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Fatty Acid Profile of *Tapinanthus bangwensis* and *Moringa oleifera* and Their Health Implications.

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Abstract

Lipid and lipophilic compounds are of great interest as bioactive additives in phytotherapy Plants contain bioactive compounds and fatty acids that have both medicinal and nutritional benefits. The aim of this research study was to evaluate the fatty acid profile of Tapinanthus bangwensis and Moringa oleifera and their health implications. The gas chromatography-mass spectroscopy technique (GC-MS) was used for the assessment. The result showed that there were saturated fatty acids, unsaturated fatty acids and methylated fatty acid esters present. The result showed that ethylacetate fraction of Tapinanthus bangwensis (ETF 1) showed the highest concentration of hexadecanoic acid (44.21%), acetone fraction of Moringa oleifera (ACF 2) had the highest concentration of both 9-Octadecenoic acid and Docosenoic acids (40.05%) respectively. The 9, 12, 15-Octadecenoic acids was most abundant in methanolic extract of Tapinanthus bangwensis (MeCE 1) (37.25%) while 9,12,15-Octadecatrienoic acid, methyl ester. was significantly concentrated in ethylacetate fraction of Moringa oleifera (16.48%) compared to the other samples. Finally, it could be concluded that Tapinanthus bangwensis and Moringa oleifera are good sources of dietary lipid of nutritional and medicinal benefits to animals and man. especially as anti-cardiovascular agent. The significance of this research study is that it provides an additional information on the fatty acid status of the two plant species investigated

Keywords: Phytotherapy, Gas chromatography/mass spectroscopy (GC-MS), *Tapinanthus bangwensis, Moringa oleifera,* Fatty acid profile

Introduction

Phytomedicine has existed since time immemoral [1]. Plants contain bioactive compounds and fatty acids that have been indicated in the treatment and management of various diseases and provides low incidence of adverse effects and this account for their worldwide use [2]. Medicinal plant constituents are the main source of new pharmaceutical and healthcare products [3]. The relevance of fatty acids in human diet and health cannot be overemphasized. The human brain and other organs are estimated to require nearly 60% [4]. Moreso, essential fatty acids usually of plant origin plays vital roles in the maintenance of optimal brain and health functions [5]. Fatty acids and essential fatty acids have been documented to regulate lymphocyte proliferation and metabolism [6], enhance autoimmunity [7], improves antioxidant activity [8], anti-inflammatory [9] and anticancer activity [10] Extraction and characterisation of several phytocompounds of these green factories have given rise to some high activity profile drugs but more knowledge of the chemical constituents of plant is desirable because such information will be valuable for the synthesis of complex chemical substances. However, researches have been carried out on the pharmacological effect(s) of *Tapinanthus bangwensis*) and *Moringa oleifera* but no much systematic research on the fatty acid profile of the leaves of the plants using gas chromatography-mass spectroscopy (GC-MS) has been reported. Therefore, the present study was undertaken to evaluate the fatty acid compositions of methanolic crude extracts and fractions of *Tapinanthus bangwensis* and *Moringa oleifera* using gas chromatography-mass spectroscopy technique. African mistletoe (*Tapinanthus bangwensis*) is an evergreen host-dependent plant of Africa origin belonging to the Loranthaceae family. It is called "all heal tree, bird lime or tree of life". Traditionally, in Nigeria, Hausas call it *Kauci (Kanchi)*, Yoruba (*Afomo onisana*) and Igbo (*Awurusie)*. It obtains its nutrients from host plant and via photosynthesis while *Moringa oleifera* on the other-hand is a fast-growing, drought-resistant and deciduous tree grown mainly in Hamalayas in India and widely cultivated in tropical and subtropical areas. It belongs to the family of Moringaceae. It is called Drumstick tree, Horseradish tree, benzoil tree, mother's best friend or miracle tree. In Nigeria, the Hausas call it *Bagaruar maka (masar)* or popularly known as *Zogale*; Yoruba- *Idagbo monoye* (tree that grows crazily) while Igbo -*Okochi egbu* (cannot be killed by drought).

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Materials and Method

Preparation of Plant Materials

Fresh leaves of African mistletoe (*Tapinanthus bangwensis*) was obtained in Lagos and was botanically identified by Mr Adeleke, Department of Pharmagnosy, University of Lagos.. *Moringa oleifera* leaves were obtained from the Co-ordinator's lodge, Nigeria Police Academy, Wudil, Kano and was botanically identified at Bayero University Kano, Kano State. The leaves of the plants were then washed and dried for five (5) days. The dried leaves were pulverized into a fluffy mass ready for extraction.

Extraction of Plant Materials

A 2150g ground leaves of *Tapinanthus bangwensis* and *Moringa oleifera* was extracted with 10,000cm^3 of methanol solvent using maceration method. The filtrates were then concentrated in an oven at 40^OC.

Solvent-partition Extraction

The concentrated methanolic crude extracts of *Tapinanthus bangwensis* and *Moringa oleifera* were further subjected to fractionation process using a separatory funnel. The solution mixture was ethylacetate-water and acetone-water in 3:2 respectively. For *Tapinanthus bangwensis*, the ethyl acetate fraction (ETF 1) was obtained by fractionating 60g of methanolic crude extract with 700cm^3 of ethyl acetate solvent while the acetone fraction (ACF 1) was obtained with fifty grams (50g) of methanolic crude extract with 700cm^3 of acetone solvent. The ethyl acetate fraction and acetone fraction obtained after dryness were 14.45g and 9.37g respectively. For *Moringa oleifera* fractionation, 72g of methanolic crude extract was fractionated with 700cm^3 of ethyl acetate solvent while 75g of methanolic crude extract was fractioned with 700cm^3 of acetone solvent respectively. The ethylacetate fraction (ETF 2) gave 8.02g while acetone fraction (ACF 2) gave 7.45g respectively.

Determination of Fatty Acids in Plant Extracts/Fractions

A 0.5grams of each crude extracts and fractions of *Tapinanthus bangwensis* and *Moringa oleifera* were dissolved in 5.0ml of their corresponding solvent of extraction and then subjected to gas chromatography-mass spectroscopy (GC-MS) technique.

Determination of Fatty Acid Methyl Esters (FAMEs)

The method of [11] was adopted. About 350mg of the plant extract/fraction was introduced into a 50ml round bottom flask. Methanolic sodium hydroxide (MeOH-NaOH) solution (5ml, 0.5M) was added to the flask together with boiling chips. The mixture was boiled under reflux for about 15mins. 7ml of methanolic boron trifluoride (MeOH-BF_3) solution was then added and boiled for 1min. A 5.0ml of heptane was added and boiled for another 1min. The flask was removed from the heat source. The condenser was removed and a small amount of saturated sodium chloride (NaCl) was added with gentle shaking by rotating several times. More saturated NaCl was added and the content was allowed to separate. The upper heptane layer was collected, dried with anhydrous sodium sulphate (Na_2SO_4) to remove traces of water and filtered. The filtrate was injected directly into the gas chromatography-mass spectroscopy (GC-MS).

Instrumentation (GC/MS)

Features	Values
Column Oven Temp.	80.0 °C
Injection Temp	250.00 °C
Injection Mode	Split
Flow Control Mode	:Linear Velocity (46.3 cm/sec)
Pressure	:108.0 kPa
Total Flow	6.2 mL/min
Column Flow	1.58 mL/min
Split Ratio	1.0
Purge Flow	3.0 mL/min
Sample volume	1µl

Results

The result showed that hexadecanoic acid was present in methanolic crude extracts and fractions of *Tapinanthus bangwensis* and *Moringa oleifera*. However the ETF 1 showed the highest concentration (44.21%) while MeCE 2 contained the lowest concentration (7.48%). Stearic acid was found in MeCE 1 and ETF 1 with concentrations as 2.92% and 9.02% respectively. Tetradecanoic acid was present in ETF 1 (1.82%), ACF 1 (1.04%) and ETF 2(2.80% respectively). The result showed that octadecanoic acid was absent in MeCE 1 and ETF 1 respectively, however, ACF 2 showed the highest concentration (14.77%) followed by ACF 1 (12.87%) while ETF 2 showed the lowest concentration (5.58%) The MeCE 2 concentration was found to be 8.00%.

Table 1: Fatty Acid Profile of Methanolic Crude Extract of *Tapinanthus bangwensis* using GC/MS

Peaks	Retention Time (min)	Injection Time	Flow Time	Area (%)	Saturated fatty acids	Mono unsaturated fatty acids	Polyunsaturated fatty acids	Fatty acid methyl esters.
9	16.98	16.900	17.117	2.41				Pentadecanoic acid, 14-methyl-, methyl ester.
10	18.382	18.00	18.858	23.32	Hexadecanoic acid(Palmitic acid)			
11	19.990	19.925	20.033	1.47				9,12-Octadecadienoic acid, methyl ester.
12	20.097	20.033	20.250	4.07				9,12,15-Octadecatrienoic acid, methyl ester.
14	21.222	20.708	21.600	37.23			9,12,15-Octadecatrienoic acid(Linonelic acid).	
15	21.700	21.600	21.950	2.92	Octadecanoic acid(Stearic acid).			

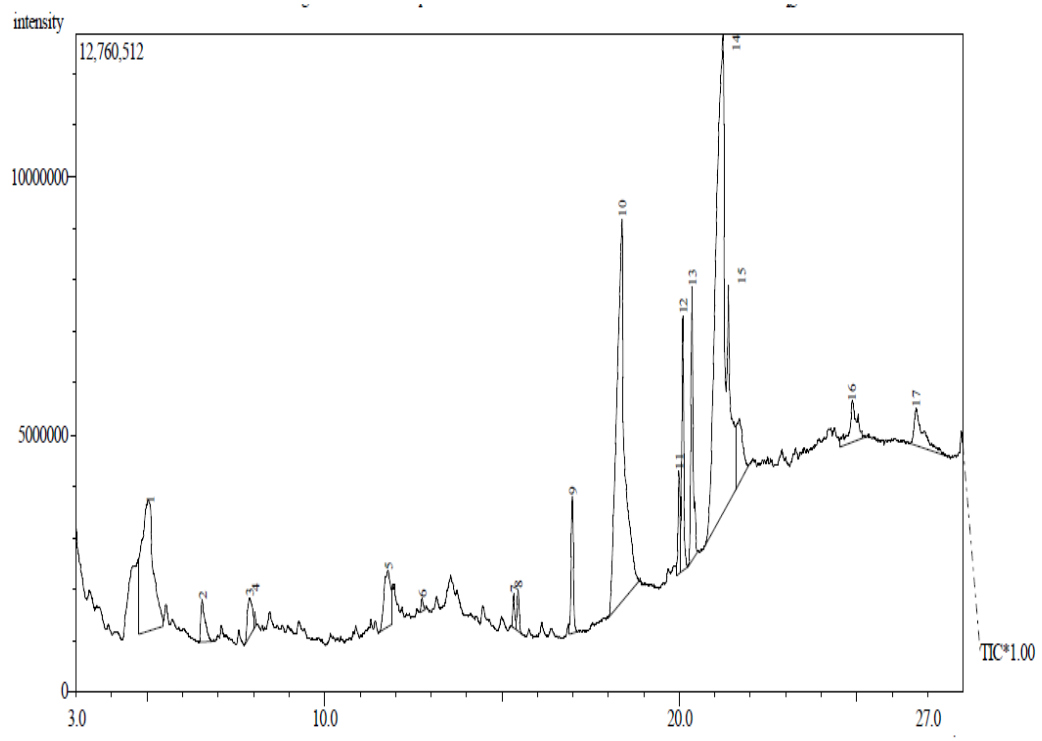

Figure 1: GC/MS chromatogram of fatty acids profile of methanolic crude extract of *Tapinanthus bangwensis.*

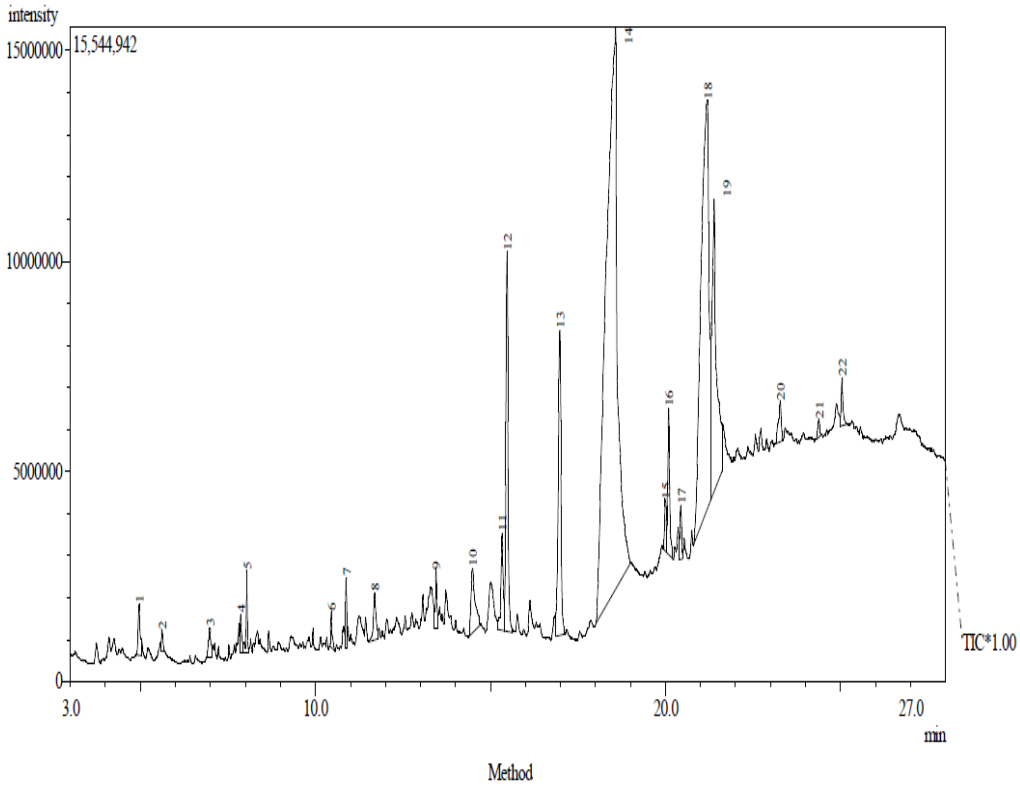

Figure 2 GC/MS chromatogram of fatty acids profile of Ethyl acetate fraction of *Tapinanthus bangwen*

Table 2 Fatty Acid Profile of Ethylacetate Fraction of *Tapinanthus bangwensis* using GC/MS

Peaks	Retention Time(min)	Injection Time	Flow Time	Area (%)	Saturated fatty acids	Mono unsaturated fatty acids	Polyunsaturated fatty acids	Fatty acid methyl esters
10	14.489	14.383	14.708	1.82	Tetradecanoic acid (Myristic acid)			
13	16.980	16.867	17.133	5.77				Heptacosanoic acid, methyl ester.
14	18.573	18.017	19.008	44.21	n-Hexadecanoic acid (Palmitic acid)			
15	19.994	19.942	20.033	0.57				9,12-Octadecadienoic acid, methyl ester (Linolelaidic acid, methyl ester).
16	20.088	20.033	20.225	1.93				11-Octadecenoic acid, methyl ester.
17	20.437	20.392	20.492	0.62				Octadecanoic acid, methyl ester.
18	21.187	20.800	21.292	22.47		9-Octadecenoic acid (Oleic acid),		
19	21.390	21.292	21.617	9.02	Octadecanoic acid (Stearic acid).			
21	24.380	24.300	24.442	0.25		(E)-13-Docosenoic acid (Erucic acid).		

Table 3: Fatty Acid Profile of Acetone Fraction of *Tapinanthus bangwensis* using GC/MS

Peaks	Retention. Time(min)	Injection Time	Flow Time	Area (%)	Saturated fatty acids	Mono-unsaturated fatty acids	Poly-unsaturated fatty acids	Fatty acid methyl esters
1	12.043	11.967	12.192	0.51	Hexadecanoic acid (Palmitic acid)			
2	14.465	14.375	14.642	1.04	Tetradecanoic acid (Myristic acid).			
4	16.541	16.467	16.667	0.54				Cyclopropanenonanoic acid, methyl ester.
5	16.991	16.875	17.200	6.27				:Pentadecanoic acid, 14-methyl-, methyl
6	18.291	18.017	18.808	19.31	n-Hexadecanoic acid (Palmitic acid).			
7	20.021	19.908	20.058	8.17				9,12-Octadecadienoic acid, methyl ester.
8	20.122	20.058	20.275	9.91				11-Octadecenoic acid, methyl ester.
9	20.437	20.375	20.583	3.11				Octadecanoic acid, methyl ester.
10	21.121	20.758	21.242	23.94		9-Octadecenoic acid (Oleic acid), 13-Docosenoic acid (Erucic acid).		
11	21.326	21.242	21.717	12.87	Octadecanoic acid (Stearic acid).			
13	24.390	24.233	24.542	6.49		(E)-13-Docosenoic acid		

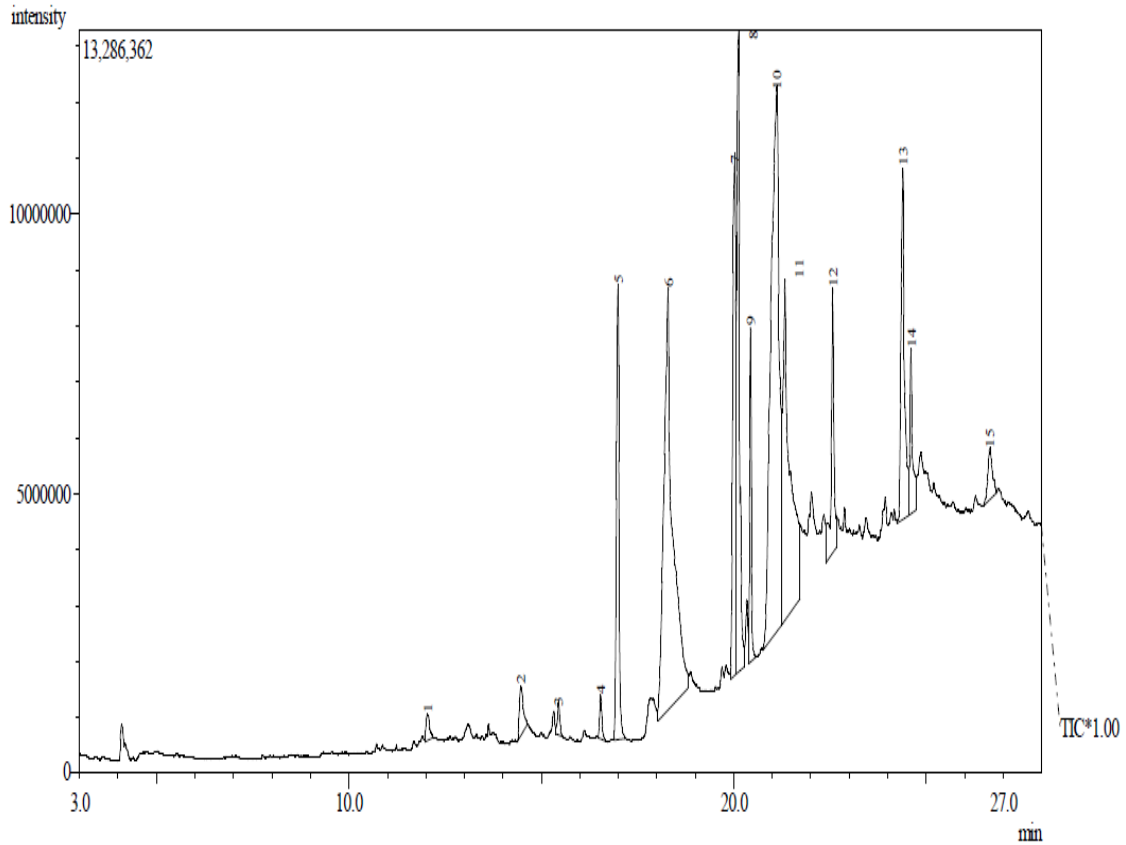

Figure 3: GC-MS chromatogram of fatty acids profile of Acetone fraction of *Tapinanthus bangwensis*

The GC-MS result also showed the presence of monounsaturated fatty acids. The most abundant were 9-octadecenoic acid and (E)-13-Docosenoic acid, however, they were found to be absent in ETF 2 and MeCE 1 respectively. For the 9-octadecenoic acid, ACF 2 showed the highest concentration (40.05%) while MeCE 2 showed the lowest concentration (15.65%). The 13-Docosenoic acid was most abundant in ACF 2 (40.05%), followed by ACF 1 (23.94%) while ETF 1 contained the lowest concentration (0.25%). The polyunsaturated fatty acid identified was 9, 12, 15-Octadecenoic acid and was found to be significantly present in MeCE 1 and ETF 2 at concentrations of 37.25% and 16.18% respectively. Methylated esters were also identified. The pentadecanoic acid. 14-methyl, methyl ester was found in MeCE 1(2.14%,), ACF 1(6.27%), MeCE 2 (0,58%) and ACF 2 (2.22%). The 9, 12-Octadecanoic acid, methyl ester was present in all the plant extracts/fractions except in ETF 2, however, MeCE 1 showed the highest concentration (14.47%) while the lowest concentration was shown by ETF 1(0.57%). The 11, 14, 17-Eicosanoic acid was found in ETF 2 (0.56%.) but absent in other samples. The 11-octadecanoic acid was present in all the extracts except in MeCE 1 and ETF 2 respectively, however, ACF 1 showed the highest concentration (9.91%) compared to others while MeCE 2 showed the lowest concentration (1.38%). The result showed that 9, 12, 15-Octadecanoic acid, methyl ester was present in MeCE 1 (4.07%) and ETF 2 (16.48%) respectively but absent in other extracts/fractions. The Octadecanoic acid, methyl ester was found to be present in ETF 1 and ACF 1 with concentrations as 0.62% and 3.11% respectively. Finally, 15-Tetradecanoic acid, methyl ester, decanoic acid, methyl ester and cyclopropanoic acid, methyl ester were present in ETF 2 (0.76%), ACF 2 (1.75%) and ACF 1 (0.54%) respectively.

Table 4: Fatty Acids Profile of Methanolic Crude Extract of *Moringa oleifera* using GC/MS

Peaks	Retention Time(min)	Injection Time	Flow Time	Area (%)	Saturated fatty acids	Mono-unsaturated fatty acids	Poly-unsaturated fatty acids	Fatty acid methyl esters.
16	16.970	16.875	17.058	0.58				Pentadecanoic acid, 14-methyl-, methyl ester.
17	18.208	17.992	18.708	7.48	n-Hexadecanoic acid (Palmitic acid).			
18	19.979	19.925	20.025	1.12				9,12-Octadecadienoic acid,methyl ester (Linolelaidic acid).
19	20.069	20.025	20.250	1.38				11-Octadecenoic acid, methyl ester.
21	21.035	20.717	21.192	15.65		9-Octadecenoic acid (Oleic acid).		
22	21.271	21.192	21.567	8.00	Octadecanoic acid			

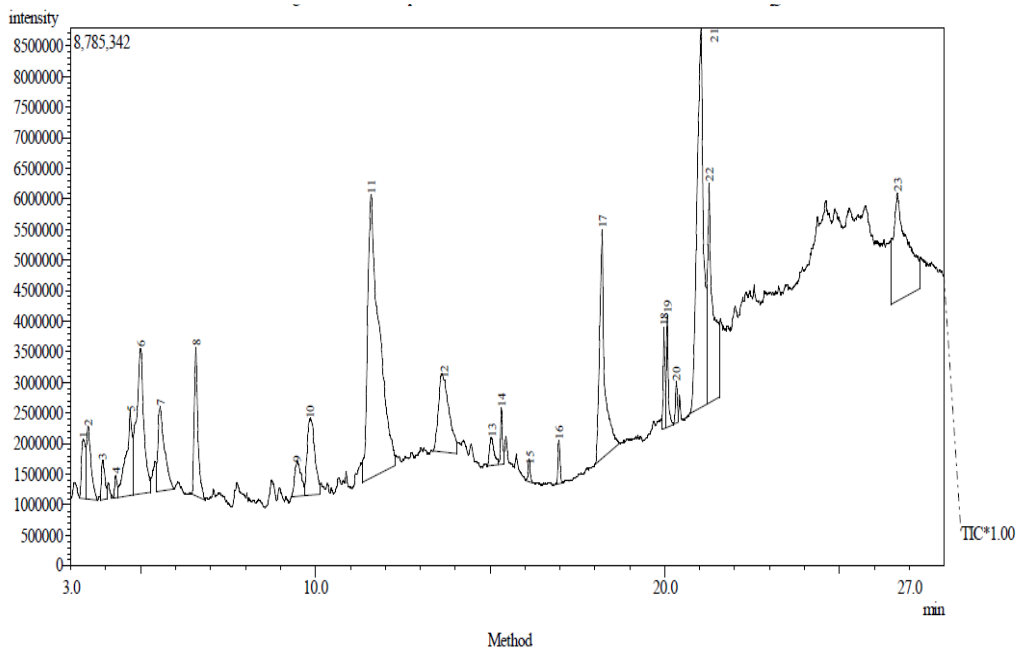

Figure 4: GC-/MS chromatogram of fatty acids profile of methanolic crude extract of *Moringa oleifera*

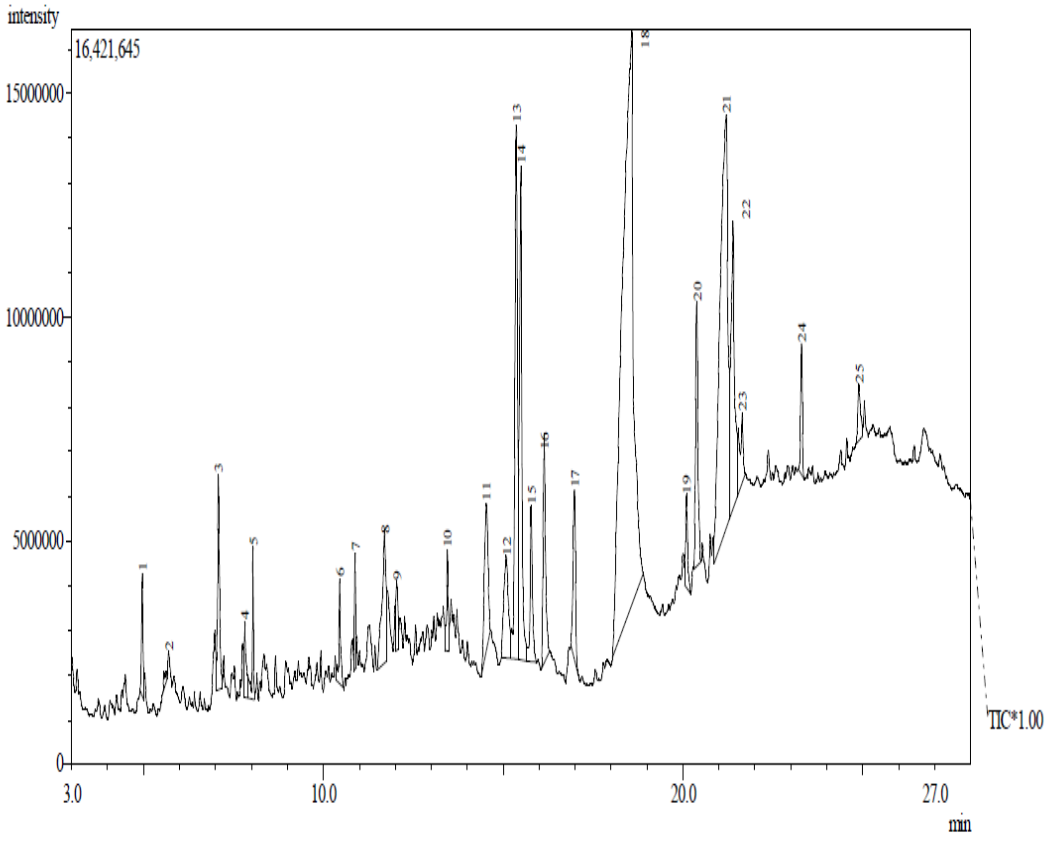

Figure 5 GC/MS chromatogram of fatty acids profile of Ethyl acetate fraction of *Moringa oleifera*

Table 5: Fatty Acids Profile of Ethylacetate Fraction of *Moringa oleifera* using GC/MS

Peaks	Retention Time (min)	Injection Time	Flow Time	Area (%)	Saturated fatty acids	Mono-unsaturated fatty acids	Poly-unsaturated fatty acids	Fatty acid methyl esters
11	14.533	14.400	14.650	2.80	Tetradecanoic acid (Myristic acid).			
18	18.576	18.042	18.892	33.41	n-Hexadecanoic acid (Palmitic acid)			
19	20.097	20.050	20.217	0.96				11,14,17-Eicosatrienoic acid, methyl ester.
21	21.197	20.867	21.292	16.48			9,12,15-Octadecatrienoic acid (Linolenic acid).	,9,12,15-Octadecatrienoic acid, methyl ester.
22	21.392	21.292	21.517	5.58	Octadecanoic acid (Stearic acid).			
25	24.886	24.808	24.992	0.76				15-Tetracosenoic acid, methyl ester.

Table 6**:** Fatty Acids Profile of Acetone Fraction of *Moringa oleifera* using GC/MS.

Peaks	Retention Time(min)	Injection Time	Flow Time	Area (%)	Saturated fatty acids	Monounsaturated fatty acids	Polunsaturated fatty acid	Fatty acid methyl esters
3	16.969	16.875	17.100	2.22				Pentadecanoic acid, 14-methyl-, methyl ester.
4	18.293	18.008	18.792	23.74	Hexadecanoic acid(Palmitic acid).			
5	19.982	19.908	20.025	3.05				9,12-Octadecadienoic acid, methyl ester.
6	20.076	20.025	20.125	4.83				11-Octadecenoic acid, methyl ester.
7	20.425	20.367	20.508	1.75				Decanoic acid, methyl este (Capric acid, methyl ester),
8	21.182	20.750	21.292	40.05		9-Octadecenoic acid (Oleic acid), 13-Docosenoic acid (Erucic acid).		
9	21.380	21.292	21.850	14.77	Octadecanoic acid(Stearic acid).			

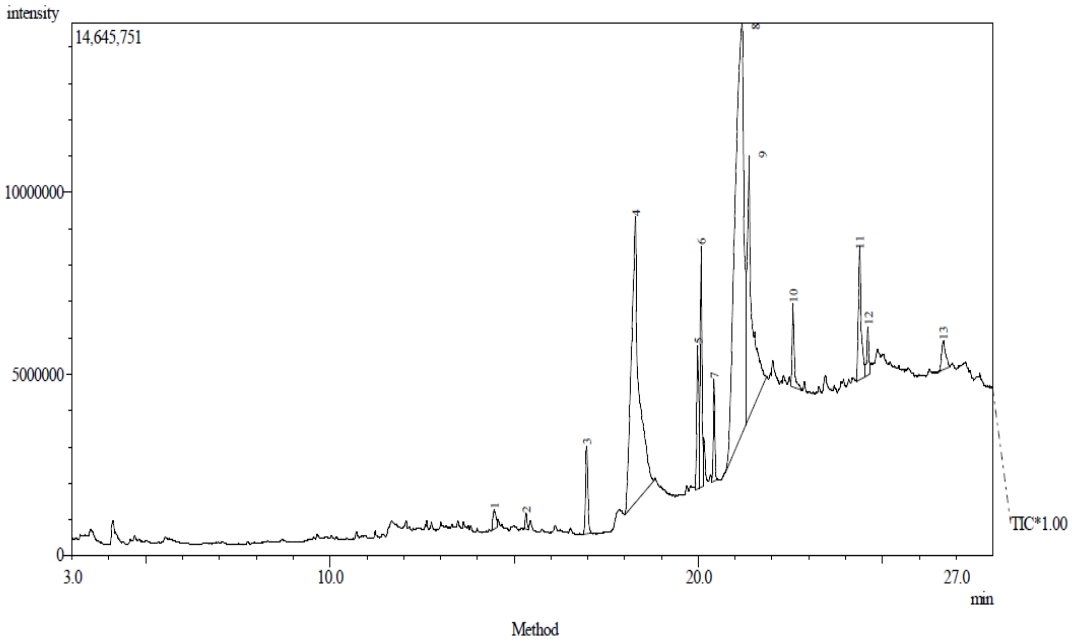

Figure 6: GC-/MS chromatogram of fatty acids profile of Acetone fraction of *Moringa olei*

Discussion

Lipid and lipophilic compounds are of great interest as bioactive additives in phytotherapy [12] The Gas chromatography/mass spectroscopiy(GC/MS) investigation of methanolic crude extracts/fractions of *Tapinanthus bangwensis* and *Moringa oleifera* revealed that they contained saturated fatty acids, unsaturated fatty acids and fatty acid methylated esters (FAMEs). The result showed that the methanolic crude extracts/fractions contained n-Hexadecanoic acids, Octadecanoic acids, tetradecanoic acid. 9-Octadecenoic acid (Oleic acids). 13-Docosenoic acid (Erucic acid) and 9,12,15-Octadecatrienoic acid in the plant samples evaluated. Some of these fatty acids have been shown to possess medicinal and nutritional benefit(s) such as anti-inflammatory, antioxidant, anticancer, hepatoprotective, hypocholesterolemic, anti-coronary, hypolipidemic properties. Theses fatty acids have been shown to exhibit hypolipidemic effect likely due to the presence of 5-α reductase inhibitor which may have blocked hydroxylmethylglutaryl CoA reductase (HMG-CoA reductase) in cholesterol biosynthetic pathway [13]. Stearic acid has been shown to have beneficial effect on liver regeneration and anti-inflammatory potentials [14; 15]). In epidemiological and clinical studies, stearic acid was associated with lowered low density lipoprotein-cholesterol (LDL-c) in comparison with other saturated fatty acids [16]. Hexadecanoic acid has been indicated to be a good anti-inflammatory agent [17]. Linoleic and oleic acids have been reported to be hypolipidemically effective [18]. The Cis Octadecanoic acid also has bioactivities of anti-metabolic syndrome and anti-cardiovascular risk factor acting by decreaseing cholesterol and triglycerides levels [19] The GC/MS analysis of the plant extracts/fractions revealed they possess methylated esters that have been reported to contribute to the medicinal quality of the plants, such as hexadecanoic acid, methyl ester, 9.12-octadecanoic acid, methyl ester. 9,12,15-Octadecanoic acid, methyl ester (Linolenic acid, methyl ester) and 11,14,17-Eicoatrienoic acid possesses anti-inflammatory, hypocholesterolemic, hepatoprotective, hypolipidemic and anti-coronary properties [20].

Conclusion

The research investigation reveals that *Tapinanthus bangwensis* and *Moringa oleifera* contain high fatty acid deposit and has shown to exhibit health promoting characteristics. It can therefore be concluded that the plants are medicinally beneficial to human health.

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