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

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

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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 $mgml^{-1}$, 10 $mgml^{-1}$, 20 $mgml^{-1}$, 30 $mgml^{-1}$ and 50 $mgml^{-1}$) 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 [1]. 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.

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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 [2]. 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 oxazolidiones [3]; due to the recurring resistance of pathogenic micro-organisms 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 [4]. 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 [5]. 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. 6It 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 [7]. 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 [9]. 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 methods10 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 [11].

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 $mgml^{-1}$, 10 $mgml^{-1}$, 20 $mgml^{-1}$, 30 $mgml^{-1}$ and 50 $mgml^{-1}$) were based on standard methods [12, 13] and [14, 15] and [16].

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 ºC for 24 h [17].

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 1×10^7 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 1×10^7 cfu/ml was seeded on the surface of a large (150 mm diameter) Mueller-Hinton agar plate. The discs containing different concentrations (5 $mgm1^{-1}$, 10 mgm^{-1}, 20 $mgm1^{-1}$, 30 $mgm1^{-1}$, 50 $mgm1^{-1}$) 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 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 [17].

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 $mgm1^{-1}$ 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 $mgml^{-1}$ concentration. The result of the MIC and MBC test showed that for the ethanolic extract of the seed both organisms, *Salmonella typhmurium* and *Esherichia coli*, had a MIC and MBC of 20 $mgml^{-1}$ and 30 $mgml^{-1,}$ respectively. The MIC and MBC of the water extract of the organisms were 10 $mgml^{-1}$ and 20 $mgml^{-1,}$ 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* [19, 7, 9]. Also had it that phytochemicals, including flavonoids, had biological functions, which include protection against microbes [8]. 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, [8] 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 ($Mgm1^{-1}$)			
Salmonella	5	10	20	30	50
typhimurium	10.0	11.0	14.0	17.0	18.0
Escherichia coli	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 ($Mgm1^{-1}$)			
Salmonella	5	10	20	30	50
typhimurium	8.0	9.0	11.0	11.0	13.0
Escherichia coli	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.

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