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Original Research Article

Towards Establishing Chemical Markers for Antidiabetic Plants: A Comparative Analysis of the Chemical Fingerprints of Three Validated Antidiabetic Plants, *Nauclea latifolia, Azadirachta indica* and *Moringa oleifera*

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Abstract

A drawback to the mainstreaming of medicinal plants into modern medicine is the issue of standardization of crude plant extracts. The aim of this study was to compare chemical fingerprints of the ethanolic leaf extracts of three known antidiabetic plants namely *Nauclea latifolia*, *Azadirachta indica* and *Moringa oleifera* and to establish if they are some common phytochemicals, with antidiabetic activities, that could be used as active biomarkers for antidiabetic plants and subsequently for standardization of the plant preparations. The dried pulverized leaves were macerated in 80% ethanol and subjected to Gas Chromatography-Mass Spectrometry (GC-MS) analysis. Chromatograms of extracts showed 51, 35 and 53 peaks of identified phytochemical compounds for *Nauclea latifolia, Azadirachta indica* and *Moringa oleifera* respectively. However, only thirteen (13) phytoconstituents namely 3,7,11,15-tetramethyl-2-hexadecen-1-ol, phytol, hexadecanoic acid methyl ester, hexadecanoic acid ethyl ester, ethyl 14-methyl hexadecanoate, cyclopropaneoctanoic acid, hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, 9,12-octadecadienoic acid, octadecanoic acid, vitamin E, campesterol, stigmasterol and gamma sitosterol were common to all three plants. All except three of these common phytoconstituents (ethyl 14-methyl hexadecanoate, cyclopropan-eoctanoic acid and campesterol) have been shown in the literature to have anti-diabetic activity. It is proposed that anyone or combination of these ten common phytochemicals be used as active therapeutic markers for standardization of the three plants and by extension other anti-diabetic plants if this same commonalities can be established for the plants. Based on the narrow percentage range in the three plants, Vitamin E(3.45- 3.79%) and 3,7,11,15-tetramethyl-2-hexadecen-1-ol(0.87- 1.99%) should be better active diabetic marker candidates for standardization of the three plants.

Keywords: Antidiabetic plants, Chemical markers, Standardization, *Nauclea latifolia, Azadirachta indica* and *Moringa oleifera*

INTRODUCTION

The use of plants for medicinal purposes is as old as the existence of man. It is estimated that 80% of rural populations in Africa, Asia and Latin America depend on medicinal plants as source of primary healthcare appa-

rently because of its ready availability, low cost and limited access to modern medicine (Farnsworth *et al*., 1985; Akerele, 1993; Bandaranayake, 2006*;* Odugbemi, 2008; Kunle *et al*., 2012; Rasheed *et al*., 2012; Mgbeje, 2015). Over the years there has been increasing popularity of herbal remedies in the western world premised on the belief that they provide a balanced and moderate approach to healing and have minimal adverse effects when the drugs are used properly(De Smet, 1997; Blumental *et al*., 1998; Calixto, 2000; Stein, 2004; Bandaranayake, 2006; Zhang *et al*., 2012). However, despite the increasing popularity of the use of medicinal plants globally, its mainstreaming into modern medicine has been rather slow because of quality, safety and efficacy issues (Efferth and Greten, 2012;Padalia, 2012, Mgbeje, 2015).Allied to this is the issue of standardization of the medicinal plant drugs as most of plant medicines are in the form of crude extracts which are a mixture of several ingredients which may vary in same species as a result of differences in intrinsic and extrinsic factors. It is to address this standardization concern for medicinal plants, specifically antidiabetic plants, using three validated antidiabetic plants namely *Nauclea latifolia, Azadirachta indica* and *Moringa oleifera* as models, that this work is being carried out.

Diabetes mellitus is a metabolic disorder resulting from absolute or relative deficiencies in insulin secretion and/or insulin action and is associated with chronic hyperglycemia and disturbances of carbohydrate, lipid, and protein metabolism (ADA, 2005; Effiong *et al*., 2013). Persistent hyperglycemia causes increased production of free radicals especially reactive oxygen species (ROS), from glucose auto-oxidation and protein glycosylation, which may ultimately result in destruction of some vital organs of the body (Halliwell and Gutteridge, 1989; Robertson, 2004; Moussa, 2008).

Nauclea latifolia Smith (syn. *Sarcocephalus latifolius*)*,* a member of the family *Rubiaceae*, and commonly known as Pin cushion tree is a straggling shrub or small tree native to tropical Africa and Asia. *Nauclea latifolia* has been employed by traditional medicine practitioners in the treatment of many ailments (Mgbeje and Abu, 2020). Its antidiabetic credentials has been established in our laboratory (Effiong *et al*., 2013) and elsewhere (Gidado *et al*., 2012; Antia and Okokon, 2014; Boucherle *et al*., 2016).

Azadirrachta indica, also known as neem, belongs to the family *Meliaceae* (Yanpallewar *et al*., 2003). *Azadirachta indica* is an average sized tree and grows to a height of 15-30 meters. It is an ever green tree but often sheds its leaves during the dry season. The tree is indigenous to the Indian sub-continent and is an exotic species in African, East Indies and Latin America (Orwa, *et al*., 2009). Each part of the Neem tree has some medicinal property and is attributed with antifungal, antiviral, antimalarial, anti-inflammatory, antioxidant and immune system properties (Kausik and Ranajet, 2002). It has been shown to have antidiabetic activity (Gupta *et al*., 2004; Essien *et al*., 2013).

Moringa oleifera Lam (also known as horseradish or drumstick plant) belongs to the family *Moringaceae*. Although native to the Himalayan tract, it is cultivated extensively in the tropics (Fahey, 2005). The plant has been used extensively for nutritional purposes and in the treatment of inflammation, cardiovascular, liver and a host of other diseases and in maintaining the integrity of hematological, hepatic and renal function (Fahey, 2005; Paliwal *et al*., 2011, Efiong *et al*., 2013; Iwara *et al*; 2013). It has also been shown to enhance sexual activity in mice (Cajuday and Pocsidio, 2010) and in the treatment of diabetes (Fahey, 2005; Efiong *et al*., 2013; Iwara *et al.,* 2013; Ebong *et al*., 2014).

MATERIALS AND METHODS

Plant Materials

Fresh mature leaves of *Nauclea latifolia, Azadirachta indica* and *Moringa oleifera* were harvested from the University of Calabar botanical farm, Calabar, Cross River State, Nigeria. The plants were authenticated at the Department of Botany, University of Calabar and were in accordance with voucher specimens Herb/Bot/ucc/017, Herb/Bot/ucc/354 and Herb/Bot/ucc/395 respectively already deposited in the department's herbarium.

Preparation of Plant Extracts

The plant extracts were prepared as previously described (Mgbeje and Abu, 2020). The harvested *Nauclea latifolia, Azadirachta indica* and *Moringa oleifera* leaves were thoroughly washed with clean water, rinsed with distilled water, allowed to drain and then dried away from direct sunlight for seven (7) days, after which it was grounded into powder with a manual blender. The pulverised leaves were weighed and then soaked in 80% ethanol solution in the ratio 1:4 (sample: ethanol) for 48 hours with intermittent agitation. The plant homogenates were doubly filtered using cheese cloth followed by Whatman filter paper and the filtrates concentrated in a water bath at a temperature of 40^{o}C. The residual mass was stored at a temperature of 4^{o}C in a refrigerator until ready for use.

Determination of Chromatographic Chemical Profile Using Gas Chromatography-Mass Spectrometry (GC-MS)

The GC-MS analysis was performed as previously described (Mgbeje *et al*., 2020). A Shimadzu GC-MS QP-2010 comprising a gas chromatogram fitted with a

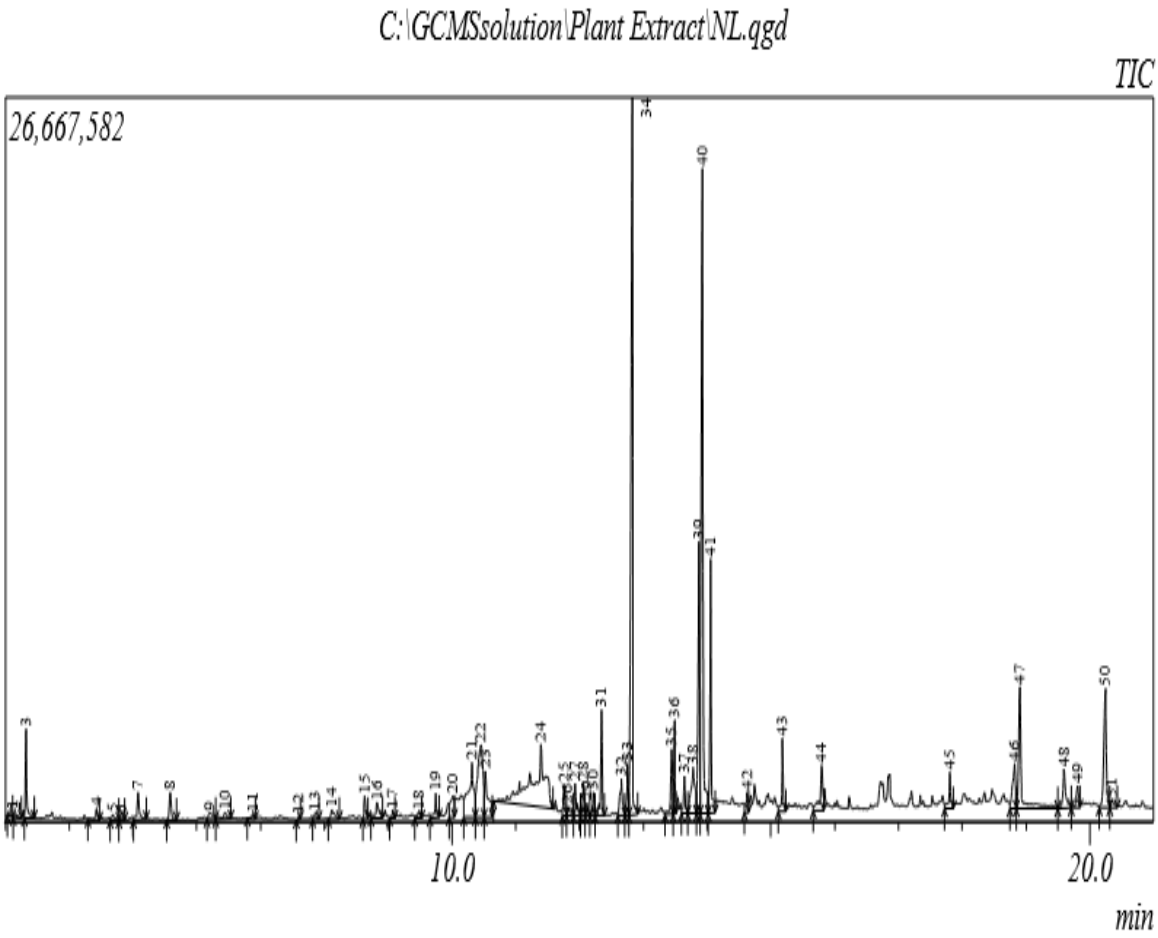

Figure 1. GC-MS full spectrum analysis of *Nauclea latifolia* ethanolic leaf extract.

25m x 0.25mm fused silica capillary column coated with CP-Sil5 and film thickness at 0.15µm and hyphenated to a mass spectrophotometer and an auto sampler was used for this analysis. The carrier gas for the chromatography was helium at 1.2ml/min. Operating conditions of the MS was: ion source temperature 230°C and ionization voltage 70ev. The identification of the components was accomplished by comparison of the retention indices, fragmentation pattern and mass spectra with spectrum of known components stored in the database of Mass spectrometry Data Center, National Institute of Standard and Technology (NIST) library (http://chemdata.nist.gov).

RESULTS

Gas Chromatography - Mass Spectroscopy (GC-MS) Profiling

The GC-MS chromatograms of *Nauclea latifolia*, *Azadirachta indica* and *Moringa oleifera* leaf extracts are shown in Figures 1-3 respectively. A complete list of the identified compounds, retention times and percentage peak areas are shown in tables 1-3. Chromatograms of extracts showed 51 peaks of identified phytochemical compounds in *Nauclea latifolia* as previously reported (Mgbeje and Abu, 2020), 35 peaks in *Azadirachta indica* and 53 peaks in *Moringa oleifera.*

However, of the 51, 35 and 53 phytoconstituents identified in *Nauclea latifolia, Azadirachta indica* and *Moringa oleifera* respectively, only thirteen (13) phytoconstituents were common to all three plants (Table 4) and include: 3,7,11,15-tetramethyl-2-hexadecen-1-ol, phytol, hexadecanoic acid methyl ester, hexadecanoic acid ethyl ester, ethyl 14-methyl hexadecanoate, cyclopropaneoctanoic acid, hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, 9,12-octadecadienoic acid, octadecanoic acid, vitamin E, campesterol, stigmasterol and gamma sitosterol. Of the thirteen (13) common phytoconstituents all except three (3) namely ethyl 14-methyl hexadecanoate, cyclopropaneoctanoic acid and campesterol, have been shown in the literature to have anti-diabetic activity (Mgbeje and Abu, 2020).

Table 1. Phytochemical compounds identified for various GC-MS peaks for *Nauclea latifolia* ethanolic leaf extract

Peak	R.Time	Area%	Height%	Name
1	3.048	0.06	0.13	2-Hexanethiol
2	3.157	0.11	0.11	2-Decanynoic acid
3	3.324	1.42	2.53	Pentanoic acid, 4-oxo-, ethyl ester
4	4.431	0.25	0.33	Butanedioic acid, diethyl ester
5	4.685	0.23	0.15	Pentanoic acid, 3-hydroxy-, ethyl ester
6	4.825	0.11	0.10	Isosorbide
7	5.083	0.74	0.78	Pentanoic acid, 4,4-dimethoxy-, ethyl ester
8	5.583	0.63	0.79	Butanedioic acid, hydroxy-, diethyl ester
9	6.206	0.38	0.17	Cyclohexanone, 2-(hydroxymethyl)
10	6.442	0.32	0.18	2-Methoxy-4-vinylphenol
11	6.880	0.13	0.13	2-Ethyl-3-hydroxy-2-methyl-succinic acid, 1ethyl
12	7.593	0.06	0.09	Nonanoic acid, 9-oxo-, ethyl ester
13	7.853	0.18	0.17	alpha.-D-Glucopyranoside, O-.alpha.-D-glu
14	8.117	0.43	0.27	l-Pyrrolid-2-one, N-carboxyhydrazide
15	8.635	0.55	0.65	3-Furanacetic acid, 4-hexyl-2,5-dihydro-2,5-dioxo
16	8.829	0.76	0.45	alpha.-D-Glucopyranoside, O-.alpha.-D-glu
17	9.072	0.16	0.18	9-Octadecenoic acid (Z)-, methyl ester
18	9.479	0.21	0.17	3-Decenoic acid, (E)-
19	9.745	0.62	0.70	Ethyl tridecanoate
20	10.011	1.03	0.59	Decanoic acid, silver(1+) salt
21	10.314	3.66	1.54	Triethyl citrate
22	10.453	4.96	2.04	Quinic acid
23	10.526	1.92	1.29	Azelaic acid
24	11.394	9.79	1.78	3-O-Methyl-d-glucose
25	11.757	0.59	0.87	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
26	11.811	0.38	0.27	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl
27	11.931	0.84	0.91	Hexadecanal
28.	12.062	0.63	0.91	Phytol, acetate
29.	12.118	0.20	0.36	Hexadecanoic acid, ethyl ester
30.	12.218	0.39	0.66	3,7,11-Trimethyl-2,4-dodecadiene
31.	12.342	1.77	2.99	Hexadecanoic acid, methyl ester
32.	12.657	1.24	1.02	Ethyl 9-hexadecenoate
33.	12.753	1.22	1.46	Ethyl 9-hexadecenoate
34.	12.823	15.80	20.29	Hexadecanoic acid, ethyl ester
35.	13.444	1.37	1.83	Ethyl 14-methyl-hexadecanoate
36.	13.488	1.62	2.66	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylclo p
37.	13.642	0.74	1.05	Methyl stearate
38.	13.781	2.41	1.30	cis-9-Hexadecenal
39.	13.871	6.46	7.69	9,12-Octadecadienoic acid, ethyl ester
40.	13.922	13.62	18.26	9,12,15-Octadecatrienoic acid, ethyl ester,
41.	14.055	4.93	7.19	Octadecanoic acid, ethyl ester
42.	14.627	0.55	0.56	Ethyl 9-hexadecenoate
43.	15.183	1.41	2.05	Hexadecanoic acid, ethyl ester
44.	15.795	1.54	1.28	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)
45.	17.803	1.04	1.04	Methyl 2-hydroxy-pentacosanoate
46.	18.816	1.53	1.27	Stigmast-5-en-3-ol, oleate
47.	18.898	4.32	3.45	Vitamin E
48.	19.594	1.48	1.09	Campesterol
49.	19.807	0.68	0.64	Stigmasterol
50.	20.239	4.11	3.39	gamma.-Sitosterol
51.	20.348	0.41	0.21	Spirost-8-en-11-one, 3-hydroxy-, (3beta.,5alpha..
		100.00	100.00	

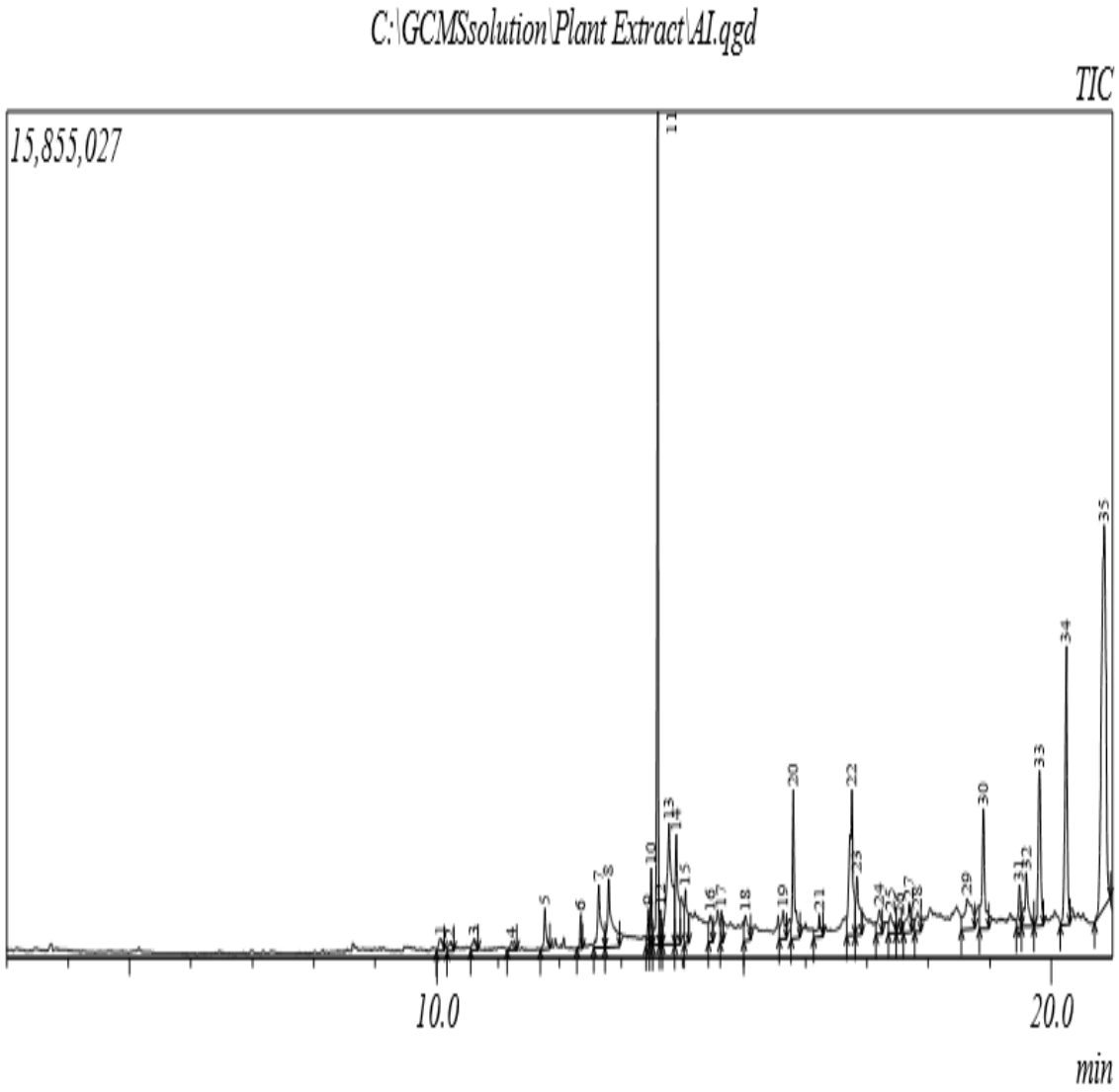

Figure 2. GC-MS full spectrum analysis of *Azadirachta indica* ethanol leaf extract.

Table 2. Phytochemical compounds identified for various GC-MS peaks for*Azadirachta indica*ethanolic leaf extract.

Peak	R.Time	Area%	Height%	Name
1	10.063	0.58	0.39	1,2,3,4-Cyclopentanetetrol, (1.alpha.,2.beta
2	10.215	0.36	0.27	Stevioside
3	10.608	0.43	0.36	Spiro[androst-5-ene-17,1'-cyclobutan]-2'-on
4	11.216	0.31	0.30	9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione
5	11.758	0.96	1.30	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
6	12.339	0.49	1.11	Hexadecanoic acid, methyl ester
7	12.633	2.52	1.97	n-Hexadecanoic acid
8	12.796	3.59	2.15	Hexadecanoic acid, ethyl ester
9	13.443	0.67	1.15	9,12-Octadecadienoic acid (Z,Z)- 10
10	13.484	1.25	2.44	9,12-Octadecadienoyl chloride, (Z,Z)-
11	13.596	14.15	26.24	Phytol
12	13.641	0.61	1.11	Tetradecanoic acid, 12-methyl-, methyl ester
13	13.778	7.67	3.82	9,12-Octadecadienoic acid (Z,Z)-
14	13.898	3.16	3.46	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-
15	14.045	1.73	1.70	Ethyl 14-methyl-hexadecanoate
16	14.452	0.99	0.84	17-Octadecynoic acid
17	14.634	0.69	0.97	Oleic Acid
18	15.024	1.09	0.78	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylc
19	15.634	1.16	0.87	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-
20	15.799	3.35	4.69	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)
21	16.227	0.81	0.72	Ethyl 14-methyl-hexadecanoate
22	16.751	5.69	4.59	Butyl 9,12,15-octadecatrienoate
23	16.836	2.18	1.87	Octadecanoic acid, 2,3-dihydroxypropyl ester
24	17.200	0.87	0.77	Ethyl Oleate
25	17.381	0.91	0.61	Z,Z-3,13-Octadecedien-1-ol
26	17.533	0.55	0.46	Heptadecafluorononanoic acid, undecyl ester
27	17.687	1.31	0.91	1-Octadecanesulphonyl chloride
28	17.825	0.92	0.65	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hex
29	18.626	2.71	0.97	18,19-Secoyohimban-19-oic acid, 16,17,20,
30	18.893	3.66	3.79	Vitamin E
31	19.479	1.03	1.31	Benzene, 1-(4-pentyl[1,1'-bicyclohexyl]-4-y
32	19.593	2.21	1.70	Campesterol
33	19.807	4.32	4.88	Stigmasterol
34	20.241	7.58	8.77	gamma.-Sitosterol
35	20.860	19.52	12.05	Astaxanthin
		100.00	100.00	

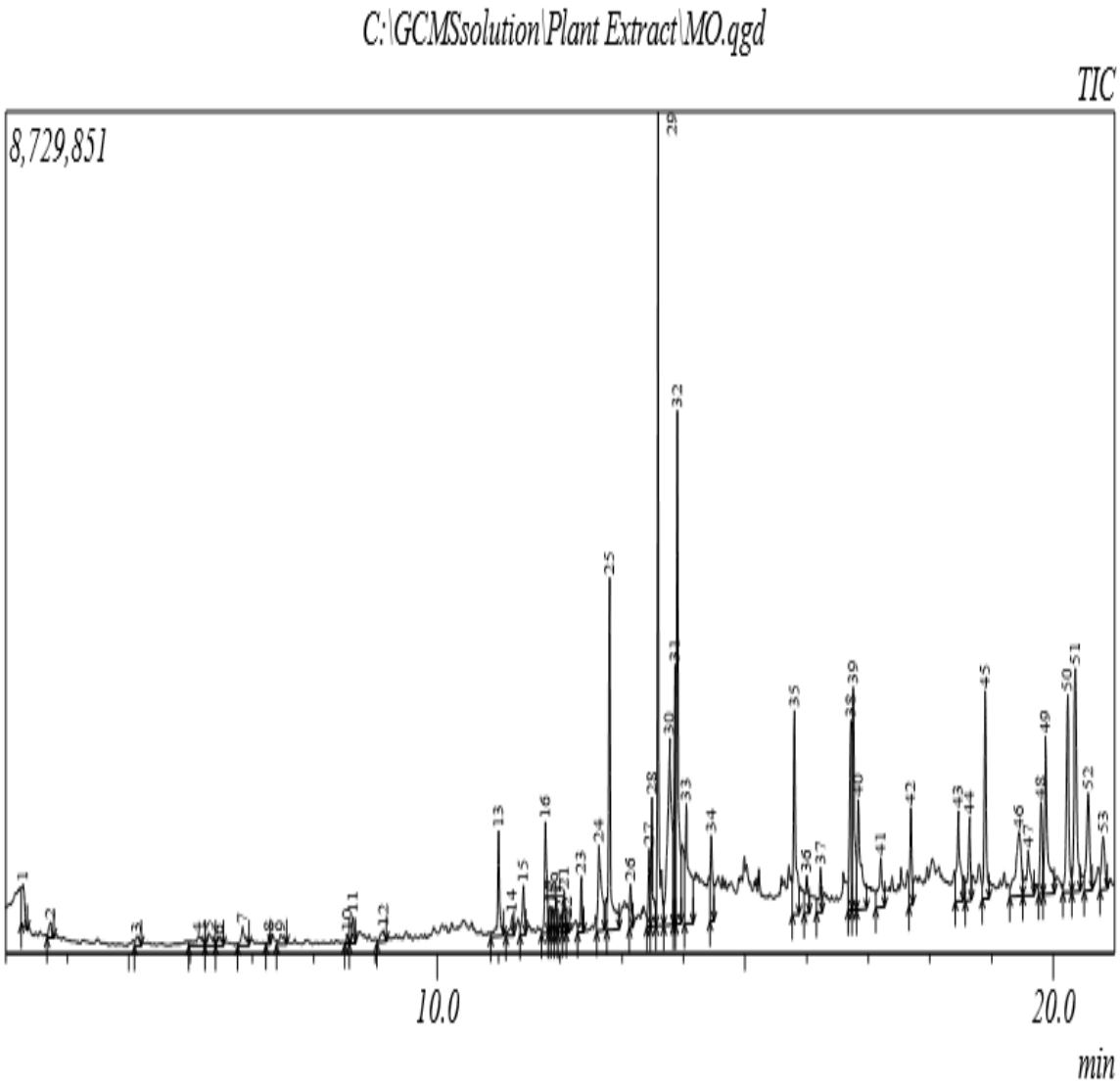

Figure 3. GC-MS full spectrum analysis of *Moringa oleifera* ethanolic leaf extract.

Table 3. Phytochemical compounds identified for various GC-MS peaks for *Moringa oleifera* ethanolic leaf extract

Peak	R.Time	Area%	Height%	Name
1	3.283	0.96	0.73	Glycerin
2	3.727	0.34	0.28	2-Butyn-1-ol, 4-methoxy-
3	5.126	0.28	0.17	2-Decanynoic acid
4	6.127	0.54	0.17	Benzyl nitrile
5	6.294	0.46	0.21	1-Deoxy-d-mannitol
6	6.46	0.27	0.13	Acetic acid, chloro-, isobutyl ester
7	6.843	0.54	0.32	Artemiseole
8	7.278	0.15	0.18	2-Decanynoic acid
9	7.460	0.40	0.18	Octanal
10	8.544	0.13	0.16	Cyclohexanecarboxaldehyde, 3,3-dimethyl
11	8.636	0.46	0.47	2-Cyclohexen-1-one, 3-(hydroxymethyl)-6
12	9.130	0.37	0.20	Cyclohexanepropanol, .alpha.,2,2,6-tetrame
13	11.000	1.59	1.87	(+)-1-Cyano-d-camphidine
14	11.216	0.42	0.34	2'-Acetonaphthone, 1',2'.alpha.,3',4',4'a,5',6',
15	11.399	0.77	0.88	Bicyclo[2.2.2]octane-1,4-diol, monoacetate
16	11.759	1.76	1.99	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
17	11.835	0.47	0.50	7-Hexadecenal, (Z)-
18	11.867	0.34	0.47	cis-1,2-Cyclododecanediol
19	11.932	0.43	0.63	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
20	12.008	0.38	0.35	1-Heptadec-1-ynyl-cyclopentanol
21	12.061	0.52	0.72	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
22	12.117	0.10	0.18	9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione
23	12.339	0.61	0.99	Hexadecanoic acid, methyl ester
24	12.626	2.34	1.52	n-Hexadecanoic acid
25	12.798	4.82	6.26	Hexadecanoic acid, ethyl ester
26	13.142	0.61	0.78	2-Propenoic acid, 3-[2-(aminocarbonyl)phen
27	13.443	0.95	1.38	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylc
28	13.483	1.72	2.29	6-Octadecenoic acid, methyl ester, (Z)-
29	13.585	8.85	14.48	Phytol
30	13.774	6.41	3.32	9,12-Octadecadienoic acid (Z,Z)-
31	13.858	2.95	4.63	Ethanol, 2-(9,12-octadecadienyloxy)-, (Z,Z)
32	13.899	6.68	9.15	9,12,15-Octadecatrienoic acid, ethyl ester, (
33	14.044	3.63	2.13	Ethyl 14-methyl-hexadecanoate
34	14.452	1.53	1.52	7-Hexadecenal, (Z)-
35	15.798	3.37	3.63	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)
36	15.997	0.83	0.67	Vinyl 10-undecenoate
37	16.227	0.80	0.80	Ethyl 14-methyl-hexadecanoate
38	16.717	3.42	3.38	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hyd
39	16.750	3.60	3.96	9,12,15-Octadecatrienoic acid, 2,3-dihydrox
40	16.835	3.04	1.96	Octadecanoic acid, 2,3-dihydroxypropyl ester
41	17.200	1.45	0.90	12-Oxododecanoic acid, ethyl ester
42	17.687	1.43	1.74	1-Decanol, 2-octyl-
43	18.458	2.26	1.61	gamma.-Tocopherol
44	18.575	1.65	1.48	3-Chloropropionic acid, heptadecyl ester
45	18.893	3.01	3.71	Vitamin E
46	19.445	2.76	1.14	Ergosterol
47	19.593	1.57	0.79	Campesterol
48	19.803	1.51	1.66	Stigmasterol
49	19.875	3.84	2.81	Octacosanol
50	20.231	4.03	3.52	gamma.-Sitosterol
51	20.357	4.99	3.98	Cholest-5-en-3-ol, 24-propylidene-, (3.beta.
52	20.565	2.25	1.74	Acetic acid, 3-hydroxy-7-isopropenyl-1,4a
53	20.811	1.40	0.94	Ergosta-7,22-dien-3-ol, (3.beta.,22E)-
		100.00	100.00	

Table 4. Common phytoconstituents in the three plant extracts and their anti-diabetic activity

Phytoconstituent	Percentage content			Anti-diabeticActivity[¥]
	NL	Al	MO	
3,7,11,15-tetramethyl-2-hexadecen-1-ol	0.87	1.30	1.99	Insulin & anti-inflammatory
Phytol	0.91	26.24	14.48	Regulates glucose metabolism
Hexadecanoic acid, methyl ester	2.99	1.11	0.99	anti-inflammatory, antioxidant,
Hexadecanoic acid, ethyl ester	20.29	2.15	6.26	and hypocholesterolemic
Ethyl 14-methyl hexadecanoate	1.83	1.70	2.13	none
Cyclopropaneoctanoic acid	2.66	0.78	1.38	none
9,12, Octadecadienoic acid	7.69	1.15	3.32	hypocholesterolemic, hepato-protective, antioxidant
Octadecanoic acid	7.19	1.87	1.96	lowers LDL cholesterol
Hexadecanoic acid, 2-hydroxy-1(OHmethyl)*	1.28	4.69	3.63	antioxidant and anti-inflammatory
Vitamin E	3.45	3.79	3.71	antioxidant, hypocholesterolemic, anti-inflammatory, hepatoprotective
Campesterol	1.09	1.70	0.79	None
Stigmasterol	0.64	4.88	1.66	anti-hepatotoxic, antioxidant, hypocholesterolemic
Gamma sitosterol	3.39	8.77	3.52	anti-inflammatory, anti-hyperlipidemic, hepatoprotective

*-(hydroxymethyl) ethyl ester
[¥]Mgbeje and Abu, 2020 and references therein

DISCUSSION

Despite increasing popularity worldwide in the use of medicinal plants in healthcare delivery on account of its affordability, accessibility, minimal side effects and the perception that it provides a balanced and moderate approach to healing, it is yet to be fully integrated into western medicine because of quality, standardization, efficacy and safety concerns.

Standardization is particularly challenging as medicinal plant extracts are a mixture of several ingredients and the question then arises as to how much a patient must ingest for an effective and safe cure. This is more so as in most cases the active principles fail to give desired activity when isolated individually.

The present study was carried out to obtain and compare chemical fingerprints of the ethanolic leaf extracts of three (3) known antidiabetic plants and to establish if they are some common phytochemicals, with antidiabetic activities, that could be used as active biomarkers for antidiabetic plants. This will also enable the standardization of the herbal preparations using the concentration levels of the biomarkers as baseline.

Standardization involves adjusting the herbal drug preparation to a defined content of a constituent or a group of substances with known therapeutic activity (referred to as true or Quantified extract); where the active ingredients are not known non active constituents or markers or, in its absence, the percentage extractable matter with a solvent may be used as a form of standardization an approach often seen in pharmacopeias (Bandaranayake, 2006; Gurib-Fakim, 2006; EMA, 2008; Efferth and Greten, 2012; Padalia, 2012; Zhang et al., 2012; Mgbeje, 2015). Accordingly and arising from the comparative analysis of the ethanolic extracts of the three validated anti diabetic plants in this study, it is proposed that anyone or combination of the ten (10) common phytochemicals with antidiabetic activities namely, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, phytol, hexadecanoic acid methyl ester, hexadecanoic acid ethyl ester, hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, 9,12-octadecadienoic acid, octadecanoic acid, vitamin E, stigmasterol and gamma sitosterol may be used as active therapeutic markers for standardization of the three plants and by extension other anti-diabetic plants if this same commonalities can be established for the plants. This is akin to standardized Ginkgo containing 26% flavones and 6% terpenes (Blumenthal *et al.*, 2000; Bandaranayake, 2006; Gurib-Fakim, 2006; World Health Organization, 2011). By virtue of their narrow concentration range in the three sample plants, 3,7,11,15-tetramethyl-2-hexadecen-1-ol (0.87-1.99%) and Vitamin E (3.45- 3.79%) will appear to be the more suitable active marker candidates for standardization of these three plants. Universal application to other anti-diabetic plants will obviously require a similar phytochemical analysis of the ethanol fraction of the plants.

CONCLUSION

In summary, a comparative GC-MS phytochemical analysis of the ethanol extract of three (3) validated antidiabetic plants namely *Nauclea latifolia*, *Azadirachta indica* and *Moringa oleifera* was carried out with a view to

unravelling common phytochemicals with known antidiabetic activities that could be used as active markers in standardization of the medicinal plant extracts. Of the Thirteen (13) common phyto-constituents, ten (10) were known to have antidiabetic activities and could be employed as active markers for standardization of the plant extracts. Based on the narrow percentage range in the three plants, Vitamin E and 3,7,11,15-tetramethyl-2-hexadecen-1-ol should be better active diabetic marker candidates for standardization of the three plants.

REFERENCES

ADA (American Diabetes Association) (2005). Diagnosis and classification of diabetes mellitus. Diabetes Care; 28(suppl. 1): S37–S43.

Akerele O (1993). Summary of WHO guidelines for the assessment of herbal medicines. *HerbalGram*; 28: 13-19.

Antia BS, Okokon JE (2014). Phytochemical composition and antidiabetic activity of ethanol root extract of Nauclea latifolia. J. Phytopharmacol.; 3: 52–56.

Bandaranayake WM (2006).Quality Control, Screening, Toxicity, and Regulation of Herbal Drugs. In *Modern Phytomedicine. Turning Medicinal Plants into Drugs. Edited by* I. Ahmad, F. Aqil, and M. Owais. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, pp25-57.

Blumenthal M, Brusse WR, Goldberg A, Gruenwald J, Hall T, Riggins CW & Rister RS (1998). *The Complete German Commission E Monographs. Therapeutic Guide to Herbal Medicines*. The American Botanical Council, Austin, TX, USA.

Boucherle B, Haudecoeur R, Queiroz EF, De Waard M, Wolfender JL, Robins RJ, Boumendjel A (2016). *Nauclea latifolia*: Biological activity and alkaloid phytochemistry of a West African tree. Nat. Prod. Rep.; 33: 1034–1043.

Cajuday LA, Pocsidio GL (2010). Effects of Moringa oleifera Lam (Moringaceae) on the reproduction of male mice (*Mus musculus*). Journal of Medicinal plants Research; 4(12):1115-1121.

Calixto JB (2000). Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). *Braz J Med Biol Res*; 33: 179-189.

De Smet PAGM (1997). The role of plant derived drugs and herbal medicines in healthcare. *Drugs*; 54: 801-840

Ebong PE, Efiong EE, Mgbeje BIA**,** Igile GO& Itam EH (2014). Combined therapy of *Moringa Oleifera* and *Ocimum gratissimum* reversed testicular damage in diabetic rats. British Journal of Medicine & Medical Research; 4(11): 2277-2290.

Efferth T and Greten HJ (2012).Quality Control for Medicinal Plants.*Medicinal and Aromatic Plants*; 1:7-9.

Effiong GS, Mgbeje BI, Igile GO, Atangwho JI, Eyong EU, Ebong PE (2013). Antioxidant enzymes activity and hormonal changes following administration of ethanolic leaves extracts of *Nauclea latifolia* and *Gongronema latifolium* in streptozotocin induced-diabetic rats. Eur. J. Med. Plants; 3: 297–309.

Efiong EE, Igile GO, Mgbeje BIA**,** Otu EA & Ebong PE (2013). Hepatoprotective and antidiabetic effect of combined extracts of *Moringa oleifera* and *Vernonia amygdalina* in streptozotocin-induced diabetic albino Wistar rats. Journal of Diabetes and Endocrinology; 4(4): 45-50.

Essien NA, Iwara AI, Mgbeje BIA, Igile GO, Egbung GE, & Ebong PE (2013). Lipid profile and hepatoprotective effects of combined leaf extracts of *Azadirachta Indica* (Neem) and *Peristrophe bicalyculata* in Alloxan-induced diabetic rats. International Journal of Medicinal Plants; 5(2): 159-162.

EMA (European Medicines Agency) (2008).Reflection paper on markers used for quantitative and qualitative analysis of herbal medicinal products and traditional herbal medicinal products. [http://www.emea.europa.eu/pdfs/human/hmpc/25362907en.pdf].

Fahey JW (2005). *Moringa oleifera*: A Review of the Medical Evidence for Its Nutritional, Therapeutic, and Prophylactic Properties. Part 1.Trees for Life Journal; 1:5.

Farnsworth NR, Akerele O, Bingel AS, Soejarto DD, Guo Z (1985) *World Health Organ.; 63*, 965.

Gidado A; Ameh DA; Atawodi SE; Ibrahim S (2012). A preliminary study of the mechanism of hypoglycaemic activity of *Nauclea latifolia* leaf ethanolic extract. J. Complement. Integr. Med.; 9: 16.

Gupta S, Kataria M, Gupta PK, Murganandan S, and Yasharoy RC (2004). Protective Role of Extract of Neem Seeds in diabetes Caused by Streptozocin in Rats. J. Ethnopharmacol.; 90(203): 185-9.

Gurib-Fakim A (2006). Medicinal plants: Traditions of yesterday and drugs of tomorrow. Molecular Aspects of Medicine; 27: 1–93.

Halliwell B, Gutteridge JMC (1989). Free Radicals in Biology and Medicine, 2nd edition.Oxford; Clarendon Press.

Iwara IA, Otu IA, Efiong EE, Igile GO, Mgbeje BIA& Ebong PE (2013). Evaluation of the nephroprotective effects of combined extracts of *Vernonia amydalina* and *Moringa oleifera* in diabetes induced kidney injury in albino Wistar rats. Scholars Journal of Applied Medical Sciences; 1(6): 881-886.

Kausik BI, Ranajet KB (2002). Biological Activities and Medicinal Properties of Neem Plant. Current Science; 82 (11): 1336-1345.

Kunle OF, Egharevba HO and Ahmadu PO (2012).Standardization of herbal medicines - A review. *International Journal of Biodiversity and Conservation*; 4(3): 101-112.

Mass spectrometry Data Center, National Institute of Standards and Technology. http://chemdata.nist.gov.

Mgbeje BIA(2015). Quality and Safety of Medicinal Plants In*Phytomedicine: Panacea for Primary health care Delivery in Nigeria* (P.E Ebong Ed.). Calabar, University of Calabar Press, pp. 293-310.

Mgbeje BIA and Abu C (2020). Chemical fingerprinting of *Nauclea latifolia*, an antidiabetic plant, using GC-MS. Journal of Complementary and Alternative Medical Research; *9*(4), 25-34.

Mgbeje BIA, Ofem-Otu J, Ugoanyanwu FO (2020). Phytochemical components of butanol fraction of *Vernonia amygdalina* leaf extract using GC-MS analysis. Merit Research Journal of Medicine & Medical Sciences; 8(4): 74-79.

Moussa SA (2008). Oxidative stress in diabetes Mellitus. Romanian J. Biophys.; 18(3): 225-236.

Odugbemi TA (2008). A textbook of Medicinal Plants from Nigeria. Lagos: University of Lagos Press.

Orwa, C., Mutua, A., Jamnadass, R. & Anthony, S. (2009). *Agroforestry database: A tree reference and selection guide*. Kenya: World Agroforestry Centre.

Padalia RC (2012). Medicinal and Aromatic plants: Chemical Goldmines. *Medicinal Aromatic plants*; 1: e113.

Paliwal R, Sharma V, Pracheta, Sharma S, Yadav S, Sharma S (2011). Anti-nephrotoxic effect of administration of *Moringa oleifera* Lam in amelioration of DMBA-induced renal carcinogenesis in Swiss albino mice. Biology and Medicine; 3(2) 27-35.

Rasheed NMA, Nagaiah K, Goud PR and Sharma VUM (2012). Chemical marker compounds and their essential role in quality control of herbal medicines. *Annals of Phytomedicine*; 1(1): 1-8.

Robertson RP (2004). Chronic oxidative stress as a central mechanism for glucose toxicity in pancreatic islet beta cells in diabetes. J. Biol. Chem.; 279(41): 42351–42354.

Stein R (2004) Alternative remedies gaining popularity. *The Washington Post*. Friday, May 28.

World Health Organization (2011). Quality control methods for herbal materials: Updated edition of Quality control methods for medicinal plant materials. *World Health Organization*, Geneva.

Yanpallewar SU, Sen S, Tapas S, Kumar M, Raju SS, Acharya SB (2003). Effects of *Azadirachta Indica* on Paracetamol Induced hepatic Damage in Albino Rats. Phytomedicine; 10(5):391-39.

Zhang J, Wider B, Shang H, Li X, Ernst E (2012). Quality of herbal medicines: challenges and solutions. *Complement Ther Med*.; 20(1-2):100-6.