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Full Length Research Article

ISOLATION AND IDENTIFICATION OF BACTERIA ASSOCIATED WITH SURFACE CONTAMINATION OF COLANUT TRADED BY VENDORS WITHIN KADUNA NORTH LOCAL GOVERNMENT AREA, KADUNA STATE, NIGERIA

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ABSTRACT
Garcinia kola (Colanut) is a well-known indigenous plant with several pharmacological activities that is widely consumed in most Northern parts of Nigeria. It is traditionally believed to serve as a remedy in the management of diarrhoeagenic bacterial infections. This study investigated the prevalence of pathogenic bacteria on the surface of colanuts sold within Kaduna North Local Government Area, Kaduna State. A total of sixty-four (64) bacteria were isolated from the samples, and were identified based on their cultural, Gram reaction, and biochemical characterizations. The pathogens were tested against selected antibiotics using Agar well diffusion method. The isolates were confirmed to be *Salmonella* specie (46%), *Escherichia coli* (32%) and *Staphylococcus aureus* (22%). People who consume colanut without proper washing are at high risk of contracting infections caused by the aforementioned pathogens.

Keywords: Garcinia cola, bacteria, antibiotics, environmental factors, and vendors.

INTRODUCTION

Garcinia kola is one such traditional medicinal plant that is an evergreen plant found in the equatorial forest of Sub-Saharan Africa where it grows wild and can also be domesticated due to its numerous medicinal values (Oze *et al.,* 2010). The plant is also known as "bitter kola" because of its seed's bitter taste or "male kola" because of its claimed aphrodisiac activity (Uko *et al.,* 2021). Garcinia kola seeds form a major part of the herbal preparation used for the treatment of various respiratory tract diseases including asthma (Okojie *et al.,* 2019). Kola nuts (Cola sp) are widely cultivated in West Africa because they serve as natural stimulants that suppress fatigue (Agbelade and Akindele, 2013). The tree is widely cultivated in Northern, Eastern, and Western parts of Nigeria.

MATERIALS AND METHODS

Study Area

The study area was Kaduna North Local Government Area of Kaduna State, Nigeria. The Local Government is known to be the metropolitan city of state otherwise known as Kaduna Capital Territory which is the economic and financial capital hub of Kaduna state with an estimated population of (677,714) and a land area of 185.2 km^2 and 158.2km (https:worldpopulationreview.com, 2022).

Sample Collection

Four (4) Kola nuts selling points were designated within the study areas which are believed to be the major consumers of kola nuts in the Local Government through pilot survey; they include the areas of (A)Badarawa , (B)Ung/Rimi , (C)Ung/Dosa , and(D) Kawo respectively. A total of one hundred and fifty (150) kola nuts samples were randomly collected from the aforementioned locations through aseptic method and was transferred into sterile containers and transported to Postgraduate Laboratory of the department of Microbiology, Faculty of Science, Kaduna State University-Kaduna, Nigeria for analysis. The samples were stored under airtight ice packs and analyzed microbiologically within 4 hours of collection.

Isolation of Bacterial from surface of colanut

Sample preparation and isolation of bacteria was done according to the method described by Afolami *et al.* (2018). Sterile normal saline was used as diluent and 1.0g of sample was weighed and transferred into 10.0mL of the diluent which is a sterile distilled water and mixed thoroughly to give a stock solution for a serial dilution process several dilutions were achieved up to 5 fold (10) and forth fold dilutions were obtained for a pour plate technique. Thereafter, 1ml of the forth dilution was used to inoculate already prepared sterile plate of nutrient agar. The inoculated bacteria plates were incubated at 37^{o}C for 24 hours.

Identification of Bacterial Isolates

Identification of bacterial isolates was done using methods of Afolami *et al.* (2018) and Aribisala *et al.* (2018). Subcultures of distinct colonies were identified by gram reaction and biochemical tests such as catalase, motility, sugar fermentation (glucose, sucrose, lactose and mannitol), triple sugar iron, methyl red/voges proskauer test, oxidase and spore staining tests. The identified isolates were freshly sub cultured on MacConkey, Salmonella Shigella agar (SSA) and Eosin Methylene Blue (EMB) agar plates respectively, and incubated at 37°C for 24 hours.

Antibiotic Susceptibility of Bacterial Isolates

The Kirby Bauer disk diffusion test was used to evaluate invitro activity of selected antibiotics against the bacterial isolates on Mueller Hinton (MH) agar. An 18h old broth culture of each isolate was standardized with 0.5 McFarland standards and plated on MH agar using sterile swabs as previously described by Onifade and Afolami O, (2018). The antibiotic disks were gently placed on the plates and incubated for 24 hours at 37^{o}C. The diameter of zone of inhibition of each isolate was measured with a calibrated ruler.

RESULTS

Four (4) different locations in the study area were mapped out for sampling. Viz: Kawo, Badarawa, Ung/ Rimi and Dosa respectively. A total of hundred and fifty (150) samples of kola nuts were randomly sampled from different vendors within the study area. Samples from Kawo recorded the highest percentage prevalence of bacteria isolates (35.94%), followed by Badarawa 28.13%), and Ung/Rimi (18.75%). However, samples from Ung/Dosa recorded the least numbers of isolates (17.19%). Similarly, percentage occurrence of bacteria isolated were identified. *Salmonella* specie was the highest (45.30%), followed by *Escherichia coli* (32.81%), and while the least recorded bacteria was *Staphylococcus aureus* (22%). The antibacterial susceptibility pattern indicated that the organisms were highly susceptible to Ciprofloxacin with the zone of inhibition ranging from: *Escherichia coli* (24 mm), *Salmonella* specie (22 mm) and *Staphylococcus aureus* (19 mm) respectively.

Table 1: Prevalence of bacterial Isolates within the Sampling Locations

Location	No of sample	No of positive Isolates	Percentage (%)
A	50	12	18.75
B	50	11	17.19
C	50	18	28.13
D	50	23	35.94
Total	200	64	100

Key:
A= Ung/Rimi
B= Ung/Dosa
C= Badarawa
D=Kawo

Table 2: Percentage Occurrence of Bacterial Isolates

Organism	No. of Isolates	Percentage (%)
Salmonella specie	29	45.30
Escherichia coli	21	32.81
Staphylococcus aureus	14	22
Total	**64**	**100**

Table 3: Antibiotic Susceptibility Pattern of the Isolates

Antibiotic (µg)	Zone of Inhibition (mm)		
	E. coli	*Salmonella* sp.	*S. aureus*
SXT (30)	11	14	10
CH (30)	13	11	15
SP (10)	15	14	13
CPX (30)	24	22	19
AM (30)	13	12	12.5
AU (10)	9	8	8.5
CN (30)	12	10	14
PEF (30)	12	11.5	11
OFX (10)	10.5	11	12
S (30)	13	11.5	12
E (10)	8	8.5	9
Z (20)	11.5	10	11
R (25)	14	13	12.5
APX (20)	12	11.5	12.5

DISCUSSION

Bacterial colonization on food samples is the first line of disease establishment. This becomes possible especially, when these foods are hawked in open air thereby accelerating the rate of contamination via aerosols if not properly covered. On the other hand, lack of personal hygiene practices by these food vendors could serves as risk factors which could lead to cross contamination of such food sample. In this present study, it was discovered that high percentages of bacterial isolates were obtained on the surfaces of kola nuts sold by vendors in the study area. *E. coli*, *Staphylococcus aureus*, have been found to be pathogenic, hence causing diseases associated by the organisms such as Salmonellosis and diarrhea among others (Jury *et al.,* 2010). In a similar study by Sonibare (2009) it was reported that local residents from Ipogun community, Akure, Western Nigeria have described clinical symptoms suggestive of acute typhoid fever and infantile food poisoning in children, attributed to *Salmonella* sp, *S. aureus* and *S. pyogenes*. Antibiotics are defined as either naturally occurring, semi-synthetic or chemically synthesized antimicrobial compounds used mainly in the treatment and prevention of diseases in both humans and animals and also as growth promoters in animal intensive industries (Jury *et al.,* 2010). The therapeutic use of an antibiotic, in either human or animal population, creates a selective pressure that favors survival of bacterial strains resistant to the antibiotic. The result is that many bacteria strains to which the antibiotic is used against become resistant to it, rendering the antibiotic ineffective as treatment of choice against that respective bacterial strain (Sonibare, 2009). Some of the mechanisms of resistance include: alteration of

permeability barriers across bacterial outer membranes, prevention of antibiotic uptake through inhibiting its corresponding transport carrier, modification of the antibiotic' s target binding sites to prevent recognition of the antibiotic, and the ability by the bacteria to chemically and/or enzymatically degrade the antibiotic (Jury *et al.,* 2010). The antibiotic susceptibility pattern of the isolates in this study revealed that the organisms were mostly resistant to the tested antibiotics, the findings in this study is similar to that of Afolami *et al.* (2020) who stated that, the susceptibility patterns of the bacteria isolates observed in their study might have resulted from exposure of termite nests on kola nut barks to active components in many herbicides used by local farmers, which may be similar to the compounds present in the antibiotics used in their study, and as such, may trigger a form of acquired antibiotic resistance in the isolates. Similar observations have been made by Brune and Dietrich (2014), Afolami *et al.* (2018) and Aribisala *et al.* (2018).

Conclusion

This study has shown that there is high prevalence of bacterial contamination on the surfaces of kola nuts with respect to the study area. In most cases, from personal interview, it is gathered that consumers of kola nuts don't wash the nuts before consuming, thereby ending up ingesting large bacterial load it is therefore important to create awareness on safe handling of kola nuts as to minimize the rate of bacterial contamination found on the sample in order to avert health risk and possible disease outbreak.

REFERENCES

Afolami, O., Aribisala, J., Oladunmoye, M., Wasiu, O. and Arogundade, I. (2018). Characterization, antibiotic sensitivity patterns, plasmid profile analysis and antagonistic potentials of microorganisms from termitaria on mango trees in Ibule-Soro, Akure, Nigeria. *Acta Sci Microbiol 1*(3): 2-7.

Agbelade, A., and Akindele, S. (2013). Land Use Mapping and Tree species diversity of Federal University of Technology Akure. *Am Int J Contemporary Res*. 3(2): 104-113.

Akingbade, O., Balogun, S., Ojo, D., Akinduti, P. Okerentugba, O., Nwanze, C., and Okonko, I. (2014). Resistant plasmid profile analysis of multidrug resistant Escherichia coli isolated from urinary tract infections 14(4): 821-826

Aribisala, J., Oladunmoye, M., Afolami, O. and Aina, T. (2018). Identification, antagonistic potentials and plasmid profiling of microorganisms associated with termitarium from cocoa trees in Ibule-soro, Akure, Nigeria, *Acta Science Microbiol.* 1(3): 8- 12

Brune, A. (2014). Symbiotic digestion of lignocellulose in termite guts. *Nat Rev Microbiol*. 12 168- 180

Jury, K.L., Vancov, T., Stuetz, R.M. and Khan, S.J. (2010). Antibiotic Resistance Dissemination and Sewage Treatment Plants. Current research, technology and education topics in applied Microbiology and Microbial Biotechnology, A. Mendez-Vilas (Ed.), pp. 509-519.

Okojie, A.K., Ebomoyi, M.I., Ekhator, C.N., Emeri, C.O., Okosun, J., Onyesu, G., Uhuonrenren, O. and Atima, J. (2019). Review of physiological mechanisms underlying the use of Garcinia kola in the treatment of Asthma. *Intern. Journal Pulmon. Med* 11(1).

Onifade, A. and Afolami, O. (2018). Antibiotic resistance patterns of Salmonella sp from clinical and water sources in Akure, Ondo State, *Nigeria. Asian J Res Med Pharm Sci*. 5(1): 1-10.

Oze, G., Okoro, I., Obi, A. and Nwoha, P. (2010). Hepatoprotective role of Garcinia kola (Heckel) nut extract on methamphetamine: induced neurotoxicity in mice. *Afr. J. Biochem. Res.* 4(3): 81-87.

Sonibare, A., Soladoye, M., Oyedokun, E., and Oluwadayo, S. (2009). Phytochemical and Antimicrobial Studies of Four Species of Cola Schott and Endl. (Sterculiaceae). *Afr J. Trad Complementary Altern Med* 6(4): 518-525

Uko, O.J., Usman, A. and Ataja, A.M. (2011). Some biological activities of Garcinia kola in growing rats. *Vet. Arhiv*. 71(5): 287-297