



Volatile constituents of hydrocolloids isolated from *Afzelia africana* and *Detarium microcarpum* seeds

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ARTICLE INFO

Article type:

Research article

Article history:

Received March 2017

Accepted May 2017

October 2017 Issue

Keywords:

Afzelia africana

Detarium microcarpum

GC/MS analysis

Hydrocolloids

Seed endosperms

ABSTRACT

The seed endosperms of *Afzelia africana* and *Detarium microcarpum* are known materials for soup thickening in Southeast and some other parts of Nigeria. The hydrocolloids responsible for the thickening effect were isolated from the seed endosperms and characterised using GC-MC technique. The volatile fraction of *A. africana* seed hydrocolloids showed the presence of seventeen constituents consisting of aromatic (0.80 %), hydrocarbons (7.67 %), phenolic (0.39 %), esters (10.19 %), fatty acids (36.35 %), alcohols (42.24 %) and steroid (2.36 %). On the other hand, twenty two compounds were identified in the seed hydrocolloids of *D. microcarpum*. They consist of hydrocarbons (20.32 %), aromatics (2.14 %), aldehyde (0.49 %), phenolic (0.37 %), fatty acids (67.80 %), esters (5.09 %) and alcohol (3.80 %). This investigation reveals that the two hydrocolloids contain appreciable amount of volatile phytochemicals that could provide certain physiological benefits to the body. Hydrocolloids from *D. microcarpum* contain more volatile phytochemicals than that of *A. africana*.

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Capsule Summary: The seed endosperms of *Afzelia africana* and *Detarium microcarpum* were characterized and aromatics, hydrocarbons, phenolics, esters, fatty acids, alcohols and steroid were detected. Hydrocolloids from *D. microcarpum* contain more volatile phytochemicals than that of *A. africana*.

Cite This Article As: Okenwa U. Igwe and Chisom Friday. Volatile constituents of hydrocolloids isolated from *Afzelia africana* and *Detarium microcarpum* seeds. Chemistry International 3(4) (2017) 286-291.

INTRODUCTION

Most plants whose parts are used as food in Southeast Nigeria are underutilized due to inadequate information on them. Some plant seed endosperms are used in the traditional food preparation to provide thickening, stabilizing and palatable properties (Abreu and Relva, 2002). *Afzelia africana* and *Detarium microcarpum* seed endosperms are among them.

A. africana popularly called African oak is the most widely distributed species in Africa. *A. africana* belongs to the family *Leguminosae* and sub-family *caesalpinaceae* (Keay et al., 1964). It is a large tree, about 15 m tall, but sometimes in damper localities up to 30 m. Bole of girth is up to 3 m, usually found in Nigeria, Senegal and across Central Africa to Sudan (Burkill, 1985). The seeds have waxy orange cup-like structure at their base and are used in Nigeria generally as soup thickening ingredient in much the same way as melon and *Irvingia gabonensis* seeds (Ejikeme et al., 2010). An

infusion of the bark of *A. africana* is used against paralysis, and a decoction against constipation. The maceration is a remedy for leprosy (Orwa et al., 2009). The crushed bark, mixed with honey, is used in veterinary medicine. The ash of the bark, prepared with Shea butter as soap, is used against lumbago. In a decoction or prepared with food, it is a treatment for back-ache. The roots are pulverised with millet-beer in Côte d'Ivoire and used to treat hernias and, in a decoction with pimento, as a remedy against gonorrhoea and stomach-ache. The ash of the fruits is rich in potassium salts and is mixed with millet for veterinary purposes (Orwa et al., 2009). The bark is also used as fish poison. Leaf decoctions and macerations are taken or applied externally against dysmenorrhoea, epilepsy, oedema, migraine, stomach-ache, asthenia, trypanosomiasis and as anodyne. Fruit preparations are taken to treat lung complaints and as aphrodisiac. Fruit ash is applied against leprosy and as soap substitute (Gerard and Loupp, 2011). It is used in local medicine for general pain relief and digestive problems. The plant like other common legumes is underutilized except as a soup thickener (Adebayo and Ojo, 2013).

D. microcarpum is a member of the *Caesalpinioaceae* special family of the larger *Leguminosae*. It is a rainforest and savannah tree of tropical Africa (Ebi and Afieroho, 2011). It is a small tree up to 10 m high, crown rather than dense and spherical. *D. microcarpum* flowering period is the rainy season and it inhabits Sudanese and Guinean Savannahs, dry forest, fallow land, from Senegal through Nigeria to Cameroon in west Africa, and as far as Sudan (Kouyate and van Damme, 2008; Reuben and Jada, 2013). In African ethno-medicine, the plant and the closely related species *Detarium senegalense* are used in the treatment of syphilis, dysentery, bronchitis, leprosy, sore throat, pneumonia, diarrhoea, malaria and meningitis (Ebi and Afieroho, 2011; Burkil, 1995). The root and stem bark are used to treat tuberculosis and itches (Eromosele et al., 1994). The barks, leaves and roots are prepared as decoctions to treat rheumatism, venereal diseases, urogenital infections, haemorrhoids, caries, biliousness, stomach ache, intestinal worms and impotence (Kouyate and van Damme, 2006). The fruit is sweet and commonly eaten fresh, while the pulp is used in the preparation of cakes and couscous. The pulp is used as a substitute for sugar. The seeds are used as frankincense and to make necklaces for women. The seeds and leaves are eaten as a condiment and vegetable. The wood is hard and tough, with a regular grain, and is easy to work. It is used for carpentry, fence poles and joinery. It is durable and long-lasting even under water (Kouyate and van Damme, 2006). The ethanol extract of the bark of *D. microcarpum* has been shown to exhibit antimicrobial action against some

pathogenic organisms including *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Citrobacter freunditis* *Staphylococcus aureus*, *Streptococcus pyrogenes* and *Listeria monocytogenes* (Abreu et al., 1998).

There is paucity of literature on the volatile chemical constituents of hydrocolloids from most of these legume seeds used as thickening and emulsifying agents in local traditional delicacies in Southeast Nigeria, especially *A. africana* and *D. macrocarpum*. This gave rise to this research, hence we report herein the volatile constituents of hydrocolloids from *A. africana* and *D. microcarpum* seeds.

MATERIALS AND METHODS

Sample Collection and Preparation

A. africana and *D. microcarpum* seeds were procured from Eha-amufu market, Enugu, Enugu State, Nigeria. The plant materials were identified and authenticated at the Plant Taxonomy Section, Forestry Department, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The seeds of *A. africana* were roasted in hot sand. With the aid of a small hammer, the ectocarp was removed. The endosperm was milled using a blender. On the other hand, the seeds of *D. microcarpum* were soaked in clean water for 24 h to remove the ectocarp. The seed endosperms were then sun-dried for 48 h. They were milled using a blender.

Extraction of Hydrocolloids

Hydrocolloids were extracted from the seed endosperm flours of *A. africana* and *D. microcarpum* by employing the method described by Igwe and Nwokocha (2014). In a typical extraction procedure, 10 g of powdered sample was dispersed in 250 ml distilled water and hydrated continuously by means of a stirrer for 2 h. This was poured into centrifuge tubes and centrifuged at 2500 rpm for 30 min. The supernatant was poured into a large beaker. The residue was reconstituted repeatedly with fresh distilled water, stirred and centrifuged again. The supernatant was pooled together and treated with isopropanol, when the hydrocolloids spooled out; the clear liquor was decanted while the trapped solvent was removed by filtration. The crude hydrocolloids were re-precipitated with isopropanol. The hydrocolloid sample was dried in a convention oven at 60 °C overnight and cooled in desiccators. This was pulverized using a blender and stored in a sealed container.

Gas Chromatography/Mass Spectrometry Analysis

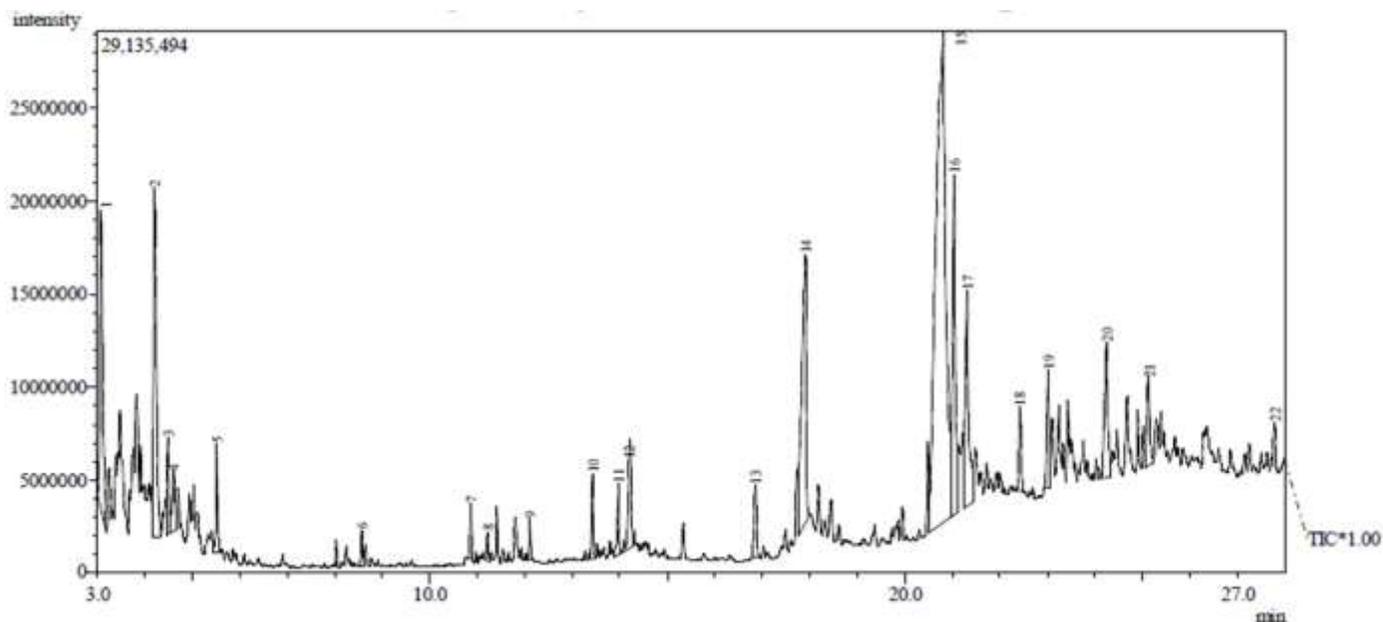


Fig. 1: GC/MS chromatogram of *D. microcarpum* seed hydrocolloids

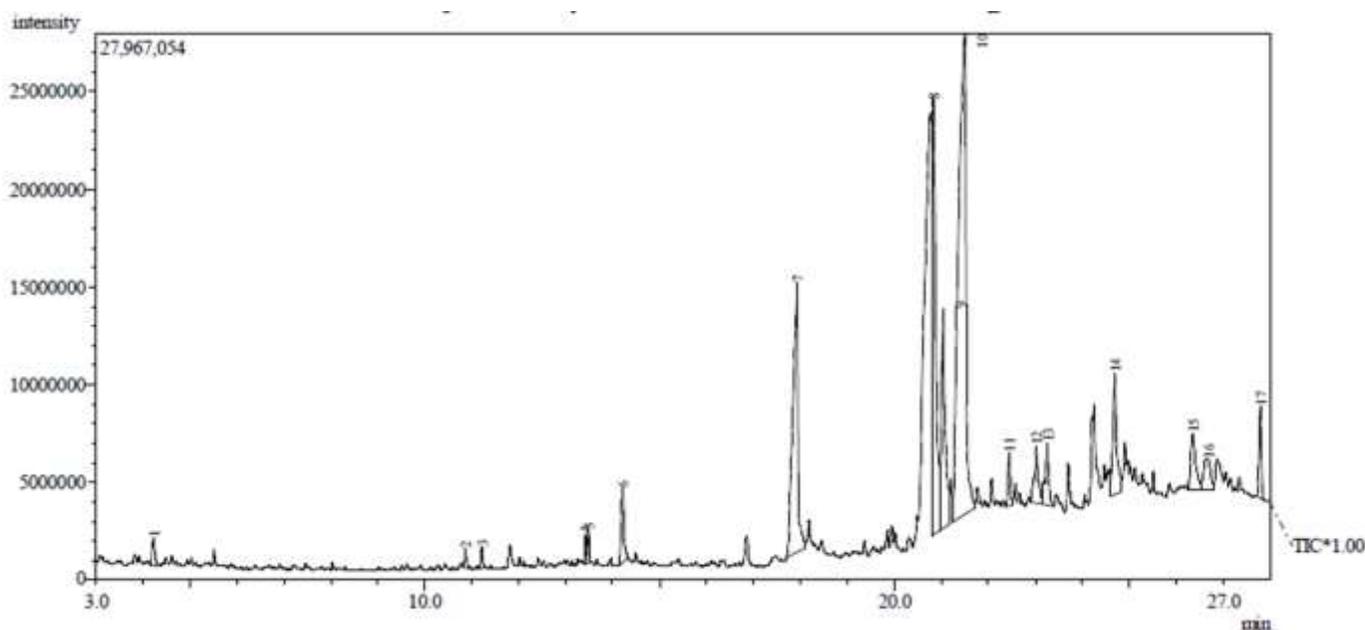


Fig. 2: GC/MS chromatogram of *A. africana* seed hydrocolloids

GC analysis was carried out in Shimadzu Japan gas chromatography 5890-11 with a fused GC column (OV-101) coated with polymethyl silicon (0.25 mm × 50 m) and the conditions were as follows: temperature programming from 80–280 °C held at 80 °C for 1 min., at 200 °C for 4 min. (rate 10 °C/min), and finally at 280 °C for 5 min. (rate 10 °C/min). The injection temperature was 250 °C. GC/MS analysis was conducted using GCMS-QP 2010 Plus (Shimadzu, Japan) with column oven temperature of 80

°C. The carrier gas was helium with a pressure of 108.2 Kpa and linear velocity of 46.3 cm/s. Total flow was 6.2 mL/min, column flow was 1.58 mL/min, injection mode was split, flow control mode was linear velocity, purge flow was 3.0 mL/min and split ratio was 1.0. Also, ion source temperature was 230 °C, interface temperature was 250 °C, solvent cut time was 2.5 min, detector gain was 0.00 KV, detector gain mode was relative and the threshold was 1000. For the mass spectrometer, start time was 3.0 min, end time was 28.0 min,

Table 1: Volatile phytochemicals from *D. microcarpum* seed hydrocolloids

Peak no	Components	Retention time(min)	Percentage composition (%)
1	Nonane	3.082	6.52
2	Decane	4.209	7.66
3	4-Methyldecane	4.494	1.27
4	Isopropylbenzene	4.615	1.55
5	Undecane	5.519	1.44
6	2,4-Decadienal	8.570	0.49
7	Hexadecane	10.866	0.60
8	3,5-Di- <i>tert</i> -butylphenol	11.215	0.37
9	3,3-Dimethyl-1-(2-carboxyphenyl)triazene	12.103	0.59
10	Hexadecane	13.422	1.06
11	2,6,11-Trimethyldodecane	13.969	1.11
12	Tetradecanoic acid	14.217	2.48
13	Sulfurous acid,2-ethylhexylisohexyl ester	16.855	1.49
14	Hexadecanoic acid	17.910	9.77
15	9-Octadecenoic acid	20.805	39.24
16	Octadecanoic acid	21.037	8.12
17	9,12,15-Octadecatrienoic acid	21.313	6.21
18	Pentafluoropropionic acid, hexadecyl ester	22.421	1.33
19	Sulfurous acid, octadecyl-2-propyl ester	23.012	2.27
20	2-Methyl-3,13-octadecadienol	24.240	3.80
21	Docosanoic acid	25.127	1.98
22	2,6,10,14,18,22-Tetracosahexaene (squalene)	27.781	0.66

Table 2: Volatile phytochemicals from *A. africana* seed hydrocolloids

Peak no	Components	Retention time(min)	Percentage composition (%)
1	Isopropylbenzene	4.228	0.80
2	4,6-Dimethyldodecane	10.866	0.32
3	3,5-Di- <i>tert</i> -butylphenol	11.213	0.39
4	4-Methylheptadecane	13.420	0.42
5	2-Thiopheneacetic acid,2-tridecyl ester	13.420	0.51
6	Octadecanoic acid	4.214	2.10
7	Hexadecanoic acid	17.930	14.40
8	9-Octadecanoic acid	20.827	17.55
9	Octadecanoic acid, 2-(2-hydroxyethoxy)ethyl ester	21.039	8.40
10	9,12,15-Octadecatrien-1-ol	21.499	37.01
11	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	22.440	1.28
12	1-Tetracosanol	23.020	2.31
13	Eicosanoic acid	23.256	2.30
14	4,6,9-Nonadecatriene	24.688	4.86
15	9,12-Octadecadien-1-ol	26.351	2.92
16	26-Nor-5-cholesten-3-beta-ol-25-one	26.690	2.36
17	2,6,10,14,18,22-Tetracosahexaene (squalene)	27.791	2.07

event time was 0.5 s, scan speed was 1250, and start m/z was 40 while end m/z was 600. All solvents used were of analytical grade and were procured from Merck, Germany. The components of the extract were identified by matching the peaks with computer Wiley MS libraries and confirmed by comparing mass spectra of the peaks and those from literature as well as using the database of National Institute of Standards and Technology (NIST) (Igwe and Okwu, 2013).

RESULTS AND DISCUSSION

GC/MS Analysis

The chloroform extracts of *D. microcarpum* and *A. africana* seed hydrocolloids were subjected to GC-MS analysis to probe their volatile phytochemical constituents. Figures 1 and 2 show the chromatogram of *D. microcarpum* and *A. africana* seed hydrocolloid extracts respectively. Twenty two compounds were identified in the seed hydrocolloids of *D. microcarpum*. They consist of hydrocarbons (20.32 %), aromatics (2.14 %), aldehyde (0.49 %), phenolic (0.37 %), fatty acids (67.80 %), esters (5.09 %) and alcohol (3.80 %). The retention time and percentage composition of each of these compounds are shown in Table 1. The most abundant compound in the hydrocolloids of *D. microcarpum* is 9-octadecenoic acid commonly known as oleic acid with an alarming composition of 39.24 %. Oleic acid has been associated with decrease in cholesterol level and reduction of high blood pressure in the body (Teres et al., 2008). This means that the seeds of *D. microcarpum* can afford high blood pressure therapy to the consumers. Hexadecanoic acid also known as palmitic acid is next in abundance with a composition of 9.77 %. Hexadecanoic acid has been proven to mediate hypothalamic insulin resistance (Benoit et al., 2009). Linolenic acid has been reported as the most abundant fatty acid in the seed endosperm of *D. microcarpum* (Kouyate and van Damme, 2006). Maybe that was why the hydrocolloids isolated from it contained high amount of linolenic acid (compound 17) i.e. 9,12,15-octadecatrienoic acid with 6.21 % composition. Linolenic acid is necessary for good health and must be acquired from our diet since it cannot be synthesized in the body. It has been reported to possess preventive effect against cardiovascular diseases (Pan et al., 2012).

On the other hand, seventeen constituents were identified in the seed hydrocolloids of *A. africana*. These compounds consist of aromatic (0.80 %), hydrocarbons (7.67 %), phenolic (0.39 %), esters (10.19 %), fatty acids (36.35 %), alcohol (42.24 %) and steroid (2.36 %). The retention times and percentage compositions of these compounds are shown in Table 2. The most abundant compound in the

hydrocolloids of *A. africana* is 9,12,15-octadecatrien-1-ol with composition of 37.01 %. This compound is the alcoholic derivative of 9,12,15-octadecatrienoic acid found in *D. microcarpum* hydrocolloids. The next are 9-octadecanoic acid (17.55 %) and hexadecanoic acid (14.40 %). More hexadecanoic acid is found in the hydrocolloids of *A. africana* than in that of *D. microcarpum*. Saturated fatty acids are engaged in the synthesis of phospholipids and spingolipids, storage and production of energy, and lipid transport (Spector, 1999; Kulkarni and Farnando, 2015). A steroidal compound, 26-nor-5-cholesten-3-beta-ol-25-one was also detected. Many derivatives of steroids possess physiological activity (Igwe and Okwu, 2013). Steroid hormones control sexual development and fertility in the human body. As a result of the vast physiological activity of steroids, many of them are used in medicine for the treatment of cancer, arthritis, allergies and infertility (Igwe and Okwu, 2013; Vollhardt, 1994). The detection of a steroidal compound in the hydrocolloid of *A. africana* seeds suggests the possibility of using the seeds in the management of fertility problems, cancer, arthritis and allergies. Other compounds found in the hydrocolloids of *A. africana* and *D. microcarpum* might possess physiological and pharmacological properties. From the GC-MS analysis results, *D. microcarpum* seed hydrocolloids contained more phytochemicals than that of *A. africana*.

CONCLUSION

The hydrocolloids isolated from the seeds of *A. africana* and *D. microcarpum* when characterised with GC-MS technique revealed they contain mainly fatty acids. These fatty acids play some vital role to maintain good health. The hydrocolloids of *D. microcarpum* contain more phytochemicals than that of *A. africana*. This research provides the more information on the chemical analysis of the volatile constituents of hydrocolloids from *A. africana* and *D. microcarpum*.

ACKNOWLEDGEMENTS

The authors are grateful to Mr. I. K. Ndukwe of Forestry Department, Michael Okpara University of Agriculture Umudike, for identifying the plant samples.

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