



Received on 30 December 2020; received in revised form, 20 February 2021; accepted, 27 February 2021; published 28 February 2021

## ANTIBACTERIAL EFFECTS OF THE SEED EXTRACTS OF ALLIGATOR PEPPER (*AFRAMOMUM DANIELLI* ROXB.) ON *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*

J. N. Uche<sup>1</sup> and K. H. Ogbonda<sup>\* 2</sup>

Department of Microbiology<sup>1</sup>, Federal University, Otuoke, Bayelsa State, Nigeria.

Department of Biology Microbiology Unit<sup>2</sup>, Ignatius Ajuru University of Education, Rumuolumeni, Port Harcourt, Nigeria.

### Keywords:

Alligator pepper, Antibacterial, Seed extract, *Salmonella typhimurium*, *Escherichia coli*.

### Correspondence to Author:

**K. H. Ogbonda**

Department of Biology Microbiology Unit, Ignatius Ajuru University of Education, Rumuolumeni, Port Harcourt, Nigeria.

**E-mail:** kemkahumphrey.ogbonda@iaue.edu.ng

**ABSTRACT:** The study investigated the antibacterial properties of the water and ethanolic extracts of the seed of alligator pepper on two bacterial species, *Salmonella typhimurium* and *Escherichia coli*, which are known human pathogens. The phytochemicals (alkaloids, flavonoids, saponins, tannins, glycosides, and phytates) in the seed extracts were estimated. The organisms were obtained from the microbiology laboratory of Federal Medical Centre (FMC), Yenagoa, Bayelsa State. The antibacterial activity of the different concentrations of the extract (5 mgml<sup>-1</sup>, 10 mgml<sup>-1</sup>, 20 mgml<sup>-1</sup>, 30 mgml<sup>-1</sup> and 50 mgml<sup>-1</sup>) were evaluated against the test organisms. Also, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined. Results showed that both the water and ethanolic extracts of the seed of the plant had inhibitory effects on the test organisms. Statistical analysis (ANOVA) demonstrated no significant difference (P=0.05) in the inhibitory effects of the extracts and the control on the organisms. It is concluded that both the water and ethanolic extracts of the seed have antibacterial properties and could be used in the treatment of common ailments and infections caused by the organisms.

**INTRODUCTION:** Medicinal plants are the richest bio-resource for drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates, and chemical entities for synthetic drugs<sup>1</sup>. A large number of plants have been reported to possess medicinal properties and are utilized extensively by various tribes and peoples of the world in the treatment of ailments.

The World Health Organization (WHO) reports that traditional medicinal substances from plant extracts have continued to provide health coverage for over 80% of the World's population, especially in the developing parts of the world<sup>2</sup>. For some time now, therefore, plant-derived substances have become of great interest owing to their versatile applications, particularly as sources of therapeutic agents.

The most important bioactive constituents of plants are secondary metabolites, which include alkaloids, flavonoids, tannins, saponins, phenolic compounds, steroids, glycosides, and terpenoids. Most are toxic to microbial cells and have been used to treat several human ailments, including those caused by



microorganisms. An antibacterial agent is a substance that kills or inhibits the growth of bacteria. Anti-bacterials that kill bacteria are said to exhibit bactericidal action, while those that merely inhibit their growth or make them static are bacteriostatic. Anti-bacterials are used in the treatment of bacterial infections and can be obtained from either natural or synthetic sources.

Those from synthetic sources include sulphonamides, quinones, and oxazolidinones<sup>3</sup>; due to the recurring resistance of pathogenic microorganisms to common antibacterial, investigations of other sources of antibacterial, particularly the plants, is gaining currency. Phytochemicals frequently act through different mechanisms than conventional antibacterials and could, therefore, be of use in the treatment of resistant bacteria<sup>4</sup>. Antibacterials act by inhibition of cell-wall synthesis, inhibition of nucleic acid synthesis, inhibition of protein synthesis, disruption of the cell membrane, or inhibition of metabolic activity<sup>5</sup>. Alligator pepper plant (*Aframomun danielli*), also known as mbongo spice or hepper pepper, is a tropical herbaceous perennial plant of the family Zingiberaceae (ginger family) of the angiosperms in the kingdom Plantae.

The seeds have a pungent peppery taste due to aromatic ketones. It is a plant that has both medicinal and nutritive values, widespread across tropical Africa, including Nigeria, Liberia, Sierra Leone, Ghana, Cameroun, Cote D' Ivoire, and Togo. The seeds of the plant possess active ingredients that can be exploited for local development of antimicrobials<sup>7</sup>. Extracts of the seed have been reported, 8 to have highly potent antiseptic and bactericidal properties and have, therefore, been used in the treatment of wounds and in the prevention of infections<sup>9</sup>. Studied the antimicrobial effects of the seed of the plant on five pathogenic bacteria, including *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium* and *Klebsiella pneumoniae*.

The study showed that the seed extract was inhibitory to the growth of *Klebsiella pneumoniae* and *Salmonella typhimurium*. He, therefore, concluded that the extracts of the seed contain phytochemicals which offer enormous potential as

a bio-control agent for the organisms and antimicrobials of therapeutic importance. This study was undertaken to investigate the antibacterial effect of the extracts of the seed of alligator pepper on *Escherichia coli* and *Salmonella typhimurium*.

## MATERIALS AND METHODS:

**Sample Collection:** Dried seeds of *Aframomum danielli* were obtained from a well-known dealer in the Yenagoa market, Bayelsa State.

**Qualitative Analysis of Phytochemicals:** Preparation of Ethanolic and Aqueous (Water) Extracts of the Seed Standard methods<sup>10</sup> were employed in the preparation of the ethanolic and aqueous extracts.

**Estimation of Phytochemicals:** Alkaloids, flavonoids, saponins, tannins, glycosides, and phytates were estimated using standard methods<sup>11</sup>.

## Evaluation of Antibacterial Activities of The Seed Extracts:

**Source of Test Organisms:** The *Salmonella typhimurium* and *Escherichia coli* used in the study were obtained from the Microbiology Laboratory of Federal Medical Centre (FMC), Yenagoa, Bayelsa State. The bacteria were subcultured and subsequently confirmed by gram-staining and other appropriate biochemical tests and then maintained on slopes of tryptone soya agar in a refrigerator at 2-80 °C.

**Preparation of the Different Concentrations of the Extracts:** The different concentrations of each of ethanolic and water extracts of the seed powder (5 mgml<sup>-1</sup>, 10 mgml<sup>-1</sup>, 20 mgml<sup>-1</sup>, 30 mgml<sup>-1</sup> and 50 mgml<sup>-1</sup>) were based on standard methods<sup>12, 13</sup> and<sup>14, 15</sup> and<sup>16</sup>.

**Preparation of Standard Bacterial Suspension (Turbidity Standard):** Loopful of each of the bacteria was inoculated into their respective culture media (*Salmonella typhimurium* in Salmonella / *Shigella mediam*; *E. coli* in Eosin methylene blue agar) and incubated at 37 °C for 24 h<sup>17</sup>.

Colonies were picked from the pure cultures of *Salmonella typhimurium* and *E. coli* using a sterile loop and transferred into test tubes containing 5 mls of normal saline. Each was properly mixed, the

absorbance and transmittance measured on a spectrophotometer, and then adjusted to that of a pre-prepared McFarland turbidity standard by adding more inoculum or normal saline. The resulting suspension contained  $1 \times 10^7$  colony forming units (cfu) per milliliter. This served as the 0.5 McFarland standard that was used as turbidity standard for the test organisms.

**Evaluation of the Antibacterial Activity:** The disc diffusion susceptibility method 18 was used. The bacterial inoculum of approximately  $1 \times 10^7$  cfu/ml was seeded on the surface of a large (150 mm diameter) Mueller-Hinton agar plate. The discs containing different concentrations (5  $\text{mgml}^{-1}$ , 10  $\text{mgml}^{-1}$ , 20  $\text{mgml}^{-1}$ , 30  $\text{mgml}^{-1}$ , 50  $\text{mgml}^{-1}$ ) of the ethanolic and aqueous extracts of the seed were placed on the correspondingly labelled parts of the inoculated agar surface.

The plates were incubated for 24 h at 35 °C prior to the determination of results. The zones of growth inhibition around each of the extract discs were measured to the nearest millimeter. The zones of inhibition of the positive control (gentamicin) at 5  $\text{mg/ml}^{-1}$  on the clinical isolates (*Salmonella typhimurium* and *Escherichia coli*) were also determined.

**Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC):** In this test, the growth or otherwise of the test organisms (*Salmonella typhimurium* and *Escherichia coli*) was investigated in a liquid medium (*Salmonella typhimurium* in Salmonella/Shigella medium; *Escherichia coli* in Eosin methylene blue) with decreasing concentrations of the seed extracts and incubated at 37 °C for 24 h<sup>17</sup>.

The minimum (lowest) concentration of the extract that inhibited the bacteria, that is, the minimal inhibitory concentration (MIC), was determined by noting the lowest concentration of the extract that inhibited the bacteria. To determine the minimal bactericidal concentration (MBC), the bacteria were each exposed to the different concentrations of the extracts and after a period of 48 h, they were sub cultured in an extract-free nutrient medium (broth) to test their capability for growth and /or reproduction. The minimum (lowest) concentration of the extract that killed the bacteria was noted.

**RESULTS AND DISCUSSION:** The inhibition zone diameter (IZD) of the ethanolic and water extracts of the seed of alligator pepper are presented in **Tables 1 & 2**. Both *Salmonella typhimurium* and *Escherichia coli* were inhibited by the ethanolic extract, *Salmonella typhimurium* being inhibited more than *Escherichia coli*. Inhibition increased as the concentration of the extract increased, with the highest inhibition occurring at the 50  $\text{mgml}^{-1}$  concentration.

The water extract had almost equal inhibition on both *Salmonella typhimurium* and *Escherichia coli* as there was no much difference in the inhibition zone diameters. As in the ethanolic extract, inhibition increased as the concentration of the extract increased, with the highest inhibition also occurring at the 50  $\text{mgml}^{-1}$  concentration. The result of the MIC and MBC test showed that for the ethanolic extract of the seed both organisms, *Salmonella typhimurium* and *Escherichia coli*, had a MIC and MBC of 20  $\text{mgml}^{-1}$  and 30  $\text{mgml}^{-1}$ , respectively. The MIC and MBC of the water extract of the organisms were 10  $\text{mgml}^{-1}$  and 20  $\text{mgml}^{-1}$ , respectively; they were also the same for both organisms.

Statistical analysis (ANOVA) showed no significant difference ( $P = 0.05$ ) in the effects of the treatments, *i.e.*, the extracts and the control in both the ethanolic and the water extracts of the seed. The findings in this study show that the extract of the seed of alligator pepper has antibacterial activity on the test organisms. The finding is in agreement with the works of the authors who worked in the antimicrobial activity of extracts of alligator pepper. In their report, they observed that the water or ethanolic extract of the seed inhibited *Salmonella* species and *Escherichia coli*<sup>19,7,9</sup>. Also had it that phytochemicals, including flavonoids, had biological functions, which include protection against microbes<sup>8</sup>. The phytochemicals in this study, therefore, may be responsible for the inhibitory effect of the seed extracts. This also might be the reason behind the use of the seeds of alligator pepper in the treatment of sore throat and other ailments in certain localities. For instance,<sup>8</sup> stated that the extract of the seed of the plant is used in the treatment of intestinal troubles in herbal medicine, as they have antiseptic and bacterial properties.

**TABLE 1: INHIBITION ZONE DIAMETER OF THE ETHANOLIC EXTRACT OF THE SEED (MM)**

	Test Organism		Concentration of Extract (Mgml <sup>-1</sup> )		
	5	10	20	30	50
<i>Salmonella typhimurium</i>	10.0	11.0	14.0	17.0	18.0
<i>Escherichia coli</i>	0.0	0.0	10.0	12.0	23.0
Total	10.0	11.0	24.0	29.0	41.0
Mean	5.0	5.5	12.0	14.5	20.5
	5	10	20	30	50
	10.0	11.0	14.0	17.0	18.0
	0.0	0.0	10.0	12.0	23.0

**TABLE 2: INHIBITION ZONE DIAMETER (IZD) OF THE WATER EXTRACT OF THE SEED (MM)**

	Test organism		Concentration of Extract (Mgml <sup>-1</sup> )		
	5	10	20	30	50
<i>Salmonella typhimurium</i>	8.0	9.0	11.0	11.0	13.0
<i>Escherichia coli</i>	8.0	9.0	11.0	11.0	12.0
Total	16.0	18.0	22.0	22.0	25.0
Mean	8.0	9.0	11.0	11.0	12.5
	5	10	20	30	50
	8.0	9.0	11.0	11.0	13.0
	8.0	9.0	11.0	11.0	12.0

**CONCLUSION:** The study has demonstrated that the water and ethanolic extracts of alligator pepper inhibited the growth of the test organisms, *Salmonella typhimurium*, and *Escherichia coli*. It is suggested, therefore, that the extract of the seed of this plant be used in the treatment of certain ailments and infections caused by these bacteria, as is the practice in some localities.

**ACKNOWLEDGEMENT:** Nil

**CONFLICTS OF INTEREST:** Nil

## REFERENCES:

1. Ncube NS, Afolayan AJ and Okoh AI: Assessment techniques of antimicrobial properties of natural compounds of plant origin current methods and future trends. African Journal of Biotechnology 2008; 7(12): 1792-06.
2. World Health Organization. Traditional medicine: growing needs and potentials. WHO Policy Perspective on Medicine. World Health Organization, Geneva. 2002; Pp 1-6.
3. Singh SB and Barrett JF: Empirical antibacterial drug discovery foundation in natural products. Biochemical Pharmacology 2006; 71(7): 1006-15.
4. Abreu AC, Mcbain AJ and Simoes M: Plants as sources of new antimicrobials and resistance-modifying agents. Natural Product Report 2002; 29: 1007-21.
5. Prescott LM, Harley JP and Klein OA: Microbiology 6th ed. McGraw Hill, New York 2005.
6. Galal AM: Antimicrobial activity of 6-paradol and related compounds. International Journal of Pharmacognosy 1996; 31: 37-43.
7. Oyagade JO, Awotoye OO, Adewunmi JT and Thorpe HT: Antimicrobial activities of some Nigerian medicinal plants, screening for antibacterial activity. Journal of Bioscience Research Communication 1999; 11: 193-97.
8. Okwu DE: Phytochemical and vitamin contents of indigenous spices of south eastern Nigeria. Journal of Sustainability and Agricultural Environment 2004; 6: 30-34.
9. Oladunmoye MK and Dada EO: Comparative studies on the antimicrobial activity of leaf extracts from *Aframomum melegueta*. Research Jou of Botany 2007; 2(2): 95-107.
10. AOAC: Official Methods of Analysis of the Association of Chemists. Analysis of the Association of Chemists, Washington DC pp 1990; 223-25, 992-95.
11. Babu HK and Savithramma N: Phytochemical screening of underutilised species of Poaceae. International Journal 2013; 1(10): 947-951.
12. Okigbo RN and Omodamiro OD: Antibacterial effects of leaf extracts of pigeon pea (*Cajanus cajan* (L) Mill sp.) on some human pathogens. Journal of Herbs Spices and Medicinal Plants 2006; 12: 117-27.
13. Karadi RV, Shah A, Parekh P and Azmi P: Antimicrobial activities of *Musa paradisiaca* and *Cocos nucifera*. International Journal of Research in pharmaceutical and biomedical sciences 2011; 2: 264-67.
14. Okorondu SI, Sokari TG, Akujobi CO and Braide W: Phytochemical and antibacterial properties of *Musa paradisiaca* stack plant. International Journal of Biological science 2010; 2: 128-32.
15. Karuppiyah P and Mustaffa M: Antibacterial and antioxidant activities of *Musa* sp. Leaf extracts against multidrug resistant clinical pathogens causing nosocomial infection. Asian Pacific Journal of Tropical Biomedicine 2013; 3: 737-42.
16. Ememobong GA and Chinweizu EU: Antibacterial and toxicity studies of the ethanolic extract of *Musa paradisiaca* leaf. Cogent biology 2016; 2(1).
17. Buris KP, Davidson PM, Stewart Jr CN and Harte FM: Antimicrobial activity of Yerba mate (*Ilex paraguariensis*) aqueous extracts against *Escherichia coli* 0157:H7 and *Staphylococcus aureus*. Journal of Food Science 2011; 76(6): M456-M62.

18. Jorgensen JH and Turnidge JD: Antibacterial susceptibility tests: dilution and disk diffusion methods. In: Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA editors. Manual of clinical microbiology. 9<sup>th</sup> ed. Washington DC. American Society for Microbiology 2007; 1152-72.

19. Akpulu IN, Dada JD, Odama EL and Galadima O: Antibacterial activity of aqueous extracts of some Nigerian medicinal plants. Nigerian Journal of Botany 1994; 7:45-48.

**How to cite this article:**

JN Uche and KH Ogbonda: Antibacterial effects of the seed extracts of alligator pepper (*Aframomum danielli* roxb.) on *Salmonella typhimurium* and *Escherichia coli*. Int J Pharmacognosy 2021; 8(2): 65-69. doi link: [http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.8\(2\).65-69](http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.8(2).65-69).

This Journal licensed under a Creative Commons Attribution-Non-commercial-Share Alike 3.0 Unported License.

This article can be downloaded to **Android OS** based mobile. Scan **QR Code** using Code/Bar Scanner from your mobile. (Scanners are available on Google Playstore)