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Evaluation of the phytochemical and GC-MS analysis of the aqueous seed extract of *Aframomum melegueta*

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Abstract
This study was designed to determine the phytochemicals present in the seed of *Aframomum melegueta* using preliminary test for secondary metabolites and Gas Chromatography Mass- spectroscopy (GC-MS) method of analysis. The results of the qualitative phytochemical screening indicated that alkaloids and saponins were moderately present; tannins, flavonoids, cardiac glycoside were slightly present while steroids were absent. The quantitative phytochemical screening using GC-MS showed the presence of thirteen (13) compounds among which are Glycerin (R/T 2.568), Caryophylene (R/T 5.858), Humulene (R/T 6.173), Cis-Vaccenic acid (R/T 7.735), Gingerol (R/T 9.848), d-Decanone,1(4-hydroxy-3-methoxyphenyl)- (R/T 11.003), Gingerol (11.527), d Manose (R/T 11.581), DL-Arabinose (R/T 11.998), Hexadecanoic acid, Methyl ester (R/T 12.180), n-Hexadecanoic acid (R/T 12.490), 9,12-Octerdecanoic acid (z,z,z)-, 9,12,15-Octerdecanoic acid (z,z,z)-. In the analysis the quantity of DL-Arabinose (R/T 11.998) is more with area percentage of (26.36%) while the least is Gingerol (R/T 9.848) with area percentage of (0.80%). Thus, the study may pose *Aframomum melegueta* to be an important source of phytochemicals of immense pharmaceutical significance.

Keywords: Phytochemical, Qualitative, Quantitative, *Aframomum melegueta*, Screening

Introduction
Plants are increasingly playing tremendous and unrestrained role in the maintenance of human health due to their medicinal value. The world health organization estimated that about 80% of the world population still depends on plants bioactive components as folk medicine in traditional therapies [1]. These health-promoting plants have stood ground as a rich source of structural novel compounds that might serve as lead for the development of synthetic drugs [2]. The availability, less side effect and the ability of phytochemicals in the plant extract to target the biochemical pathway and low cost of medicinal plants have resulted in its popularity and have been globally used for prevention and cure of human diseases [3-5]. Medicines in the traditional settings are prepared majorly from a single plant or combination of number of plants; and its efficacy is largely dependent on the use of appropriate plant part as well as the biological potency which in turn depends upon the presence of necessary quantity and nature of secondary metabolite in a raw drug [6, 7]. Consequently, traditional societies and ethnic nationalities have over the years employed medicinal plants in ethno-medicine for the treatment of various diseases without any scientific knowledge of the physiologically active ingredients known as phytochemicals, which were responsible for the plant's medicinal and pharmacological potentials [8-10]. Additionally, the WHO has emphasized the need to ensure the quality of medicinal plants products using modern controlled technique and applying suitable standards [11].

Aframomum melegueta which is a popular plant used in rain forest area of Africa especially Nigeria as a spice in food and also as kola with kola nuts during ceremonies, is a medicinal plant which belongs to the family Zingiberaceae. In Nigeria, its local names include: 'ose-oji' (Igbo), 'atare' (Yoruba), 'Cita' (Hausa) [12]. Its aqueous extract has been shown pharmacologically to reduce gestational weight in pregnant rats [13]. The spice is used in West Africa for the purposes of alleviating stomach ache and diarrhoea, cardiovascular diseases, diabetes and inflammation [14], as an aphrodisiac [15] and a remedy for snakebites and scorpion stings [16]. The bioactive constituents of aqueous seed extract of *Aframomum melegueta* have not been properly elucidated. Hence, the present study was to determine the bioactive constituents present in the aqueous seed extract of *Aframomum melegueta* by Gas chromatography and Mass spectroscopy (GC-MS) technique.

Materials and Methods

Collection of the plant material

The seed of *Aframomum melegueta* were harvested fresh from of Umuobasi Nnentu in Okporoenyi autonomous community, Aba South Local Government Area, Abia State, Nigeria dried and ground to powder.

Qualitative Phytochemical screening

The phytochemical screening was performed on the pulverized seeds of *Aframomum melegueta* for identification of phyto-constituents. The constituents tested include: Alkaloids, Tanins, saponins, phenolic compounds, cardiac glycoside, flavonoids and steroids as described by [17-21].

GC-MS Analysis

The samples for GCMS were prepared by dissolving 3g of extracted powder in aqueous solvent. For the analysis, GC-MS-QP 2010 SHIMADZU instrument was used. To analyze the sample, the column oven temperature and Injector temperature were set at 800°C and 200°C respectively. The flow control mode was maintained in linear velocity with a split injection mode split ratio of 20. The column flow was 1.46ml/min with a helium carrier gas of 99.9995% purity. The column oven temperature program was set as follows:-

The temperature was set at 80°C with 2 minutes hold time by the rate of 10. The temperature was 300°C with 10 minutes hold time. The column at 5 minutes was used with a length of 30 milimeters and diameter of 0.25mm and its film thickness will be 0.25 µm. The ion source temperature for MS condition was 200°C and interface temperature was 240°C. Starting m/z (Mass to charge) ratio was 40 and ending with m/z ratio of 700. (40-700 m/z).

Identification of the Phytochemical Constituents

To identify the unknown phytochemical components present in the extract, their individual mass spectral peak value was compared with the data base of National Institute of Standard and Technology (NIST) which holds about 62,000 patterns. Then, the phytochemical was identified based on the hits returned after comparing the unknown peak value and the chromatogram from GCMS against the known chromatogram, peak value from the NIST library data base. Subsequently, the detail about their molecular formula, molecular weight, structures was obtained.

Results

Table 1: Phytochemical Screening on *Aframomum melegueta* seeds

Phytochemicals	Presence
Steroids	-
Saponins	+
Tanins	+
Flavonoids	+
Cardiac glycosides	+
Phenolic compounds	++
Alkaloids	++

Keys: + = slightly present, ++ = moderately present and - = absent.

Quantitative phytochemical composition of *Aframomum melegueta* using GC-MS method of analysis is shown in Table 2. From the study, thirteen (13) compounds were eluted and found present in the plant seed extract.

Table 2: Quantitative phytochemical composition of *Aframomum melegueta* using GC-MS method of analysis

S/No	RT	Compound Name	MW	Formular	Area %
1	2.568	Glycerin	92	$C_3H_8O_3$	2.02
2	5.858	Caryophylene	204	$C_{15}H_{24}$	3.43
3	6.173	Humulene	204	$C_{15}H_{24}$	8.95
4	7.735	Cis-Vacceni acid	282	$C_{17}H_{26}O_4$	18.65
5	9.848	Gingerol	294	$C_{17}H_{26}O_4$	0.80
6	11.003	d-Decanone,1(4-hydroxy-3-methoxyphenyl)-	278	$C_{17}H_{26}O_4$	2.38
7	11.527	Gingerol	294	$C_{17}H_{26}O_4$	9.43
8	11.581	d-Manose	294	$C_{17}H_{26}O_4$	7.39
9	11.998	DL-Arabinose	294	$C_{17}H_{26}O_4$	26.36
10	12.180	Hexadecanoic acid, Methyl ester	294	$C_{17}H_{26}O_4$	10.36
11	12.490	n-Hexadecanoic acid	294	$C_{17}H_{26}O_4$	5.18
12	12.961	9, 12-Octerdecanoic acid (z, z, z)-	294	$C_{17}H_{26}O_4$	3.87
13	13.014	9,12,15-Octerdecatrienoic acid (z, z, z)-	294	$C_{17}H_{26}O_4$	1.16

Keys: RT = Retention time,
MW = Molecular weight

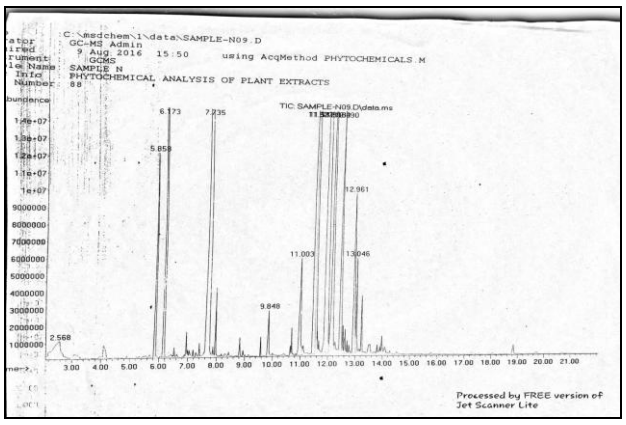

Fig 1: Chromatogram of GC-MS analysis of *Aframomum melegueta* seed

Discussion

The potential usefulness of medicinal plants in health and the concomitant implication cannot be overemphasized. The relevance of a plant to pharmacy lies in the ability of the plant to possess elaborate organic compounds that possess pharmacological properties or compounds that are of use in pharmaceutical formulations as flavoring agents or formulation aids. For instance, some plant secondary metabolites such as alkaloids, phenols, glycosides, terpenoids, saponins, flavonoids and steroids have been implicated in their ability to inhibit the formation of pro-inflammatory signaling molecules such as prostaglandin or leukotrienes [22]. Screening of the dried seeds sample of *Aframomum melegueta* as carried in this study revealed the presence of saponins, tanins, flavonoids, phenols, cardiac glycosides and alkaloids in various quantities while steroid was absent. These

phytochemical agents have indeed been implicated in the treatment of various diseases [23, 24]. The presence of flavoniods may be attributed to the anti-inflammatory properties [25] and as such offers bodily protection against allergies. It has also been reported that flavonoids exhibit free radical scavenging property, super antioxidants and with strong anticancer activity [26].

Alkaloids are of therapeutic significance. Pure isolated alkaloids and the synthetic derivative are used as the basic medicinal agents due to their analgesic, antispasmodic and antibacterial potentials [27]. The result of the preliminary phytochemical analysis of this present study may give credence to its ethnomedicinal usage. Saponin is used as a mild detergent and in intracellular his to chemistry staining to allow antibody access to intracellular proteins. In medicine, it is used in hyperchlolesterolaemia, hyperglycaemia, antioxidant, anticancer, anti-inflammatory, and weight loss among others. It is also known to have antimicrobial properties [28].

The GC-MS analysis of *Aframomum melegueta* seeds revealed different bioactive components which accounts for the various properties of the *Aframomum melegueta* seed. The presence of thirteen compounds as revealed in this study also provides some biochemical basis for its ethnopharmacological uses in the treatment and prevention of various diseases and disorders. The individual names of compounds with respect to their individual peak number, retention time and area % are represented in (Table 2) above. Among the compounds present are Glycerin 2.02% with (Retention Time 2.568), Caryophylene 3.43% with (Retention Time 5.858), Humulene 8.95% with (Retention Time 6.173), Cis-Vacceni acid 18.65% with (Retention Time 7.735), 3-Decanone,1(4-hydroxy-3-methoxyphenyl)- 2.38% with (Retention Time 11.003), Gingerol 9.43% with (Retention Time 11.527), d-Mannose 7.39% with (Retention Time 11.581), n-Hexadecanoic acid 5.18% with (Retention Time 12.490), 9,12-Octadecanoic acid (z,z,z)- 3.87% with (Retention Time 12.961), 9,12,15-Octadecanoic acid (z,z,z)- 1.16% with (Retention Time 13.014) and Hexadecanoic acid, methyl ester 10.37% with (Retention Time 12.180). Other phytochemical present include DL-Arabinose 26.36% with (Retention Time 11.998) which has the highest percentage area and Gingerol 0.80% with (Retention Time 9.848) which has the least percentage area.

The n-Hexadecanoic acid - palmitic acid possesses an antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant, antiandrogenic activities [29]. Humulene, also known as α-humulene or α-caryophyllene, is a naturally occurring monocyclic sesquiterpene ($C_{15}H_{24}$) that produce anti-inflammatory effects in mammals, and has potential to be a tool in the management of inflammatory diseases. It was also found to decrease the edema formation caused by histamine injections [30]. Humulene produced inhibitory effects on tumor necrosis factor-α (TNFα) and interleukin-1 β (IL1B) generation in carrageenan-injected rats [31]. Cis-vaccenic acid, is an omega-7 fatty acid that possess hypolipidemic effect in rats [32]. Gingerols have been reported to possess blood glucose lowering effect [33] and anti-inflammatory [34, 35] and antidiabetic activities [36]. Caryophyllene was shown to be selective agonist of cannabinoid receptor type-2 (CB_2) and to exert significant cannabimimetic antiinflammatory effects in mice [37], Antinociceptive [38], neuroprotective [39], anxiolytic, antidepressant and anti-alcoholism [40, 41].

Conclusion

In the present study, thirteen phytochemical constituents were identified from the dried sample of seeds of *Aframomum melegueta* by Gas Chromatography - Mass Spectrometry (GC - MS) analysis. The presence of these phytochemical constituents justifies the use of this plant for various ailments by traditional practitioners. Isolation of individual phytochemical constituents and subjecting it to biological activities are being undertaken.

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