PREPONDERANCE OF PALMITOLEIC ACID IN *MORINGA OLEIFERA* Lam. (Moringaceae) SEEDS AND LEAVES FROM CHEMICAL ANALYSIS AND GAS CHROMATOGRAPHY

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ABSTRACT

Moringa oleifera is the most widespread species of the genus Moringa, the only genus in the family Moringaceae. It is a very popular plant used in traditional herbal medicine. Different parts of the plant contain a profile of important minerals and phytochemicals. The leaves and seeds are good sources of proteins, vitamins, beta-carotene, amino acids, and phenolic compounds. Study was carried out to check chemical constituents of leaves and seed oil of M. oleifera obtained from Ikorodu, Lagos State. Nigeria. Dried plant parts were pulverized and subjected to proximate analysis while the oils were extracted from the seeds with hexane using Sohxlet apparatus and analyzed with gas chromatography. The chemical contents of M. oleifera leaves obtained through proximate analysis showed it had more protein than the seeds with 45.28% protein while the seeds had 40.10%. the most prevalent mineral elements in M. oleifera are magnesium and calcium which were found to be 49.50 and 54.85 (mg/100g) in the seed, 42.80 and 54.95 (mg/100g) in the leaves respectively. Gas chromatographic analysis of the oils showed the presence of various fatty acids and other organic compounds with palmitoleic acid being the most abundant with 48.41% yield of total oils and oleic acid being 11.45% much less than earlier reported. Palmitoleic acid has shown possible influence in fatty liver deposition/production, insulin action and fatty acid synthase. This makes M. oleifera seeds very important new source of natural therapy for hyperglycemia and hypertriglyceridemia.

Keywords: *Moringa oleifera*, gas chromatography, proximate analysis, palmitoleic acid

INTRODUCTION

Oils in plants are usually classified in two ways; fixed oils e.g. Shea butter, olive oil and essential or volatile oils e.g. citrus oil, mint oil. They are used extensively in herbal medicine practice and serve diverse purposes in amelioration of health and can be applied to a wide spectrum of diseases. Oils also find application in the flavour and fragrance industry, pharmaceutical industry, and in aromatherapy. They can also function as antimicrobials (Ogbolu *et al*., 2007, Warnke *et al*., 2009). Essential oils are usually obtained by steam or hydro distillation of botanicals while fixed oils are obtained by de-fatting procedures. Extracts from hexane and other hydrophobic solvent are called *concretes*, which are a mixture of essential oil, waxes, resins, and other lipophilic (oil soluble) plant material (Marzouki,2008). Different parts of plants can yield oils, including the flowers, leaves, seeds, roots, stems, bark, and wood. The oil of the same plant can vary strongly in composition depending on the species, location, soil and weather conditions, and level of expertise and care given by farmers and distillers. For such reasons, the characterization of the oils through chemical analysis is a mandatory step in the production chain, to be carried out by both researchers and quality control labs. Practically all oils consist of chemical mixtures that are often quite complex; they vary widely in chemical composition. Almost any type of organic compound may be found in oils (hydrocarbons, alcohols, ketones, aldehydes, ethers, oxides, esters, and others). (Shakhashiri, 2008).

Interest in the oil extracted from *Moringa oleifera*, (Fig.1 and 2) known commercially as 'Ben' or 'Behen' oil, has existed for well over a century. The first recorded study of the composition of the oil was carried out in 1848 which revealed a fatty acid with a high melting point (Anon, 1904). This was subsequently called behenic acid from which the commercial name for *M. oleifera oil* came (Anon, 1904), *M. oleifera* is the most common of the genus and considered a multipurpose tree native to the foothills of the Himalayas in northwestern India (Olson, 2010) and cultivated throughout the tropics (Janick and Paull, 2008). Schill, 2008 reported high content of oleic acid in *M. oleifera*. It is considered a potential oil seed feed stock for bio diesel (Schill, 2008). *M. oleifera* leaves and seeds are greatly popular for management of many and diverse health problems, spanning from anaemia to high

blood pressure. Study interest is the chemical constituents of leaves and oils of seeds of *M. oleifera* obtained from Ikorodu, Lagos State. Nigeria

MATERIALS AND METHODS

Extraction of oil from *M. oleifera* seeds and leaves

Solvent extraction

M. oleifera seeds and leaves were collected from Ikorodu area in Lagos state 6.6000° N, 3.5000° E and 86% humidity. They were authenticated at the department of Botany, University of Lagos and assigned with Herbarium number LuH 5061. They were dried in an oven at 40-50ºC. Seeds were dehusked, weighed and pulverized to coarse powder. The seed powder was transferred into a glass jar and soaked in petroleum-ether for about 72 hours for extraction of the oil from the seed. The leaves were also extracted in the same manner. The oil extracts were collected in a beaker and exposed to dry air at room temperature to ensure the solvent evaporates completely and then stored in dark bottles till required.

Proximate analysis of seeds and leaves.

The proximate analysis (carbohydrate, fats, protein, moisture and ash) of *M. oleifera* seeds and leaves were determined by using the methods of the Association of Official Analytical Chemists (AOAC, 1990) were used for proximate analysis. Moringa flour samples (5 grams) was used for determination of moisture content by weighing in crucible and drying in oven at 105oC, until a constant weight was obtained. Determination of ash content was done by ashing at 550ºC for 3h. The Kjeldah method was used to determine the protein content. The crude fibre content of the samples was determined by digestion method and the fat was done by Soxhlet extraction method. All determinations were done in Triplicate All the proximate values were reported in percentage.

Mineral Contents

The levels of the mineral elements calcium, phosphorus, sodium, magnesium, potassium and nitrogen were determined using the wet digestion extraction methods as described by Ojuwale (1998), Andrew (1999). 0.2 g of the samples were weighed into a 15 ml flask. 5 ml of the extraction mixture (H_2SO_4-Selenium salicylic acid was added to the sample and allowed to stand over night). The mixture was heated initially at 20°C for 3 h and 5 ml of concentrated perchloric acid ($HCIO_4$) added. This was then heated vigorously until digestion was completed. The solution was allowed to cool and filtered using an acid washed filter paper into 50 ml volumetric flask and finally made up to mark with distilled water. The potassium and sodium content were determined using the flame photometer method, phosphorus by the vanado-molybdate yellow method using the spectrophotometer method. Calcium and magnesium determined by the versanate EDTA complexometric titration method and nitrogen by the semi micro distillation method using the Markham apparatus. Mineral contents (calcium, sodium, magnesium, potassium, copper, cobalt, nitrogen,iron and zinc) were recorded as mg/100g.

Phytochemical tests

Tests for tannins, glycosides, anthraqinones, saponins, alkaloids, sterols and triterpenes were carried out according to Harbone, 1998.

Gas chromatography

This utilized a PerkinElmer AutoSystem XL GC with an Equity-5 fused silica capillary column (60 m x 0.32 mm i.d., film thickness 0.25 µm; Supelco Bellefonte, PA, USA). The oven temperature program ranged from 70 ºC to 250 °C, programmed at 3 °C/min, with initial and final hold time of 2 min, carrier gas: Helium at 10 psi constant pressure, a split ratio of 1:40; injector, transfer line and source temperatures were 250 °C; ionization energy 70 eV; mass scan range 40-450 amu. Characterization was achieved on the basis of retention time, elution order, co injection with standards in GC-FID capillary column (Aldrich and Fluka), mass spectra library search (NIST/EPA/NIH version 2.1 and Wiley registry of mass spectral data 7th edition).

The chemical constituents were identified by matching mass spectra with spectra of reference compounds in mass spectral library of the National Institute of Standards and Technology (NIST). The name, molecular weight and structure of the components of the test materials were to be identified.

Refractive index

This was carried out using the Abbe refractometer at room temperature of 25°C, while the reference value was found to be 1.4671 at 20°C. The surfaces of the lower and upper prism were first wiped with methanol to remove impurities which may interfere with the reading. The oil sample was mounted on the lower prism. The dark region and the light region were examined using the eye piece. The adjustment knob was adjusted so that the intersection between the two bands coincide with the point of intersection of the 2 cross lines seen under the eye piece then the reading is taken and result noted

RESULTS

Fig. (1) Life form picture of *M. oleifera* plant in a garden

Fig. (2) Life form picture of *M. oleifera* seeds

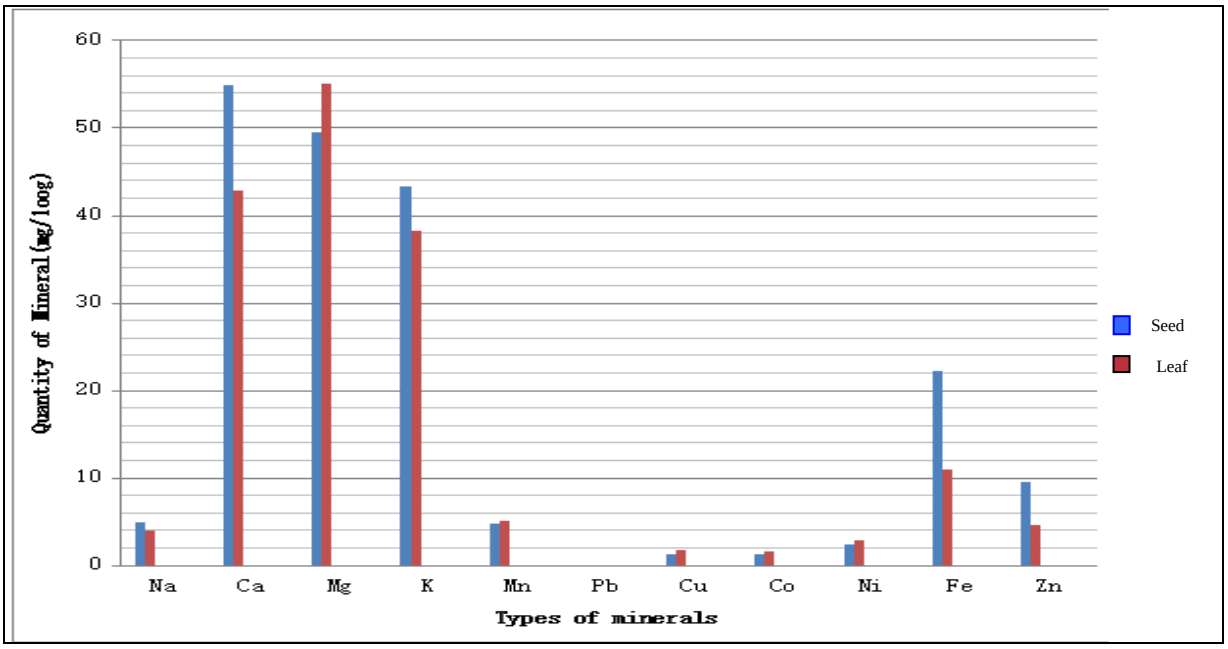


Fig. (3) Mineral content of *M. oleifera* seeds and leaves

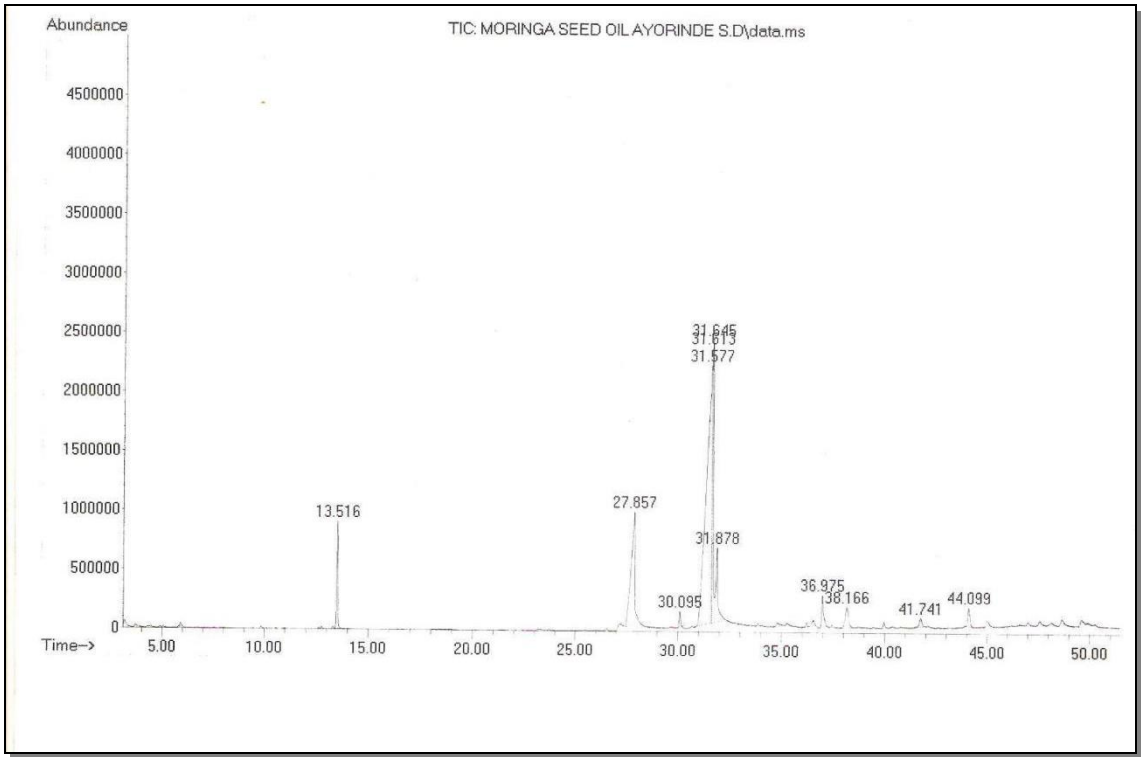


Fig. (4) Gas Chromatograph of *M. oleifera* seed oil

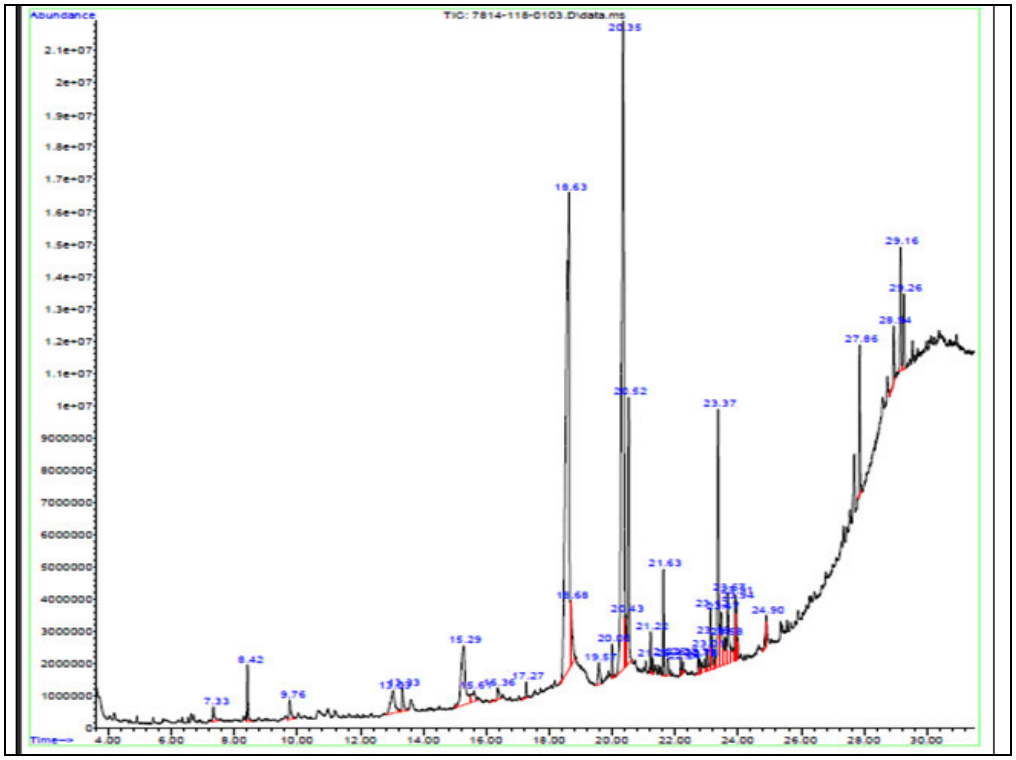


Fig. (5) Gas Chromatograph of *M. oleifera* leave oil [Bhattacharya, 2015]

Table 1 Gas chromatography of *M. oleifera* seed oil

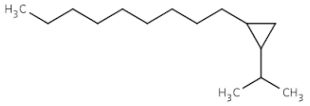
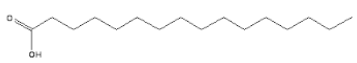
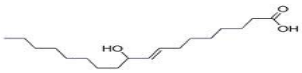
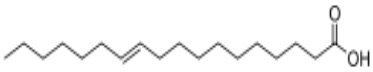
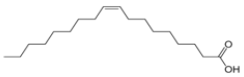
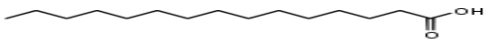
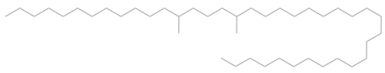
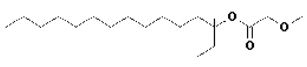
Peak Number	Retention Time	Percentage of Total	Compound
1	13.516	4.495	1-Isopropyl-2-nonyl-cyclopropane 
2	27.857	16.209	n-hexadecanoic acid/ Palmitic acid 
3	30.095	0.677	8-octadecenoic acid 
4	31.577	48.401	9-hexadecenoic acid/Palmitoleic acid 
5	31.613	5.815	Cis-vaccenic acid 
6	31.645	11.450	6-octadecenoic acid/oleic acid 
7	31.878	5.815	Pentadecenoic acid 
8	36.975	2.236	9-octa decenal 
9	38.166	2.544	Tetracontane 
10	41.741	0.847	Octadecamethyl-hexsiloxane 
11	44.099	1.481	Pentadecyl, methoxyacetic acid 

Table 2. Chemical components of *M. oleifera* seeds from proximate analysis

Proximate analysis	Average (%)
Carbohydrate	31.63±0.09
Protein	40.10±0.05
Crude fat	3.09±0.00
Moisture content	9.42±0.00
Ash value	6.19±0.17
Crude fibre	9.57±0.16

Mean ± Standard deviation (n=3)

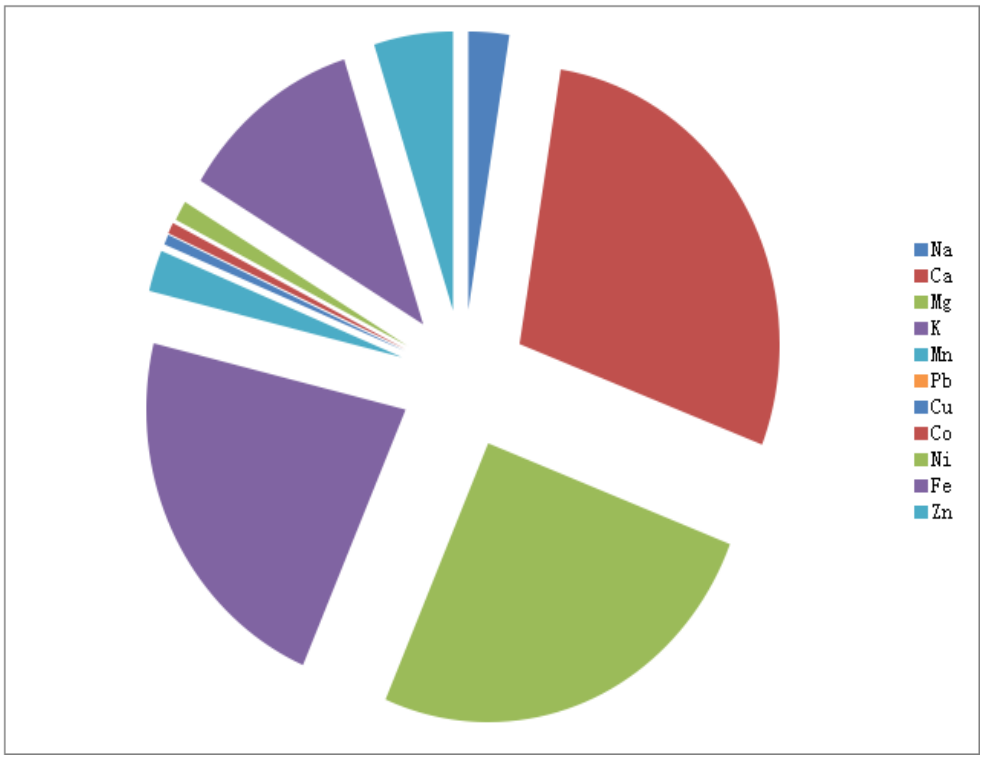


Fig (6) Mineral contents of *M. oleifera* seeds

Table (3) Proximate analysis of *M. oleifera* leaves

Proximate analysis	Average (%)/SD
Carbohydrate	18.92±0.10
Protein	45.28±0.02
Crude fat	6.40±0.01
Moisture content	12.76±0.00
Ash value	10.63±0.01
Crude fibre	6.00±0.04

Mean ± Standard deviation (n=3)

DISCUSSION

Previous studies on *Moringa oleifera* (Fig. 1 and 2) species found an abundance of oleic acid and probably leading to the plant being assigned the specific name of *oleifera*. The seeds used for these studies showed a preponderance of palmitoleic acid (Table 1 and Fig. 4) also known as 9-hexadecenoic acid, and is an omega-7 monounsaturated fatty acid. It is known to be biosynthesized

from palmitic acid by the action of the enzyme delta-9 denaturase. It has also been shown to increase insulin sensitivity by suppressing inflammation, as well as inhibit the destruction of insulin-secreting pancreatic beta cells (Yang *et al*., 2011). This may be the reason for the success of the seeds in lowering cholesterol levels in consumers.

The proximate analysis (Table 2 and 3) showed that the seed is rich in carbohydrate and protein. This is contrary to the result obtained by Nzikou *et al.*, 2009 which showed a higher percentage of crude fat.

This variation of result could be due to variation in climate of the various geographical locations from which the pods were harvested, as the *M. oleifera* species used for the analysis of (Nzikou *et al.*, 2009), was obtained from Brazzaville whereas the species used for this analysis was obtained from Ikorodu area of Lagos, Nigeria. It is equally of interest to note that the most prevalent mineral elements in *M. oleifera* are magnesium and calcium (Fig. 3) which were found to be 49.50 and 54.85 (mg/100g) in the seed, 42.80 and 54.95 (mg/100g) in the leaves respectively. Magnesium plays a major role in photosynthesis, carbohydrate metabolism, nucleic acids metabolism etc. The presence of calcium in high concentrations therefore makes *M. oleifera* a good dietary source of the mineral for patients with calcium deficiency. The presence of flavonoids can be used to explain the anti-inflammatory, anti-oxidant and hypoglycaemic effects of these seeds (Bhattacharya, 2015).

Oils have many properties which are used for their identification. One of such property is the refractive index. The refractive index of *M. oleifera* seed oil was carried out, and compared with the reference value. The refractive index was found to be 1.470 the slight difference in the value obtained compared to the reference might be due to the variation in the sensitivity of the refractometers, as well as slight variation the temperature at which the readings were taken.

CONCLUSION AND RECOMMENDATIONS

This study has confirmed again the dietary importance of *M. oleifera* and the Gas chromatographic analysis of the oils has shown the presence of various fatty acids and other organic compounds with palmitoleic acid being the most abundant. Palmitoleic acid has shown possible influence in fatty liver deposition/production, insulin action and fatty acid synthase. This makes *M. oleifera* seeds very important new source of natural therapy for hyperglycemia and hypertriglyceridemia adding to existing knowledge that some species of the plant have a preponderance of palmitoleic acid a highly useful compound for carbohydrate metabolism.These results therefore makes it relevant that people incorporate *M.oleifera* leaves and seeds to their diet.

ACKNOWLEDGEMENT

The authors are grateful to the laboratory staff of Department of Pharmacognosy for their help and cooperation during the course of the work.

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