Research Article

Study on Thin Layer Chromatograghy (TLC) and Gas Chromatography-Mass Spectrometry (GC-MS) analysis of methanolic extract of *Moringa oleifera* root

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Abstract

Background: Active compounds from plants have been shown to exert some biological activities and this has been the bedrock for modern medicine and synthetic drugs. **Objective:** Is to isolate the bioactive compounds present in the root of *Moringa oleifera*. **Materials and Methods:** The samples were identified and authenticated by a plant taxonomist. The samples were air-dried at room temperature for 7 days and then grounded into coarse form, kept in an air-tight container until required. Soxhlet extraction method was used with methanol of analytical grade. Fractions of compounds were separated using thin layer chromatography (TLC) and gas chromatography- mass spectrometry (GC-MS) for the detection of bioactive compounds. **Results:** The qualitative TLC analysis of the methanolic root extract of *M. oleifera* revealed the presence of seven (7) aglycans with R_f value of 0.83, 0.77, 0.71, 0.48, 0.60, 0.34 and 0.64.The fractions were subjected to preparative TLC and pooling of similar compounds was done to obtain compounds A and B from the first four bands and the last three bands respectively.The pooled compounds A were further subjected to Gas chromatography – Mass spectroscopy (GC-MS) analysis for further separation, identification and elucidation. Nineteen (19) compounds were identified from the GC-MS analysis. **Conclusion:** Nineteen compounds have been isolated from the root of *Moringa oleifera*. Some of these compounds have known biological activities against microbes, cancer, oxidative stress, pain and inflammation. This confirms its use in traditional and herbal preparations for the treatment of various ailments.

Keywords: Thin layer chromatography, Gas Chromatography-Mass Spectrometry, bioactive compounds, *Moringa oleifera*

Introduction

Plants have been used as food and for medicinal purposes since prehistoric times with medicinal plants as the bedrock for modern medicines (Evans, 2009). Access to plants of medicinal value to the rural and developing countries is constantly increasing and the study of pharmacological activities of the active principles of plants is on the rise. WHO promoted the gardening of *Moringa oleifera* among other leafy and green vegetables in India to combat deficiency of iron, folic acid, vitamins A and B_{12} (WHO, 1999). *Moringa oliefera* Lam also known as *Moringa pterygosperma* belongs to the family Moringaceae; a single genus with 14 known species (Ram, 1994). The tree is found in the tropic and sub-tropic regions. It is said to have originated from Agra and Oudh in the North West region of India, south of the Himalayan Mountains (Marcu, 2006). In Nigeria, the plant is common in the northeast and middle belt regions. *Moringa oleifera* has

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various synonyms in different languages, in English it is known as Drum stick, Horseradish tree, Mother's best friend, Miracle Tree and West African Bean. In Hausa language it is known as Zogale and Bagaruwa maka, in Igbo it is Ikwe oyibo and in Yoruba language it is called Ewe ile, Ewe Igbale, Idagbo monoye. The Idoma and Kanuri people call it E'ge' rige'du and Alum respectively (Lowell and Sreeja, 2001). The Marghi people call it Kabi. Its use as an antibacterial, antifungal, anti-inflammatory, anti-tumor, anti-diabetic, antispasmodic and antitubecular among other usage traditionally earned it the name "Miracle Tree" (Anwar et al., 2007). Nutritionally, it is agood source of various nutrients such as Vitamins A, C, calcium and proteinswith an excellent ability to naturally boost energy. The oil of *M. oleifera* is highly medicinal, it subsides the inflammation caused by chameleon bite of a family member (personal communication). It has been shown to alleviate anaemia and aid in improving the health of malnourishednursing mothers (Folkard and Sutherland, 1996). Currently, Moringa is being used raw, unprocessed by many countries including Nigeria, Australia, Fiji, Senegal and Brazil (John et al., 1986). Every part of the plant (leaves, flowers, fruits) is edible and has been shown to alleviate or cure diseases (Anwar et al., 2007). The roots offer a concentrated form of many of the chemical compounds found throughout the other parts of the plant and can provide therapeutic benefits for many conditions and ailments (Fahey, 2009). Additionally, it has been used to treat impotence, sexual dysfunction, and to bring on menstruation. In poultice form, they are used in rheumaticarthritic pains. They are also use as diuretic and may have some antiseptic qualities in topical use as well (Fahey, 2009).

Materials and methods

The roots of *Moringa oleifera* was collected within Maiduguri Metropolitan. The samples were identified and authenticated by a plant taxonomist at the Department of Biological Sciences, University of Maiduguri and a voucher specimen No. VPP/12/002 was prepared and deposited in the Department of Veterinary Physiology and Biochemistry. The samples were air-dried at room temperature for 7 days and then grounded into coarse form, kept in an air-tight container until used.

Extraction of the Plant Materials

The soxhlet extraction method was used with methanol of analytical grade. About 1000g of the grounded plant sample was extracted using methanol according to the method described by (Harwood and Moody, 1989) as modified by (Usman et al., 2007). The extractive was then filtered and concentrated at low pressure to obtain a mass called crude methanol extract (CME) and thereafter, kept in a tight container until use.

Analytical Thin Layer Chromatography (TLC)

All the fractions collected from the column separation of the methanol extract were spotted on commercially available already activated aluminium back coated silica gel GF_{254} chromatoplates, 20 × 20 cm × 0.25 mm thick (Sigma, U.S.A.) containing a fluorescence indicator (fluorescein, 15%). This allows detection of compounds which quench the fluorescence indicator when the plate was observed in U.V. light of 254 nm and 366 nm wavelength (Olaniyi and Ogungbamila, 1993; Ogundaini et al., 2000). The solvent system used was chloroform, 20 ml using a Shandon chromotank (a micro one) and visualized with U.V. light at wavelengths of 254 nm and 366 nm. The substances which quench this fluorescence at 254 nm appeared as yellow spots (Ogundaini et al., 2000). The margin between compounds was 0.5 mm and the amount applied manually using a capillary tube was 0.25µl. The recombined fractions were then air-dried in the laboratory, weighed and stored in clean, dried, bottles.

Gas Chromatography-Mass Spectrometry (GC-MS) of the Methanol Extract

Gas Chromatography-Mass Spectrometry (GC-MS) is a hyphenated technique. It combines chromatography with spectrometry. This hyphenated technique, GC-MS has the important characteristic in that identification is accomplished with unique spectral information rather than retention time data and does not have the limitations inherent in chromatography or spectrometry, applied alone. Therefore, complex mixture often can be analyzed in detail with little or no prior chemical information about the sample. It can provide conformation of structure, identify unknown drugs and their metabolites in body fluids and tissues, quantitate drugs and their metabolites in body fluids and tissues and be of great value in the analysis of therapeutic agents (Ayim et al., 2000).

Procedure

The GC-MS spectrometer model used was QP 2010PLUS SHIMADZU, Japan. The analysis was carried out at the National Research Institute for Chemical Technology (NARICT), Zaria. The GC-MS was equipped with a split injector and an ion-tap mass spectrometer detector, together with a fused-silica capillary column having a thickness of 1.00µm, dimension of 30 m × 0.25 mm and temperature limits of 60°C to 325°C. The column temperature was programmed between 60°C and 250°C at a rate of 3.0 ml/min. The temperature of the injector and the detector were at 250°C and 260°C respectively. Helium gas was used as a carrier gas at a flow rate of $46.3 cmsec^{-1}$. Compounds were identified by computer-aided matching of their spectra with spectra of known compounds from the library of spectra from the National Institute of Standards

and Technology (NIST, 2009).

Results

T.L.C. Analysis

The result of the thin layer chromatography (T.L.C.) examination of methanolic root extract of *M. oleifera* revealed the presence of seven (7) aglycans. The R_f value of the spots observed on the analytical plates were 0.83, 0.77, 0.71, 0.48, 0.60, 0.34 and 0.64 (table 1). Their respective colours were light blue, light green, light yellow, pinkish light color, light brown, light purple and dark purple respectively.

After qualitative T.L.C, the fractions were subjected to preparative T.L.C. after which pooling was done. The first four bands (1, 2, 3 and 4) that appeared similar were pooled together and referred to as compound A, while the last three bands (5, 6 and 7) were also pooled together and given the name as compound B. The pooled compounds A were further subjected to Gas chromatography – Mass spectroscopy (GC-MS) analysis for further separation, identification and elucidation. Nineteen (19) compounds were identified from the GC-MS analysis; their retention time and heat quality are shown in table 2, while the compound name, molecular formula and molecular weight

$$R_f \text{value} = \frac{distance\ travelled\ by\ the solute\ from\ origin\ (spot distance)}{distance\ travelled\ by\ the\ solvent\ front\ from\ the\ origin}$$

Table 1. R_f values for various fractions of methanol root extract of *Moringa oleifera*

Spots	Distance travelled by spot (cm)	R_f Value
A	5.3	0.82
B	5.0	0.77
C	4.6	0.71
D	3.1	0.48
E	3.4	0.52
F	2.2	0.34
G	4.0	0.62

Solvent system: Ethylacetate, chloroform and methanol (15: 8: 4);Running time was 35 ; solvent front travelled from origin was 6.5

Table 2. GC-MS analysis of methanol extract showing scan number, retention time and heat quality

#Peak	Scan No.	Retention time	Heat quality
1	13	3.1	89
2	30	3.2	89
3	41	3.3	89
4	81	3.7	93
5	92	3.8	88
6	127	4.1	95
7	159	4.3	96
8	174	4.4	86
9	212	4.8	79
10	224	4.9	78
11	280	5.3	94
12	384	6.2	76
13	445	6.7	88
14	1213	13.1	95
15	1557	16.0	88
16	1758	17.6	89
17	1909	19.0	85
18	2087	20.4	86
19	2111	20.6	84

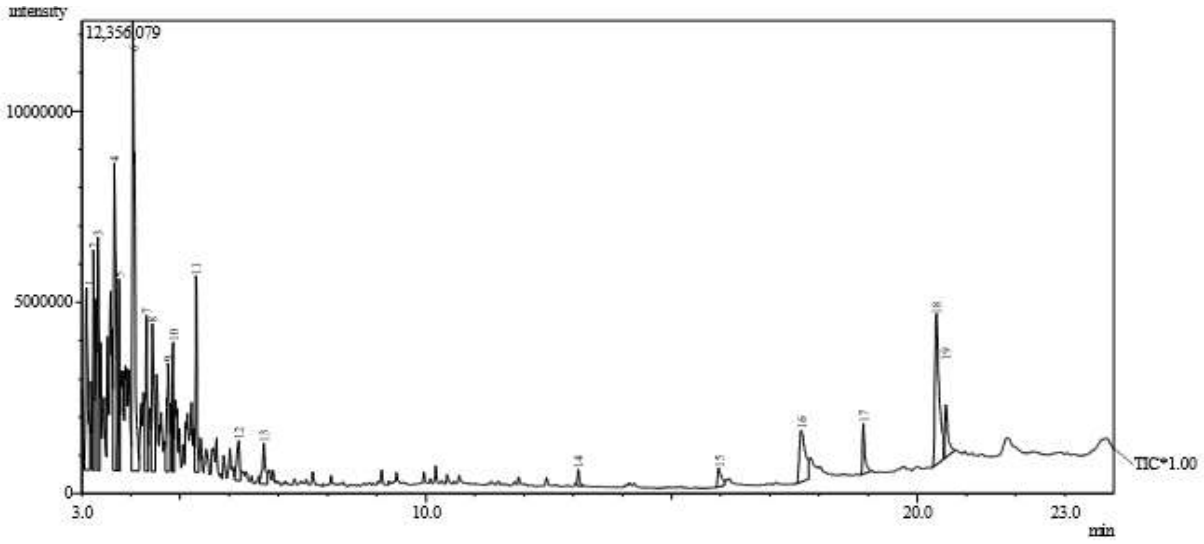

Figure 1. GC-MS chromatogram of the 19 compounds isolated from the root of methanolic extract of *Moringa oleifera*

Table 3. GC-MS of isolated compounds showing molecular formula, molecular weight and bioactivity

Peak No	Compounds	RT(mins)	MF	MW	Peak Area (%)	Compound nature	Bioactivity
1	Cis 1-ethyl-4-methyl-cyclohexane	3.1	C_9H_{18}	126	7.03	Alkanes (cycloalkane compound)	
2	3-methyl-1-cyclooctene	3.2	C_9H_{16}	124	4.90	Alkanes (cycloalkane compound)	
3	Isopropylcyclohexane	3.3	C_9H_{18}	126	6.73	Cyclohexane. Mostly found in fruits such as Pawpaw	Acts as a nutrient also has analgesic effects
4	1-ethyl-2-methylbenzene	3.7	C_9H_{12}	120	11.36	Aromatic hydrocarbon	Antibacterial
5	1,2,3-trimethylbenzene	3.8	C_9H_{12}	120	4.36	Aromatic hydrocarbon	It decreases pain sensitivity in male wister rats.
6	n-decane	4.1	$C_{10}H_{22}$	142	18.25	Alkane hydrocarbon	
7	4-methyldecane	4.3	$C_{11}H_{24}$	156	4.55	Branched Alkanes	Isolated from aroma volatiles of coconut, cooked beef and wheat. Has antimicrobial activity.
8	1,2,3-trimethyl	4.4	C_9H_{12}	120	5.21		
9	2-phenyl-3-propyl	4.8	$C_{15}H_{24}$	204	3.93	Cyclohexanes	Antimicrobial and antibacterial activity.
10	1,2-dimethyl-4-ethylbenzene	4.9	$C_{10}H_{14}$	134	2.96	Aromatic hydrocarbon	
11	Decane	5.3	$C_{10}H_{22}$	142	4.56	Alkanes	
12	1-isopropanyl-4-methyl-1,3-cyclohexadie	6.2	$C_{10}H_{14}$	134	1.98		Used in food industries. Antimycotic properties and antibacterial activity.
13	Dodecane	6.7	$C_{12}H_{26}$	170	1.87	Alkanes	
14	2,6,11-trimethyldodecane	13.1	$C_{15}H_{32}$	212	0.54	Branched alkane	Antibacterial activity.
15	1-pentadecanocarboxylic acid	16.0	$C_{16}H_{32}O_2$	256	1.15	Alcohol and a straight chain carboxylic acid	Antimicrobial and topical anti-inflammatory activity.
16	13-decosenoic acid	17.6	$C_{22}H_{42}O_2$	338	4.94	It is an omega-9-fatty acid	Studies performed on lab. animals showed that it has a toxic effects on the heart at high doses.
17	5-(hydroxymethyl) undecane	19.0	$C_{12}H_{26}O$	186	2.00	2-Butyl-1-octanol	Antioxidant
18	5-tetradecane	20.4	$C_{14}H_{28}$	196	10.88	Long-chain alkane	Acts as plant metabolite and as a component of volatile oils,
19	n-Nanodecanol	20.6	$C_{19}H_{40}O$	284	2.79	Long-chain alcohol	Antibacteria

Bioactivity copied from Korsak *et al.,* (2000); Rudback *et. al*., (2012); Yue *et al.,* (2017)

as well as the compound nature and bioactivity are shown in figure1 and table 3.

Discussion

The identification of novel compounds from natural sources using high throughput techniques is valuable to drug discovery and development. The purification of such compounds has led to commercialization of plant-based drugs in the treatment of several diseases and as supplements to man. This research has therefore utilized one of the techniques (Gas Chromatography-Mass Spectrometry) to identify bioactive compounds from the root of methanolic extract of *Moringa oleifera*, nineteen compounds were isolated. Most of the compounds found have known medicinal activities and have been shown to illicit anti-inflammatory, anticancer, antimicrobial, antimycotic, antioxidant and analgesic effects as shown in table 3.

Most isolation studies were carried out using the leaves, seed, flowers or bark of the plant, which also reveals the presence of various compounds that are known to have anti-inflammatory, antifungal, antihypertensive, antispasmodic, anti-ulcer, diuretic, and pesticidal effects (Mahdi et al., 2017; Igwe et al. 2015; Kadhim and AL-Shammaa, 2014; Inbathamizh and Padmini, 2012). 1, 2, 3, trimethylbenzene, decane and docosenoic acid that are shown to be present in this study were also isolated from the seed oil of *moringa oleifera*as reported by Adegbe et al., 2016. Docosenoic acid (Behenic acid) is used in the cosmetic industry as a hair conditioner or moisturizer (Warra, 2014). Earlier studies on the root revealed the presence of compounds such asmethylhexadecanoate, methyl 14-hydroxy-5-tetradecenoate, 1, 11 diphenyl undecane and cyclopentanyl hexadecane, with the extracts showing hypotensive activity in rats (Sana et al., 2015; Faizi et al., 2014). This study also revealed the presence of like compounds. *Moringa oleifera* is not just a tree, it is a "miracle tree' that is full of therapeutic activities. The plant is made available by nature to both the rich and poor. The isolation of the bioactive compounds from the plants should be further utilized in the pharmaceutical and cosmetic industries. To avoid undesirable effects; toxicity studies needs to be carried out on the bioactive compounds in both man and animal.

Conclusion

In conclusion the bioactive compounds isolated from the root of *Moringa oleifera* using GC-MS Analysis are used against microbes, cancer, oxidative stress, pain and inflammation. Further investigation on toxicity studies were needed to know its effects on vital organs.

Conflicts of interest

Authors declare that there is no conflicting interest amongst us**.**

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