

World News of Natural Sciences

An International Scientific Journal

WNOFNS 37 (2021) 18-30

EISSN 2543-5426

Evaluation of phytochemical constituents of Methanol extract of *Moringa oleifera* Lam. whole leaf by Gas Chromatography-Mass Spectrometry and Fourier transform infrared spectroscopy analysis

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ABSTRACT

The research study evaluated the phytochemical constituents of Methanol extracts of *Moringa oleifera* whole leaf by Gas Chromatography-Mass Spectrometry (GC-MS) and Fourier transform infrared spectroscopy (FT/IR) analysis. The leaves were washed, air dried for 2 weeks, then ground into a fine powder and extracted using methanol by maceration for 24 hours using standard procedures. After the contact time elapsed, the solvent filtered to recover the extract. The functional groups and the chemical constituents of the methanol extract of *Moringa oleifera* leaves investigated using Fourier transform infrared spectroscopy (FT/IR) and Gas Chromatography-Mass Spectrometry (GC-MS) respectively. The FT/IR analysis confirmed the presence of O-H, C=C, C-H, C-O, CH₃ and C=C-H bond stretching functional groups, which indicates that the substance is an aliphatic alcohol, ester, aldehyde and carboxylic acid. However, the fingerprint region had a pattern that is specific for every molecule, the presence of -OH function and N-O stretch; suggest that or alcohols and nitrogen or aromatic or aliphatic phenols containing molecules are major components of *the Moringa oleifera* leaf studied. GC-MS analysis of the extract reveals the identification of twenty compounds, in which two compounds were identified in each peak. N,N'-Pentamethylenebis[s-3-aminopropyl thiosulfuric acid and 2-Myristinoyl pantetheine (100%), 2-Myristinoyl pantetheine and Deoxyspergualin (92.05%), 5-Octadecenal and 9-Hexadecenoic acid (27.94%). N,N'-Pentamethylenebis[s-3-aminopropyl thiosulfuric acid and Pentetic Acid are the major phytoconstituents. Most of the compounds in the list are bioactive and possess medicinal properties, which further justify the application of *Moringa oleifera* traditional plant in the discovery of novel therapeutics.

Keywords: *Moringa oleifera*, methanol extract, phytochemical constituents, GC-MS analysis, FT-IR analysis

1. INTRODUCTION

Plants are the richest sources of drugs for traditional and modern medicines, therefore they are unique source of medicinal artefacts, food, energy and shelter for human and animal, many useful harvests obtained from plants directly or indirectly validate their importance to the human and other living organisms [1, 2]. Plants that produce constituents mostly as secondary metabolites having medicinal values called medicinal plants. These constituents differ from plant to plant with likely therapeutics used in treatment of human and animal diseases including pains-relieve, convulsion and cardiovascular diseases [3, 4]. World Health Organization (WHO) has estimated that 4 billion people “80% of the world population use herbal medicines” for some aspect of primary health care and the Food and Agriculture Organizations (FAO) report, about 25 % of the synthesized drugs are manufactured from the medicinal plant sources (roots, leaves, barks and seeds) examples include aspirin and quinine [5-7].

Moringa oleifera belongs to the family Moringaceae, commonly known as the ‘drumstick’ or ‘miracle’ tree, which has extensive been grown and utilized for variety of purposes across the tropics, most especially in Northern Nigeria. All the plant parts has nutritious and non-nutritious supplements due to bioactive components called phytochemicals. Bamishaiye *et al.* found that the leaves extract contained phenols, saponins, steroids, alkaloids, flavonoids and tannins [8, 9]. The leaves of the plant are edible in nature and very nutritious and hence, consumed in Nigeria as vegetable [10].

However, apart from its curative and nourishing uses, there are several reports on the biological and physiological activities to remediate hypertensive, cholesterol and glycaemic effects, also used as painkiller, treat heartburn stomach ache and ulcers [11-13]. Its reputation as a “miracle tree” – nutritionally speaking – has sparked academic interest in this species, yet research on *Moringa oleifera* has only picked up over the last few years, mostly with regard to its possible use as a novel food source. As such a source, mainly the leaves – be it fresh or in dried form – are of most interest.

However, climatic factors, soil content and maturity can impacts the distribution of its phytochemicals in leaves of *Moringa oleifera* [8-9, 14]. In this view, the study is aim to evaluate the Gas Chromatography-Mass Spectrometry (GC/MS) and (FT-IR) analysis of the phytochemical constituents of the Methanolic extract of *Moringa oleifera* leaves grown in Ihitte Ezihe in isiala mbano, South-Eastern Nigeria.

2. MATERIALS AND METHODS

2. 1. Plant Sampling

Fresh leaves of *Moringa oleifera* were collected from a local market in Isiala Mbano, Imo State, Nigeria and were later identified by taxonomist in the Department of Plant Science and Biotechnology, Abia State University, Uturu, Nigeria. A part was deposited in the herbarium for reference purposes.

2. 2. Preparation of Samples

Moringa oleifera leaves were destalked, washed and shade dried at ambient temperature for 21 days with constant turning to prevent fungal growth. The dry leaves were later milled into fine powder using a manual grinder to obtain the vegetable leaf meals and weighed with a Meter balance and were stored in 4 °C temperature in refrigerator in well-labelled airtight containers for analysis (**Figure 1**).



Figure 1. Whole and sliced leaves of *Moringa oleifera* Lam.

2. 3. Preparation of Extract

Moringa oleifera whole leaves extract was prepared according to the method as described by [8]. 80 grams of the sample were extracted using 600 ml of methanol in an orbital shaker and allowed for 72 hours at room temperature. The supernatant was separated and kept in a beaker. The extracts were filtered using Whatman No.1 filter paper to remove extractable substances, at every 3 hours interval. The combined extracts were then evaporated with rotary evaporator and the dried extracts were transferred to sample bottles, which were placed in a desiccator to remove traces of water that could have been present and were later kept in tightly stoppered bottles at 4 °C until further analysis.

2.4. Preliminary phytochemical investigation

The methanol extracts were subjected to phytochemical screening to identify the presence of alkaloid, tannins, saponin, flavonoid, terpenoid, glycosides and phenol. Presence of bioactive compounds was determined using the standard methods [15].

2. 5. Gas Chromatography Mass Spectrometer (GC-MS) Analysis

5 ml of methanol extract of *Moringa oleifera* whole leaves was evaporated to dryness and reconstituted into 2 ml methanol. The extracts were subjected to GC-MS analysis using GC-MS-QP 2010 (SHIMADZU instrument) with Db 30.0 column (0.25 μ m diameter \times 0.25 μ m

thickness). The oven temperature was pre-set from 70 °C (5 minutes for), with an increase of 10 °C/min to 200 °C, then continued to 280 °C at 5 °C/min interval and allowed to continuous range of 35 minutes isothermal. Mass spectra were at 70 eV with scan intermission of 0.5 seconds and scan range from 40–1000 m/z. Helium was carrier gas at 99.99 % pressure with flow rate of 1.0 ml/min. and electronic pressure control. Samples were dissolved in methanol and injected automatically [16-17].

2. 6. Fourier Transform Infrared Spectrophotometer (FT/IR) Analysis

Dried powder of the plant extracts of *Moringa oleifera* leaves was used for FT-IR analysis. 10 mg of the dried samples were capsulated in 100 mg of Potassium bromide pellet. It was transferred to FT-IR spectrometer (Shimadzu, Japan) for analysis with scan range from 401 to 4001 cm^{-1} with a resolution of 4 cm^{-1} [13].

3. RESULTS AND DISCUSSION

3. 1. Preliminary phytochemical analysis

The results of qualitative analysis of the methanol extract of *Moringa oleifera* whole leaf are shown in **Table 1**. It revealed the presence of alkaloid, terpenoids, saponins, phenols, glycosides. Phytochemical constituents of plants serves as defense mechanism against many microorganisms. The therapeutic properties of medicinal plants are possibly due to the presence of various secondary metabolites [13, 18]. Methanol extract of *Moringa oleifera* whole leaf revealed the presence of phenol, alkaloids, saponins, terpenoid and glycoside compounds, which are known to have remedial activity against diseases producing pathogen. The presence of some of these bioactive components confirms similar results by 19-22, who in their study reported that *Crassula argentea* and *Bryophyllum pinnatum* contain tannins, phenols, glycosides, alkaloids, terpenoids as their phytochemicals. Phenols are acting as antimicrobial agents [21]. Alkaloid exhibited promising antidiarrheal, anti-inflammatory, anticancer and antidiabetic activities and cure urinary disorders [19, 23-24]. Steroids are naturally occurring lipids, or fat-soluble chemical, with a great range of physiological activity. Thus, the initial screening test may be suitable in the discovery of the bioactive components

Table 1. Phytochemical constituents present in Methanolic extracts of *Moringa oleifera* whole leaf.

S/No	Phytochemicals	<i>Moringa oleifera</i> methanol extract
1	Tannins	-
2	Terpenoids	+
3	Flavonoid	-
4	Phenols	+
5	Alkaloids	+

6	Saponins	+
7	Glycosides	+

+ = Positive, - = Negative

3. 2. Fourier Transform Infrared Spectroscopic Analysis

The data on the peak values, the probable functional groups and remark assignment (obtained by FT/IR analysis) present in the methanol extracts of *Moringa oleifera* are represented in **Table 2**.

Table 2. Absorption Frequencies of FT/IR Result Obtained from methanol extract of *Moringa oleifera* whole leaf.

S/No	Absorption frequency (cm ⁻¹)	Functional groups	Remark assignment
1	3632.3	O-H group (Alcohol).	OH- Stretching, H- bonded.
2	3337.8	O-H group (Alcohol).	OH- Stretching
3	2929.7	-CH ₃ (Alkane)	C-H Stretching
4	2830.9	=C-H (Aldehyde)	C=O Stretching
5	2571.9	-CO-OH (Carboxylic acid)	Unknown
6	2432.1	CH ₃ (Alkyl group)	C-H Stretching
7	1707.1	C=O (Ketone or carboxylic acid)	C=O stretching
8	1541.3	C=C (Aromatic group)	C=C Stretching
9	1507.7	C=C (Aromatic group)	C=C Stretching
10	1448.1	C=C (Aromatic group)	C=C Stretching
11	1420.1	C=C (Aromatic group)	C=C Stretching
12	1364.2	-CH ₃ (Trimethyl)	C=C Bending
13	1228.2	Ar-OH (Acids)	C-O Stretching
14	1164.8	CH ₂ (Methylene group)	CH ₂ Wagging
15	1094.0	C=C-CR ¹ R ¹ -OH	C=O Stretching
16	1023.2	C-O (Ester)	Unknown
17	672.8	Cis RCH=CHR ¹	Unknown

The FT/IR spectrum of methanol extract of *Moringa oleifera* whole leaf is presented in **Figure 2**.

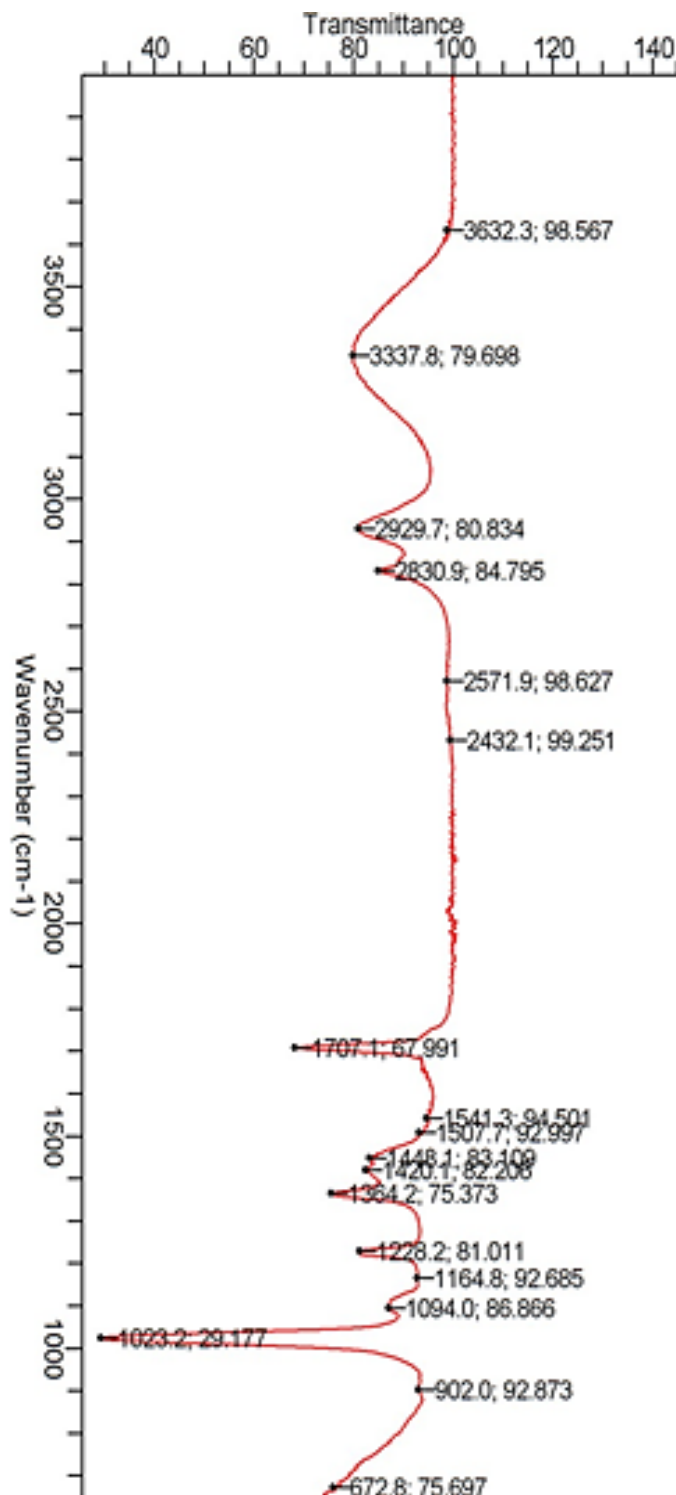


Figure 2. FTIR- Spectrum wave numbers of methanol extract of *Moringa oleifera* whole leaf.

The infrared region is useful in functional group identification of the active components present in extract via the peaks FT/IR values. When the extract was passed into the FT/IR, the functional groups of the components were separated based on its peaks ratio.

The FT/IR spectroscopic studies revealed the presences of alcohol, alkanes, aldehydes, carboxylic acid, aromatics and esters were observed from methanol extract of *Moringa oleifera* whole leaf. The peaks therefore, between 3500-3200 cm^{-1} , 3500- 3100 cm^{-1} , 2750-250 cm^{-1} and the top at 900 – 675 cm^{-1} are diagnostic marker for the presence of OH, NH, C=O and C=C functions respectively. The C-H stretch associated with H-C=O usually differed a little in frequency because of the inductive effect of oxygen attached to the carbon atom and hydrogen atom, thereby making it weaker. The broad band, therefore, in this region of the spectrum is diagnostic of the presence of OH group. The results of IR analysis (1541.3- 420.1 cm^{-1}) also reveal that, the components of *Moringa oleifera* leaf could be aliphatic or aromatic. It may therefore be inferred that aromatic or aliphatic alcohols or phenols, amine, ketones, esters and some nitrogen containing compounds are some of the constituents of the extract of *Moringa oleifera* whole leaf. The characterization of Methanoic extract of *Moringa oleifera* leaf reveals the presence of C=O, C-O, C=C, -CH₃ –CO-OH etc. band stretching, suggesting that components of *Moringa oleifera* leaf may be aromatic or aliphatic. This is also in agreement with the opinion of [25-29] and [30], who conducted a fluorescence spectroscopic study of a coagulating protein extracted from *Moringa oleifera* seeds.

3. 3. Gas Chromatography-Mass Spectroscopy analysis

The components present in the methanol extract of *Moringa oleifera* whole leaf were identified by GC-MS analysis. The GC-MS chromatogram with peak area is given in **Figure 3**. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and Area (%) are presented in **Table 3**. Twenty major compounds were detected in methanol extract of *Moringa oleifera* whole leaf. Compounds 6 and 10 were the same (Pentetic acid) as predicted by comparing with the standard mass spectral databases. However, they varied in their respective retention time, area and area percentage.

The results revealed that, N,N'-Pentamethylenebis[s-3-aminopropyl thiosulfuric acid and 2-Myristynoyl pantetheine (100.0%) was found as major component followed by 2-Myristynoyl pantetheine and Deoxyspergualin (92.05%), 5-Octadecenal and 9-Hexadecenoic acid (27.24%) , N,N'-Pentamethylenebis[s-3-aminopropyl thiosulfuric acid and Pentetic Acid (26.29%). N,N'-Pentamethylenebis[s-3-aminopropyl thiosulfuric acid and 2,6-Bis[2-[2-S thiosulfuroethylamino] ethoxy] pyrazine (8.70%), Acetamide,N-methyl-N-[4-[4-fluoro-1-hexahydropyridyl]-2-butynyl]- and N-Methoxy-1-ribofuranosyl-4-imidazolecarboxylic amide (6.39%), 3-Dodecen-1-ol (6.44%). Glucobrassicin and Pentetic Acid (5.64%), N,N'-Pentamethylenebis[s-3-aminopropyl thiosulfuric acid and 2-Myristynoyl pantetheine (5.37%), 5 α -androstane-3,17-dione 17-monooxime and Pyrrolo[3,2k]anthracene-4,6-diol, 3-methoxy-4b,5,6,7,8,9,10,11,11a,12-decahydro-11-methyl- (0.11%) and Morphinan-3-ol-6-one, 4-methoxy and 5 α -androstane-3,17-dione 17-monooxime (0.04%).

Most of the compounds identified in the methanol extract of *Moringa oleifera* whole leaf seem to possess medicinal properties and some of them are commonly present in many medicinal plants. The presence of Pentetic acid, 5-Octadecenal, Glucobrassicin, tetrapentacotane, 2-propenoic acid, pentadecyl ester, 3,4- dihydroxymandelic acid has acted as a various therapeutic and pharmaceutical benefits such as hydroxylation of liver, enzymes antipyretic analgesic, ant rheumatism and antimicrobial activity during phase I metabolism, hair

growth promoter, inhibit production of uric acid and arachidonic acid inhibitor in human body respectively [25].

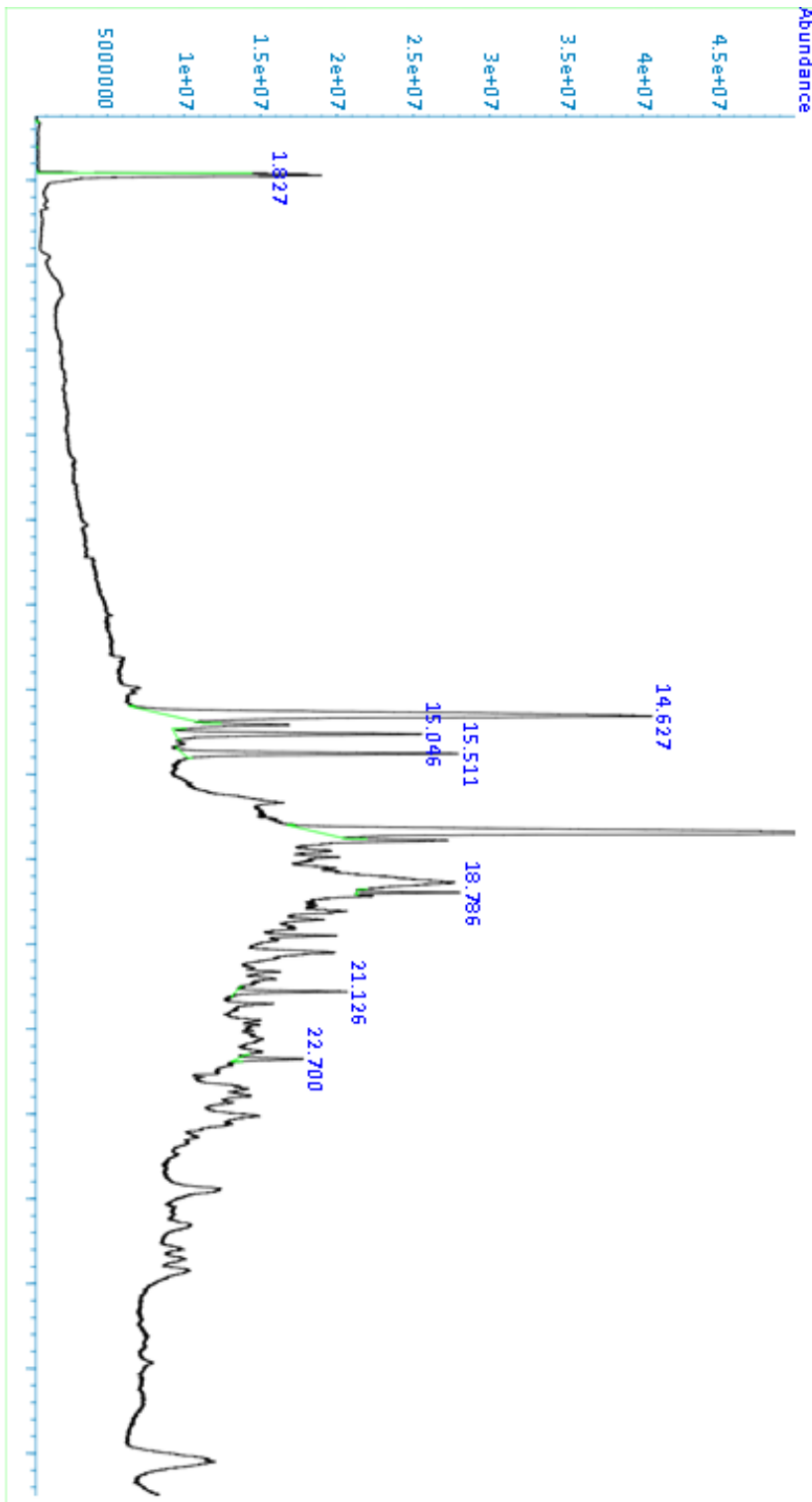


Figure 3. Chromatogram of methanol extract of *Moringa oleifera* whole leaf by GC-MS

Earlier studies on GC-MS analysis of ethanol extract of *Moringa oleifera* leaves have identified 9- Octadecen –1- ol, cis - 9 – Octadecen–1–ol, Oleol, Satol, Ocenol, Sipo, Decanoic acid, Dodecanal as the major compounds [17], which is similar to the present work.

Table 3. Phytocomponents identified in the plant Methanolic extracts of *Moringa oleifera* whole leaf by GC-MS.

S/No	Retention Time (mins)	Peak Area (%)	Molecular Weight	Name of compound	Molecular Formular
1	0.622	0.11	303	5 α -androstande-3,17-dione 17-monooxime	C ₁₉ H ₂₉ NO ₂
			303	Pyrrolo[3,2-k]anthracene-4,6-diol, 3-methoxy-4b,5,6,7,8,9,10,11,11a,12-decahydro-11-methyl-	C ₁₈ H ₂₅ NO ₃
2	1.210	0.04	287	Morphinan-3-ol-6-one, 4-methoxy-	C ₁₇ H ₂₁ NO ₃
			303	5 α -androstande-3,17-dione 17-monooxime	C ₁₉ H ₂₉ NO ₂
3	1.827	6.39	226	Acetamide, N-methyl-N-[4-[4-fluoro-1-hexahydropyridyl]-2-butynyl]-	C ₁₂ H ₁₉ FN ₂ O
			273	N-Methoxy-1-ribofuranosyl-4-imidazolecarboxylic amide	C ₁₀ H ₁₅ N ₃ O ₆
4	14.627	92.05	484	2-Myristynoyl pantetheine	C ₂₅ H ₄₄ N ₂ O ₅ S
			387	Deoxyspergualin	C ₁₇ H ₃₇ N ₇ O ₃
5	15.046	27.24	266	5-Octadecenal	C ₁₈ H ₃₄ O
			254	9-Hexadecenoic acid	C ₁₆ H ₃₀ O ₂
6	15.511	26.29	410	N,N'-Pentamethylenebis[s-3-aminopropyl thiosulfuric acid	C ₁₁ H ₂₆ N ₂ O ₆ S ₄

			393	Pentetic Acid	C ₁₄ H ₂₃ N ₃ O ₁₀
7	17.380	100.00	410	N,N'-Pentamethylenebis[s-3-aminopropyl thiosulfuric acid	C ₁₁ H ₂₆ N ₂ O ₆ S ₄
			484	2-Myristynoyl pantetheine	C ₂₅ H ₄₄ N ₂ O ₅ S
8	18.380	5.37	410	N,N'-Pentamethylenebis[s-3-aminopropyl thiosulfuric acid	C ₁₁ H ₂₆ N ₂ O ₆ S ₄
			484	2-Myristynoyl pantetheine	C ₂₅ H ₄₄ N ₂ O ₅ S
9	21.126	8.70	410	N,N'-Pentamethylenebis[s-3-aminopropyl thiosulfuric acid	C ₁₁ H ₂₆ N ₂ O ₆ S ₄
			478	2,6-Bis[2-[2-S-thiosulfuroethylamino]ethoxy]pyrazine	C ₁₂ H ₂₂ N ₄ O ₈ S ₄
10	22.700	5.64	448	Glucobrassicin	C ₁₆ H ₂₀ N ₂ O ₉ S ₂
			393	Pentetic Acid	C ₁₄ H ₂₃ N ₃ O ₁₀

4. CONCLUSIONS

The present study shows that the Methanol extract of *Moringa oleifera* whole leaves possesses phytochemicals, such as alkaloid, terpenoids, saponins, phenols, and glycosides that possess high therapeutic value. The infrared characterization revealed the presence of C=O, C=C, C-O, CH₃, -CO-OH etc. bond stretching's and C=C-H that are diagnostic markers of aliphatic as well as aromatic compounds. Twenty major compounds were detected from methanol extract of *Moringa oleifera* whole leaf by GC.MS. The compounds possess various potential health benefits and can be consumed as a source of nutrition food by human to treat different ailments. It is recommended as the production of nutraceutical based food manufacturers and pharmaceuticals development for antipyretic, antiepileptic, anti-inflammatory, antiulcer, antispasmodic, antihypertensive, cholesterol lowering antioxidant, antidiabetic, antibacterial and antifungal treatments.

Acknowledgement

The authors wish to acknowledge laboratory technicians and students of Pure and Industrial Chemistry, Abia State University, Uturu, Nigeria for their assistance during the research work.

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