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Composition of Alligator Pepper (*Aframomum melegueta*) obtained from Yenagoa in Bayelsa State, Nigeria

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Abstract
Alligator pepper (Aframomum melegueta) is a spice from the Zingiberaceae family, a traditional snack with great pharmacological potentials. Its seeds components (proximate parameters, minerals and bioactive compounds) were determined in samples obtained from a market in Yenagoa the capital city of Bayelsa State, Nigeria. The proximate results were 66.58 % carbohydrate, 8.28 % fibre, 5.41 % protein, 8.91 % fat, 2.34 % ash and 8.53 % moisture content. Results for Minerals analyzed were 0.99 % K, 0.003 % Fe, 0.004 % Mn, 0.02 % Ca and 0.23 % P. The GC-MS analysis of the methanol extract of Aframomum melegueta revealed the presence of 15 bioactive componds with different retention times and peak areas. The bioactives included: Humulene (9.13 %), 3-Decanone (16.76 %), 4-Hydroxy-3-methoxyphenylglycol (26.61 %) and 1-(4-Hydroxy-3-methoxyphenyl)decane-3,5dione, (16.29%). Many of the bioactive compounds are known for their potential antibacterial, antitumor and anti-inflammatory properties.The investigation also revealed that the seeds of Aframomum melegueta contains a moderate amount of 2-butanone (16.37 %), a nose, throat, skin and eye irritant. This research therefore affirms the medicinal potential of Aframomum melegueta, however, it recommends caution in its application for medicinal purposes considering the nose, throat, skin and eye irritant present in it.

Keywords: *Aframomum melegueta, proximate, minerals, bioactive compounds*

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I. Introduction

Medicinal plants, also called medicinal herbs, have been discovered and used in traditional medicine practices since prehistoric times. Plantssynthesize hundreds of chemical compounds for functions such as defense against insects, fungi, diseasesand herbivorous mammals. Numerous phytochemicals with potentials or established biological activities have been identified. However, since a single plant contains widely diverse phytochemicals, the effects of using a whole plant as medicine are uncertain. Further, the phytochemical content and pharmacologicalactions, if any, of many plants having medicinal potential remain unknownby rigorous scientific research to define efficacy and safety [1].Medicinal plants are widely used in non-industrialized societies, mainly because they are readily available and cheaper than modern medicines. The annual global export value of the thousands of types of plants with medicinal properties was estimated to be US$377.63 million in 2020-2021 [2].In 2017, the potential global market for botanical extracts and medicines was estimated at several hundred billion dollars. In many countries, there is little regulation of traditional medicine, but the World Health Organization coordinates a network to encourage safe and rational usage. Medicinal plants face both general threats, such as climate change and habitat destruction, and the specific threat of over-collection to meet market demand [3].

Aframomum melegueta(Alligator pepper) is a spice from the ginger family. Itis a perennial plant native to swampy habitats along the West African coast and humid areas, chiefly Ghana and Nigeria (Plate 1)

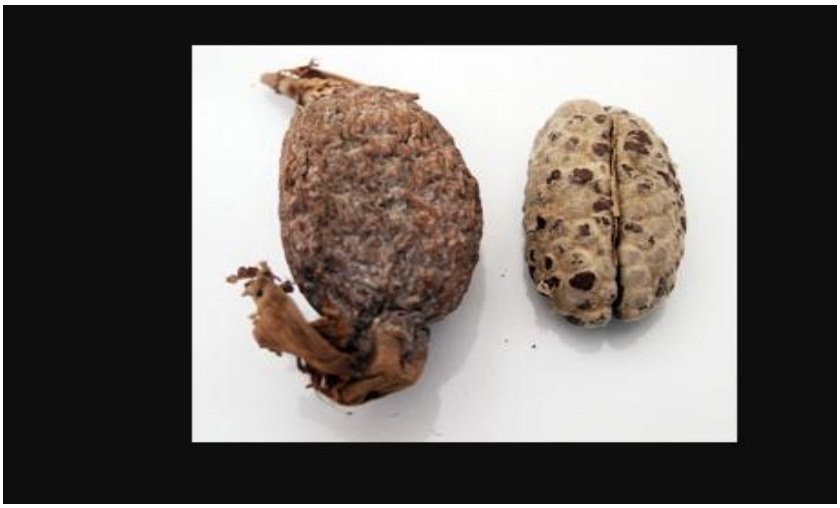

Plate 1: : Dried Fruits of *Afromomum meleguta*[4]

The seeds of *Aframomum melegueta*are used as a spice in food due to the aromatic flavor and pungent taste or as ingredients of ethnomedical preparations for the treatment of snakebites, stomachaches, and diarrhea, hypertension, aphrodisiac measles and leprosy[5][6] [7]. They are also taken for excessive lactation and post partem hemorrhage, and are used as a purgative, galactogogue, anthelmintic and hemostatic agent [7]. Pharmacological investigations have demonstrated that the seeds have anti-ulcer, antimicrobial and cytoprotection effects [8]. Antibacterial and anti fungal effects result from 6-paradol and 6-shogoal, compounds found in the seeds[2]. The effects of the aqueous extract of seeds of *Afromomum melegueta*on some parameters of the reproductive function of mature male rats have also been reported [9].*Aframomum melegueta* aqueous seeds extract has been reported to possess abortifacient properties[10].

Gingerol, an important active compound isolated from the seed of this plant, was shown to inhibit prostaglandin and leukotriene biosynthesis [11]. The chemical composition of the oils from the leaves was dominated by hydrocarbon compounds such as pinene, caryophyllene, humulene, selinene, -selinene and germacrene A [12].Tijani and Luka [13] studied the bioactive and antimicrobial effects of the ethanol extracts of the seeds of alligator pepper, and Ocimumgrassitimumon causative agents of post-harvest decay of carrots. The seed extract of alligator pepper showed higher inhibition of mycelia growth in Penicillium (83.33%). Some biochemical and haematological Parameters in rats fed with high lipid diet revealed that aqueous extracts of Afromomum melegueta, can be used in weight management as well as in improvement of lipid profile. Inegbenebor and Ebomoyi [14] studied the effect of saline extract of Alligator Pepper (*Aframomum melegueta*) on Serum Progesterone in Pregnant Sprague Dawley Rats. Their results showed that progesterone level decreased significantly on days 7 and 21 of pregnancy among rats administered with 6.7 mg/kg body weight. Omoboyowa *et al*[15] alsostudied the effects of methanol seed extract of *Aframomum melegueta* (Alligator Pepper) on Wistar Rats with 2,4-Dinitrophenylhydrazineinduced Hemolytic Anemia, and the study was able to deduce that the extract of *Aframomum melegueta* has a negative effect on wistar rats. Although there were no recorded side effects of the consumption of alligator pepper, however, pregnant and lactating mothers are encouraged not to consume it based on an experiment by Inegbenebor *et al.*[16]. Their studyshowed that high dosage of alligator pepper administered to pregnant rats led to the termination of their first trimester pregnancies. Based on this report, pregnant women in their first trimester are highly adviced to refrain from eating the alligator pepper seeds to avoid miscarriages. [17].

Although, manifold uses of *Aframomum melegueta*seeds especially those relative to its medicinal effects have been reported in literature, there is still limited knowledge on the biochemical composition of the seeds of *Aframomum melegueta* seeds consumed in Yenagoa, Bayelsa State.

II. Materials And Methods

2.1Materials

All chemicals used were of analytical grades and obtained from BDH, Labtech chemicals, Ken Light Laboratories, Kermel.

2.2 Methods

Aframomum melegueta fruits were purchased from Opolo market, Yenagoa, Yenagoa Local Government Area, Bayelsa State, Nigeria and properly identified at the Biological Sciences Department of the University of Africa, Toru-Orua. *Aframomum melegueta*seeds were manually removed from the pods and air dried for fourteen days. The seeds were pulverized with an electronic blender and stored in a desiccator for analysis.

2.2.1 Proximate and Mineral Analyses

Standard procedures as described by the Association of Official Analytical Chemists [18] were used in the determination of the following Proximate Parameters: Moisture Content, Fat Content, Ash Content, Crude Fibre, Crude Protein and Carbohydrate Content; and the following Minerals:

2.2.2Determination of Bioactive Compounds

Gas Chromatography-Mass Spectroscopy (GC-MS) analyses of methanol extract of *Aframomum melegueta* seed powder was performed with GC (Agilent 6890) and MS (5973 MSD) equipped withRestek capillary column (30 m × 0.53 mm; film thickness 0.12 µm), operating in electron impact mode at 60 eV. Helium (88.799 %) was used as a carrier gas at a constant flow of 1 ml /min withan injection volume of 0.5l (split ratio of 10:2); injector temperature was 200^0 C and ion source temperature was 230 °C. The oven temperature was kept130 ^{0} C (isothermal for 5min). The temperature was ramped at 30^0 C /min to 180 ^{0} C and then by 5 ^{0} C /minto 250 ^{0} C(isothermal for 9 min). Mass spectra were recorded at 60 eV with an interval of 0.5 seconds and fragments from 50 to 650 Da. Gas Chromatography (GC) running time was 45 minutes and 20 seconds.

III. Results And Discussion

The results for proximate and mineral analyses are presented in Table 1 while the result of the GC-MS analysis is presented in Table 2. The chromatogram is shown in Figure 1.

Table 1: Results of Proximate and Mineral Analysis of*Aframomum melegueta*seeds

Proximate	**Mean ± S.D of Composition of dry sample (%)**	**Mineral Composition**	**Mean ± S.D of Composition of dry sample (%)**
Moisture (%)	8.53 ± 0.02	Potassium (K)	0.99 ± 0.03
Ash (%)	2.34 ± 0.03	Iron (Fe)	0.003 ± 0.01
Protein	5.41 ± 0.01	Manganese (Mn)	0.004 ± 0.03
Fat	8.91± 0.02	Calcium (Ca)	0.02 ± 0.01
Fibre	8.28 ± 0.01	Phosphorus (P)	0.23 ± 0.01
Carbohydrate	66.58 ± 0.02		

Table 2: Result of GC-MS analysis of bioactive components in *Aframomum melegueta*seed powder

S/N	**Retention Time**	**Name of compound**	**Molecular formula**	**Molecular weight**	**Peak Area %**
1	1.919	Silane	$C_3H_{10}O_2Si$	106	0.94
2	3.005	Hexanal	$C_6H_{12}O$	100	3.09
3	4.108	Octanal	$C_8H_{16}O$	128	0.56
4	7.000	Caryophyllene	$C_{15}H_{24}$	204	3.62
5	7.314	Humulene	$C_{15}H_{24}$	204	9.13
6	7.611	(3S,3aS,8aR)-6,8a-Dimethyl-3-(prop -1-en-2-yl)-1,2,3,3a,4,5,8,8a-octa hydroazulene	$C_{15}H_{24}$	204	1.20
7	9.497	Spiro[4.4]nonan-2-one 3-Octyne	$C_9H_{14}O$	138	0.84
8	10.572	2-Butanone	$C_{11}H_{14}O_3$	194	16.37
9	16.001	3-Decanone	$C_{17}H_{26}O_3$	278	16.76
10	16.253	2,3-Divanillyl-1,4-butanediol	$C_{20}H_{26}O_6$	362	0.48
11	16.790	(E)-1-(3,4-Dimethoxyphenyl)dec-4-en-3-one	$C_{18}H_{15}N_3O_4$	337	0.83
12	17.099	4-Hydroxy-3-methoxyphenylglycol	$C_9H_{12}O_4$	184	26.61
13	15.910	(E)-1-(4-Hydroxy-3-methoxyphenyl)d-en-5-one	$C_{17}H_{22}O_4$	290	0.83
14	12.024	Butan-2-one, 4-(3-hydroxy-2-methoxyphenyl)	$C_{11}H_{14}O_3$	194	2.46
15	17.973	1-(4-Hydroxy-3-methoxyphenyl)decane-3,5dione	$C_{17}H_{24}O_4$	292	16.29

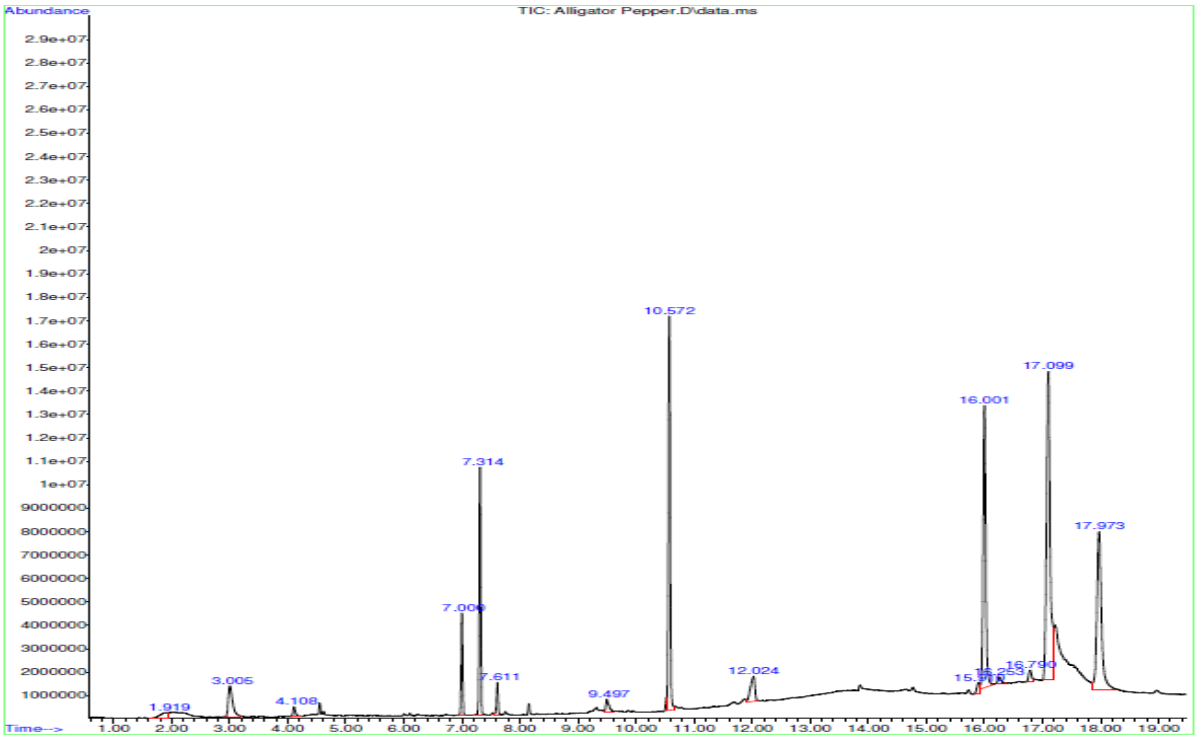

Figure 1: Gas Chromatogram of Ethanol Extract of *Aframomum melegueta*Seeds

The proximate and mineral contents of the seeds of *Aframomum melegueta* in Yenagoa, Nigeria, were compared with *Aframomum melegueta* in other regions in Nigeria. The results were also compared with results of other species that belong to the Zingiberaceae family. The moisture content of the seeds of *Aframomum melegueta*was8.53 % (Table 1). Moisture content is a determining factor in the physical appearance, shelf-life and resistance to bacterial contamination [19].The moisture content value obtained in this study is much lower than results obtained from seeds of *Aframomum melegueta* inWestern Nigeria, 13.66 %[20].The ash content of the seeds of *Aframomum melegueta* was 2.34 %, a little higher compared to Aframomum chrysanthum specie in the same Zingiberaceae family, 2.28 % [21]. Ash content analysis is the burning away of organic content, leaving inorganic minerals. This analysis is important because, it helps in determining the physiochemical properties of foods, fruits and seeds as well as retard the growth of microorganisms[22].The protein content of the seeds of *Aframomum melegueta* was5.41%. This is relatively low compared to that of *Aframomum chrysanthum*, 13.10 % [21] a little lower than the minimum requirement of 6 % necessary for maintenance of good animals condition [23]. The fat content of the seeds of *Aframomum melegueta* was 8.91 %. This was lower than that of white and yellow *Zingiber officinale*, 17.11 % and 9.89 % [24]. The fiber content of the seeds of *Aframomum melegueta* was 8.28 %. Fibre is a special type of carbohydrate that helps promote good digestive health [25]. This is high compared to that of a close relative,*Zingiber officinale*, 0.92 % [26]. The carbohydrate content of seeds of *Aframomum melegueta* was 66.58 %. Carbohydrates are the primary fuel source for the brain's high energy demands [25]. This is also relatively low compared to thatZingiber officinale 71.46 %[26].
Results for the analysis of mineral composition of the seeds of *Aframomum melegueta*are also presented in Table 1. The amount of potassium (K) in the seeds of *Aframomum melegueta*was 0.99 %. This amount is high compared with the concentration of potassium (K) in the white and yellow varieties of *Zingiber officinale* **(**0.000098 % and 0.000138 %) [24].Concentration of Fe in the seeds of *Aframomum melegueta*was 0.003 %.The concentration is high compared with the concentration of Fe in the white and yellow *Zingiber Officinale*, (0.000029 % and 0.000014 %) [24]**,** but still within the normal range for normal body functions. Iron is an essential element for blood production [27]. The concentration of manganese (Mn) in theseeds of *Aframomum melegueta*was 0.004 %. This was higher compared to the concentration of Mn in white and yellow ginger species,0.000003 % and 0.00007 %[24].Mn is a trace mineral required for the normal functioning of the brain and nervous system [28].The concentration of Ca in the seeds of *Aframomum melegueta* was 0.02 %. This is far higher compared with the Ca concentration in white and yellow species of ginger 0.000068 % and 0.000041 %[24].Calcium (Ca) provides structure and strength to the body skeleton [29].The concentration of Phosphorus (P) in the seeds of *Aframomum melegueta* was 0.23 %. This amount is higher than the concentration of Phophorus in the white and yellow ginger species, 0.000042 % and 0.000047 % [24].

The Gas chromatography-Mass spectrometry (GC-MS) analysis of the methanol extract of the seeds of *Aframomum melegueta* is presented in Table 2. Fifteen (15) secondarymetabolites with different retention times and peak area percentages are presented.The secondary metabolites that had significant area percentage are Humulene (9.13 %), 2-Butanone (16.37 %), 3-Decanone (16.76%), 4-Hydroxy-3-methoxyphenylglycol (26.61 %) and1-(4-Hydroxy-3-methoxyphenyl)decane-3,5dione, (16.29%). Many of the bioactive components including Humulene have antibacterial, antitumor and anti-inflammatory properties [29],although the seeds of *Afromomum meleguata*contain a high amount of 2-butanone, a compound that causes nose, throat, skin and eye irritation. Swallowing high levels of 2-butanone can result in birth defects, fainting and death [31],It also contains a high amount of 3-Decanone, a chemical considered hazardous by the 2012 OSHA Hazard Communication Standard[32].The result of GC-MS analysis of methanol extract of the seeds *of Afromomum melegueta* has shown the presence of many secondary metabolites that possess a range of biological activities, justifying its use in folk medicine

IV. Conclusion

This study focusedon the proximate, minerals and bioactive components in methanol extract of the seeds of *Aframomum melegueta*. Proximate content was higher than mineral content in the seeds of *Aframomum melegueta*. Gas chromatography-mass spectrometry (GC-MS) analysis of methanolic extract of the seeds of *Aframomum meleguetas*howed 15 phytochemicals. Many of the bioactive components including Humulene possess antibacterial, antitumor and anti-inflammatory properties. Seeds of Afromomum meleguata also contain a high amount of 2-butanone, a compound that causes nose, throat, skin and eye irritation. Swallowing high levels of 2-butanone can result in birth defects, fainting and death.

Optimistically, this study will provide additional biochemical information on the seeds of *Aframomum melegueta* in Bayelsa State.

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