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Original Research Article

HPLC and FT-IR Analyses of Nigerian Alligator Pepper (*Aframomum melegueta)* Methanol Seed Extract

Folasade M. Makinde[1*], Etido F. Udo[2] , Dolapo D. Olugbile[2] , Oduola O. Abiola[3]

[1]*Food Science and Technology Programme, College of Agriculture, Engineering and Science, Bowen University, Iwo, Osun State, Nigeria.*
[2,3]*Medical Laboratory Sciences Programme, College of Health Sciences, Bowen University, Iwo, Osun State, Nigeria.*

ABSRTACT

Traditional knowledge had been established to successfully preserve and restore biodiversity across the continent since it accommodates indigenous herbalism. Traditional medicine had been adopted in Nigeria prior to the colonialism era. The study examined the phytochemical constituents in methanolic seed extract of alligator pepper (*Aframomum melegueta*) grown in Nigeria by Fourier Transform Infrared Spectrophotometer (FT-IR) and High Performance Liquid Chromatography (HPLC) analyses. *Aframomum melegueta seeds* were extracted with 70% methanol at room temperature for 48 h. The functional groups in aqueous methanolic seed extract were determined using FT-IR spectroscopic method while the bioactive compounds were determined using HPLC. The FT-IR result established the presence of N-H, C=N, C=C, C-O and O-H functional groups in the methanolic extract. The HPLC analysis of the methanolic extract identified ten compounds. The major components were farnesyl (37.11%), lycopene (34.15%), dihydrocapsiacin (9.55%), capsaicin (8.40%) and delphenidin (7.91%). Other compounds present in the extract at low levels were phytoene (0.74%), β-lonone (0.57%), limonene (0.56%), 6-methoxymellen (0.56%) and trans-beta-ocimene (0.45%). In essence, this study established the ethnobotanical use of *Aframomum melegueta* as functional food ingredient.

Keywords: *Aframomum melegueta, phytochemical, FT-IR, HPLC*

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Introduction

Traditional medicine is globally accepted for health care. It is related to the knowledge and practice adopted in diagnosing, preventing, and treating disease to enhance general health and wellness. Traditional medicine has been used to treat common diseases since ancient times.[1] A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for the management of diseases or for the synthesis of drugs. Specifically, the medicinal value of these plants lies in some chemical substances that produce a definite physiological action in human or animal system.[2] The bioactive constituents are majorly the alkaloids, flavonoids, tannins and phenolic compounds.

Aframomum melegueta is a tropical herbaceous perennial plant of the genus *Aframomum* belonging to the family *Zingiberaceae* (ginger family) of the angiosperms in the Kingdom plantae. This plant has orange coloured lips and pinkish-orange upper flowers that can develop into fleshly indehiscent pods. The pod contains numerous small, reddish brown seeds.

*Corresponding author. E mail: maria.makinde@bowen.edu.ng
Tel: +234-9060717567

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Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

This plant is also known as *alligator pepper, grains of paradise, guinea pepper or melegueta pepper*. It is native to tropical African countries such as Ghana, Nigeria, Sierra Leone, Liberia, Togo and Cote D'Ivoire.[3] This traditional plant which is well known in Nigeria as "*Ataare*" has been used for years in the management of diseases.

The advances in analytical techniques including High Performance Liquid Chromatography (HPLC) and Fourier Transform Infrared Spectrophotometer (FT-IR) are techniques for the identification and determination of phytochemical compounds. HPLC analytical technique is used for the isolation of various plant materials. It is a technique that can separate a mixture of compounds and identify, quantify and purify the individual components in a mixture. Over the years, this technique is gaining popularity for fingerprinting study for the quality control of herbal plants.[4] FT-IR is most recognized tool for identifying the types of chemical bonds (functional groups) present in compounds. The wavelengths of light absorbed characterized the chemical bond presented in an annotated spectrum. The study determined the bioactive compounds present in methanol extract of *Aframomum melegueta* using HPLC and FT-IR techniques, which may provide an explicate to its use in folklore medicine.

Materials and Methods

Plant material

Aframomum melegueta pods containing the seeds were purchased from Oja Oba market in Iwo, Osun State, Nigeria. The seeds are identified

using iNaturalist and authenticated by a taxonomist in the Biology Programme of the Bowen University.

Plant sample

The alligator pepper pods were shade dried for 7 days. After drying, the seeds were manually separated from pods and winnowed to remove adhering shafts. The seeds were ground with laboratory blender (MX-795N, National, Japan), screened through 70 mesh sieve and stored in sealed glass container prior to use. Plate 1 shows *Aframomum melegueta* pods, seeds and powder, respectively.

Extraction procedure

The ground sample (450g) was extracted with 2250 mL of 70% methanol. The mixture was shaken for 48 h using bath shaker at room temperature, filtered using Whatmans No. 1 filter paper and then preserved at 4 ºC for further process.

FT-IR spectroscopic analysis

Crude extract of *Aframomum melegueta* was used for the FT-IR Analysis. The crude extract was loaded in the crystal surface of FT-IR spectroscopic (Cary 630, Agilent Technologies), with a scan range from 650 to 4000cm^{-1} with a resolution of cm^{-1} to generate a spectrum. Spectral data obtained were compared with literature data.[5]

HPLC analysis

The HPLC analysis of the methanolic crude extract was carried out using chromatographic system (Shimadzu, Nexera MX) with a UV detector. The separation was performed on a uBondapak C18 (100 mmx4.6 mm, 7 µm) column at ambient temperature. The mobile phase consists of Acetonitrite to water (70:30 v/v). The known weight of the sample (10 g) was extracted with Acetonitrite. The extract was stabilized with ethyl acetate and introduced into 25 mL standard flask, and made up to the mark. A known sample volume (5 µl) was injected at a flow rate 2 mL/
min. The sample was run for 15 min and detection was done at 254 nm by UV detector. The data generated was recorded and processed using autochro-3000 software.

a b c

Plate 1. *Aframomum melequeta* (a) pods (b) seeds (c) powder.

Results and Discussion

FT-IR spectrum of Aframomum melegueta

The identified functional groups in *Aframomum melegueta* methanolic seed extract (AMMSE) are as shown in Table 1.The spectrum as presented in Figure 1 identified the functional groups in the extract based on the peaks values represented in the region of infrared radiation.The FT-IR results established the presence of N-H, C=N, C=C, C-O and O-H functional groups. Over the years, FT-IR spectroscopy has proven to be sensitive and reliable for the detection of biomolecular composition.

HPLC spectrum of Aframomum melegueta

The compounds present in the AMMSE identified by HPLC analytical technique are as presented in Figure 2. Research interest on the standardization and characterization of herbal drugs is ever increasing with the advent of modern chromatographic systems which offer rapid, convenient and cost effective analyses. Ten compounds were identified in the AMMSE using HPLC. The major components were farnesyl (37.11%), lycopene (34.15%), dihydrocapsiacin (9.55%), capsaicin (8.40%) and delphenidin (7.91%). Other compounds present in the extract at low levels were phytoene (0.74%), β-lonone (0.57%), limonene (0.56%), 6-methoxymellen (0.56%) and trans-beta-ocimene (0.45%).

The seed extract has highest concentration of farnesyl (37.11%) as indicated in Figure 2. A variety of eukaryotic proteins has the potential to undergo post-translational modification by the attachment of a farnesyl group to a cysteine residue. This process results in the formation of a thioether bond. Farnesylated proteins perform a variety of functions in cells such as enzyme catalysts, structural proteins and components in signal transduction networks.[6] Most importantly, since constitutive activation of target protein such as Ras, is a major contributory factor in a number of alignment human tumors, its inactivation by interfering with the farneslylation step has been extensively studied as a strategy to develop new anticancer agents. [7]

The seed extract contains lycopene (34.15%) as indicated in Figure 2. Lycopene is the red carotenoid found in fruits and vegetables, especially those with a red color. It is often considered to be primary bioactive carotenoid that mediates health benefits.[8] Asides, the extract also contain other colourless precursor carotenoid such as phytoene though in much lower level (9.55%) compared to the lycopene. Phytoene are linear hydrocarbons, more specifically they are alkenes with 9 double bonds. It has three of its double bonds conjugated (3c.d.b). However, it is considered that at least 7c.d.b.are needed for a carotenoid to exhibit color; hence phytoene with lower value is considered colorless carotenoid.[9] Phytoene are present in common Western diet such as tomato, grapefruits, watermelon etc and in some peppers and it is readily absorbed by humans. Animal models of carotenoid absorption even suggested preferential of phytoene in some tissues.[8] Most importantly, lacking color, this carotenoid precursor can be especially useful in food development where such attribute is not desired. There is evidence that lycopene or its metabolites could protect from light damage and oxidative stress, exhibit anti-inflammatory activity or exert anti carcinogenic activity. [10]

Delphenidin is a purple- coloured plant pigment that is highly active in its aglycone form. Several animal and human clinical studies have shown that delphenidin exerts beneficial effects on gut microbiota.[11] Most importantly, increasing evidence shows its potential pharmaceutical and nutraceutical applications, as indicated by exhibiting the activities of anti-oxidation, anti-inflammation, anti-microorganism, anti-diabetes, cardiovascular protection and anti-cancer.[12] *β*-Ionone is a natural plant volatile compound, and it is the 9,10 and 9',10' cleavage product of β-carotenoid by the carotenoid cleavage dioxygenase.[13] It is widely distributed in flowers, fruits and vegetables and could also be endogenously produced. *β*-Ionone, whether of an endogenous or exogenous origin, possesses anti cancer, anti inflammatory and anti microbial effects.[13] Trans-beta-ocimene is one of the most common monoterpenes found in nature. In the field of botanical medicine, there is an association of β-ocimene in essential oils with anticonvulsant activity, antifungal activity, antitumor activity and pest resistance. [14, 15, 16]

Limonene is found in the essential oils of aromatic plants. The therapeutic effects of limonene have been extensively studied, proving anti-inflammatory, antioxidant, antinociceptive, anticancer, antidiabetic, antihyperalgesic, antiviral, and gastroprotective effects, among other beneficial effects in health.[17] Pepper fruits and their products contain capsaicinoids responsible for the pungency. The two most abundant capsaicinoids in peppers are capsiacin and dihydrocapsiacin, both constituting about 90%, with capsiacin accounting for approximately 71% of the total capsaicinoids in most of the pungent pepper varieties.[18] The AMMSE had 8.40% and 9.55% of capsiacin and dihydrocapsiacin, respectively. Capsiacin had been reported to possess analgesic, anticancer and anti-cardiovascular potentials.[19] Plants possess various biochemical and structural defense mechanisms which protect them against infection such as 6-methoxymellen reported in AMMSE. The compound is a phytoalexin that is inhibitory to *Botrytis cinerea* and *Sclerotinia sclerotiorum*. It is however imperative to note that it has recently been demonstrated that phytoalexins may also display health-promoting effects in humans. For instance, resveratrol produced by *Vitaceae* has been reported as a cardioprotective, antitumor, neuroprotective and antioxidant agent as well as an antifungal and antibacterial. [20]

Table 1: Functional groups in *Aframomum melegueta*

S/N	Frequency cm^{-1} (Test sample)	Frequency range cm^{-1} (Reference article)	Functional group assignment	Phyto compounds identified
1	3378.8	3300-3500	N-H stretching	Primary and Tertiary Amines
2	3350.2	3010-3350	N-H stretching	Secondary Amines
3	2948.3	2800-3000	N-H stretching	Primary Amine Salt
4	2838.4	2700-3000	N-H stretching	Secondary Amine Salt
5	2031.4	2000-2222	N-H bending	Primary Amino acids
6	1653.1	1471-1689	C=N stretching	Imines, oxides, thiazoles,carbonates, guanidines
7	1449.5	1400-1500	C=C stretching	Aromatic compounds
8	1410.8	1400-1500	C=C stretching	Aromatic compounds
9	1112.6	1000-1260	C-O stretching	Alcohol
10	1013.8	1000-1260	C-O stretching	Alcohol
11	684.0	650-769	O-H bending	Alcohol
12	659.7	650-700	O-H bending	Alcohol

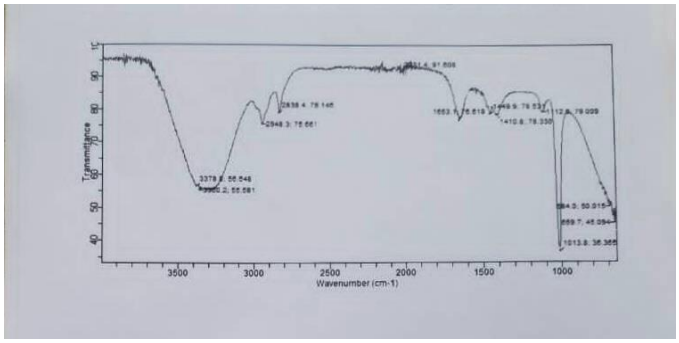

Figure 1: Fourier Transform Infrared spectrum of *Aframomum melequeta*.

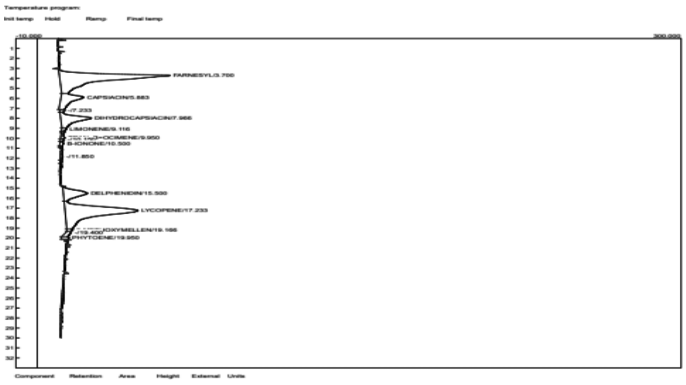

Figure 2: HPLC chromatogram of *Aframomum melequeta*.

Conclusion

The present work established the various phytochemicals and FT-IR parameters in methanolic seed extract of *Aframomum melegueta* which could be of interest in therapeutic applications in Nigeria. This primary information will facilitate studies on the safety and efficacy of the extract in clinical trials.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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