Antimicrobial Activity of *Ocimumgratissimum* Extract Against Bacterial Isolates Associated with Spoilt Tomatoes Sold in Lafia Metropolis

Peter, U.U.[1], Mfonido, I.B.[2], Gladys, A.A.[1], Naja'atu, S.H.[1], Janet, C.G.[1], Maryam, A.O.[1], Kosiso, V.A.[1] and Ogadegbor, A.O.[1]

[1]*Department of Microbiology, Federal University of Lafia, Nigeria*
[2]*Department of Microbiology, University of Uyo, Nigeria*
**Corresponding author*

Abstract:- **This study was conducted to investigate bacterial isolates associated with spoilt tomato fruits (*Lycopersicunesculentum*) obtained in Lafia metropolis, Northern Nigeria, and their susceptibility pattern on extract of *Ocimumgratissimum*. A total of 75 pieces of spoilt tomato fruits were purchased from Alamis, Kasuwan tomato, and Modern markets. Samples obtained were transported to the laboratory using sterile polyethylene bags. Spread plate methods were adopted for isolation. Colonies from each agar plates were examined both macroscopically and microscopically. Two different extracts, ethanol and methanol were obtained from *Ocimumgratissimum*. Agar well diffusion and micro-broth dilution technique were used for antibacterial susceptibility test and minimum inhibitory concentration, respectively. Five species of bacteria were identified of which *Staphylococcus aureus* was the dominant organism with percentage frequency of 19 (30.2%), *Escherichia coli*15 (23.8%), *Salmonella* spp12 (19.0%), *Proteus*spp9 (14.3%), and the least was *Pseudomonas*spp8 (12.7%). The methanol and ethanol extract of *O. gratissimum* showed activity against the test organisms. *S. aureus* was more susceptible to the extract with a mean zone of inhibition of 21.5±0.7mm and 23±2.5mm, respectively. The least activity was recorded in *E. coli* and *Salmonella*spp with a mean zone of inhibition of 13.5±0.7mm and 2.5±0.7mm, respectively, in 100mg/mL.The mean zone of inhibition differs significantly at *P*<0.05 against the test organisms. The minimum inhibitory concentration of *O. Gratissimum* against the test organisms in ethanol and methanol extract was between 0.19-12.5mg/mL and 0.39-50mg/mL.*O. gratissimum* could be a potential drug for the treatment of infections. Having identified the presence of harmful bacteria such as *S. aureus, Salmonella*spp, *Pseudomonas*spp *Proteus*spp *Escherichia coli* in spoilt tomatoes, the consumption of spoilt and unhealthy tomatoes among members of the populace should be discouraged.**

Key words: **Tomato, *Ocimumgratissimum*, Inhibitory, *L.esculentum*, diffusion**

I. INTRODUCTION

For many centuries, the tomato has been a part of different recipes consumed globally, and this is because of its excellent nutritional value. In addition to their nutritional value, are also their medical values as well as their economic importance [1]. Tomato cultivation is highly susceptible to various pathogens, which usually result in significantyield loss and spoilage in the quality of fruits. Tomato (*Lycopersiconesculentum*) is an important vegetable crop in Nigeria for the domestic purpose, which accounts for about 18% of the daily consumption of vegetables, which averages out at 50% per person [2]. Tomato can be consumed in different ways, blended into tomato juice, can be taken raw or cooked or fried. Several species of tomato are reported globally with different sizes, shapes, as well as colors. Some species of tomato assume round, spherical, or cylindrical shape. The colors of tomatoes range between pink, green, yellow, and red. But the most widely grown in Nigeria is the red colored ones because it has a high amount of lycopene which is an antioxidant,despite being of many species, they all are almost of the same nutritional value [3]. Despite its importance, tomato fruits have been faced with diseases causing rots, which lead to loss of quality and also substantial postharvest loss [4]. During harvest, transportation as well storage, they are several pathogenic microorganisms that could cause damage and disrupt the structure of the fruit serving as a point of entry for different opportunistic microbes;this includes fungi, bacteria, as well as viruses. In Nigeria, tomatoes are usually grown in the Northern part of the country such as Niger, Jos, Jigawa, Benue, Katsina, Zamfara, Kano, Kebbi, Taraba, Yobe, Kaduna, Sokoto, Nasarawa, Gombe and Bauchi state [5]. Lack of storage facilities, poor marketing infrastructure, and inadequate processing havecaused the country always to experience a high level of post-harvest losses to infestation by pathogenic microorganism as well as low yield [5].*Ocimumgratissimum* contains many phytochemicals and contain a large quantity of flavonoid, tannins, glycosides, amides, steriodes, and alkaloids [6]. These phytochemicals have been reported to exhibit antibacterial activity. The study is conducted to determine the antibacterial activity of *Ocimumgratissimum* against some bacteria isolated from spoilt tomato.

II. MATERIALS AND METHODS

Collection of samples and preparations

Seventy-five (75) spoilt tomato samples were procured from table stands of tomato vendors from three different local Lafia

markets, namely: Kasuwan tomato, Modern Market, and Alamis market into sterile containers. Containers were correctly labeled and transported to the laboratory for analysis. The samples were washed with sterile water to reduce microbial load and kept free from dust and insets at room temperature. Sterile Scapula and forceps were used to cut spoilt portions on the tomato and then put in a beaker containing 10mL of sterile water in other to make a stock solution. Three beakers were used, one for each market [7]. Ten test tubes containing 9mLof sterile normal distilled water were placed in a test tube rack. 1mL from the prepared stock solution was pipetted aseptically into the first tube and mixed, 1mL was then transferred from the first tube to the 2nd tube, the second tube was mixed,then 1mLwas transferred to the next tube. It was repeated till the last.

Preparation of media

All media used in this research work were prepared according to the manufacturer's instructions. They were autoclaved at 121°C for 15minutes. After sterilization, the media were aseptically dispensed into sterile Petri dishes and allowed to solidify [8].

Culture

Approximately 0.1LM was transferred from the test tubes into an agar plate and then spread evenly with a glass spreader. The plates were incubated for 24hrs.

Isolation

Using sterile inoculating loop and a streak plate method, newly prepared sterile EMB, MSA, XLD, agar plates were used to subculture distinct bacteria colonies in other to obtain pure cultures. After sub-culturing, pure isolates of bacteria were collected and preserved using an agar slant in a bijou bottle. Samples were stored under 4°C for subsequent identification.Distinct colonies that developed after sub-culturing were examined for the morphology and cultural characteristics, including the margin, elevation, shape, color, and transparency.

Characterization and identification of bacteria isolates

Bacteria isolates were characterized and identified by the Gram staining method, microscopic examination, and Biochemical tests[9].

Preparation of plant materials

Ocimumgratissimum leaves were procured at Lafia modern market Nasarawa State and then transported to the Microbiology laboratory of the Federal University of Lafia. The leaves were washed using sterile water from the laboratory and air-dried at room temperature for 7 days and then crush using laboratory mortar into a fine powder. The crushed sample wasextracted using methanol and ethanol. 20g of the sample was dissolved in 200mL of ethanol and methanol for 48hrs. The dissolved sample was filtered using Whatman no 1 filter paper. The filtered sample was concentrated using a rotary evaporator and then resuspended by dissolving them in methanol and ethanol.

Antimicrobial activity

Agar well diffusion technique was adopted as described by Peter *et al*., [6]. Wells were made on Mueller Hinton agar plates using a sterile cork borer. 40µL of the extract was added to the wells. The plates were labeled correctly and incubated for 24hrs at 370C.

Minimum inhibitory concentration

Minimum inhibitory concentration was determined by the broth micro-dilution method in tryptone soya broth (TSB) using a 96 well microtitre plates as described by Peter *etal*., [6].

III. RESULTS

Table 1:Frequency (%) of bacterial isolates from spoilt tomato

Bacterial isolates	Frequency	Percentage frequency (%)
Escherichia coli	15	23.8
Salmonella spp	12	19.0
S. aureus	19	30.2
Proteus spp	9	14.3
Pseudomonas spp	8	12.7
Total	**63**	**100**

Table 2: Mean zone of inhibition (MZI) of bacterial isolates on methanol extract of *O.gratissimum*

	MZI (mm)						
Conc.	*E.coli*	*S.aureus*	*Salmonella*	*Proteus*	*Pseudomonas*	F-Value	P-Value
100mg/mL	13.5±0.7	21.5±0.7	00±0.0	19.5±2.1	18.5±2.1	64.70	<0.000
50mg/mL	11.5±0.7	18±0.7	00±0.0	15±1.4	12±1.4	84.25	<0.000
25mg/mL	10±4.2	14.5±0.7	00±0.0	12±1.4	9.5±0.7	14.44	<0.006
N.C	00±0.0	00±0.0	00±0.0	00±0.0	00±0.0		

***P*<0.05,** Reference control, Gentamycin (31mm), Ciprofloxacin (36mm), N.C = Negative control (methanol)

Table 3: Mean zone of inhibition (MZI) of bacterial isolates on an ethanol extract of *O.gratissimum*

MZI (mm)							
Conc.	*E.coli*	*S.aureus*	*Salmonella*	*Proteus*	*Pseudomonas*	F-Value	P-Value
100mg/mL	17.5±2.1	23±2.8	10±1.4	20±1.4	19±1.4	12.73	<0.008
50mg/mL	13±1.4	19.5±0.7	7±1.4	16±1.4	14.5±0.7	30.17	<0.001
25mg/mL	12±1.4	17±1.4	2.5±0.7	14.5±0.7	12.5±0.7	55.13	<0.000
N.C	00±0.0	00±0.0	00±0.0	00±0.0	00±0.0	-	-

P<0.05**,** Reference control, Gentamycin (31mm), Ciprofloxacin (36mm), N.C = Negative control (ethanol).

Table 4: Minimum inhibitory concentration (MIC) of bacterial isolates on ethanol and ethanol extract of *O.gratissimum*

MIC (mg/mL)		
Organisms	Ethanol extract	Methanol extract
Escherichia coli	0.39	0.78
Staphylococcus aureus	0.19	0.39
Salmonella spp	12.5	50
Proteus spp	0.78	3.12
Pseudomonas spp	0.39	3.12

IV. DISCUSSION

Tomato is a succulent fruit with high nutritional value. The nutrient content supports the growth of microorganisms, such as bacteria and fungi. These organisms can produce enzymes that degrades the fruits in the process utilizing the nutrients. Thus, given risingto the deterioration and spoilage of these fruits. This has become a severeproblem because tonnes of the fruits are lost per annum to the proliferation and activities of these microorganisms

Tomato fruit possesses a uniquebarrier that acts effectively against most plant spoilage microorganisms. However, thenatural barrier can be removed and the tomato fruits may become contaminated during their growing in fields or during harvesting, post-harvest handling and distribution [10].

The bacterial isolates were *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* spp, *Proteus* spp, and *Pseudomonas* spp. The presence of *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas* spp in this study is in line with the findings reported by Jushi*et al.*, [11]. Percentage occurrence of bacterial isolates of spoilt tomato presented in table **1**. Sixty-three (63) bacteria were isolated from 75 spoilt tomatoes collected. *Staphylococcus aureus* was the dominant organism with a frequency occurrence of 19(30.2%), *Escherichia coli* 15(23.8%), *Salmonella*spp 12(19%), *Proteus*spp 9(14.3%), and the least occurrence was *Pseudomonas* spp 8(12.7%). This finding also is in line with the work of Pandukur*et al*., [12], who also isolated *Salmonella*spp, *Pseudomonas* spp, *Staphylococcus aureus*, *Proteus* spp.The result of the mean zone of inhibition of bacterial isolates from spoilt tomato on methanol extract of *Ocimumgratissimum*as presented in table **2** showed that the extract was active against the test organisms from 100mg/mL to 25mg/mL.*S. aureus* was more susceptible to the extract with the highest zone of inhibition of 21.5±0.7mm in 100mg/mL, *proteus*spp 19.5±2.1mm, *Pseudomonas* 18.5±2.1mm in 100mg/mL and no activity was recorded in *Salmonella spp* 00±0.0mm.

The result of the mean zone of inhibition of bacterial isolates from spoilt tomato on an ethanol extract of *Ocimumgratissimum* as presented in table **3** showed that the extract was active against the test organisms from 100mg/mL to 25mg/ml.*S. aureus* was more susceptible to the extract with the highest zone of inhibition of 23±2.8mm in 100mg/mL, *proteus*spp 20±1.4mm, *Pseudomonas*spp19±1.4mm in 100mg/mL and the least activity was recorded in *Salmonella* spp 2.5±0.7mm. The was a significant difference among the various concentrations of the extract at *P*<0.05 against the test organisms.

Result of a minimum inhibitory concentration of bacterial isolates on ethanol and methanol extract of *Ocimumgratissimum* as presented in table **4** showed that ethanol extract of *Ocimumgratissimum*has an inhibitory activity of 0.39mg/ml for *E. coli*, *S. aureus* 0.19mg/mL, *Salmonella*spp 12.5mg/mL, *Proteus* spp 0.75mg/mL, *Pseudomonas*spp 0.39mg/ml. While methanol extract of *Ocimumgratissimum* has an inhibitory activity of 0.78mg/mL for *E. coli*, *S. aureus* 0.39mg/mL, no activity found in *Salmonella*spp, *Proteus*spp 3.12mg/ml, and *Pseudomonas* spp 3.12mg/mL.The result from the study showed that tomato fruit samplesfrom the Modern market recorded the highest occurrence of bacteria, while the samples from Alamis market recorded the lowest occurrence of bacteria. The bacterial recorded indicated a highlevel of contamination of the tomato fruit samples fromthe Modern market. The isolation of soil-dwelling bacteria such as *Pseudomonas* spp, from the tomato

fruit, was evidence of opportunistic contamination from human activity. Also, the presence of *Escherichia coli*, often associated with fecal matter, indicates that the samples may have been contaminated through poor human handling processes. However, the occurrence of this bacteria in the spoilt tomato fruit samples investigated wassimilar to the report by [13]. The presence of bacterial isolates in the spoilage of tomato fruits is a source of potential health hazard to man. This is due to their production of toxins thatare capable of inducing endotoxin in man following ingestion. However, these toxins differ in their manner and degree of toxicity.

V. CONCLUSION

Several genera of bacteria have been identified in this study as being associated with the spoilage of tomato fruits. Therefore, intensive efforts should be made by the appropriate health workers to discourage or stop the display and sales of spoilt tomato fruits in local markets. The general public should also be sensitized on the health risks that may be associated with the consumption of spoilt tomatoes, although they could be relatively cheaper. These microorganisms could be agents in foodborne bacterial diseases.

VI. RECOMMENDATIONS

Consumption of spoilt tomato fruits among members of the public should be discouraged.

Members of the public should avoid handling spoilt tomato fruits as this could expose them to harmful bacteria. Members of the public who insists on consuming spoilt tomato fruits should endeavor to boil them properly in other to kill the bacteria that could be present in such tomato fruits.

CONFLICT OF INTEREST

There was no conflict of interest

REFERENCES

[1]. Abdel-Aziz, H., Windeck T., Ploch, M., and Verspohl, E. J. (2006). Mode of action of gingerols and shogaols on 5-HT3 receptors: binding studies, cation uptake by the receptor channel and contraction of isolated guinea-pig ileum. *European Journal of Pharmacology*.**530**:136–143.

[2]. Adesegun, E.A., Ajayi, E.O., Adebayo, O.S., Akintokun A.K., Enikumehin O.A. (2012) Effects of *Ocimumgratissimum* and *Aframomummelegueta* (K.schum) Extracts on the Growth of *Sclerotium rolfsii*(Sacc). *International Journal of Plant Pathology*. **3**:74-81

[3]. F.A.O. (2010). Fruits and Vegetables: An Overview on Socio economical and Technical issues. www.fao.org/docrep/005/y4358E/Y435 04.htmF

[4]. Uzeh, R.E., Alade, F.A., Bankole, M. (2009). The microbial quality of pre-packed mixed vegetable salad in some retail outlets in Lagos, Nigeria. *African Journal of Food Science*. **3**:270-272

[5]. Adaskaveg, J. E., Forste, H., and Sommer, N. F. (2000). Principles of post-harvest pathology and management of decays of edible horticultural crops. *In: Post-harvest technology of horticultural crops*.**331**: 163-195

[6]. Peter, U.U., Emmanuel, M.I., Felix, A. Lawrence, B.E., Bashiru, S. Mfonido, U. Inyang, I.H., Fatimah, A. Gladys, A.A. (2020). Antibacterial Activity of *Ocimumgratissimum* Against Drug Resistant Bacteria Isolated from Drinking Water Quality in Calabar, Cross River State. *International Journal of Innovative Studies in Sciences and Engineering Technology***. 6**:20-25.

[7]. Block, E. (2010). Garlic and other Alliums: The Lore and the Science. *The Royal Society of Chemistry*, **13**:200-210

[8]. Bhuiyan, M. K., Shovan, L. R., Begum, J. A. and Pervez, Z. A. (2008). *In vitro* control of *Colletotrichum dematium*causing anthracnose of soybean by fungicides, plant extracts and *Trichoderma harzianum. International Journal of Sustainable Crop Production*, **3**:10-17.

[9]. Cheesbrough, M.C. (2006). Different identification methods for fungi and bacteria. *District laboratory practice*.**6**:128-195.

[10]. Al-Hindi, R.R., Al-Najada, A.R., Mohamed S.A. (2011). Isolation and identification of some fruit spoilage fungi: screening of plant cell wall degrading enzymes. *African Journal of Microbial Research*. **5**:443-448.

[11]. Jushi, A.P., Pate,l S.P. (2008). Microbiological analysis of fresh fruits and vegetables and effects of antimicrobial agent on microbial load. *Journal of Food Protection*. **14**:23-24

[12]. Pandukur, S.G., Onyimba, I.A., Itelima, J.U., Plangnan, G.A., Duru, V. (2017). Antibacterial potential of orange peels extracts against some bacterial isolates associated with tomato in Jos metropolis. *Nigeria. Nigerian Journal of Microbiology*. **31**:3953-3961

[13]. Ghosh, A. (2009). Identification of microorganismsresponsible for spoilage of tomato (*Lycopersicumesculentum*) fruit. *Journal of Phytology*. **1**:414-416