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Original Contribution

TERPENOID CONSTITUENTS' CONCENTRATION OF *AFRAMOMUM MELEGUETA* USING HYDRODISTILLATION AND SOLVENT METHODS OF EXTRACTION

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ABSTRACT
Terpenoid constituents' concentration of *Aframomum melegueta* using hydrodistillation and solvent methods of extraction were carried out using Gas Chromatography (GC) . Forty seven compounds were detected in each method with α-cardinol(15.75%), cis-calamenen-10-ol(7.76%) β-panasinsene(6.37%) caryophylene oxide (6.85%), α-muurolol (5.70%) and cubenol (5.53%) as major constituents in hydrodistillation method and α-cardinol (21.79%) , α-muurolol (9.56%) , cis-calamenen-10-ol (4.62%), β-panasinsene(4.52%), α-ylangene (3.04%) and γ-cadinene (3.14%) as major terpenoids for solvent extraction method. The result showed that the yield and concentration of compounds obtained depend on extraction method, with solvent extraction having higher yield and higher concentrations of the terpenoids.

Key words: Terpenoids, concentration, *Aframomum melegueta*, hydrodistillation and extraction method.

INTRODUCTION

Aframomum melegueta is a species in the ginger family, zingiberaceae. This spice is commonly known as "grains of paradise, melegueta pepper, alligator pepper, guinea grains or guinea pepper". It is obtained from the plant's ground seeds, it gives a pungent, peppery flavor. It is a native of West Africa but is an important cash crop in the Basket special words of Southern Ethiopia (1). In West African folk medicine, grains of paradise are valued for their warming and digestive properties among Efik people in Nigeria, also is being used for divination and ordeals determination (2).

Due to wide occurrence of terpenoids in nature, all terpenoids could not be isolated from plants by a general method. However, mono- and sesqui-terpenoids have a common source and therefore, their isolation has been generalized. Generally, isolation of essential oils can be carried out using the following methods: cold pressing or scarification, which is used to extract citrus, lemon and grass oil; effleurage for plants such as jasmine or tuberose which have very low contents of essential oil; steam distillation and solvent extraction methods (3).

The two methods, steam distillation or hydrodistillation and solvent extraction are the most popular methods of isolation of essential oils. These two methods have their merits and demerits. Steam distillation is used for isolation of oil with high yields but some terpenoids undergo decomposition. Also, some constituents of the terpenoids like esters, which are responsible for the odour and fragrance of the oil may undergo decomposition, resulting in inferior quality odour. Solvent extraction- is used to isolate essential oils from plants which yield low quality oil on steam distillation due to decomposition. However, the solvent will extract compounds that are not essential oils

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like lipids, ethers, esters, etc. These can cause an increase in yield and also affect its chemical properties. In this work, we have investigated the effects of isolation methods (hydrodistillation and solvent methods) on the chemical composition of the oil of *Aframomum melegueta* found in Ilasa- Ekiti, Nigeria.

MATERIALS AND METHODS

Sample collection and identification

Aframomum Melegueta used for this work was collected from Ilasa-Ekiti in Ekiti State, Nigeria in the month of December, 2009. The sample was identified by Dr. Onovo, J of the Department of Plant Science and Biotechnology of Nasarawa State University, Keffi. Nigeria

Extraction of the essential oils

The sample was grounded into powdery form and 150g of it was subjected to hydrodistillation for 5 hours using all glass Clevenger apparatus. The oils obtained were dried over anhydrous sodium sulfate and transferred into sample bottles and stored in the refrigerator until they were ready for analysis. Also petroleum ether (40-60^{o}C) was used to extract the oil from the sample using Soxhlet extractor.

Identification of the Component

The identification of the chemical constituents was carried out at Multi-Environmental Management Consturtium Limited, Lagos using Gas Chromatographic (GC) separation technique by direct injection method in the split mode under the following conditions; Hewlett-Packard 6890 GC equipped with a flame ionization detector (FID) and a quartz capillary column; 30m x 0.25mm x 0.25µm; Hydrogen as a carrier gas at 1.3ml/min flow rate; oven temperature 40^{o}C (initial) ramped at 50 ^{o}C/min to 200 ^{o}C and ran at 200^{o}C for 2 mins, injector temperature of 300^{o} C; ionization energy of 70 electron volt. A digital integrator was used to integrate the area of the signal from the detector. The integrated area, retention time and percentage composition were printed automatically at the end of each peak. The qualitative identification of different constituents was performed by the comparison of their retention times and mass spectra with those of the library.

RESULTS AND DISCUSSION

Tables 1 and 2 show the identified chemical constituents of *Aframomum melegueta* using hydrodistillation and solvent extraction methods respectively. Forty-six components were identified. The oils were rich in monoterpenoid and sesquiterpinoids with α-cadinol (15.75%), cis-calamenenol (7.76%) ,β-panasinsene (6.37%), caryophyllene (6.85%), α-muurolol (5.70%) and cubenol (5.53%) as major constituents in hydrodistillation method of isolation and α-cardinol (21.79%), α-muurolol (9.5%), cis-calamenenol (4.62%), β-panasinsene (4.52%), α-ylengene (3.04%) and γ-cadinene (3.14%) as major terpenoids for solvent extraction method. The two most abundant compounds were α-cardinol and cis-calamenenol and these accounted for 23.55% in hydrodistillation. For solvent extraction, α-cardinol and muurolol accounted for 31.35%. When considering ten most abundant compounds it was discovered that apart from these first, all the other eight compounds i.e calamenenol, caryophyllene, β-panasinsene, cubenol, γ-cadinol, spathulenol , α-ylangene and 2-nerolidol acetate were more abundant in hydrodistillation than in the solvent extraction methods as shown in tables 1 and 2; calamenenol (7.76% vs., 4.62%), caryophyllene oxide (6.85% vs., 1.44%), β-panasinsene (6.37% vs., 4.53%) , cubenol (5.53% vs., 1.80%), cadinene (4.84% vs., 3.15%) , cpathulenol (4.17%vs., 2.27%), α-ylangene (3.64% vs., 3.04%) and 2-nerolidol acetate (3.20% vs., 1.76%) respectively for hydrodistillation and solvent extraction methods.

Earlier work on this plant by Ajaiyeoba and Ekundayo (1999) (4) using hydrodisllation showed that 27 compounds were obtained with humulene and caryophyllene which accounted for 82.6% of the volatile oils.

However, from the current work, humulene was not found and caryophyllene concentration found was low. More compounds were discovered in this recent work (forty seven compounds) compared to seventeen found in Ajaiyieoba and Ekundayo (1999) (4). The observed differences may be due to geographical origin (5), genetic factors, culture conditions and environment (6), crops and past crop processing (7), and different changes as nutrition status of the plant as well as other factors that can influence the oil composition.

Table 1. *Identified chemical constituents in essential oil of Aframomum melegueta using hyrodistillation.*

s/n	Compound	Concentration(%)
1.	Isoamyl acetate	0.53
2.	Tricyclene	0.53
3.	Camphene	0.53
4.	Sabinene	0.68
5.	Limonene	0.69
6.	α-pinene	0.68
7.	β-pinene	0.53
8.	Benzyl alcohol	1.28
9.	Cis ocimene	0.59
10.	Myrcene	0.45
11.	Allo ocimene	0.52
12.	α-Thujene	0.59
13.	γ-Terpinene	1.38
14.	Fenchone	1.24
15.	Neral	1.14
16.	Geranial	0.79
17.	Isoartemisia	0.53
18.	1,8 – Cineole	0.28
19.	Nerol	0.75
20.	Linalool	0.23
21.	N – Nonanal	0.07
22.	α- Terpineol	0.58
23.	α -Ylangene	3.64
24.	α- Bourbonene	1.59
25.	β- Panasinsene	6.37
26.	α- Neo – Clovene	0.97
27.	Sesquisabinene	1.27
28.	γ-Himachalene	0.53
29.	α- Caryophyllene	0.53
30.	γ- Cadinene	4.84
31.	Trans – Calamenene	1.00
32.	Cis Myrtanyle Isobutyrate	1.07
33.	Germacrene B	2.22
34.	Germacrene D	1.21
35.	Perillyle Isobutyrate	1.01
36.	Spathulenol	4.17
37.	Caryophyllene Oxide	6.85
38.	Elemicin	0.27
39.	B-Copaen – 4 α – ol	0.85
40.	Cubenol	5.53
41.	Epi – α – Cadinol	2.26
42.	Epi – α-Muurolol	2.18
43.	α – Muurolol	5.71
44.	α – Cadinol	15.78
45.	Selin – 11 – En – 4 – α – ol	1.50
46.	Cis- Calamenen- 10 – ol	7.76
47.	2 – Nerolidol Acetate	3.20

Table 2. *Identified chemical constituents in essential oil of Aframomum melegueta using solvent.*

s/n	Compound	Concentration(%)
1.	Isoamyl acetate	0.78
2.	Tricyclene	0.77
3.	Camphene	0.76
4.	Sabinene	0.99
5.	Limonene	1.00
6.	α-pinene	0.99
7.	β-pinene	0.78
8.	Benzyl alcohol	1.86
9.	Cis ocimene	0.66
10.	Myrcene	0.65
11.	Allo ocimene	0.75
12.	α-Thujene	0.86
13.	γ-Terpinene	2.02
14.	Fenchone	1.81
15.	Neral	1.58
16.	Geranial	1.11
17.	Isoartemisia	0.77
18.	1,8 – Cineole	1.86
19.	Nerol	1.08
20.	Linalool	0.33
21.	N – Nonanal	1.57
22.	α- Terpineol	0.85
23.	α -Ylangene	3.04
24.	α- Bourbonene	1.53
25.	β- Panasinsene	4.52
26.	α- Neo – Clovene	1.41
27.	Sesquisabinene	1.83
28.	γ-Himachalene	2.30
29.	α- Caryophyllene	0.74
30.	γ- Cadinene	3.15
31.	Trans – Calamenene	1.05
32.	Cis Myrtanyle Isobutyrate	1.57
33.	Germacrene B	1.06
34.	Germacrene D	1.77
35.	Perillyle Isobutyrate	1.47
36.	Spathulenol	2.27
37.	Caryophyllene Oxide	1.44
38.	Elemicin	0.38
39.	β-Copaen – 4- α – ol	1.24
40.	Cubenol	1.80
41.	Epi – α – Cadinol	1.19
42.	Epi – α-Muurolol	2.52
43.	α – Muurolol	9.56
44.	Selin – 11 – En – 4 – α – ol	2.00
45.	α – Cadinol	21.79
46.	Cis- Calamenen- 10 – ol	4.62
47.	2 – Nerolidol Acetate	1.77

Moreover, it was observed that alkanol derivative of the terpenoids found in this seed accounted for about 45% of the total compounds in the oil using hydrodistilation and solvent extraction methods. This high percentage of alkanol may be responsible for diplopia and blurred vision in haealthy Igbo men as reported by lgwe *et al.* (1999) (8) and loss of appetite, in rats as reported by lnegbenebor *et al* (2009) (1).

CONCLUSION

It can be concluded that the methods of extraction of essential oil affects the concentration of terpenoids: It was observed that solvent extraction method achieved higher concentration and yield of terpinoids than the hydrodistilation method.

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