

CHARACTERIZATION OF ALLIGATOR PEPPER OIL

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Abstract

Aframomum melegueta seed is used as a spice in food due to their aromatic flavor and pungent taste or as ingredients of ethnomedical preparations for the treatment of snakebites, stomachaches, and diarrhea and other ailments. Oil from *Aframomum melegueta* seeds was analyzed for its phytochemicals, ant-repellent potentials and constituent chemicals. Oil was extracted from dry seeds and subjected to phytochemical screening, ant-repellent screening. The oil was also analyzed using Gas Chromatography-Mass Spectrometry. The oil was found to be rich in phytochemicals (saponins, tannins, steroids, glycosides, flavonoids and alkaloids). The oil also has repellent quality towards ants and mosquitoes. The GC-MS analysis of the oil showed some organic compounds (Octanol, Limonene, Methone, Menthol, Dihydrocarvone, Methylacetate, β -Bourbonene, Trans-caryo—phyllene, Piperitone, d20-BHT, β -Cubebene, BHT, Eicosane, Heneicosane, Docosane, Tricosane) that have use in the pharmaceuticals and the industries producing flavors in food and odor in perfumes.

Keywords: *Aframomum melegueta*, GC-MS analysis, Ant, Repellant, Phytochemical screening, oil

INTRODUCTION

Alligator pepper (*Aframomum melegueta*) 'grain of paradise', is a perennial plant native to western Africa, and its seeds are used as a spice in food due to their aromatic flavor and pungent taste or as ingredients of ethnomedical preparations for the treatment of snakebites, stomachaches, and diarrhea [1, 2,]. Antimicrobial, anti-inflammatory, anticancer, and antioxidant properties have been reported for alligator pepper [3]. Concerning seed volatile constituents, they showed repellent activity against adults of the maize weevil *Sitophilus zeamais* [4].

Sonibare [5] evaluated the antimicrobial, phytochemical properties of *Aframomum melegueta* and reported positive results for these medical conditions. **Lawal** [6] reported the potent effect on the blood pressure of normotensive and hypertensive patients.

Essential oils are naturally occurring substances with a broad spectrum of bioactivity because of the presence of several oils [7]. Essential oils have recently attracted particular attention as alternative pest control agents because of their specificity of action against insect pests, biodegradable nature, and potential for commercial application [8].

The chemical composition of seeds of *A. melegueta* has been well studied. A methanolic extract of the seeds was reported to contain gingerdione, paradol, shogaol as the major compounds. The seeds essential oils have been reported to consist of humulene, β -caryophyllene and their oxides as the major constituents. The supercritical CO₂ extracted essential oil of *A. melegueta* had been analysed by GC/MS. Forty-three components were detected and identified with the major components asparadol, shogaol, gingerdione, α -humulen, gingerol [9].

Pharmacological investigations have demonstrated that the seeds have anti-ulcer, antimicrobial and cytoprotection effects. Antibacterial and anti fungal effects result from 6-paradol and 6-shogol, compounds found in the seeds. The seeds of *A. melegueta* contain also gingerol, inhibitor of prostaglandins and leukotriens synthesis, justifying the anti-inflammatory effect of the plant. Aqueous extract of *A. melegueta* has aphrodisiac effect [10].

Previous studies had indicated that the extracts of the seeds are used to treat diarrhea and others gastro-intestinal disorders, snake bite and intestinal worms and pains. Traditionally, aqueous extract of the seeds is applied topically for abscesses and joint distortions [11].

Diomandé [12] gave the GC/MS analysis of Essential Oil of Five *Aframomum* species from Côte D'ivoire. The results of the analysis of volatile oils by GC/MS have showed 52 identified components with the total proportion ranged from 96.3 to 97.9%. The chemical composition of the oils from the leaves was dominated by hydrocarbon compounds such as -pinene, -caryophyllene, -humulene, -selinene, -selinene and germacrene A. Meanwhile, the rhizomes oil characterized with oxygenated components, namely eucalyptol, linalool and caryophyllene oxide accompanied by a few hydrocarbon constituents.

Tijjani [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%)

Tijjani [13] studied the effects of *Aframomum melegueta*, *Zingiberofficinale* and *Piper nigrum*. Some biochemical and haematological Parameters in rats fed with high lipid diet. The study

therefore revealed that aqueous extracts of *Aframomum melegueta*, can be used in weight management as well as in improvement of lipid profile.

Gbenga and Shakpo [14] studied the antimicrobial activity and physical characteristics of oil extracted from alligator pepper seed (*Aframomum melegueta*) cultivated in Owo, Ondo State, Nigeria. Antimicrobial activity of oil from alligator pepper (*Aframomum melegueta*) was tested against five pathogenic organisms, *Klebsiella pneumonia*, *Salmonella typhi*., *Bacillus cereus*, *Escherichia coli* and *Staphylococcus aureus*.

Inegbenebor and Ebomoyi [15] studied the effect of saline extract of Alligator Pepper (*Aframomum Melegueta*) on Serum Progesterone in Pregnant Sprague Dawley Rats. Results showed that progesterone level decreased significantly on days 7 and 21 of pregnancy among rats administered with 6.7 mg/kg body weight, but day 7, only those treated with 13.3 mg/kg body weight of saline extract of Alligator pepper.

Owokotomo [16] studied the chemical constituents of the leaf, stem, root and seed essential oils of *Aframomum melegueta* by GC-MS. **Angaye and Inengite** [17] gave the spectral and antimicrobial studies of alligator pepper oil.

Omoboyowa [18] studied the effects of methanol seed extract of *Aframomum melegueta* (Alligator Pepper) on Wistar Rats with 2,4-Dinitrophenylhydrazineinduced Hemolytic Anemia.

The aim of the study was to assess the medicinal and insect-repellent characteristics of oil extracted from alligator pepper. The objectives of the study are; extraction of oil from *A. melegueta* using organic solvent, analyses of the oil using GC/MS, and assessing the efficacy of the oil in controlling mosquitoes and ants.

MATERIALS AND METHOD

Reagents used

Hydrochloric acid, sulphuric acid, olive oil, ethyl acetate, ferric Chloride, potassium hydroxide, glacier acetic acid, Mayers reagent, chloroform, n-hexane, HP 5890 Series II GC with Electronic Pressure Control, interfaced to a HP 5989 Mass Spectrometer.

Sample

The seeds for this study (*Aframomum melegueta*) were bought in Amassoma, Bayelsa State, Nigeria. The outer coats of the seeds were removed, the seeds dried to constant weight, ground to powder, and extracted with n-hexane.

METHODS

Oil Extraction Procedure

100 g of the powdered sample was put into an air tight extraction bottle and 200 cm³ of n-hexane as extracting solvent was used to soak the sample for 72 hours after which it was decanted. The oil was obtained after evaporation using Water bath at 70°C to remove the excess solvent from the extracted oil. The oil was then stored in refrigerator prior to the analysis proper.

Phytochemical Screening

Basic qualitative phytochemical screening of *Aframomum melegueta* seed oil was carried out by testing the presence or absence of the following plant constituents: flavonoids, tannins, saponins, glycosides, alkaloids and steroids by using the methods outline by **Trease and Evans** [19].

Test for Saponins

0.5 g of oil extract was added to 5 mL of distilled water in a test tube and the solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3

drops of olive oil and agitated vigorously after which it was observed for the formation of an emulsion.

Test for Tannins

About 0.5 g of the oil extract was boiled in 10 mL of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and the solution observed for brownish green or a blue-black colouration.

Test for Steroids

0.5 g of the oil extract was dissolved in 10 mL of chloroform and equal volume of concentrated H_2SO_4 was added by the sides of the test tubes. Reddish upper layer and yellowish sulphuric acid layer with green fluorescence indicate the presence of steroids.

Test for Cardiac Glycosides (Keller-Killiani Test)

0.5 g of oil extract was dissolved in 5 mL water and 2 mL of glacial acetic acid solution containing one drop of ferric chloride solution was also added. This was underlayered with 1 mL of concentrated H_2SO_4 . A brown ring at the interface indicated the presence of a deoxysugar characteristics of cardenolides. A violet ring may appear below the brown ring while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Test for Flavonoids

A portion of the extract was heated with 10 mL of ethyl acetate over a steam bath for 3 minutes, the mixture was filtered and 4 mL of the filtrate was shaken with 1mL of dilute ammonia solution. A yellow colouration indicates the presence of flavonoids.

Test for Alkaloids

Extracts were dissolved individually in dilute HCl and filtered. Filtrates were treated with Mayer`s reagent (potassium mercuric iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Ant Repellent Test

Sugar ants were collected in a transparent plastic container (with a lid to prevent the ants from escaping) containing sugar particles. 3 portions of cotton wool were spiked with alligator pepper oil and kept inside the container in three positions. It was allowed to stand for 30 minutes and this set up was observed for 30 min.

Mosquito Repellent Test

The extracted *Aframomum melegueta* seed oil was sprinkled on a white plain paper and lit in a mosquito-infested room. Three volunteered persons went into the room and stayed for 30 min. The experiment was repeated without addition of *Aframomum melegueta* seed oil, as the control and observations were made.

GC-MS analysis

The analysis was done with the following analytical conditions. The GC injection port temperature was at 250 °C and the GC/MS transfer line was at 250 °C. A SGE BPX 35 GC capillary column was used with the following dimensions: 0.25 mm x 0.25 µ x 60 m. The

analytical process was programmed with an initial temp of 30 °C for 5 min, a 6 °C min⁻¹ ramp to 260 °C and a final temp of 260 °C for 5 min. Carrier gas (He) flow was 0.90 mL per min.

RESULTS AND DISCUSSION

This section presents results of chemical analyses of oil from *Aframomum melegueta* seed. Table 1 presents data of the phytochemical analysis and Table 2 presents the organic components of the oil under investigation. The ant-repellant activities of the oil was also investigated and the results are presented in Figure 1.

Table 1. Phytochemical analytical results of *Aframomum melegueta* seed oil

Alkaloids	Saponins	Flavonoids	Tannins	Glycosides	Steroid
++	++	+++	++	—	++

+, ++, and +++ indicate low, moderate, and high concentrations respectively

Many common plant-based foods and herbs contain powerful phytochemical substances that can improve the quality of our health. Phytochemicals protect us against many diet related diseases [20]. Results of the phytochemical screening of *Aframomum melegueta* seed oil showed the absence of glycosides while saponins, tannins, alkaloids, steroids were present in moderate quantity. The test showed high quantity of flavonoids. These phytochemicals exhibit various pharmacological and biochemical actions when ingested by animals [20]. Plants used in the treatment of diseases are said to contain bioactive components with biological activity some of which are responsible for the characteristic odor, pungencies and color of plant, while others give the particular plant its culinary, medicinal or poisonous virtue [19]. The qualitative phytochemical screening of *Annonamuricata* was in agreement with the works of [21].

It has been reported that flavonoids and phenolics are free radical scavengers that prevent oxidative cell damage, and have strong anticancer activities [22, 23] and they might induce mechanism that affect cancer cells and inhibit tumor invasion [24]. These activities could be attributed to their ability to neutralize and quench free radicals [23, 22, 25]. It can also be due to their redox properties, presence of conjugated ring structures and carboxylic group which have been reported to inhibit lipid peroxidation [26].

Herbs that have tannins as their component are astringent in nature and are used for the treatment of intestinal disorders such as diarrhoea and dysentery [27], thus supporting the reasons why *Aframomum melegueta* seed has position among medicinal plants used for the treatment of microbial infection. Tannins are known to be useful for the prevention of cancer as well as treatment of inflamed or ulcerated tissues [28].

Alkaloids are beneficial chemicals to plants serving as repellent to predators and parasites. This probably endows these group of agents its antimicrobial activity. Several alkaloid containing medicinal plants are reported to have been used by the early man as pain relievers, as recreational stimulants or in religious ceremonies to enter a psychological state to achieve communication with ancestors or God [29]. Saponins are believed to react with the cholesterol rich membranes of cancer cells, thereby limiting their growth and viability [29]. Saponins have the property of precipitating and coagulating red blood cells [30]. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness [31]. Saponins in medicinal plants are responsible for most biological effects related to cell growth and division in humans and have inhibitory effect on inflammation [32].

The chemical components present in the oil extracted from *Aframomum melegueta* seed are shown in Table 2; they are mainly organic compounds with functional group ranging from complex alkane, alkenes, alcohols, ketones, aldehydes and esters. The major compounds are natural chemical products having isoprene units as the mode of classification, i.e. monoterpenes. The presence of these compounds could be arranged in decreasing order of Carvone (13.47%) > Menthol (11.88%) > methone (9.07%) > Butylated hydro toluene (BHT) (8.83%) > β -Cubebene (7.81%) > d20-BHT (7.11%), others are of lesser weights. Menthol and methone are used in flavoring, perfume and cosmetics because of their characteristic aromatic and minty odor. D-Carvone is present in the seeds of caraway (*Carumcarvil.*), in the aerial parts and the seeds of dill plants (*AnethumgraveolensL.*), in the oil from the leaves and flowers of *Chrysanthemum indicum L.* (Compositae), mandarin peel oil (*Citrus reticulataBlanco*, Rutaceae), ginger-grass oil, kuromoji oil, lavender oil, and many other essential oils [33]. D-Carvone may be used as food flavouring, feed flavouring, in feed additive, in personal care products, as (veterinary) medicine [34], natural insect repellent and in cosmetics.

Table 2. Organic components in *Aframomum melegueta* seed oil

Components	% Weight
Octanol	1.07
Limonene	3.89
Methone	9.07
Menthol	11.88
Dihydrocarvone	3.91
Carvone	13.47
Methylacetate	6.39
β -Bourbonene	7.93
Trans-caryo—phyllene	6.31
Piperitone	1.28
d20-BHT	7.11
β -Cubebene	7.81
BHT	8.83
Eicosane	1.25
Heneicosane	3.28
Docosane	4.39
Tricosane	2.08

Aframomum melegueta seed oil showed great repellence effect towards ants as the sugar ants were repelled immediately the oil-spiked cotton wool was introduced into the container as seen in Figure 2. The repellent test of *Aframomum melegueta seed* oil on mosquitoes was moderate as mosquito bites and buzzing were experienced after 10-15 min. The control showed no repellent activity on mosquitoes.



Figure 1. Ant-repellent activity of oil from *Aframomum melegueta* seed

CONCLUSION

Aframomum melegueta seed oil is rich in phytochemicals (saponins, tannins, steroids, glycosides, flavonoids and alkaloids) suggestive of its application in medicine. The oil also has repellent quality towards ants and mosquitoes. The GC-MS analysis of the oil showed some organic compounds (Octanol, Limonene, Methone, Menthol, Dihydrocarvone, Methylacetate, β - Bourbonene, Trans-caryo—phyllene , Piperitone, d20-BHT, β -Cubebene, BHT, Eicosane, Heneicosane, Docosane, Tricosane) that have use in the pharmaceuticals and the industries producing flavors in food and odor in perfumes.

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