



GCMS analysis of Phytochemicals in the Methanolic Extract of *Moringa oleifera* Leave.

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Received 29 October, 2015; Accepted 20 November, 2015 © The author(s) 2015. Published with open access at www.questjournals.org

ABSTRACT:- A preliminary phytochemical study of leaves of *Moringa oleifera* was done using Gas chromatography-Mass spectrometry for the determination of the constituents. Chromatogram of the GCMS analysis carried out for the identification of the phytochemicals present in the metabolic leave extract of indicated the presence of eleven compounds. The main compounds were methyl (11E)-11-octadecanoate, 30.15% and cis octadecanoic acid, 19.16% with bioactivities of antimetabolic syndrome and anticardiovascular risk factor. Phytochemicals produced by plants could act as alternatives to antibiotics, antihelminthics, antiviral of several infectious agents or act as nutritional factors for non infectious diseases. They can also act as antioxidants and flavouring agents.

Keywords:- Anticardiovascular risk factor, Antimetabolic syndrome, 5-alpha reductase, GC-MS analysis, *Moringa oleifera*, 5-alpha reductase.

I. INTRODUCTION

It has been shown that *in vitro* screening methods could provide the needed preliminary observations required to select crude plant extracts with potentially useful properties for further investigations [1]. Plants are important medicinal sources in different countries and therefore are used as potent and efficacious drugs. Plants have used traditionally as medicine for several years in folk medicine [2].

Moringa oleifera (family *Moringaceae*) popularly called Horse radish tree, Drumstick tree or ben oil tree in English, Zogalle by the Hausas, Ikwe Oyibo by the Igbos, Eweile by the Yorubas and Gawara by the Fulanis, all of Nigeria [3] is a fast growing evergreen deciduous, perennial tree which grows to a height of 10-12 meters with trunk which may reach 45cm. The plant is slender with drooping and brittle branches. The leaves are feathery, pale green, compound trippinnate and 30-60cm, with many small leaflets. Flowers are white or creamy with fragrant smell and are bisexual [3,4]. The plant is reported to be used in phytomedicine as antioxidant, antimicrobial, anti-inflammatory, antipyretic, antiulcer, anti-diabetic, antitumor and as a hypocholesteromic agent [5,4]. The aim of this research is to identify phytochemicals in plants by GC-MS analysis as preliminary study. Then structural-functional relationship to bioactivity will be established thereby charting a new road map for drug discovery by molecular docking. The compounds with the highest peak area percentage in the extract could be adopted for molecular modelling because of the presence of particular bioactive compounds. Furthermore our focus is to chart a new way for plant based drug which will be docked and modelled so that it could be used to control metabolic and other diseases.

II. MATERIALS AND METHODS

2.1 Plant Material

Fresh leaves of *Moringa oleifera* was harvested at Ohafia town in Abia State, Nigeria. The plant leaves were identified by Prof M C Dike at the Taxonomy section of College of Natural and Environmental Management, Michael Okpara University of Agriculture, Umudike, Nigeria.

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2.2 Preparation of Plant Extract

The plant material of *Moringa oleifera* was collected from wild, shade dried for 10 days and pulverized to powder using mechanical grinder. The plant extract was prepared using Soxhlet method described by Jensen, [6]. Thirty five grams (35 g) of powdered sample was introduced into the extraction chamber of the Soxhlet extractor using methanol as solvent. Temperature was maintained at 70° C throughout the extraction period of 48 hrs. At the end of the extraction period, the extract was concentrated using oven at 35° C to obtain dried extract which was sent for GCMS analysis.

2.3 GCMS analysis of *Moringa oleifera*

The characterization of the Phytochemicals in *Moringa oleifera* was done using GC-MS QP2010 Plus (Shimadzu, Japan). The identification of the phytochemicals in the sample was carried out using a QP2010 gas chromatography with Thermal Desorption System, TD 20 coupled with Mass Spectroscopy (Shimadzu). The ionization voltage was 70eV. Gas Chromatography was conducted in the temperature programming mode with a Restek column (0.25 mm, 60 m, XTI-5). The initial column temperature was 80°C for 1min, and then increased linearly at 70°C min⁻¹ to 220°C, held for 3 min followed by linear increased temperature 10°C min⁻¹ to 290°C for 10 min. The temperature of the injection port was 290°C and the GC-MS interface was maintained at 290°C. The sample was introduced via an all-glass injector working in the split mode, with helium carrier gas low rate of 1.2 ml min⁻¹. The identification of compounds was accomplished by comparison of retention time and fragmentation pattern, as well as with mass spectra of the GC-MS.

2.4 Identification of Phytochemicals in *Moringa oleifera*

Identity of the active components in the extract was by comparison of their retention indices, peak area percentage and mass spectra fragmentation pattern with those stored on the National Institute of Standards and Technology (NIST) digital library data and also with published literature. NIST08.LIB [7], WILEY8.LIB [8], library sources were used for matching the identified components from the plant material. The name, molecular weight, formula, structure and bioactivities of the compounds were ascertained.

III. RESULT AND DISCUSSION

3.1 Results

GCMS chromatogram of the methanolic extract of *Moringa oleifera* (Figure 1) showed eleven peaks which indicated the presence of eleven phytochemicals constituents. Figure 2 shows the mass spectra of methanolic extract of *Moringa oleifera*

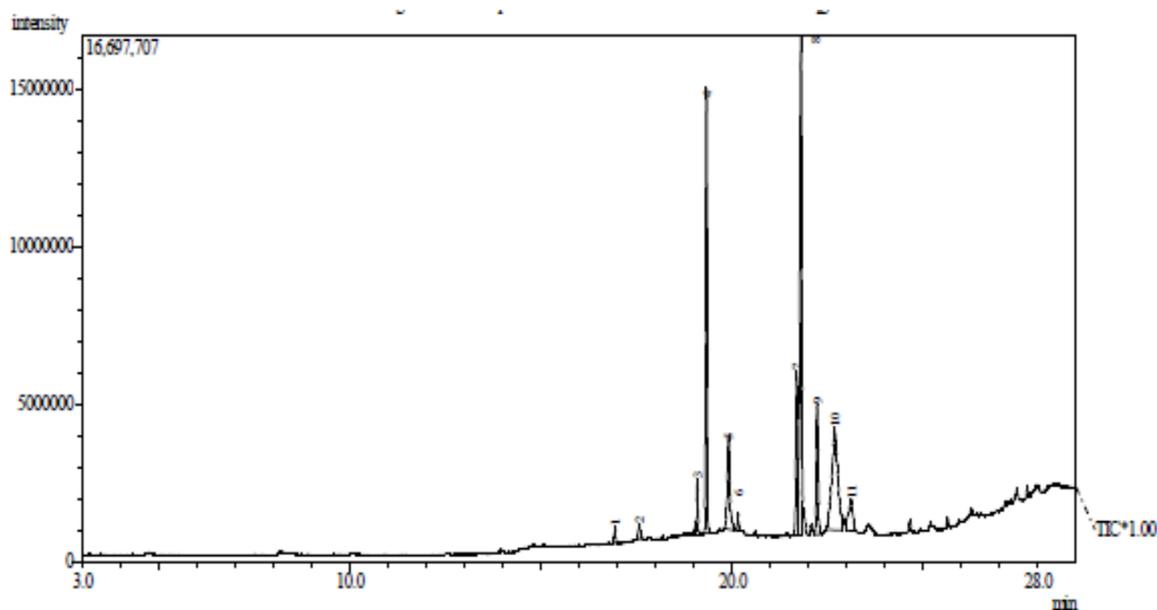


Fig 1 Shows GCMS chromatogram of methanolic extract of *Moringa oleifera*

The mass spectra of the phytochemicals in *Moringa oleifera* were compared with that in the NIST Library database, then the eleven compounds were characterized and identified Figure 2.

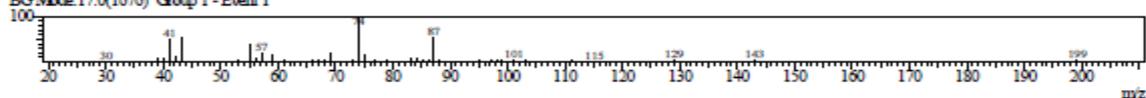
GCMS analysis of Phytochemicals in the Methanolic Extract of *Moringa oleifera* Leave.

Line# 1 R.Time:16.9(Scan#:1674)

MassPeaks:46

RawMode:Single 16.9(1674) BasePeak:74(72801)

BG Mode:17.0(1676) Group 1 - Event 1

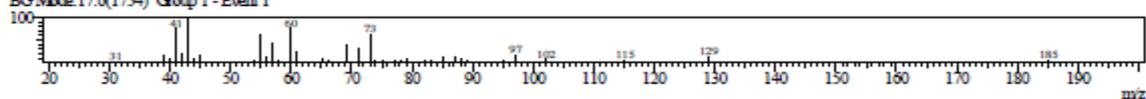


Line# 2 R.Time:17.6(Scan#:1749)

MassPeaks:57

RawMode:Single 17.6(1749) BasePeak:43(37717)

BG Mode:17.6(1754) Group 1 - Event 1

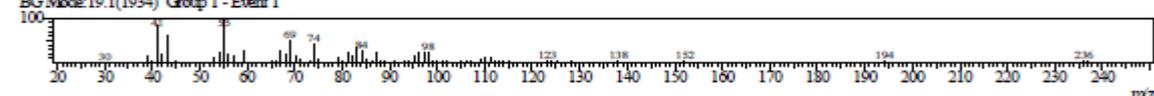


Line# 3 R.Time:19.1(Scan#:1932)

MassPeaks:78

RawMode:Single 19.1(1932) BasePeak:55(111387)

BG Mode:19.1(1934) Group 1 - Event 1

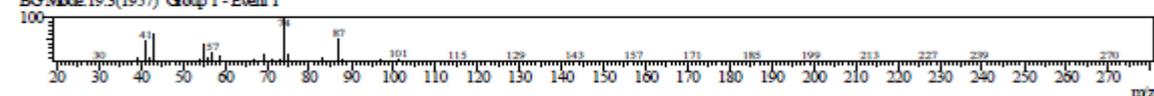


Line# 4 R.Time:19.3(Scan#:1961)

MassPeaks:101

RawMode:Single 19.3(1961) BasePeak:74(2274285)

BG Mode:19.3(1957) Group 1 - Event 1

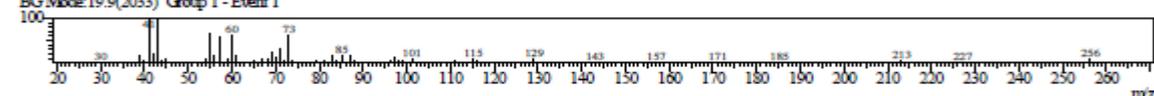


Line# 5 R.Time:19.9(Scan#:2029)

MassPeaks:80

RawMode:Single 19.9(2029) BasePeak:41(110048)

BG Mode:19.9(2033) Group 1 - Event 1

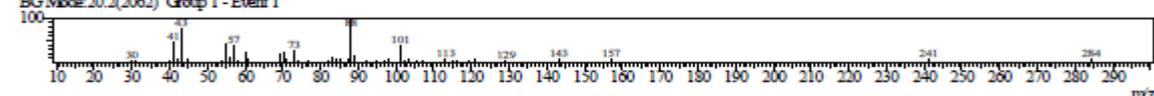


Line# 6 R.Time:20.2(Scan#:2060)

MassPeaks:60

RawMode:Single 20.2(2060) BasePeak:88(42493)

BG Mode:20.2(2062) Group 1 - Event 1

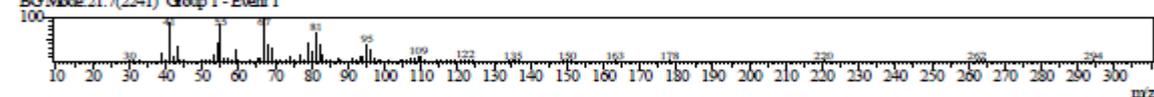


Line# 7 R.Time:21.7(Scan#:2244)

MassPeaks:96

RawMode:Single 21.7(2244) BasePeak:67(245945)

BG Mode:21.7(2241) Group 1 - Event 1

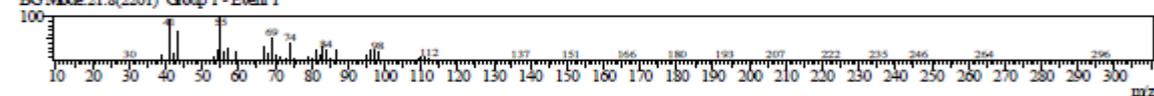


Line# 8 R.Time:21.8(Scan#:2257)

MassPeaks:137

RawMode:Single 21.8(2257) BasePeak:55(1220373)

BG Mode:21.8(2261) Group 1 - Event 1

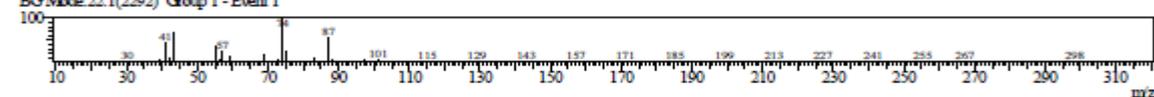


Line# 9 R.Time:22.2(Scan#:2308)

MassPeaks:90

RawMode:Single 22.2(2308) BasePeak:74(774917)

BG Mode:22.1(2292) Group 1 - Event 1

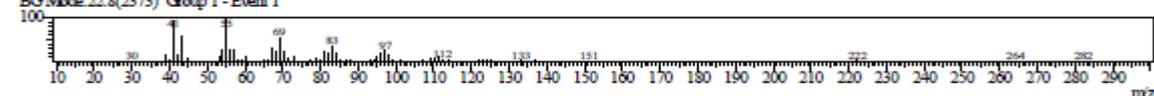


Line# 10 R.Time:22.7(Scan#:2363)

MassPeaks:86

RawMode:Single 22.7(2363) BasePeak:55(183221)

BG Mode:22.8(2373) Group 1 - Event 1



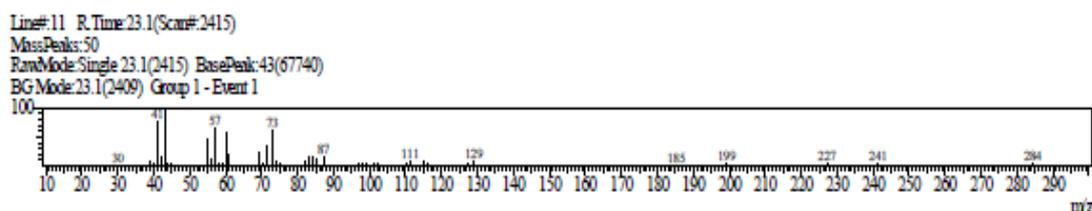


Fig 2 shows the mass spectra of methanolic extract of *Moringa oleifera*

The active principles with their retention time (RT) and concentration (Peak area %) are presented in (Table 1). The molecular weight, formula, structure and bioactivities of phytochemicals that contributed to the medicinal activity of *Moringa oleifera* are shown in (Table 1). Among the eleven compounds identified, the main abundant compounds were, methyl (11E)-11-octadecanoate, 30.15% and cis octadecanoic acid, 19.16% with bioactivities of antimetabolic syndrome and anticardiovascular risk factor.

Table 1 Shows the name, retention time, molecular weight, formula, structure and bioactivities of <i>Moringa oleifera</i>							
S. No	Name of Compound	Retention time	Peak area %	Molecular weight	Molecular formula	Molecular structure	Bioactivity
1	Methyl undecanoate	16.93	0.57	200.31	C ₁₂ H ₂₄ O ₂		Natural flavoring substances
2	Undecanoic acid	17.56	0.98	186.29	C ₁₁ H ₂₂ O ₂		Antifungal agent, antiseborrhoeic
3	methyl tetradeca-8,10,12-trienoate	9.090	2.08	236.34	C ₁₅ H ₂₄		Protective against metabolic syndrome and cardiovascular disease risk factors [9].
4	Methyl -14- methyl pentadecanoate	9.328	17.67	270.45	C ₁₇ H ₃₄ O ₂		Flavoring agent
5	n-Hexadecanoic acid or Palmitic acid	19.909	7.46	256.42	C ₁₆ H ₃₂ O ₂		Mild antioxidant and anti-atherosclerotic activity [10]
6.	Ethyl hexadecanoate or Ethyl palmitate	0.157	0.86	284.47	C ₁₈ H ₃₆ O ₂		Mild antioxidant and anti-atherosclerotic activity [10]
7	Methyl linolealidate or Methyl trans,trans-9,12-octadecadienoate	21.691	9.21	294.47	C ₁₉ H ₃₄ O ₂		Protective against metabolic syndrome and cardiovascular disease risk factors [9].
8	Methyl (11E)-11-octadecenoate	21.802	30.15	296.48	C ₁₉ H ₃₆ O ₂		Protective against metabolic syndrome and cardiovascular disease risk factors [9].
9	Methyl hexadecenoate	22.229	7.64	268.43	C ₁₇ H ₃₂ O ₂		Antioxidant, hypocholesterolemic nematicide, pesticide, flavor, lubricant, antiandrogenic, hemolytic 5-Alpha reductase inhibitor
10	cis-.Octadecenoic acid or cis-Oleic Acid	22.683	19.16	282.46	C ₁₈ H ₃₄ O ₂		Protective against metabolic syndrome and cardiovascular disease risk factors [9].
11	Octadecanoic acid or Stearic acid	23.112	4.23	284.47	C ₁₈ H ₃₆ O ₂		Antiinflammatory, Antiandrogenic Cancer preventive, Dermatitogenic Hypocholesterolemic, 5-Alpha reductase inhibitor, anemiagenic insectifuge, flavor [11]

3.2 Discussion

Lipid lowering drugs work in several ways including decreasing cholesterol production, decrease cholesterol absorption from the intestine and removing cholesterol from blood stream [12]. Drugs that act directly to decrease cholesterol levels also have the beneficial effect of further lowering cholesterol levels by stimulating the production of additional LDL receptors [13]. There are currently five major types of medications available for treating hypercholesterolemia. HMG-CoA reductase inhibitor (Statins), bile acid-binding resins, cholesterol absorption inhibitor agents, niacin and its congeners and the fibrates [14]. HMG-CoA reductase (eg atorvastatin, rosuvastatin, simvastatin) a key enzyme in the cholesterol biosynthetic pathway can reduce or block hepatic synthesis of cholesterol and are the cornerstone of LDL reducing therapy. Statins also reduce triglyceride levels [14]. From GCMS result *Moringa oleifera* contain octadecanoic acid (stearic acid) $C_{18}H_{36}O_2$ and cis octadecenoic acid (cis oleic acid) $C_{18}H_{34}O_2$ which had hypolipidemic activity may be because it contains 5-alpha reductase inhibitor which may have blocked HMG-CoA reductase which is a major enzyme in the cholesterol biosynthetic pathway (Gopalakrishnan and Vadivel 2011). Atherosclerosis which is the hardening of the arteries leads to cardiovascular heart disease (CHD) which is the leading cause of death among men and women in USA [15]. The main compounds were methyl (11E)-11-octadecanoate, 30.15% and cis octadecanoic acid, 19.16% with bioactivities of antimetabolic syndrome and anticardiovascular risk factor acting by decreasing cholesterol and triglyceride (Gillingham et al 2011). The resultant decrease in cholesterol and triglycerides are important in treatment of the metabolic syndrome [16]. The major complications of atherosclerosis includes ischemic heart disease, stroke and peripheral vascular disease [17].

IV. CONCLUSION

From the GCMS analysis it shows that *Moringa oleifera* contain bioactive compounds with medicinal potentials. The plant is therefore recommended for further research because of its antimetabolic syndrome and anticardiovascular risk factor. It could therefore be beneficial in the management of hypertension, heart diseases, arteriosclerosis, stroke and heart failure. Compounds and several synthetic analogs of mavalonate are competitive inhibitors of HMG-CoA reductase which inhibits cholesterol synthetic pathway [18]. Octadecanoic acid (stearic acid) and octadecenoic acid or (oleic acid) identified in *Moringa oleifera* have hypocholesterolemic activity because of the presence of 5-alpha reductase inhibitor. Further research is required to corroborate the activity of 5-alpha reductase inhibitors activity found in *Moringa oleifera* and enzymes of synthetic pathway of cholesterol.

ACKNOWLEDGEMENT

We acknowledge with thanks, the grant from EUNISELL that made it possible for the experimental works to be completed.

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