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Quantitative Analysis of Phytochemicals Constituent of Melegueta Pepper Seed

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Abstract:- The *Aframomum melegueta* seed is a spice, commonly used in the management of various health conditions. The quantitative phytochemicals constituent of the extract was evaluated in this study. The seeds used in this study were obtained from the New Market (*Oja-tuntun)* in Ilorin. The healthy seeds were hand-picked and small-grained. Their quantitative phytochemicals and composition were evaluated using a Spectrophotometer and GC-MS. The study showed that the seeds extract had phenolic content (77.94 ± 0.83 mg/g), tannin (73.52 ± 0.79 mg/g), flavonoid (23.41 ± 0.65 mg/g) and saponin (38.29 ± 0.65 mg/g). The phytochemicals composition also revealed the presence of eleven bioactive compounds among which are Humulene (7.54% and 25.104 Retention Time), Dihydrojasmone (1.38% and 39.203 Retention Time) and Caryophylene (3.66% and 24.040 Retention Time). Other phytochemicals present include, 3-Decanone, 1-(4-hydroxy-3-methoxy-phenyl) (31.30% and 39.053 Retention Time) which happens to be the best proportion space and 3-Pyridineacetic acid (1.01% and 40.117 Retention Time) which has the smallest proportion space. Thus, this study justifies that the seed is an important source of phytochemical that may contribute in management of different disease and health conditions.

Keywords:- *Phytochemical, Retention Time, Spectroscopy.*

I. INTRODUCTION

Medicinal plants are widely distributed to immense pharmaceutical significance and contribute in the maintenance of various health conditions. It was reported that 80% of the global populace rely on traditional medicine (Dhanalakshmi and Manavalan, 2014).The composition of medicinal plants is a source of different structural compounds which will help in the creation of artificial medicine (Cowan, 1999).

The less side effect and low costs of natural products obtain from the plant has focused on metabolic disorder which enhance its contribution in management of malady conditions (Dhanalakshmi and Manavalan, 2014). Savithramma *et al.* (2010) reported that medicines are prepared by traditional practitioner by combining appropriate plant parts. Its biological efficiency depends on the amount and quality of phytochemicals in the extract. It was reported that secondary metabolites have immensely contribute to thepharmacological potentials and also enhance in management of ailments (Aja *et al*. 2010).

Aframomum melegueta is a cosmopolitan plant employed in Federal Republic of Nigeria as a spice in foods. Its locally called atare in Yoruba and 'ose-oji in Igbo and as well (Tijani and Luka, 2013).Inegbenebor *et al*. (2009) reported its aqueous extract to cut back physiological state weight in pregnant rats. It is able to alleviate stomach ache diarrhea and polygenic disease (Ilic *et al*., 2010). The quantitative phytochemicals screening constituents of the extract were not determined. Hence, this study aims to quantify and determine the phytochemicals constituent of *Aframomum melegueta.*

II. MATERIALS AND METHODS

➢ *Materials*

Folin–Ciocalteu reagent, gallic acid, Folin-Denis reagent, tannic acid, quercetin, 1,10-phenanthroline, methanol and UV/Visible spectrophotometer.

➢ *Collection and Identification of Seeds*

The *Aframomum melegueta* seeds were bought from the New Market (*Oja-Tuntun)*, Ilorin, Kwara State, Nigeria. The seeds were deposited and authenticated at the herbarium, Federal University of Ilorin with voucher number (UILH/001/1166).

➢ *Preparation of Extracts*

The *Aframomum melegueta* seeds were air dried and grounded to a powdery form. A portion of 100g was weighed and packed into a beaker and extracted with 70% v/v methanol for 24h. The methanolic extracts were then dehydrated in the oven at 45^{o}C. The dehydrated extract was kept in refrigerator during the experiment.

➢ *Determination of Percentage Yield of the Extracts*

The percentage yield of extract was expressed as follows: Where W_1represents the weight of extract and empty beaker and W_2 is the weight of concentrated extract and beaker weight of sample.

$$\text{Yield (\%)} = \frac{W_2 - W_1}{\textbf{Weight of sample}} \times 100$$

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III. QUANTITATIVE SCREENING OF PHYTOCHEMICAL

➢ *Phenol Content Determination*

The phenol composition of the methanolic extract was determined used the method described by Chan *et al*. (2007). 300µL of the extract was dispensed in a test tube, 1.5 mL of Folin–Ciocalteu reagent, and 1.2 mL of 7.5% sodium bicarbonate solution was added respectively. It is allowed to stand for 30mins at 37 ºC, the absorbance was measured in a UV/Visible spectrophotometer at 765nm against a blank.

➢ *Tannin Content Determination*

To get the tannin content of the samples, the procedure described by Padmaja (1989) was used. 0.1mL the sample was dispensed in a test tube , 7.5mL of distilled water, 0.5mL of Folin-Denis reagent, 1mL of 35% sodium carbonate solution and diluted to 10mL with distilled water were added respectively. It is allowed to stand for 30mins at 37 ºC, and then the absorbance was measured in a UV/Visible spectrophotometer at 760nm against a blank.

➢ *Flavonoid Content Determination*

To get the flavonoid content of the samples, the procedure described by Kale *et al*.(2010) was used. A portion of 0.5mL of sample was poured into a test tube, followed by 1.5mL of methanol, 0.1mL of aluminium chloride (10%), 0.1mL of 1M potassium acetate and 2.8mL of distilled water was added respectively. The reaction mixture was shaken, allowed to stand at room temperature for 30mins, and then the absorbance was measured in a UV/Visible spectrophotometer at 514 nm against a blank.

➢ *Saponin Content Determination*

The saponin content was determined using the process described by Makkar *et al*. (2007). An aliquot 0.25mL of extract was dispensed in a test tube along with 0.25mL vanillin reagent (8% vanillin in ethanol) and 2.5mL of 72% aqueous Tetraoxosulphate (VI) acid (H_2SO_4). The tube was subjected to heat in a water bath at 60°C for 10min. The tube was cooled in ice for 4 min and allowed to acclimatize to room temperature and then the absorbance was measured in a UV/Visible spectrophotometer at 544 nm.

➢ *Gas Chromatography Coupled with Mass Spectrometer (GC-MS) Analysis*

1µL of extract was injected into the SE 30 column with split mode of (10:1). Nitrogen gas was used as a carrier at rate of 0.8 mL/min and the total execution of 24min. The information was compare with database of National Institute Standard and Technology MS library (NIST-MS library) for identification of unknown bioactive constituents.

IV. RESULT

Samples	Extraction Solvent	Seed Powder(g)	Extracted Seed Powder(g)	Yield (%)
Aframomum melegueta	Methanol	100	2.44	2.44

Table 1:- Percentage yield of the extract

Samples	Phenolic (mg/g)	Flavonoids (mg/g)	Saponin (mg/g)	Tannin (mg/g)
Aframomum melegueta	77.94 ± 0.83	23.41±0.65	38.29 ±0.65	73.52 ± 0.79

Table 2:- Quantitative Phytochemicals Screening
Mean ± standard deviation

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Peak Number	RT	Compound Name	Area %
1	24.040	Caryophyllene	3.66
2	25.104	Humulene	7.54
3	39.053	3-Decanone, 1-(4-hydroxy-3-methoxy phenyl)-	31.30
4	39.203	Dihydrojasmone	1.38
5	39.303	2H-Pyran-2-one, 5,6-dihydro-6-[2-(3-hydroxy-4methoxyphenyl)ethyl]-4-methoxy-, (S)-	2.08
6	39.366	6-(3,5-Dimethyl-furan-2-yl)-6-methyl-hept-3-en-2-one	21.70
7	39.572	Propan-2-one, 1-(4-isopropoxy-3-methoxyphenyl)-	16.29
8	39.854	2-Butanone, 4-(4-hydroxy-3-methoxy phenyl)-	11.18
9	40.117	3-Pyridineacetic acid	1.01
10	40.486	Phenol, 4-ethyl-2-methoxy-	2.76
11	40.761	Dihydrocapsaicin	1.01

Table 3:- GC-MS Analysis of Bioactive Compounds
Key: RT = Retention Time

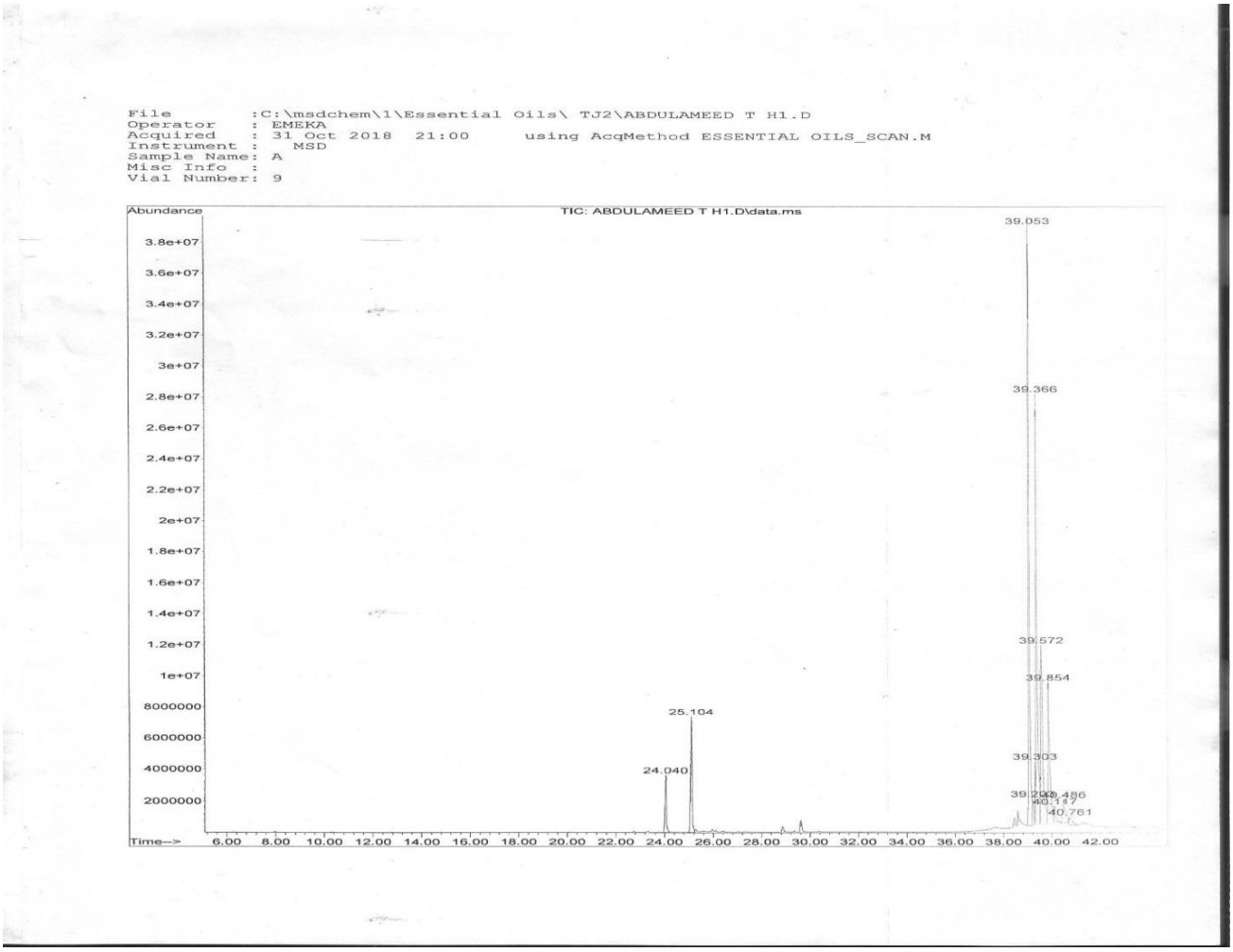

Fig 1:- Chromatogram of *Aframomum melegueta* seed using GC-MS analysis

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V. DISCUSSION

The benefits of healthful plants in health and also their concomitant effect cannot be undervalued. Phytochemicals are mainly responsible for the plant pharmacological potentials and also facilitate the incorporation in ethno-pharmacology in prevention of metabolic disorder. For example, phenolics, flavonoids, tannins and saponins have been associated with their restriction regarding pain reliever markers like leukotrienes (Edeoga *et al*., 2005). The phenolics content are also attributed to its anti-inflammatory properties, antioxidant and anticancer activity by exhibit free radical scavenging property (Njoku and Akumefula, 2007). The presences of tannins in Guinea grains seed at an awfully high concentration enhance its use in management and treatment of ulcers condition (Echo *et al*., 2012). Saponins has outstanding antiprotozoal activity, anti-carcinogenic properties and other health benefits which have reported by (Adesokan and Akanji, 2010).

In this study the phytochemical constituents of medicinal seed, were evaluated. The quantitative phytochemical analysis reveals the presence of the following products such as flavonoids, phenolics, saponins, and tannins. The phenolic content was significantly high when compared to other phytochemical and this present result was similar to Ukpabi *et al*. (2012) which had considerably high amounts of polyphenols. Fajobi *et al*. (2017) documented high considerable amount of phenolics and flavonoids content when compared with the present result of *Piper guineense* seed.

Mahmood *et al*. (2008) observed that factors just like the age, sort of material and solvent used might have an effect on percentage extract yield. Variation in active ingredients may be genetic or environmental. Damilola*et al*. (2017) reported that methanolic seed extract of *Aframomum melegueta* was observed to have a percentage yield of 2.60% which was similar to the present result. Stephen *et al*. (2014) reported 7.94% for the methanolic seed extract of *Aframomum* meleguetawhichwas high compared to the present result. Ogbonna*et al*. (2013) reported 13.5% for the extract of *P. guineense*which was significantly different with the present result.

The phytochemicals composition disclosed the presence of eleven (11) bioactive compounds among which are Humulene (7.54% with 25.104 Retention Time), Dihydrojasmone (1.38% with 39.203 Retention Time) and Caryophylene (3.66% with 24.040 Retention Time).Other phytochemicals present include, 3-Decanone, 1-(4-hydroxy-3-methoxy-phenyl) (31.30% with 39.053 Retention Time) which happens to be the best proportion space and 3-Pyridineacetic acid (1.01% with 40.117 Retention Time) which has the smallest proportion space.

Passos *et al*. (2007) reported that Humulene is a monocyclic sesquiterpene ($C_{15}H_{24}$) which exhibits anti-inflammatory properties and decreasing the skin allergy caused by allergen intake. According to Fernandes *et al*. (2007) the mechanism involves inhibition of cancer makers include tumor necrosis factor-α (TNFα), interleukin-1 β (IL1B) generation and as well having anti-inflammatory characteristics (Joshua *et al.*, 2016).

Caryophyllene is associated in nursinganti-fungal (Cakir*et al*., 2004). Its mechanism of action was shown to be agonist against cannabinoid receptor type-2 (CB_2) and as well have anti-inflammatory effects in mice (Gertsch*et al*., 2008). Neuro-protective, anxiolytic, antidepressant and anti-alcoholism (Bah *et al*., 2014). Gingerols (2-Butanone, 4-(4-hydroxy-3-methoxy phenyl)-) have been reported to possess low blood sugar impact and anti-inflammatory activities (Jolad*et al*., 2005).

3-Decanone, 1-(4-hydroxy-3-methoxy phenyl)- is the active ingredient of the floral seeds of guinea pepper.The paradol was recognized to possess anti-oxidative property and growth promotion effects(Yanna and Shmuel, 2004). 6-(3,5-Dimethyl-furan-2-yl)-6-methyl-hept-3-en-2-one is a campesterol compound and is assumed to possess anti-inflammatory effects. It is known to inhibit many pro-inflammatory and matrix degradation mediators that are concerned in animal tissue (Gabay*et al*., 2010).

VI. CONCLUSION

The quantitative phytochemical analysis revealed eleven (11) bioactive compounds identified from *Aframomum melegueta* seed extract and this establishes the beneficiary attribute which contribute immensely in metabolic disorder. The structural elucidation of 3-Decanone, 1-(4-hydroxy-3-methoxy phenyl)- as a novel compound and subjecting it to biological activities can be recommended for further study.

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