GC-MS ANALYSIS OF BIOACTIVE COMPOUNDS FROM THE FRUIT PULP AND SEED EXTRACTS OF *DIOSPYROS MESPILIFORMIS* (JACKAL BEERY)

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ABSTRACT
Diospyros mespiliformis is ubiquitously found plant in northern Nigerian farms and forests. Previously, various parts of this plant were reported for treatment of different types of diseases and there was neither a report of collection of the plant's fruit pulp and seed from Dutsin-Ma nor on GC-Ms analysis. Preliminary phytochemical screenings of n-hexane and methanol extracts were carried out according to standard procedures. Various bioactive compounds' characterization of the extracts was done by GC-MS technique. The phytochemical analysis of the crude extracts of the n-hexane and methanol on the seed plant revealed the presence of alkaloids, flavonoids, phlobatanins, saponins, steroidal nucleus, tannins, cardiac glycosides, anthraquinones, cardenolides, and terpenoids. The GC-MS analysis of pulp showed 26 peaks of different phytoconstituents of which major components are: octanoic acid, decanoic acid, dodecanoic acid, tetradecanoic acid, octadecanoic acid, 6-octadecenoic acid and eicosanoic acid; while the GC-MS analysis of seed showed 22 peaks of different phytoconstituents of which major components are: hexadecanoic acid, n-hexadecanoic acid, 9-octadecenoic acid and 9, 12-octadecadienoic acid. In conclusion, both the presence of phytochemicals detected from the phytochemical screening and the high unsaturated fatty acid content analyzed from GC-MS confirm the reports of the applications of Diospyros mespiliformis for the treatment of various diseases.

Keywords: *GC-MS, Extract, compounds, Diospyros Mespiliformis*

Introduction

Fruits represent an abundant source of nutritive and non-nutritive bioactive compounds which are mostly associated with reduced risk of many non-communicable/chronic diseases (Wolfe and Ijeoma, 2008). Global increase in chronic diseases has necessitated the research into diverse fruits and vegetables in order to increase their consumption for the potential health beneficial constituents of the underutilized plants. Generally, the health benefits of fruits are mainly attributed to their antioxidant activity which is largely characterized by the number and diversity of their phytochemical composition. Thus, the more diverse the phytochemicals available in a diet, the better the health-beneficial potential derived.

Therefore, seeking for possible solutions to curb the growing phenomenon of low consumption of unrefined plant-based diets and rising chronic diseases, Padulosi *et al*. (2006), recommended plant biodiversity with emphasis on indigenous but underutilised species to significantly contribute to the realization of domestic food security as well as help curtail the double burden of malnutrition especially in low/middle income nations. Similar calls have been made for the numerous lesser known Nigerian fruits including *Annona muricata, Annona senegalensis, Detarium microcarpum*, *Diospyros mespiliformis, Gardenia erubescens, Irvingia gabonensis*, *Senna alata*, *Trichosanthes anguina* and *Piliostigma thonningii* (Abubakar *et al*., 2015a; Hassan *et al*., 2004; Jimoh and Oladiji, 2005; Ojiyako and Igwe, 2008; Onimawo *et al*., 2003; Onyechi *et al*., 2012; Otori and Mann, 2014).

The above scenario has led to a paradigm shift of researchers into exploring the maximum use of underutilised tree fruits with emphasis on their health-beneficial phytochemicals. This is with the aim of sensitizing a number of low/middle income countries to explore the value of most of their indigenous but underutilised fruits in order to aid productive inputs in encouraging their increased consumption, value addition, probable use in food formulations and fortifications, and their subsequent commercial production in ensuring food security (Abubakar *et al*., 2015a, b; Adekunle and Oyerinde, 2004; Dosunmu and Ochu, 1995; Otori and Mann, 2014).

Hassan *et al*. (2004) and Ietidal *et al*. (2009) reported bioactive phytochemicals such as alkaloids, tannins and saponins with a corresponding high antioxidant activity (Ndhlala *et al*., 2008) of *Diospyros mespiliformis* (L). Kone and Atindehou, (2008) and Luseba and Van der Merwe, (2006) also reported uses of *Diospyros mespiliformis* in traditional veterinary medicine in Africa and its extract display muscular activity of experimental rat (Belemtougri *et al*., 2006). Investigating key parameters for assessing the health potential of the underutilized fruits will complement literature in making known the potential of the Nigerian underutilized species as well as provide a stepping-stone for further research works in other uses of the fruits.
There is dearth of information on the health beneficial constituents of the edible portions of indigenous underutilised fruits in Nigeria to make any substantial claim for their optimal use (Akintayo *et al*., 2002; Adekunle and Oyerinde, 2004; WHO/FAO, 2004). Endeavours in research works to enhance the

commercial production and subsequent value addition of fruits, have been tilted towards only the highly known plants leaving no room for variety for consumers. The aim of this work is to analyze and characterize the phytochemical compounds of fruits pulp and seed samples of *Diospyros mespiliformis*, collected for the first time from Dutsin-Ma, using GC-MS.

Materials and Methods

Sample Collection and Identification

Healthy-looking matured but fruits pulp and seed of *Diospyros mespiliformis* were collected from the habitat of this tree on Federal University Dustin-Ma, Old Campus. The plant was identified by a plant taxonomist from the Department of Biology of this University. A voucher specimen was deposited for future reference. Samples were collected from the area within the morning period and transported to the laboratory. They were immediately washed under running water and air-dried. The unbruised and infection-free fruits were hand-picked and kept in plastic sealable bags for further processing and analyses.

Extraction

Fruits pulp and seed samples were extracted with hexane and methanol for 72 h using percolation method (Tijjani *et al.,* 2011).

Determination of the Phytochemical Components in the Extracts

The phytochemical screenings were done at the Chemistry Laboratory of the Department of Applied Chemistry, Federal University Dutsin-Ma, Nigeria. The hexane and methanol extracts were subjected to phytochemical analysis to screen for the presence of secondary metabolites: alkaloids, saponins, anthraquinones, cardenolides, flavonoids, phlobatanins, cardiac glycoside, terpenoids, steroidal nucleus and tannins. The phytochemical screening methods reported by Edeoga *et al.* (2005) were adopted as standard procedure for this study.

GC-MS Analysis and Identification of Components

GC-MS analysis was carried out on a Shimadzu (Kyoto, Japan) GC-MS model QP 2010 at National Research Institute for Chemical Technology, Zaria, according to the EN 14103 standard method (Adams, 2007). The GC column oven temperature (70°C), injecting temperature (250°C), flow control mode (linear velocity), total flow (40.8 ml/min) column flow (1.80 ml/min), pressure (116.9 kpa), linear velocity (49.2 cm/s) and purge flow (3.0 ml/min) were employed for this analysis. A sample volume of 8.0 µl was injected using split mode (split ratio of 20:0). The peak area, that is, the percentage amount of each component was calculated by comparing its average peak area to the total areas. Software was used to handle mass spectra and chromatogram. Interpretation of mass spectrum was conducted by comparing the database peaks of National Institute of Standard and Technology (NIST) library with those of the unknown compounds (Stein *et al.*, 2002). This is done by computer taking the spectrum of the unknown component and comparing with the spectrum of the known components stored in its library. Component relative percentages were calculated based on GC peak areas without using correction factors. The name, molecular weight and structure of the components of the test materials were ascertained based on this comparison.

Results and Discussion

Table 1: Percentage Recovery per 400g of Pulp and Seed of *Diospyros mespiliformis*

Extract Type	Extract Mass(g)	Percentage recovery (%)
n-hexane	50.2	12.55
Methanol	50.5	12.63

Table 2: Phytochemical Test of Fruits Pulp and Seed of *Diospyros mespiliformis*

S/No	Metabolites	Pulp	Seeds
1.	Alkaloids	-	++
2.	Flavonoids	++	+
3.	Phlobatannins	-	+
4.	Saponins	-	+
5.	Steroidal nucleus	++	+
6.	Tannins	-	++
7.	Cardiac Glycosides	-	++
8.	Anthraquinones	-	++
9.	Cardenolides	++	+
10	Terpenoids	++	+

Key: (+) = presence; (-) = absence

The phytochemical analysis of the crude extracts of the *n*-hexane and methanol on the pulp plant revealed the presence of steroidal nucleus, flavonoids, cardenolides and terpenoids.

The phytochemical analysis of the crude extracts of the *n*-hexane and methanol on the seed plant revealed the presence of alkaloids, flavonoids, phlobatanins, saponins, steroidal nucleus, tannins, cardiac glycosides, anthraquinones, cardenolides and terpenoids.

Discussion of the Phytochemical Screening Results

The detection of tannins in the samples is in accordance with reports by Etebu (2012), Onyechi *et al*. (2012) and Adekunle and Oyerinde (2004). Tannins are compounds that have the ability to react with proteins to form stable water insoluble components. Since bacteria cell wall are made up of proteins, tannins, are seen as active detoxifying agents by precipitating the protein components and hence inhibiting their growth. This gives an indication of the potential health benefits of the fruit seeds, as tannins are acclaimed for their antimicrobial and antiviral activity, and also in the treatment of tonsillitis (Akiyama *et al*., 2001). Furthermore, tannins are reported to possess free-radical scavenging activity and anti-inflammatory properties; used as astringents and being use to treat skin eruptions (Tijjani *et al.,* 2011)

The presence of alkaloids in seeds of the medicinal plant made it possible to ascertain its potential

antimicrobial activity ranging from anti-bacterial, anti-malarial and antifungal activities. Other uses of medicinal uses of alkaloids include their use as anti-amoebic agents and astringents (Abdu, 1990).
The presence of saponins serves as an indicator for possible antibacterial activity of the plant seed (Tijjani *et al.,* 2011).

Glycosides, on hydrolysis, yield non-sugar component (aglycone or genin) .These compounds possess strong antibacterial activities according to reports by Wolfe *et a*. (2010), Onyechi *et al.* (2012) and Etebu (2012).
Specific tests for cardiac glycosides had negative results for seeds and pulp samples of the plant. The absence of cardiac glycosides in the fruits pulp and seed is desired because of the toxicity associated with the intake of these compounds; though Onike (2010) suggested relaxant and calming effects by the chemical compounds on the heart and muscles when it is consumed in small doses.

Flavonoids which are significantly recognized for their antimicrobial, anti-oxidant and anti-tumor properties (Tijjani *et al.,*2011) were detected in fruits (pulp and seeds). The finding corroborates earlier detections for some Nigerian varieties by Wolfe *et al*. (2010) for fruits pulp and seeds (Etebu, 2012).

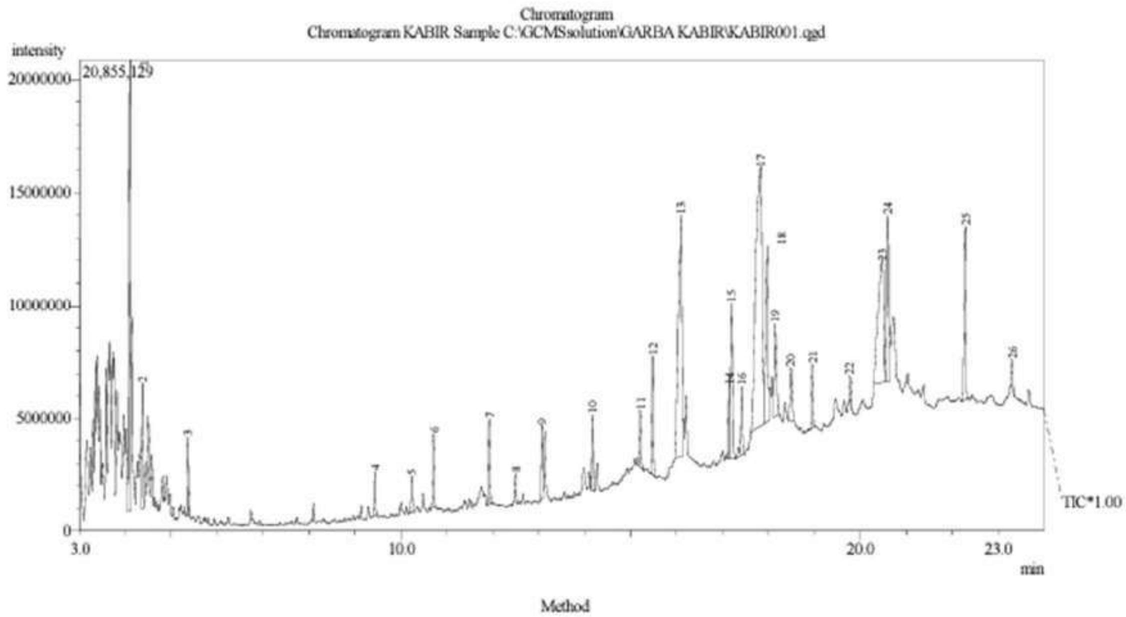

Figure 1: GC-MS of Fruit Pulp of *Diospyros mespiliformis*

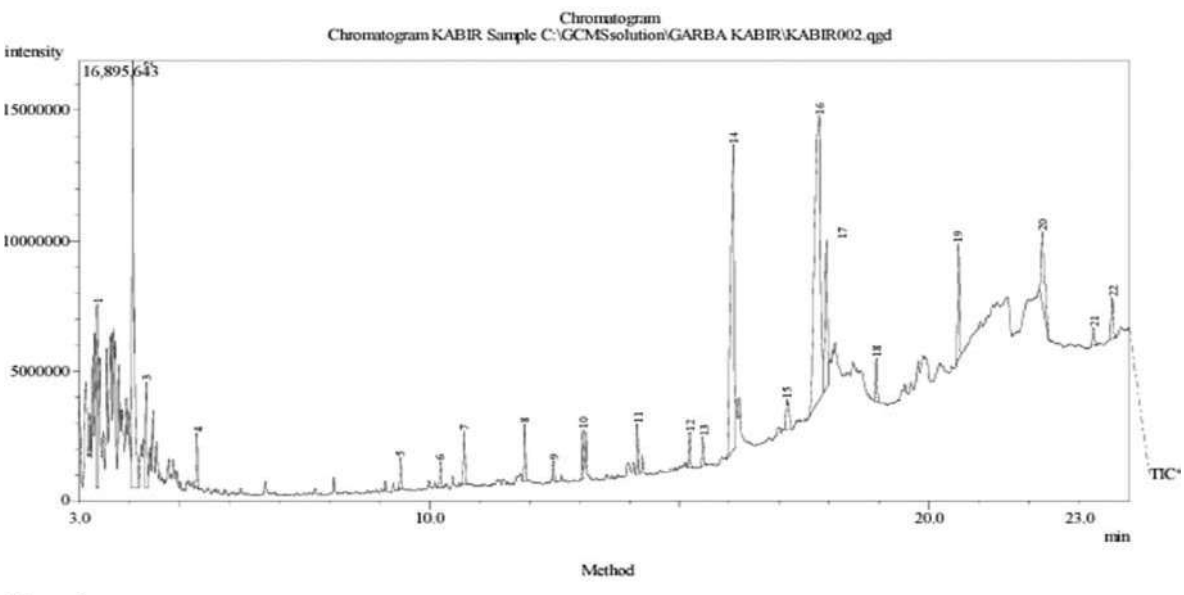

Figure 2: GC-MS of Fruit Seed of *Diospyros mespiliformis*

Table 3: Compounds Identified through GC-MS/MS with their Molecular Weight and Molecular Formula

No.	R.T.	Name of the compounds	Molecular formula	Molecular weight	Peaks Area %
1	4.1	*n*-Decane	$C_{10}H_{22}$	`142	9.31
2	4.4	Nonane,2,6-dimethyl	$C_{11}H_{24}$	156	2.19
3	5.4	*n*-Undecane	$C_{11}H_{24}$	156	1.24
4	9.4	*n*-Tetradecane	$C_{14}H_{30}$	198	0.76
5	10.2	*n*-cetane	$C_{16}H_{34}$	226	0.73
6	10.7	*n*-Hexadecane	$C_{16}H_{34}$	226	1.40
7	11.9	*n*-Hexadecane	$C_{16}H_{34}$	226	1.65
8	12.5	Pentadecane, 2,6,10-trimethyl	$C_{18}H_{38}$	254	0.63
9	13.1	*n*-Tridecane	$C_{13}H_{28}$	184	1.43
10	14.2	*n*-Octadecane	$C_{18}H_{38}$	254	1.45
11	15.2	*n*-Hexadecane	$C_{16}H_{34}$	226	1.09
12	15.5	Pentadecanoic acid,14-methyl-methyl ester	$C_{17}H_{34}O_2$	270	2.55
13	16.1	*n*-Tetradecanoic acid	$C_{14}H_{28}O_2$	228	12.25
14	17.1	9,12-Hexadecadienoic acid, methyl ester	$C_{17}H_{30}O_2$	266	1.93
15	17.2	14-Octadecenoic acid,methyl ester	$C_{19}H_{36}O_2$	296	3.67
16	17.4	*n*-Octadecanoic acid, methylester	$C_{19}H_{38}O_2$	298	1.71
17	17.8	9-Octadecenoic acid (Z)	$C_{18}H_{34}O_2$	282	23.34
18	18.0	*n*-Octadecanoic acid	$C_{18}H_{36}O_2$	284	5.83
19	18.2	cis-9-cis-12-Octadecadienoic acid	$C_{18}H_{32}O_2$	280	3.21
20	18.5	3-Tetradecyne	$C_{14}H_{26}$	194	1.64
21	18.9	*n*-Eicosane	$C_{20}H_{42}$	282	1.33
22	19.8	*n*-Nonadecane	$C_{19}H_{40}$	268	0.48
23	20.5	Tetracosane	$C_{24}H_{50}$	338	10.21
24	20.6	n-Octadecane	$C_{18}H_{38}$	254	4.30
25	22.3	n-Eiscosane	$C_{20}H_{42}$	282	4.89
26	23.3	n-Tetracosane	$C_{24}H_{50}$	338	0.77

Table 4: Compounds Identified through GC-MS/MS with their Molecular Weight and Molecular Formula

No.	R.T	Name of the Compounds	Molecular Formula	Molecular Weight	Peaks Area %
1	3.4	*n*-Propylcyclohexane	C_9H_{18}	126	5.87
2	4.1	*n*-Decane	$C_{10}H_{22}$	142	15.46
3	4.4	Nonane, 2,6-dimethyl	$C_{11}H_{24}$	156	2.50
4	5.4	*n*-Undecane	$C_{11}H_{24}$	156	1.17
5	9.4	*n*-Tetradecane	$C_{14}H_{30}$	198	0.74
6	10.2	4,6-Dimethyldodecane	$C_{14}H_{30}$	198	0.68
7	10.7	*n*-Tridecane	$C_{13}H_{28}$	184	1.32
8	11.9	*n*-Cetane	$C_{16}H_{34}$	226	1.47
9	12.5	2,6,10-trimethylpentadecane	$C_{18}H_{38}$	254	0.56
10	13.1	*n*-Tridecane	$C_{13}H_{28}$	184	1.28
11	14.2	*n*-Tetradecane	$C_{14}H_{30}$	198	1.32
12	15.2	2-Methylnonadecane	$C_{20}H_{42}$	282	0.93
13	15.5	*n*-Hexadecanoic acid methyl ester	$C_{17}H_{34}O_2$	270	0.82
14	16.1	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	19.09
15	17.2	3,8-Dimethylundecane	$C_{13}H_{28}$	184	1.62
16	17.8	13-Docosenoic acid	$C_{22}H_{42}O_2$	338	28.40
17	17.9	*n*-Octadecanoic acid	$C_{18}H_{36}O_2$	284	6.15
18	18.9	*n*-Eicosane	$C_{20}H_{42}$	282	1.15
19	20.6	*n*-Eicosane	$C_{20}H_{42}$	282	3.09
20	22.3	Octadecanoic acid,2-oxo-methyl ester	$C_{19}H_{36}O_3$	312	4.19
21	23.3	*n*-Eicosane	$C_{20}H_{42}$	282	0.57
22	23.7	5.9,13-Trimethyl-4,8,12-tetradecatrienenitrile	$C_{17}H_{27}N$	245	1.61

Discussion of the GC-MS Results

The *n*-hexane extract of *Diospyros mespiliformis* fruit pulp and seed were sent for GC-MS/MS analysis due to the indication of possessing some biological components. The phytochemical composition analysis by mass spectrometry is considered as a proper way to convey the pharmacological importance and therapeutic nature of the plant species. It is very clear from the present study, the n-hexane extract of the fruits pulp and seed powder contains terpenes, fatty acids, alcohols and steroids. Some of the metabolites were clinically proven for its various pharmacological activities such as anti-inflammatory, anti allergic, antioxidant, antidiabetic, anti-microbial and many more. The list of compounds identified in the pulp and seed extracts through GC-MS are presented in Tables 5 and 6.

The GC-MS spectrum of pulp revealed the presence of major components, namely: octanoic acid, decanoic acid, dodecanoic acid, tetradecanoic acid, octadecanoic acid, 6-octadecenoic acid and eicosanoic acid. While for the seeds, major components present were; hexadecanoic acid, n-hexadecanoic acid, 9-octadeccenoic acid and 9, 12-octadecadienoic acid. The pulp and seeds had 100% unsaturated fatty acids and this makes it desirable, particularly, for coronary heart diseases patients; most especially if the oils are proven to possess zero cholesterol.

Conclusion

The presence of phytochemicals detected from the phytochemical screening and the high unsaturated fatty acid content analyzed from GC-MS in the pulp, fruit and seed extracts of *Diospyros mespiliformis* indicate that the plant parts contains compounds of different medicinal values and hence could be used as antibacterial, antifungal, anti-protozoa, antioxidant, anti-inflammatory, anti-allergic, anti-diabetic, antitumor, astringents; also used to treat skin eruptions, in the treatment of tonsillitis and coronary heart diseases.

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