in food preservation are yet to be fully explored. Since tomato fruit is endowed with several bio-active compounds including alkaloids and antioxidants, it might be an effective and efficient antimicrobial agent against food spoilage microorganisms. Hence, this study investigated the preservative potential and antimicrobial activities of *S. lycopersicum* phenotypes' juice on selected spoilage microorganisms isolated from meat.

## MATERIAL AND METHODS

### Sample collection

For the purpose of this study, samples of three phenotypes of ripe and unripe Tomato fruits grown in Nigeria namely round-globular, round-undulated, and elongated were purchased from Osiele market, Odeda Local government, Ogun State ($7^0$13'N$3^0$31' E coordinates: $7^0$13'N$3^0$31'E).

### Authentication of the sample

One of the three Tomato samples, round-undulated phenotype, being a wild type, was authenticated at the Herbarium Section of the Department of Botany, University of Lagos, with a voucher number LUH 8150. The other two samples, round- globular and elongated phenotypes were regarded as hybrids and therefore were not authenticated.

### Study site

The research was conducted at the Biology Department Laboratory, Federal College of Education, Osiele, Abeokuta, Nigeria, while lyophilization of the Tomato Juice was carried out in Central Research Laboratory, Ahmadu Bello University, Zaria, Kaduna, Nigeria.

### Grouping of the plant samples

| Phenotype (Shape) | Sample code |
|---|---|
| Round-Undulated Ripe | YRR |
| Round- undulated Unripe | YRU |
| Round- globular Ripe | HRR |
| Round-globular Unripe | HRU |
| Elongated- Ripe | HER |
| Elongated- unripe | HEU |

### Sample Preparation

### Preparation of Tomato Juice

Tomato juice of ripe and unripe round-undulated, round-globular and elongated phenotypes was extracted from their fruits using a manual juice extractor. The juice extract was collected into labelled sterile bottles, and stored in a refrigerator set at -4 ºC prior to the analysis.

### Lyophilization of the Tomato Juice

Using a lyophilizer to eliminate its moisture contents under a very low temperature, the extracted juice was freeze-dried into a powdery form suitable for solvent extraction.

### Solvent Extraction of the Lyophilized Tomato Juice

Solvent extraction of the lyophilized tomato juice was carried out as described by Gul and Safdar [23]. Briefly, 20g of lyophilized Tomato juice sample was weighed and transferred into four clean well labelled Erlenmeyer flasks. About 300mL of ethyl acetate, petroleum ether, acetone and ethanol solvents were separately added to the weighed lyophilized Tomato juice sample respectively. The mixtures were shaken vigorously, sealed and left on standing for 48 hours.

Thereafter, the filtrate was collected and concentrated under a temperature below 40 ºC using a rotary evaporator. The resultant solvent extracts obtained were stored in a refrigerator set at -4 ºC for future analysis.

## Screening of Food Preservative potential of the solvents' extracts

### Experimental setup

One hundred grams (100g) of fresh meat samples were placed in a set of sterile plastic containers of equal size, labelled according to the names of the tomato phenotypes' juice under investigation -YRR, YRU, HRR, HRU, HER and HEU- as well standard groupings including salt and boil treatment groups. Ten percent (10 %) w/w lyophilized juice of each of the tomato phenotypes was sprinkled over the meat sample in corresponding sterile containers. A set of sterile plastic containers containing untreated fresh meat served as control, while another set of meat samples in plastic containers subjected to salt and boiling water treatments served as standard groups respectively for the experiment. All plastic containers were adequately perforated for aeration, and were left at 28 °C for 5 days. The setup for each group was done in triplicate. Sampling was done every day for microbiological analysis including total heterotrophic counts.

## Antimicrobial Potential Investigation

### Isolation of microorganisms from the meat

Ten gram of each of the treated meat samples was added with 90 ml sterile peptone water and was shaken. Serial dilution was performed to fourth dilution factor and then 0.1 mL of the 3rd and 4th diluents was inoculated into sterile Plate Count Agar (PCA), MacConkey Agar (MAC), Mannitol Salt Agar (MSA), De Man Rogosa and Sharpe Agar (MRSA), and Sabouraud Dextrose Agar (SDA) using the pour plate method. The plates were incubated accordingly: PCA, MAC and MSA were incubated at 37 ºC for 24 hours. MRSA was incubated in an anaerobic jar at 37 ºC for 24 h while SDA was incubated at 28 ºC for 48-72 h. The colonies were counted using an electronic colony counter and distinct colonies were sub-cultured accordingly to obtain pure colonies.

### Storage of the isolates

The pure colonies of bacterial isolates were maintained on Nutrient Agar (NA) slants, while fungal isolates were maintained on SDA slants. Both were stored in the refrigerator at 4 °C.

### Identification of the isolates

The isolates were identified based on their morphological and biochemical characteristics. The bacterial isolates were identified with reference to the Bergey's Manual of Determinative Bacteriology. The fungal isolates were identified with reference to microscopic and macroscopic evaluations as reported by Barnett and Hunter [24].

### Antimicrobial Susceptibility Test

Initially, the lyophilized Tomato juice solvent extracts were reconstituted in their corresponding solvents as 2 g of the various lyophilized solvent extracts were weighed into a set of well-labelled beakers containing 20 mL of solvent each. The antimicrobial activity of the extracts was tested against selected isolates using agar-well diffusion method as described by Irobi *et al.* [25]. Five isolates (2 Gram positive and 3 Gram negative) were selected for the test. The bacterial isolates were standardized by adjusting to 0.5 McFarland standards. Then 0.1 mL was inoculated on sterile Mueller Hinton Agar (MHA), and the inoculum was spread over the surface of the agar using sterile spreader. The plates were left on the workbench for 30 minutes, and sterile cork borer (6 mm) was used to make well on the agar. The well was filled up with the prepared solution of each extract. The plates were left on the workbench for 1 hour to allow diffusion of the extracts and were then incubated at 37 °C for 24 h. After incubation, the zones of inhibition in metres were measured and recorded.

The susceptibility of the bacterial isolates was also tested against conventional antibiotics serving as standards for comparison. This was carried out using the Kirby Bauer disc diffusion method. The antibiotics used for Gram-negative bacteria were Amoxicillin (30 µg), Streptomycin (30 µg), Ciprofloxacin (10 µg), Perfloxacin (10 µg), Gentamycin (10 µg), Chloraphenicol (30 µg), Sparfloxacin (10 µg), Aµgmentin (25 µg), and Tarivid (30 µg). Meanwhile, Ampliclox (20 µg), Zinnacef (20 µg), Amoxacillin (20 µg), Rocephin (20 µg), Ciprofloxacin (10 µg), Streptomycin (30 µg), Septrin (30 µg), Erythromycin (10 µg), Perfloxacin

(10 µg), and Gentamycin (10 µg) were used for Gram-positive bacteria. The zones of inhibition were measured and interpreted with reference to Clinical Laboratory Standard Institute (CLSI) [26].

### Evaluation of sensory characteristics of the Meat sample

The organoleptic characteristics of the treated meat samples were determined using a structured questionnaire as described by Ayinde *et al.* [27]. Briefly, colour, aroma and general acceptability assessment of the meat samples treated with and without lyophilized Tomato juice solvent extracts was conducted for 72 hours by consented ten unrelated assessors. The assessors comprised of equal number of male and female habitual meat consumers of age range 18-55 years, who had been trained prior to the commencement of the study. The aroma and colour of the meat were evaluated using a 7-point Hedonic scale scoring graded as follows: 7 = excellent; 6 = very good; 5 = good; 4 = average; 3 = fair; 2 = poor; 1 = very poor). However, scoring of general acceptability of the meat by the assessors was based on how much they liked or disliked the meat samples under investigation. Scoring was done using a 9-point Hedonic scale graded as follows: 1 = like extremely; 2 = like moderately, 3 = like very much, 4 = like slightly; 5 = neither like or dislike; 6 = dislike slightly; 7 = dislike very much; 8 = dislike moderately, and 9 = dislike extremely. The assessors scored the meat samples from various setup at designated time-points, and the mean scores of the observed characteristics were determined after the third day of sampling and scoring. Average total score $\geq 4$ was considered generally good for colour and aroma, while average total score$< 4$ was considered generally bad. Likewise, Average total score $< 4$ was considered "like" for general acceptability, while average total score$\geq 4$ was considered generally as "dislike."

### DATA ANALYSIS

Continuous variable data were subjected to descriptive and inferential statistics using SAS version 9.4. The mean values were subject ed to analysis of variance (ANOVA) for multiple comparisons, and the level of significance was considered at $P< 0.05$.

### RESULTS

The morphological and biochemical characteristics of the pure bacteria cultures isolated from the fresh meat samples revealed their probable identity as *Staphylococcus aureus, Micrococcus species, Staphylococcus saprophyticus, Escherichia coli, Bacillus subtilis, Bacillus mycoides, Citrobacter freudii, Klebsiella specie, Enterobacterspp, Proteus vulgaris, Pseudomonas specie, Lactobacillus plantarum, Lactobacillus fermentum* and *Lactobacillus alimentarius* (Table 1-1.1). Meanwhile, microscopic and macroscopic characteristics of the isolated pure fungi revealed their identity as *Alternaria alternaria, Sporothrix schenckii and Acremonium species* (Table 1.2). The yeast isolated was morphologically and biochemically identified as *Saccharomyces cerevisiae* (Table 1.3).

The microbial counts including total bacterial load, total coliform count and total fungi count were presented in Table 2-4. Findings from the study revealed significant decrease ($p< 0.05$) in the total bacteria count at Day 2 incubation period of YRR treated meat as compared with the count at Day 1, Day 3, Day 4 or Day 5 (Table 2). However, YRU and HRU treated meat were also attributed with nonsignificant decreased ($p> 0.05$) total bacteria count at Day 2 incubation period as compared to the count at Day 1. Meanwhile, no bacteria growth was observed in meat preserved with boiling at Day 1 incubation period. Generally, time-dependent increase in the total bacteria counts, most especially, at Day 3, Day 4 and Day 5 incubation periods was observed to be the general trend of the bacterial load in all the experimental groups including the untreated meat (control group) (Table 2).

In the same way, significant decrease ($p< 0.05$) in the total Coliform count in meat treated with YRR, YRU, HRR, HRU and HER lyophilized tomato juice extracts at Day 2 as compared to that of other incubation time including the first day of the experiment, was revealed in the study (Table 3). Meanwhile, no total coliform growth was observed in the first two days of the incubation period in the boiled meat. But the total coliform bacteria growth began to set in at Day 2 incubation period and significantly increased ($p< 0.05$) continuously with increasing incubation period, most especially, at Day 3, Day 4 and Day 5 as compared to Day 1 (Table 3). Contrarily, meat treated with HEU extract, salted meat as well as untreated meat (Control) showed

time-dependent increase in total coliform count in all the incubation periods considered for the experiment (Table 3).

Furthermore, outcomes from the study also showed that meat treated with YRR, YRU, HER and HEU had mean total fungal counts which was significantly lower ($p< 0.05$) at Day 2 incubation period as compared to the rest of the incubation periods considered in the study (Table 4). However, nonsignificant ($p> 0.05$) decreased mean total fungal counts in HRR treated meat at Day 2 incubation period as compared to the count at Day 1 was also observed (Table 4). Nonetheless, significant increase in the mean total fungal count of untreated meat (control) group, salted meat group as well as boiled meat group at Day 2 to Day 5 as compared to the count at Day 1 incubation period was recorded (Table 4).

Generally, the microbial counts including total bacterial count (TBC), total coliform count (TCC) and total fungal count (TFC) of the meat sample treated with the juice of different Tomato phenotypes increased with increasing time (0 – 120 hours) of incubation except at Day 2 in some of the treatment groups. However, the increase in the microbial counts (TBC, TCC and TFC) observed for the juice of different Tomato phenotypes (YRR, YRU, HRR, HRU, HER, HEU) treated meat sample is significantly ($p< 0.05$) lower in comparison with the untreated meat sample (positive control). Meanwhile, taking into consideration different methods of preservation, it was found that the magnitude of increase in the microbial counts observed for Tomato phenotype juice treated meat samples was significantly ($p< 0.05$) lower as compared to meat samples treated by salting method; but it was significantly ($p< 0.05$) higher in comparison to meat samples treated by hydrothermal (boiling) method (Table 2-4).

More so, it was observed that the gram negative bacteria were mostly resistant to a wide spectrum of antibiotics (Amoxicillin, Augmetin, Gentamycin, Perfloxacin, Tarivid, Septrin, Streptomycin, Chloramphenicol and Sparfloxacin) but are sensitive to Ciprofloxacin alone (Table 5). However, the gram positive bacteria were mostly resistant to a wide spectrum of antibiotics (Amoxicillin, Augmetin, Gentamycin, Tarivid, Septrin, Streptomycin, Chloramphenicol and Sparfloxacin) and are sensitive to just few (Ciprofloxacin and Perfloxacin) (Table 6). Meanwhile, given the zone of inhibition, different solvent extract (ethylacetate, ethanolic, petroleum ether and acetone) of the lyophilized Tomato phenotypes juice were found to display varying degrees of antimicrobial potential against some of the gram negative and positive bacteria that were isolated from the meat sample (Table 5-6). The gram negative bacteria (*Escherichia coli*, *Pseudomonas species* and *Proteus vulgaris*) were widely resistant to the Tomato extracts (Table 5) while the gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) were largely sensitive to the Tomato extracts (Table 6). The ethylacetate extract of YRR had the highest inhibitory potential against *Staphylococcus aureus* with zone of inhibition of 16.2 ± 0.40 mm relative to other Tomato phenotypes within the group. However, the ethanolic extract of HER had the highest inhibitory effect against *Staphylococcus aureus* with zone of inhibition of 12.0 ± 0.25 mm and *Bacillus subtilis* with 1.5 ± 0.20 mm zone of inhibition among other Tomato phenotypes within the group (Table 6).

Additionally, the results of sensory qualities assessment of the meat samples treated with and without lyophilized Tomato juice extracts revealed a very high score/values above 6.0 for meats treated with YRR, YRU, HRR and HER lyophilized juice extracts at first day of treatment, which corresponds to very good grade according to the Hedonic scale. However, a significant increase ($p< 0.05$) in the mean values of sensed aroma and general acceptability qualities of the meat treated with YRR, YRU, HRR and HER Tomato juice extracts among others as compared with untreated meat (Control) at Day one of the treatments was evident from our observation (Table 7). On the other hand, there was no significant difference ($p> 0.05$) in the colour attributes of the YRR, YRU, HRR and HER treated meat as compared to the control at day one of the treatment. Meanwhile, a progressive time-dependent decrease was observed for the overall sensory qualities including colour, odour and general acceptability of meat treated with different Tomato juice extracts as well as for the control.

**Table 1** The Morphological and Biochemical Characteristics of the Bacteria Isolated from Fresh Meat

| LABEL | Gram | Growth @15 | Growth @37 | Growth@45 | Arabinose | Glucose | Lactose | Mannitol | Maltose | Cellulobiose | Raffinose | Ribose | Salicin | Rahmnose | Galactose | Sorbitol | Xylose | Treahalose | Catalase | ISOLATE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A1 | SR | + | + | + | - | + | - | + | + | + | + | + | + | - | + | - | - | - | - | *L. plantarum* |
| B1 | SR | - | + | - | + | + | + | + | + | + | + | + | + | - | - | + | + | + | - | *L. fermentum* |
| C1 | LR | + | + | + | + | + | - | + | + | + | - | + | + | + | + | + | + | + | - | *L. alimentarius* |

GPC= Gram Positive Cocci; GNB= Gram Negative Bacilli; NA= Not Applicable; + = Positive; - = Negative

**Table 1.1** The Morphological and Biochemical Characteristics of the Lactobacilli Bacteria Isolated from Fresh Meat

| LABEL | Gram | Motility | Glucose | Lactose | Mannitol | Maltose | Indole | Methyl Red | Vogesproskauer | Citrate | $H_2S$ | Sucrose | Urea | Oxidase | Coagulase | Catalase | ISOLATE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | GPC | NA | + | + | + | + | NA | - | + | NA | NA | + | - | - | + | + | *Staphylococcus aureus* |
| 2 | GPC | NA | + | + | + | + | NA | - | + | NA | NA | + | - | + | - | + | *Micrococcus species* |
| 3 | GPC | NA | + | + | + | + | NA | - | + | NA | NA | + | - | - | - | + | *Staphylococcus saprophyticus* |
| 4 | GNB | + | + | + | + | + | + | + | - | - | - | NA | - | - | NA | + | *Escherichia coli* |
| 5 | GPB | + | + | + | + | + | NA | - | + | NA | NA | + | - | - | NA | + | *Bacillus subtilis* |
| 6 | GPB | + | + | + | + | + | NA | + | + | NA | NA | + | - | - | NA | + | *Bacillus mycoides* |
| 7 | GNB | + | + | + | + | + | - | + | - | + | + | - | - | - | - | + | *Citrobacter freudii* |
| 8 | GNB | - | + | + | + | + | - | - | - | + | - | + | + | - | - | + | *Klebsiella specie* |
| 9 | GNB | + | + | + | + | + | - | - | - | + | - | + | - | - | - | + | *Enterobacter spp* |
| 10 | GNB | + | + | - | - | - | - | + | - | + | - | + | + | - | NA | + | *Proteus vulgaris* |
| 11 | GNB | - | + | + | + | - | - | - | - | + | - | + | + | - | - | + | *Pseudomonas spp* |

SR= Gram Positive Short Rod; LR=Gram Positive Long Rods; + = Positive; - = Negative

**Table 1.2** The Morphological and Biochemical Characteristics of the Moulds Isolated from Fresh Meat

| Label | Macroscopy | Microscopy | Identity |
|---|---|---|---|
| E | Grayish black, showing floccus appearance. Reverse is brown | Branched acropetal chain multi-celled conidia emerging from elongated Conidiophores. Conidia are short, ovoid, brown smooth walled | *Alternaria alternaria* |
| K | Moist and glabrous, with a wrinkled andfolded Surface, creamy grey reverse | Short aerial hyphae, Conidiophores are ovoid smooth walled darkly-Pigmented, septatehyphae | *Sporothrix schenckii* |
| J | Moist yellowish powdery, suede-like white | Hypha are erect phialides, conidia is single-celled, globose, cylinderica | *Acremonium specie* |

**Table 1.3** The Morphological and Biochemical Characteristics of the Yeast Isolated from Fresh Meat

| SN | Macroscopy | Microscopy | GTT | UREA | CY-HEX | Growth @37 | Glu | Yeast |
|---|---|---|---|---|---|---|---|---|
| M | Creamy colour, smooth glaborous | Large globose budding blastoconidia | - | - | - | + | - | *Saccharomyces cerevisiae* |

GTT= Germ Tube Test; CYHEX= Cyclohexime; GLU= Glucose

**Table 2** The Total Bacterial Count (TBC) of the Tomato Phenotypes Juice Treated Meat Samples

| Sample | Time (Days) | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| YRR | 2.03 ± 0.3 b | 1.60 ± 0.4 a | 2.12 ± 0.2 b | 4.74 ± 0.3 c | 5.03 ± 0.5 d |
| YRU | 0.95 ± 0.2 a | 0.81 ± 1.0 a | 2.03 ± 0.3 b | 3.60 ± 0.5 c | 5.22 ± 0.7 d |
| HRR | 3.51 ± 0.3 a | 4.34 ± 0.2 ab | 5.20 ± 0.2 bc | 5.81 ± 0.4 c | 6.72 ± 0.3 c |
| HRU | 1.18 ± 0.8 a | 1.06 ± 0.2 a | 1.93 ± 0.3 ab | 2.73 ± 0.3 b | 5.09 ± 0.2 c |
| HER | 1.35 ± 0.5 a | 1.99 ± 0.1 b | 3.44 ± 0.3 c | 4.38 ± 0.3 d | 6.12 ± 0.4 e |
| HEU | 1.10 ± 0.2 a | 1.14 ± 0.3 a | 2.55 ± 0.5 b | 3.01 ± 0.1 b | 5.77 ± 0.4 c |
| Control Positive | 5.74 ± 0.3 a | 7.87 ± 0.5 b | 8.04 ± 0.5 b | 11.43 ± 0.2 c | 14.03 ± 0.5 d |
| Control Salt | 3.83 ± 0.2 a | 4.24 ± 0.3 a | 7.37 ± 0.3 b | 8.17 ± 0.3 c | 12.62 ± 0.2 d |
| Control Boil | NG | 0.03 ± 0.1 a | 0.89 ± 0.1 b | 2.16 ± 0.2 d | 1.52 ± 0.2 c |

Mean values with different superscript letter within a column are significantly different at $p< 0.05$; N.B.: YRR - Round-undulated Ripe; YRU - Round globular Unripe; HRR - Round- globular Ripe; HRU - Round globular Unripe; HER - Elongated Ripe

**Table 3** The Total Coliform Count (TCC) of the Lyophilized Tomato Phenotypes Juice Treated Meat Samples

| Sample | Time (Days) | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| YRR | 2.21 ± 0.4 b | 1.43 ± 0.1 bc | 2.19 ± 0.2 b | 3.58 ± 0.2 a | 5.84 ± 0.3 c |
| YRU | 1.17 ± 0.1 c | 1.12 ± 0.2 a | 2.07 ± 0.5 b | 2.81 ± 0.2 c | 4.44 ± 0.5 a |
| HRR | 2.91 ± 0.3 a | 2.68 ± 0.1 ab | 4.26 ± 0.2 c | 4.87 ± 0.5 c | 5.21 ± 0.2 d |
| HRU | 1.6 ± 0.5 b | 1.24 ± 0.1 ab | 3.21 ± 0.4 a | 5.02 ± 0.2 b | 5.83 ± 0.3 c |
| HER | 2.11 ± 0.1 a | 2.00 ± 0.5 b | 3.65 ± 0.3 b | 4.26 ± 0.5 c | 6.31 ± 0.4 d |
| HEU | 1.87 ± 0.2 a | 2.16 ± 0.3 a | 2.75 ± 0.6 b | 4.10 ± 0.4 c | 4.82 ± 0.2 c |
| Control Positive | 3.56 ± 0.4 a | 4.28 ± 0.5 b | 6.35 ± 0.3 a | 7.19 ± 0.2 ab | 10.26 ± 0.2 c |
| Control Salt | 2.79 ± 0.3 a | 3.49 ± 0.2 c | 5.12 ± 0.3 a | 6.23 ± 0.1 c | 8.15 ± 0.2 b |
| Control Boil | NG | NG | 0.02 ± 0.1 b | 0.30 ± 0.2 a | 1.50 ± 0.1 c |

Mean values with different superscript letter within a column are significantly different at $p< 0.05$; N.B.: YRR - Round-undulated Ripe; YRU - Round globular Unripe; HRR - Round- globular Ripe; HRU - Round globular Unripe; HER - Elongated Ripe

**Table 4** The Total Fungal Count of the Lyophilized Tomato Phenotypes Juice Treated Meat Samples

| Sample | Time (Days) | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| YRR | 1.21 ± 0.2 a | 1.09 ± 0.1 c | 1.94 ± 0.3 bc | 2.63 ± 0.2 b | 3.42 ± 0.1 c |
| YRU | 1.18 ± 0.1 b | 1.05 ± 0.3 ab | 2.59 ± 0.4 a | 3.11 ± 0.2 b | 3.87 ± 0.1 c |
| HRR | 1.63 ± 0.1 a | 1.46 ± 0.1 a | 2.36 ± 0.3 b | 3.72 ± 0.2 ab | 4.21 ± 0.2 b |
| HRU | 1.53 ± 0.2 a | 1.74 ± 0.3 ab | 2.70 ± 0.1 c | 3.91 ± 0.5 b | 4.36 ± 0.1 d |
| HER | 1.27 ± 0.4 a | 1.21 ± 0.2 b | 2.74 ± 0.1 bc | 3.83 ± 0.2 c | 5.42 ± 0.2 cd |
| HEU | 1.67 ± 0.1 a | 1.49 ± 0.3 b | 2.35 ± 0.2 bc | 2.94 ± 0.3 c | 4.18 ± 0.1 |
| Control Positive | 2.76 ± 0.3 ab | 3.91 ± 0.2 b | 5.24 ± 0.2 c | 7.43 ± 0.4 b | 9.89 ± 0.3 c |
| Control Salt | 1.38 ± 0.2 a | 2.95 ± 0.1 b | 4.11 ± 0.2 c | 5.48 ± 0.2 cd | 8.77 ± 0.1 c |
| Control Boil | NG | NG | 0.02 ± 0.1 a | 0.30 ± 0.2 a | 0.50 ± 0.1 b |

Mean values with different superscript letter within a column are significantly different at $p< 0.05$; N.B.: YRR - Round-undulated Ripe; YRU - Round globular Unripe; HRR - Round- globular Ripe; HRU - Round globular Unripe; HER - Elongated Ripe

**Table 5** Antimicrobial Susceptibility of Gram Negative Bacteria against Lyophilized Tomato Phenotypes Juice Solvent Extracts and Conventional Antibiotic Discs

| Antimicrobial Agent | Zone of Inhibition (mm) | | |
|---|---|---|---|
| | *Escherichia coli* | *Pseudomonas specie* | *Proteus vulgaris* |
| Antibiotics | | | |
| Amoxicillin | 1.0 ± 0.03 (R) | 3.4 ± 0.32 (R) | 2.2 ± 0.22 (R) |
| Augmentin | 0.0 ± 0.00 (R) | 1.0 ± 0.02 (R) | 0.0 ± 0.00 (R) |
| Gentamycin | 0.0 ± 0.00 (R) | 0.3 ± 0.01 (R) | 0.0 ± 0.00 (R) |
| Pefloxacin | 0.0 ± 0.00 (R) | 1.2 ± 0.06 (R) | 0.0 ± 0.00 (R) |
| Tarivid | 0.0 ± 0.00 (R) | 1.2 ± 0.04 (R) | 0.6 ± 0.02 (R) |
| Septrin | 0.0 ± 0.00 (R) | 0.0 ± 0.00 (R) | 0.0 ± 0.00 (R) |
| Streptomycin | 0.0 ± 0.00 (R) | 0.0 ± 0.00 (R) | 0.0 ± 0.00 (R) |
| Chloramphenicol | 0.0 ± 0.00 (R) | 0.0 ± 0.00 (R) | 1.0 ± 0.01 (R) |
| Sparfloxacin | 1.1 ± 0.04 (R) | 2.7 ± 0.05 (R) | 2.9 ± 0.10 (R) |
| Ciprofloxacin | 18.5 ± 0.50 (S) | 19.4 ± 0.42 (S) | 17.3 ± 0.30 (S) |
| Ethylacetate Extract | | | |
| YRR | 0.0 ± 0.00 | 0.6 ± 0.22 | 1.0 ± 0.20 |
| YRU | 2.0 ± 0.40 | 0.0 ± 0.00 | 0.5 ± 0.10 |
| HRR | 1.0 ± 0.20 | 0.0 ± 0.00 | 0.0 ± 0.00 |
| HRU | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 |
| HER | 0.0 ± 0.00 | 0.8 ± 0.20 | 0.0 ± 0.00 |
| Ethanolic Extract | | | |
| YRR | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 |
| YRU | 0.0 ± 0.00 | 2.3 ± 0.02 | 0.0 ± 0.00 |
| HRR | 2.5 ± 0.30 | 0.0 ± 0.00 | 0.0 ± 0.00 |
| HRU | 0.2 ± 0.02 | 0.0 ± 0.00 | 0.0 ± 0.00 |
| HER | 0.0 ± 0.00 | 1.0 ± 0.20 | 0.0 ± 0.00 |
| Petroleum Ether Extract | | | |
| YRR | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.5 ± 0.08 |
| YRU | 0.0 ± 0.00 | 0.3 ± 0.02 | 0.0 ± 0.00 |
| HRR | 1.4 ± 0.07 | 0.8 ± 0.01 | 0.0 ± 0.00 |
| HRU | 0.0 ± 0.00 | 0.5 ± 0.04 | 0.4 ± 0.02 |
| HER | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 |
| Acetone Extract | | | |
| YRR | 0.0 ± 0.00 | 1.0 ± 0.45 | 0.0 ± 0.00 |
| YRU | 0.6 ± 0.05 | 0.5 ± 0.04 | 0.5 ± 0.02 |
| HRR | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 |
| HRU | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 |
| HER | 0.4 ± 0.02 | 0.5 ± 0.02 | 0.0 ± 0.00 |
| Control | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 |

N.B.: “R” - Resistant; “S” – Sensitive N.B.: YRR - Round- undulated Ripe; YRU - Round globular Unripe; HRR - Round-globular Ripe; HRU - Round globular Unripe; HER - Elongated Ripe

**Table 6** Antimicrobial Susceptibility of Gram Positive Bacteria against Lyophilized Tomato Phenotypes Juice Solvent Extracts and Conventional Antibiotic Discs

| Antimicrobial Agent | Zone of Inhibition (mm) | | | |
|---|---|---|---|---|
| | *Staphylococcus aureus* | | *Bacillus subtilis* | |
| Antibiotics | | | | |
| Ciprofloxacin (10 µg) | 11.2 ± 0.30 | (S) | 15.5 ± 0.50 | (S) |
| Streptomycin (30 µg) | 2.3 ± 0.08 | (R) | 2.0 ± 0.20 | (R) |
| Septrin(30 µg) | 0.3 ± 0.01 | (R) | 0.5 ± 0.05 | (R) |
| Erythromycin (10 µg) | 0.4 ± 0.02 | (R) | 0.2 ± 0.02 | (R) |
| Pefloxacin(10 µg) | 19.1 ± 0.25 | (S) | 15.3 ± 0.30 | (S) |
| Gentamycin (10 µg) | 0.4 ± 0.04 | (R) | 0.1 ± 0.01 | (R) |
| Ampiclox(20 µg) | 0.0 ± 0.00 | (R) | 0.0 ± 0.00 | (R) |
| Zinnacef(20 µg) | 0.0 ± 0.00 | (R) | 0.0 ± 0.00 | (R) |
| Amoxicillin(20 µg) | 0.0 ± 0.00 | (R) | 0.2 ± 0.03 | (R) |
| Rocephin(20 µg) | 4.4 ± 0.02 | (R) | 3.0 ± 0.30 | (R) |
| Ethylacetate Extract | | | | |
| YRR | 16.2 ± 0.40 | | 0.0 ± 0.00 | |
| YRU | 1.5 ± 0.20 | | 1.0 ± 0.20 | |
| HRR | 1.0 ± 0.20 | | 0.5 ± 0.00 | |
| HRU | 2.6 ± 0.20 | | 0.0 ± 0.00 | |
| HER | 3.0 ± 0.00 | | 1.0 ± 0.20 | |
| Ethanolic Extract | | | | |
| YRR | 7.0 ± 0.20 | | 0.0 ± 0.00 | |
| YRU | 0.0 ± 0.00 | | 0.0 ± 0.00 | |
| HRR | 1.5 ± 0.02 | | 0.0 ± 0.00 | |
| HRU | 2.6 ± 0.02 | | 0.8 ± 0.02 | |
| HER | 12.0 ± 0.25 | | 1.5 ± 0.20 | |
| Petroleum Ether Extract | | | | |
| YRR | 0.5 ± 0.02 | | 0.0 ± 0.00 | |
| YRU | 1.5 ± 0.20 | | 0.5 ± 0.02 | |
| HRR | 0.0 ± 0.00 | | 0.7 ± 0.06 | |
| HRU | 0.6 ± 0.04 | | 1.2 ± 0.04 | |
| HER | 0.0 ± 0.00 | | 0.5 ± 0.08 | |
| Acetone Extract | | | | |
| YRR | 1.8 ± 0.04 | | 0.5 ± 0.07 | |
| YRU | 0.0 ± 0.00 | | 0.0 ± 0.00 | |
| HRR | 0.5 ± 0.06 | | 0.0 ± 0.00 | |
| HRU | 0.8 ± 0.09 | | 0.0 ± 0.00 | |
| HER | 0.0 ± 0.00 | | 0.0 ± 0.00 | |
| Control | 0.0 ± 0.00 | | 0.0 ± 0.00 | |

N.B.: “R” - Resistant; “S” – Sensitive; YRR - Yoruba Round Ripe; YRU - Yoruba Round Unripe; HRR - Hausa Round Ripe; HRU - Hausa Round Unripe; HER - Hausa Elongated Ripe

**Table 7** Sensory Qualities of Meat Treated with or without Lyophilized Tomato Phenotypes Juice

| Treatment | First Day | | | Second Day | | | Third Day | | |
|---|---|---|---|---|---|---|---|---|---|
| | Colour | Aroma | G.A | Colour | Aroma | G.A | Colour | Aroma | G.A |
| YRR | 6.5±0.384 ab | 6.0±0.592 bc | 6.4±0.726 bcd | 5.8±0.537 a | 5.3±0.557 a | 4.9±0.726 b | 4.5±0.300 a | 3.6±0.963abc | 4.1±0.823 ab |
| YRU | 6.3±0.677 abc | 6.9±0.687 a | 6.8±0.600 ab | 5.7±0.793 ab | 5.6±0.758 a | 6.1±0.977 a | 4.3±0.966 a | 3.9±0.836ab | 4.2±0.386 ab |
| HRU | 5.8±0.748 cd | 6.3±0.756 abc | 6.5±0.590 abc | 5.2±0.798 abc | 5.4±0.735 a | 5.1±0.829 b | 3.1±0.852 b | 4.1±0.861a | 4.3±0.934 a |
| HRR | 6.6±0.847 ab | 5.9±0.84 c | 6.3±0.829 cd | 5.8±0.536 a | 5.1±0.875 a | 4.8±0.872 b | 4.5±0.635 a | 3.2±0.300bc | 3.3±0.848 c |
| HER | 6.3±0.502 ab | 6.2±0.916 bc | 6.5±0.654 abc | 5.5±0.385 abc | 5.1±0.875 a | 4.7±0.463 b | 3.5±0.909 b | 3.5±0.987abc | 3.6±0.837 bc |
| HEU | 5.5±0.860 d | 6.6±0.943 ab | 6.7±0.572 abc | 5.3±0.625 abc | 5.3±0.458 a | 4.9±0.853 b | 3.5±0.901 b | 3.5±0.922abc | 3.3±0.848 c |
| BOIL | 6.1±0.651 bc | 6.9±0.451 a | 6.9±0.452 a | 5.3±0.622 abc | 5.5±0.931 a | 5.1±0.820 b | 3.2±0.902 b | 4.2±0.866a | 4.6±0.922 a |
| SALT | 5.5±0.487 d | 5.2±0.593 d | 6.0±0.973 de | 5.1±0.588 bc | 4.3±0.690 b | 4.6±0.972 b | 2.9±0.995 b | 3.0±0.698cd | 3.1±0.448 c |
| MEAT ONLY | 6.8±0.600 a | 5.0±0.63 d | 5.6±0.513 e | 4.9±0.783 c | 3.0±0.837 c | 2.6±0.670 c | 2.8±0.960 b | 2.3±0.965d | 1.6±0.600 d |

Mean values with different superscripts letter within a column are significantly different at $p< 0.05$; N.B.: YRR - Round- undulated Ripe; YRU - Round globular Unripe; HRR - Round- globular Ripe; HRU - Round globular Unripe; HER - Elongated Ripe; G.A- General Acceptability; 7- Excellent; 6- Very Good; 5-Good; 4-Average; 3-Fair; 2-Poor; 1-Very poor.

## DISCUSSION

The search for natural agents with remarkable inhibitory potential against foodborne infectious organisms as well as food spoilage microbes is generally on increasing demand. This is due to consumers' awareness and concern about health implications of synthetic food preservatives frequently employed in food processing as well as their preference for green and additive-free foods [28]. Plant extracts and their bioactive constituents exhibiting antimicrobial potentials are showing promising applications for bio-preservation of food. Thus, this study investigated the food preservative potential of S. lycopersicum (Tomato) juice via antimicrobial activity evaluation.

Microorganisms are ubiquitously found in nature [29-31]. In this study, the isolation and identification of several microbial species including gram positive bacteria, gram negative bacteria and fungi revealed baseline microbial diversity in fresh meat samples. The putative microbial species identified including Escherichia coli, Staphylococcus aureus, Pseudomonas spp., Enterobacter spp., Bacillus subtilis, Proteus vulgaris, Micrococcus spp. are commonly reported culture or unculture microorganisms found in foods and environment [29-33]. Moreover, the existence of bacteria in fresh meat has been widely documented from different parts of the globe [34-36]. Likewise in support of our findings, Clarence et al. [26] highlighted Staphylococcus aureus, E coli, Bacillus spp., Pseudomonas spp, Enterobacter spp., and Klebisiella spp as major isolated microorganisms identified in meat-pie samples. Additionally, Escherichia coli, Staphylococcus aureus, Pseudomonas spp, and Enterobacter spp. are reportedly identified as predominant isolates in fresh meat samples collected from several abattoirs and traditional open markets [29,37-42].

Gram negative bacteria, most especially, Escherichia coli is found almost everywhere in the environment and discriminately remains one of the major causes of food contamination. Although meat is a highly desirable and excellent source of protein, it is highly perishable since it presents favourable medium and nutrient composition that supports the growth of various microorganisms [43]. On this premise, meat is reportedly prone to microbial contamination at various stages of preparation from animal slaughtering to the point of consumption [42]. During animal slaughtering, dressing and cutting, wide range of microbes gain entry into the carcass surfaces from the exterior of the animal to its intestinal tract, but more are introduced by air, butchery equipment and abattoir workers. These present possible reasons meat harbours some biodiversity of microorganisms amongst which might be potential spoilage microbes that could thrive through their metabolic activities [43,44].

In a bid to control meat spoilage, several methods from refrigeration to boiling and frying are employed to reduce the rate of microbial metabolic activities. The significant reduction of microbial load recorded for meats treated with S. lycopersicum juice extracts

relative to untreated meat implies that the growth of bacteria, coliform and fungi may have been inhibited largely by the Tomato juice extracts. In addition, the significant reduction in microbial load observed in meat samples treated with the juice of different Tomato phenotypes as compared to salted meat, but not boiled meat, implies that Tomato juice might be more effective for microbial growth inhibition in comparison to the application of salt. However, it could be less potent in comparison to boiling (hydrothermal) method.

The growth and development of microorganisms in culture media occur in phases which are time dependent [45]. The quality of meat deteriorates as a result of metabolic activities that leads to production of chemicals that may be bring about rancidity and spoilage, which may increase with incubation time of the microbes. Although microbial activities increases with time of microbial incubation, no noticeable effects on the sensory qualities of meat sample would be observed inasmuch as the microbial counts has not reached a threshold load that can initiate meat spoilage through discolouration of meat, formation of slime on meat surfaces and offensive odour generation [41]. Hence inhibition of microbial growth in meat can prolong its shelf- life [41]. In this study, though microbial count increase with increased incubation time was the general trend recorded for all the meat treatment groups, the observed slower rate of increase associated with Tomato juice treated groups in comparison to the untreated and salting groups might reduce the rate of attaining the microbial load threshold that could initiate meat spoilage. This evidence further suggests the preservation potency of Tomato juice over salting.

Furthermore, evidence of short length of zone of inhibition (<4.0 mm) of tested microbial species by majority of the conventional antibiotics investigated revealed that most of the gram positive and gram negative bacteria isolated from meat sample might possess antibiotic resistance trait. Hence, poor sanitary practices and improper preparation of meat prior to consumption could harbour these microorganisms and predispose consumers to foodborne infection as well as antibiotic resistance. Similarly, the little or no sensitivity of representative gram negative bacteria investigated for antimicrobial susceptibility to solvent extracts of the Tomato phenotypes juice may be due to low concentration of the extracts considered in the study. Higher concentrations of these extracts might exhibit higher inhibitory activities against the tested microorganisms. Moreover, since some ethylacetate and ethanolic Tomato phenotypes juice extracts (especially YRR and HER) yielded better sensitivity against some gram positive bacteria in comparison to petroleum ether and acetone extracts, solvent specificity might be indicated for better or optimum extraction of the Tomato juice active ingredient.

Moreover, the somewhat preservation of organoleptic properties (colour, aroma and general acceptability) with increasing time of incubation until the fifth day of the meat treatment with the Tomato juice extracts in comparison to untreated meat (control) implies that the juice extracts might have little or no alteration effects on the sensory properties of the meat. Hence, a natural food additive that maintains or retains the colour, aroma and other sensory qualities of meat might be considered as better and desirable food additive by consumers.

Although we have presented some baseline or elementary findings, it is quite important to address some limitations of our study in order to inform future perspectives. Firstly, microbial identification was solely based on morphology and biochemistry. Since we did not introduce molecular techniques including polymerase chain reaction, electrophoresis and genetic sequencing, our microbial identity may have just been probable or putative. Secondly, due to limited volume of juice extracted from the Tomato sample batch obtained initially, we could not investigate preservative potential in a concentration-dependent manner. Therefore establishing a range of concentration of the Tomato juice extract with meat preservative potential will be quite important. Thirdly, we only tested few representative microorganisms for the antimicrobial susceptibility and no antifungal testing was examined. It would be pertinent to consider investigating the antimicrobial susceptibility of all the microbial isolates from fresh meat. Since some yeasts moulds were isolated from fresh meat samples, it would also be important to investigate and compare antifungal susceptibility to wide range of standard antifungal drugs and different concentrations of Tomato juice extracts.

## CONCLUSIONS

Tomato juice extracts possess higher antimicrobial potentials as compared to salting method but lower than hydrothermal method. This is owing to the fact

that meat samples treated with the juice of different Tomato phenotypes exhibited significant reduction in the total microbial load in comparison to untreated meat (control) and salted meat, but higher in comparison to boiled meat (hydrothermal). Moreover, the preservation of organoleptic properties over a longer period of time in the meat treated with the juice extracts as compared to untreated and salted meat showed the effectiveness of the preservative potential of Tomato juice extracts. Lastly, solvent specificity might result in effective active ingredient extraction. Among the solvent extracts of the lyophilized Tomato juice investigated in this study, ethyl acetate and ethanolic extracts of lyophilized juice of YRR and HEU Tomato phenotypes exhibited stronger growth inhibitory potentials on the isolated microbes associated with meat spoilage and foodborne infections. Therefore, lyophilized Tomato juice can serve as a potential meat preservative.

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## Conflict of Interests

The authors declare no conflict of interest

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