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PHYTOCHEMICAL SCREENING AND GC-MS ANALYSES OF AQUEOUS EXTRACTS OF *Aframomum melegueta* (ALLIGATOR PEPPER) SEEDS AND FRUITS AS POTENTIAL AGENTS FOR MANAGING FISH HEALTH

[*1]Abe Grace Osisienemo, [2]Oniye Sonnie Joshua, [3]Auta Jehu, [4]Onimisi Hassan Umaru, [3]Yusuf Abdullateef

[1]Bioresources Development Centre, National Biotechnology Research and Development Agency (NBRDA), Bayero University New Site, Kano State, Nigeria.
[2]Department of Biological Sciences, National Open University of Nigeria, Abuja, Nigeria.
[3]Department of Biology, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.
[4]Livestock and Fisheries Department, National Agricultural Extension and Research Liaison Services (NAERLS), Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

*Corresponding authors' email: osisienemo@gmail.com

ABSTRACT

Aframomum melegueta (Roscoe) K. Schum is a tropical herbaceous perennial plant of the genus *Aframomum* belonging to the family Zingiberaceae (ginger family) of the angiosperms in the Kingdom Plantae. The seeds and whole fruit of *Aframomum melegueta* were extracted separately with distilled water (1:10 w/v) by maceration for 24 hours at room temperature. The extracts were filtered, concentrated at ≤50 ˚C and stored at 4 °C until use. The resulting aqueous extract was subjected to qualitative and quantitative phytochemical analysis. The qualitative phytochemical screening revealed the presence of alkaloids, cardiac glycosides, saponins, phenolic compounds, tannins, steroids, carbohydrates, flavonoids and terpenoids. Quantitative analysis demonstrated significant concentrations of carbohydrates (13.94 and 13.94%); phenolic compounds (5.18 and 6.80%) and flavonoids (5.64 and 4.15%) from the seeds and whole fruits respectively. The GC-MS analysis eluted twenty-two (22) bioactive compounds from *A. melegueta* seeds extract and seventeen (17) bioactive compounds from the whole fruit aqueous extract. These bioactive compounds may act synergistically to offer strong antimicrobial, antioxidant and anti-inflammatory potentials in aquaculture nutrition as well as managing the health of fish.

Keywords: *Aframomum melegueta*, Proximate, Phytochemical, GC-MS, Aqueous Extract

INTRODUCTION

Aframomum melegueta (Roscoe) K. Schum is a tropical herbaceous perennial plant of the genus *Aframomum* belonging to the family Zingiberaceae (ginger family) of the angiosperms in the Kingdom Plantae. It is a perennial herbaceous plant known for its pungent, aromatic seeds, which are used as spice (Aguda & Gbadamosi, 2019). It is known as grains of paradise or alligator pepper as English common names (Owokotomo *et al*., 2014; Bamidele, 2019). Locally, it is known as '*ataare*' in Yoruba, '*ose oji*' in Igbo and '*chitta mai ko ko'* in Hausa language. It is native and most abundant across tropical West and Central Africa mainly in Nigeria, Ghana, Liberia, Benin, Sierra Leone, Cote D' Ivoire and Togo (Bennett and Inengite, 2022). Studies have shown that the seeds (Plate I) contain important phytochemicals namely, alkaloids, glycosides, tannins, flavonoids, sterols, triterpenes, and oils, some of which are responsible for its antimicrobial properties (Ogwu *et al*., 2024). The most important of these bioactive constituents which are mainly secondary metabolites are alkaloids, flavonoids, tannins and phenolic compounds which are toxic to microbial cells (Yerinbide *et al*., 2024). The seeds have pungent peppery taste due to aromatic ketones (Osuntokun, 2020). It is a plant with both medicinal and nutritive values, found commonly in rain forest.

The indiscriminate use of various feed additives in aquaculture such as antibiotics, hormones and other synthetic compounds, has raised significant concerns despite their role in enhancing growth performance in fish. These substances can produce several adverse effects, including the emergence of drug resistant bacterial strains, bioaccumulation of chemical residues in fish tissues, immune suppression, and environmental contamination. The use of medicinal plants have great effectiveness in disease preventive measures because of their broad spectrum activity, cost effectiveness and eco-friendly disease preventive measure (Anjusha *et al.*, 2019). Some work has been done on the use of *Aframomum melegueta* seed powder and extracts in humans, rats (Adefegha *et al*., 2016, Olatunji *et al*., 2018), poultry (Afolabi and Eko, 2016; Eko *et al*., 2022) and very little documented on fish (Kwankwa *et al*., 2020). However, information is currently unavailable on the proximate composition, quantitative phytochemicals and gas chromatography-mass spectrometry (GC-MS) of *A. melegueta* whole fruits.

The aim of this study is to make use of the nutritional and medicinal effects of this natural spice as a useful natural additive and an alternative to synthetic chemicals in managing the health of fish, mitigating oxidative stress, as well as modulating their immune system.

MATERIALS AND METHODS

Collection of Plant Materials

Mature dried *Aframomum melegueta* were obtained in bulk from Sheik Abubakar Gumi market in Kaduna North Local Government Area of Kaduna State in Nigeria and authenticated by a Taxonomist at the Department of Botany, Ahmadu Bello University, Zaria with voucher specimen number: ABU01251. The whole fruits were carefully cleaned, air-dried and divided into two portions. The seeds were separated from the pulp and pericarp for the first portion, while the other portion was whole. The two portions were grinded with a blender (Kenwood owBL440001) separately and sieved to obtain 500 g each and stored in airtight containers.


(a)


(b)

Figure 1: *Aframomum melegueta* Seeds (a) and whole Fruit (b)

Preparation of Plant Extracts
The finely ground powders of *A. melegueta* seeds and whole fruit were extracted separately with distilled water (1:10 w/v) by maceration for 24 hours at room temperature. The extracts were filtered, concentrated at ≤50 ˚C and stored at 4 °C until use.

Qualitative Screening for *Aframomum Melegueta* Seed and Whole Fruit
The qualitative screening for the presence of phytochemical constituents in aqueous extracts of *Aframomum melegueta* seeds and whole fruit were carried out using standard procedures as described by Sofowora (1993) and Trease and Evans (1989).

Quantitative Screening for *Aframomum Melegueta* Seed and Whole Fruit
Standard methods were used for the quantitative screening of *A. melegueta* seeds and whole fruits. Cardiac glycosides (Solich, 1992), Steroids (Harbone, 1973), total triterpenoid content (Harbone, 1973), total carbohydrate (Dubois *et al*., 1956), Anthraquinone (Sakuloanich and Gritsanapan, 2001), total phenolics content (Ainsworth and Gillespie, 2007; Alhakmani *et al*., 2013), total flavonoid content (Zhishen *et al*., 1999), total alkaloid content (Shamsa *et al*., 2008; Sharief *et al*., 2014), tannin content (Marinova *et al*., 2005) and saponin content (Makka *et al*., 2007).

Determination of Bioactive Compounds in *Aframomum Melegueta* Seed and Whole Fruit Aqueous Extracts
The gas chromatography-mass spectroscopy (GC-MS) (Model: GC7890B 5977A) analysis was carried out to separate and identify bioactive compounds present in the seeds and whole fruit of *A. melegueta* aqueous extract respectively. Identification of the compounds was carried out by comparing the mass spectra obtained with those of the standard mass spectra from National Institute of Standard and Technology (NIST) database.

RESULTS AND DISCUSSION

Phytochemical Screening of Aqueous Extracts of *Aframomum Melegueta* Seeds and Whole Fruit
The results of the qualitative and quantitative phytochemical profile of aqueous extracts of *A. melegueta* seeds and whole fruit are presented in Tables 1 and Figure 2 respectively. The qualitative phytochemical screening of aqueous extracts of *A. melegueta* seeds and whole fruit revealed the presence of alkaloids, cardiac glycosides, saponins, phenolic compounds, tannins, steroids, carbohydrates, flavonoids and terpenoids (Table 1) respectively.

Table 1: Qualitative Phytochemical Screening of *Aframomum Melegueta* Seeds and Whole Fruits

Phyto-constituents	Seed	Whole Fruit
Alkaloids	+	+
Cardiac Glycosides	+	+
Saponins	+	+
Phenolic compounds	+	+
Tannins	+	+
Steroids	+	+
Carbohydrates	+	+
Flavonoids	+	+
Terpenoids	+	+
Anthraquinones	-	-

Keys: + = present, - = below detection limit

The phytochemical compounds revealed in the qualitative phytochemical screening of the aqueous extract of *Aframomum melegueta* seed and whole fruit is quite common across many plants, but with varying concentrations. Agim *et al*. (2017) reported the absence of steroids in the phytochemical screening of *A. melegueta* seeds which is contrary to this study. However, this study was similar to the reports of Doherty *et al.*, (2010), which revealed the presence of alkaloids, tannins, saponin, steroids, cardiac glycoside, flavonoid, terpenoids and phenol in *A. melegueta* seeds. According to Jeruto *et al*. (2017) and Behl *et al*. (2021), the presence of these phytochemicals supports its use and efficacy as an antimicrobial agent.

The quantitative phytochemical analysis of aqueous extracts of *A. melegueta* seeds (Figure 2) showed that carbohydrates contents (%) was the highest (13.94), followed by flavonoids (5.64), Phenols (5.18), cardiac glycosides (3.07), tannins (2.14), alkaloids (1.75), triterpenes (1.56), steroids (1.51) and the least was saponins (1.23). While the aqueous extracts of *A. melegueta* whole fruit (Figure 2) showed that carbohydrate was the highest (13.94), followed by phenols (6.80), flavonoids (4.15), cardiac glycosides (3.07), triterpenes (1.56), steroids (1.51), saponins (1.06), alkaloids (0.68), and the least was tannins (0.65).

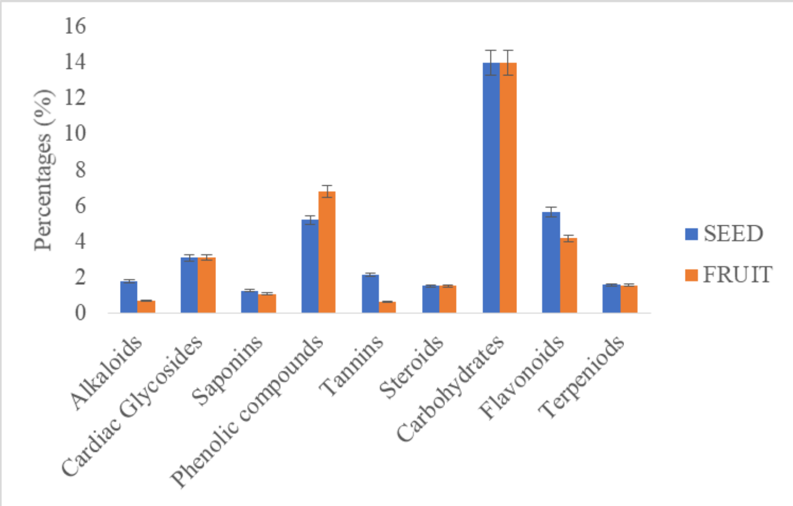

Figure 2: Quantitative Phytochemical Screening of *Aframomum melegueta* Seeds and Whole Fruit

The higher content of alkaloids, tannins and flavonoids in the seeds in this study, indicates a stronger potential for antioxidant and antimicrobial activity, while the fruits with a higher content of phenolic compounds are key antioxidants too. Agim *et al.* (2017) stated that alkaloids are of therapeutic significance and isolated alkaloids and the synthetic derivative are used as basic medicinal agents due to their analgesic, antispasmodic and antibacterial potentials. The higher value of flavonoids in the seeds indicate a stronger antioxidant activity and has also been attributed to the anti-inflammatory properties which offers bodily protection against allergies, exhibit free radical scavenging property, super antioxidants and with strong anticancer activity (Umukoro & Ashorobi, 2001; Njoku & Akumefula, 2007). Tannins exhibit a high level of biological activity and with a higher percentage in the seeds compared to the whole fruit, is an indication of its astringent, antimicrobial and antioxidant effects (Ozogul *et al*., 2025).

The higher content of phenolic compound in the whole fruits may indicate a higher antioxidant activity, immunostimulatory effect, better gut health, anti-microbial effects, increased palatability and growth (Beltran & Esteban, 2022). Furthermore, cardiac glycosides, steroids and carbohydrates present in equal proportionate concentrations suggest that *A. melegueta* seeds and whole fruits may possess potent and diverse biochemical properties that may have a comparable potential for cardiovascular modulation, hormonal effects and nutritional value. Saponin content in the seeds suggests a more concentrated source which could enhance its antibacterial properties, but also increase potential toxicity if included in excess to fish feed. According to Rezaei (2020), the presence of saponin in the diet of male and female convict cichlid (*Amatitlania nigrofasciata*) led to the increase in specific growth rates, improved body weight and increased food efficiency at 700 mg/kg and 300 mg/kg of food respectively.

Okugbo and Oriakhi (2015) in their study, reported that methanolic extract of *A. melegueta* leaves revealed the presence of medically active compounds including terpenoids, reducing sugars, flavonoids, saponins, alkaloids and cardiac glycoside, however, steroid was below the detectable level. This report contradicts the current study which had the presence of all the phytochemicals tested including steroid in the seed and whole fruit samples tested. Also, Yerinbide *et al.* (2024) reported the presence of eight phytochemicals in *A. melegueta* seeds and pods in which flavonoid content had the highest percentage of 4.29% alongside others which varied with this study. These variations in phytochemical presence and quantity can differ between different parts of the same plant, such as seeds and leaves (Okugbo & Oriakhi, 2015; Agidew, 2022). In addition, the types of solvents and extraction methods used can influence which compounds are extracted and detected during phytochemical screening (Doherty & Olaniran, 2010).

Gas Chromatography-Mass Spectroscopy (GC-MS) Profile of *Aframomum Melegueta* Seeds and Whole Fruit

The GC-MS analysis of the aqueous extracts of *Aframomum melegueta* revealed different bioactive compounds of the seeds and whole fruit. The aqueous extract of the seed eluted twenty-two (22) compounds (Fig. 3) and the names of the compounds, retention times, percentage peak area and molecular weights are shown in Table 2. Also, the aqueous extract of the whole fruit eluted seventeen (17) compounds (Fig. 4) and the names of the compounds, retention times, percentage peak area and molecular weights are shown in Table 3.

The gas chromatography-mass spectroscopy (GC-MS) profile of *A. melegueta* seed revealed several bioactive compounds with well documented nutritional implications for aquaculture that may initiate certain physiological and immunological responses in *C. gariepinus*. The four compounds with highest peak areas possess diverse bio-functional properties. For instance, Urs-12-en-3-ol, acetate, (3.beta) - compound is a pentacyclic triterpenoid acetate, structurally related to ursolic acid derivatives (Khwaza *et al*., 2020; NCBI, 2025). It exhibits potent anti-inflammatory, antioxidant, anti-cancer and anti-microbial activities. It is also known to inhibit pro-inflammatory cytokines such as TNF-∝ and IL-6 through the down regulation of the NF-kB signaling pathway (Arulnangai *et al*., 2025). Additionally, it enhances antioxidant enzyme activity by up-regulating the expression of endogenous enzymes such as superoxide dismutase and catalase (Jia, *et al*, 2021). Its antimicrobial activity is attributed to disruption of microbial membrane integrity and inhibition of pathogenic proliferation (Liu *et al*., 2024). According to Ugokwe *et al*. (2025), in the context of aquaculture, the presence of this compound may contribute to enhanced immune function, reduced oxidative stress and improved disease resistance in *C. gariepinus*.

3-Decanone, 1-(4-hydroxy-3-methoxyphenyl) commonly known as 6-paradol is a phenolic ketone that is structurally analogous to zingerone, commonly found in pungent spices and sharing the 4-hydroxy-3-methoxyphenyl core, but differing by its longer C_{10} alkyl substituent (Kim *et al*., 2017; Seba *et al*., 2020; NCBI, 2025). It exhibits antioxidant properties via its phenolic hydroxyl group, thereby enabling effective neutralization of reactive oxygen species (ROS) (Wei *et al*., 2017). In aquatic organisms, such compounds have been shown to modulate immune responses and improve tolerance to pathogenic invasion, suggesting a supportive role in maintaining homeostasis during immunological challenges in fish culture (Mansoori *et al*., 2024). 13-Octadecenal, (Z) - also known as cis-13-octadecenal is an unsaturated aliphatic aldehyde, which is a long chain fatty derivative with both structural and biological relevance (NCBI, 2025). Terpenoids and aldehydes of this nature are known to participate in antimicrobial defense through their ability to form Schiff bases with microbial proteins, thereby disrupting essential cellular functions (Shettima *et al*., 2024; Mala *et al*, 2024). Although, its specific role in fish immunity is not fully clear, its presence suggests potential antibacterial and modulatory effects that may contribute to gut health and external pathogen control (Bartolome *et al*, 2013).

2-Methyl-Z, Z-3, 13-octadecadienol is a polyunsaturated long chain fatty alcohol with two cis double bonds (Adeyemi *et al*., 2017; Ahmad & Sirajo, 2024; NCBI, 2025). Fatty alcohols has been associated with membrane-modulating, antioxidant and anti-inflammatory properties which may enhance mucosal integrity and modulate lipid metabolism, thereby supporting cellular defense mechanisms (Ahmad & Sirajo, 2024). In aquaculture species, long chain alcohols has demonstrated beneficial roles in maintaining epithelial barrier function and preventing bacterial colonization, which are crucial for resistance to opportunistic pathogens such as *Vibrio parahaemolyticus* (Nimalan *et al*., 2022). The identification of these compounds in *A. melegueta* seeds suggests that their inclusion in fish diets can enhance antioxidant status, modulate immune responses and improve resistance in a case of microbial infection. These bioactive metabolites may likely act synergistically and could offer physiological benefits, making them viable candidates for use in functional fish feeds and immune-nutrition strategies.

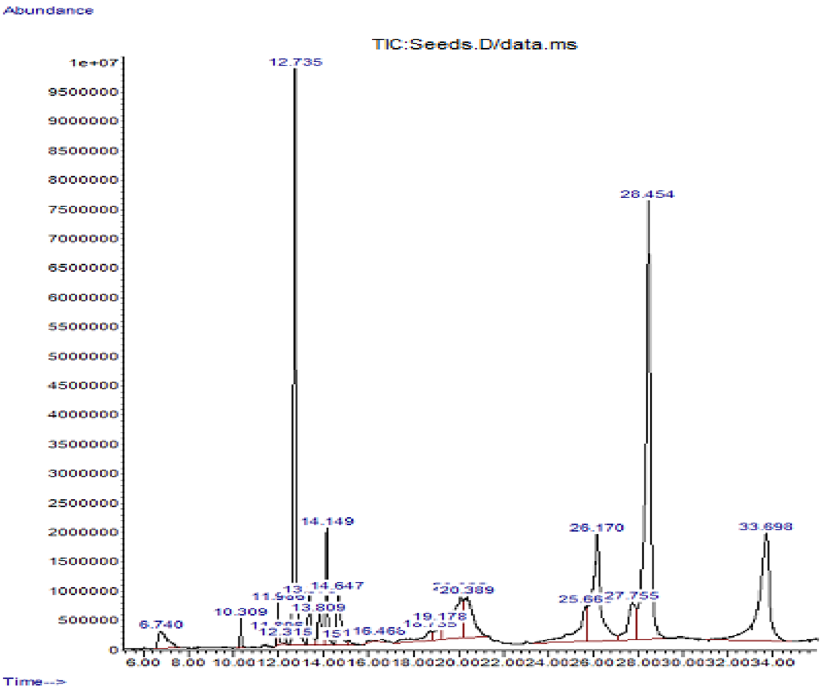

Figure 3: GC-MS Spectra of Aqueous Extract of *Aframomum melegueta* Seeds

Table 2: GC-MS Profile for the Aqueous Extract of *Aframomum Melegueta* Seeds

Retention Time	% Peak Area	Name of Compound	MW (g/mol)
6.7405	1.7713	Butan-2-one, 4-(3-hydroxy-2-methoxyphenyl)-	194.23
10.3088	0.401	Hexadecanoic acid, ethyl ester	284.48
11.896	0.1552	Linoleic acid ethyl ester	308.50
11.9863	0.6336	(E)-9-Octadecenoic acid ethyl ester	310.52
12.3155	0.0891	Octadecanoic acid, ethyl ester	312.53
12.7345	18.0957	3-Decanone, 1-(4-hydroxy-3-methoxyphenyl)-	278.39
13.3778	1.9625	1-(4-Hydroxy-3-methoxyphenyl)dec-4-en-3-one	276.37
13.8092	1.3485	1-(4-Hydroxy-3-methoxyphenyl)decane-3,5-dione	294.39
14.1491	2.6575	9-Octadecenoic acid, 12-hydroxy-, ethyl ester, [R-(Z)]-	326.51
14.6472	2.6474	5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)decan-3-one	294.39
15.141	0.1849	1-(4-Hydroxy-3-methoxyphenyl)dodecan-3-one	306.44

Retention Time	% Peak Area	Name of Compound	MW (g/mol)
16.0189	0.122	1-(4-Hydroxy-3-methoxyphenyl)dec-4-en-3-one	276.37
16.4682	0.2428	1-(4-Hydroxy-3-methoxyphenyl)dodecane-3,5-dione	322.45
18.7347	1.2518	Octadecane, 1-(ethenyloxy)-	296.54
19.1778	0.9639	Oleic Acid	282.47
20.0847	5.8917	2-Methyl-Z,Z-3,13-octadecadienol	280.49
20.3889	4.3081	Oleic Acid	282.46
25.6681	4.102	(R)-(-)-(Z)-14-Methyl-8-hexadecen-1-ol	254.46
26.1698	10.2378	13-Octadecenal, (Z)-	266.46
27.7555	3.5472	12-Oleanen-3-yl acetate, (3.alpha.)-	468.77
28.4536	26.0711	Urs-12-en-3-ol, acetate, (3.beta.)-	468.75
33.6976	13.3149	13-Octadecenal, (Z)-	266.46

MW= molecular weight

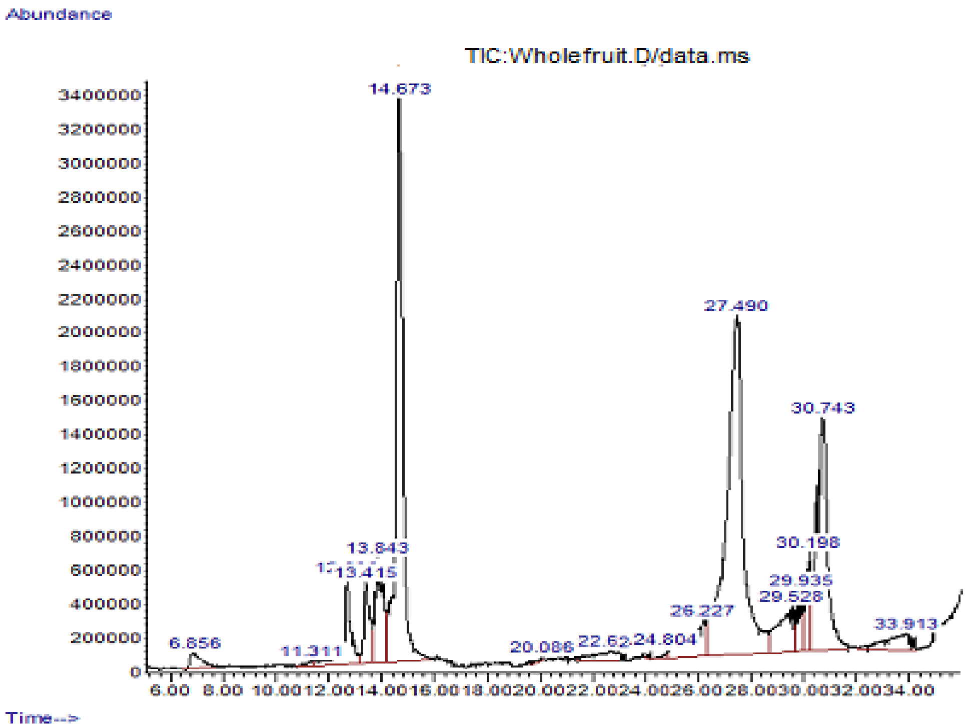

Figure 4: GC-MS Spectra of Aqueous Extract of *Aframomum melegueta* whole Fruit

Table 3: GC-MS Profile for *Aframomum Melegueta* Whole Fruit

Retention Time	% Peak Area	Name of Compound	MW (g/mol)
6.8558	1.0416	Butan-2-one, 4-(3-hydroxy-2-methoxyphenyl)-	**194.30**
11.3113	0.2098	Spiro[5.6]dodecane-1,7-dione	**194.27**
12.6896	3.5492	3-Decanone, 1-(4-hydroxy-3-methoxyphenyl)-	**278.39**
13.4149	2.9944	1-(4-Hydroxy-3-methoxyphenyl)dec-4-en-3-one	**276.37**
13.8425	4.7186	1-(4-Hydroxy-3-methoxyphenyl)decane-3,5-dione	**294.39**
14.6734	22.6264	5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)decan-3-one	**294.39**
20.0865	0.1453	Bicyclo[3.1.1]heptan-3-one, 2-(but-3-enyl)-6,6-dimethyl-	**178.27**
22.6756	1.3137	2-Methyl-Z,Z-3,13-octadecadienol	**280.59**
24.1875	0.083	Oleic Acid	**282.47**
24.8043	0.186	2-Methyl-Z,Z-3,13-octadecadienol	**280.59**
26.2268	3.811	Oleic Acid	**282.47**
27.4901	33.6479	2-Methyl-Z,Z-3,13-octadecadienol	**280.59**
29.528	4.1158	6-Octadecenoic acid, (Z)-	**282.47**
29.9349	1.8429	2-Methyl-Z,Z-3,13-octadecadienol	**280.59**
30.1975	2.1792	cis-11-Hexadecenal	**238.41**
30.7427	15.1618	n-Propyl 11-octadecenoate	**324.54**
33.9133	2.3733	9-Octadecenal, (Z)-	**266.46**

MW= molecular weight

The GC-MS profile of *A. melegueta* whole fruits eluted several compounds, highlighting four major compounds with the highest peak areas. 2-Methyl-Z, Z-3, 13-octadecadienol is a terpenoids that acts as a natural immune booster which may enhance mucosal defenses in fish via antioxidant activity (Godara *et al*., 2019). This compound is also useful in aquaculture as an insect repellant in controlling parasites such as fish lice or fly vectors near hatcheries (Buchmann, 2022). The implication of this is that it can serve as a functional feed additive or pond water treatment for disease prevention. 5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl) decan-3-one is a phenolic ketone (gingerol derivative) (NCBI, 2025). It is an anti-inflammatory and antioxidant (NCBI, 2025), thereby reducing oxidative stress and inflammatory responses in stressed or infected fish. As a growth promoter, it may improve appetite and nutrient uptake, thereby enhancing growth performance. As a stress modulator, this compound can help fish recover from transport, vaccination or pathogen challenge. The implication of this compound is that it is valuable for boosting innate immunity (NCBI, 2025), thereby reducing mortality during disease outbreaks and improving feed conversion ratios.

N-Propyl 11-octadecenoate is a waxy 18-carbon saturated fatty acid ester, meaning that it is formed by a fatty acid and an alcohol (Ohiri *et al*., 2023; NCBI, 2025). It has also been identified in some plant extracts such as in the methanolic extract of *Juncus maritimus* (Alamre *et al*., 2020) It is associated with antimicrobial and antifungal properties which suggests its potential use in disease prevention or as a feed supplement to enhance fish health in intensive culture systems (Onanuga and Oloyede, 2022). 1-(4-Hydroxy-3-methoxyphenyl) decane-3, 5-dione is a curcumin-like diketone, also known as gingerdione (a natural product found in *Zingiber officinale*) (NCBI, 2025). It is a compound with potential relevance due to its known antimicrobial, anti-parasite, immunostimulatory, anti-inflammatory, anti-oxidative and growth promoting properties, thereby suggesting that it could be explored as a treatment or preventative measure for bacterial infections in aquaculture (Mao, *et al*., 2019).

The six (6) bioactive compounds that were found in both seeds and the whole fruits of *A. melegueta* could be as a result of the seeds also been present in the whole fruits matrix indicating that the seed and whole fruit share common biosynthetic pathways for creating these specific compounds, leading to their presence in both. Agim *et al*. (2017) reported that thirteen (13) compounds were eluted and found present in the plant seed extract as against twenty-two (22) compounds that were found present in *A. melegueta* seeds and seventeen (17) compounds in the whole fruit in this study. Oladunmoye (2019) reported different organic compounds that was eluted from *A. melegueta* seeds after a broad analysis of five different solvents used (10% sodium hydroxide, methanol, ethanol, petroleum ether and n-hexane) and the result of the study revealed that organic compounds present in the extracts of *A. melegueta* depends on the type of extraction solvents used and that only few compounds were similar in all the extracts analyzed by the GC-MS.

CONCLUSION

Aframomum melegueta seeds and whole fruit contain different phytochemical compounds such as carbohydrates (13.94 and 13.94), phenolic compounds (5.18 and 6.80), flavonoids (5.64 and 4.15) and cardiac glycosides (3.07 and 3.07) amongst others. The gas chromatography-mass spectrometry (GC-MS) profile of the aqueous extracts of the seeds (22 compounds eluted) and whole fruit (17 compounds eluted) revealed several bioactive compounds which it possesses, with diverse bio-functional properties that have potent implications for fish health particularly with respect to growth and immune competence. Further studies on the use of *A. melegueta* seeds and whole fruit as potential phytobiotic additives and its effects on oxidative stress and immunocompetence in *Clarias gariepinus* is upcoming.

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