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# GC-MS, CHEMICAL CHARACTERIZATION AND ANTI-MICROBIAL ACTIVITY OF MORINGA *OLEIFERA* LEAF EXTRACT

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**ABSTRACT:** *The analysis of the leaf of Moringa oleifera was carried out using Gas Chromatography Mass Spectroscopy (GC-MS). Result obtained showed the presence of alkaloids, flavonoids, steroids, tannins, terpenoids and cardiac glycoside. The GC-MS analysis showed that the leaf extract contained 16 compounds which included; Dodecanoic acid ($C_{12}H_{24}O_2$), Tetradecanoic acid, ($C_{14}H_{28}0_2$), Hexadecanoic acid, ($C_{17}H_{34}O_2$), n-Hexadecanoic acid, ($C_{16}H_{32}0_2$), 9-Octadecanoic acid ($C_{19}H_{36}O_2$), cis-Vaccinic acid, ($C_{18}H_{34}O_2$), Oleic acid, ($C_{18}H_{34}O_2$), Eicosanoic acid, ($C_{21}H_{42}O_2$), Oleic acid, 3-hydroxylpropyl ester ($C_{21}H_{40}O_3$), Oleic acid, ($C_{18}H_{34}O_2$), Docosanoic acid, Methyl ester, ($C_{23}H_{46}O_2$), Vitamin E ($C_{29}H_{50}O_2$), Trioxa-5-phosphaheptacos-18-en-1-aminium,4-hydroxyl-N,N,N-trimethyl-10-oxo-{(1-oxo-9-ocl)}, ($C_{44}H_{84}NO_8P$), Oleic acid, 3-(octadecyloxy) propyl ester, ($C_{39}H_{76}O_3$), Oleic acid, 3-(octadecyloxy) propyl ester, ($C_{39}H_{76}O_3$), 9-Octadecanoicacid [Z]., 2-hydroxyl-1,3-propanedyl ester, ($C_{39}H_{72}O_5$). Similarly, the antimicrobial result showed that the extract had activities against six selected human pathogens such as; Staphylococcus aureus with diameter of inhibition 3.8 and minimum inhibition concentration (MIC) of 50 mg/g, Streptococcus spp with diameter of inhibition 5 and (MIC) 25mg/g, Escherichia coli with diameter of inhibition 20 and (MIC) of 25mg/g, Salmonela. typhi with diameter of inhibition 3 and (MIC) 50, P. aureginosa with diameter of inhibition 3 and (MIC) 50.*

**KEYWORDS:** Moringa, chromatogram, microbial, spectroscopy inhibition



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## INTRODUCTION

Moringa *oleifera Lam. (Moringaceae)*, commonly known as drumstick or horseradish is a small, fast, growing, evergreen, or deciduous tree that usually grows up to 10 or 12 m in height, native to the Sub-Himalaya tracts of India, Pakistan, Bangladesh, Central America, Afghanistan, and Africa, Anwar and Rashid, (2007). Over the past two decades, many reports have appeared in the mainstream scientific journals describing its nutritional and medicinal properties (Akerele., 1993). *Moringa oleifera*, which is rich in vegetable oil and high in nutritional values, is used in Asia as a vegetable and medicinal plant. This is attributed to the presence of proteins, vitamins, and various phenolic compounds in the oil, Anwar, (2007). Nevertheless, all parts of the *Moringa Oleifera tree* are edible and have been consumed for many years by humans. The diverse range of medicinal uses of *Moringa oleifera*, include its use as an antioxidant, Verma, Diaz-Gonzalaz and Gonzalez-Remirez., (2009), anti-carcinogenic, Bharadi, Tabassum and Azad, (2003), anti-inflammatory, antispasmodic, diuretic, Caceres, Saravia, Rizzo, Zabala, De Leon and Nave, (1992), antiulcer, antibacterial, antifungal Caceres, Cabrera, Morales, Mollinado and Mendia, (1991), Sulaiman, Somchit, Israt, Ahmad and Moin, (2008), as well as its wound healing ability has been demonstrated, Rathi, Bodhanker and Baheti., (2006). Additionally, the root bark has been used as an analgesic, alexeteric, anthelmintic, and treatment for heart complaints, as well as for eye diseases, inflammation and dyspepsia, Nadkarni, (1954). Phytochemical screening is of paramount importance in identifying new source of therapeutically and industrially valuable compound having medicinal significance, to make the best and judicious use of available natural wealth. Plants are the richest source of drugs for traditional medicine, modern medicines, nutraceuticals food supplements, folk medicine, pharmaceutical intermediates and chemical entities for synthetic drugs, Hammer, Carson and Riley, (1999). The use of plant product as medicines could be traced as far back as the beginning of human civilization. Moringa *oleifera* is a short, slender, deciduous, perennial tree, grows to about 10 m tall, rather slender with drooping branches; branches and stem are brittle, with corky bark; leaves are feathery, pale green, compound, tripinnate, (30-60 cm long), with many small leaflets, 1.3-2 cm long, 0.6-0.3 cm wide, lateral ones somewhat elliptic, terminal ones obviate and slightly larger than the lateral ones; flowers are fragrant, white or creamy-white, (2.5cm in diameter), borne in sprays, with five (5) at the top of the flower; stamens are yellow; pods are pendulous, brown, triangular, splitting lengthwise into 3 parts when dry, (30-120 cm long, 1.8 cm wide), containing about 20 seeds embedded in the pith. The pod is tapering at both ends, nine (9) ribbed; seeds are dark brown, with 3 papery wings, James, (1983).

### Experimental

The fresh leaves and seeds of *Moringa oleifera* were collected from Abakaliki Area of Ebonyi State, Nigeria and were identified by taxonomist in the Department of Applied Biology, Ebonyi State University, Abakaliki, Nigeria. Plant sample collected was also deposited in the herbarium for reference purposes. The leaves were destalked washed and shade dried at ambient temperature with constant turning averts fungal growth. The leaves were later milled to obtain the vegetable leaf meals (VLMs) using an electric blender was stored in 40 º C in well labeled airtight containers for analysis. 40.0 gm of dried powdered leaves and seeds of *Moringa oleifera* were extracted successively with 300 ml of methanol in an orbital shaker for 24 hrs at room temperature. The extracts were filtered using Whatman No.1 filter paper to remove extractable substances, at every 3 hrs interval. The extracts were then evaporated with rotary evaporator and the dried extracts were stored at 4°C in two


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different sterile containers. GC-MS analysis of the methanolic extract of *M. oleifera* leaves were performed using Shimadzu Japan gaschromatography QP2010PLUS with a fused GC column (2010) coated with polymethyl silicon (0.25 nm x 50 m) and the conditions were as follows: Temperature programming from 80–200oC held at 80oC for 1 min, rate 5oC/min and at 200 $^{o}$C for 20 mins. Field ionization detector (FID) temperature 300 $^{o}$C, injection temperature 220 $^{o}$C, carrier gas nitrogen at a flow rate of 1 ml/min, split ratio 1:75. Gas chromatography mass spectroscopy was conducted using GCMS –QP 2010 Plus Shimadzu Japan with injector temperature of 220 $^{o}$C and carrier gas pressure of 116.9 kpa. The column length is 30 m with a diameter of 0.25 mm and flow rate of 50 ml/min. The elutes were automatically passed into a mass spectrometer with a dictator voltage set at 1.5 kv and sampling rate of 0.2 sec. The mass spectrum was also equipped with a computer fed mass spectra data bank. Hermlez 233 M-Z centrifuge (Germany) was used. Component Identification Chemical constituent components of the extracts were identified by matching the peaks with Computer Wiley MS libraries and confirmed by comparing mass spectra of the peaks and those from literature. Qualitative test were then carried out on the cool solution. 13.0 g of iodine crystal and 2.0 g of potassium iodide were dissolved in water in a 100 ml volumetric flask and the solution was made up to 100ml as Wagner Reagent. 1.30 g of mercuric chloride and 5.0g of potassium iodide were dissolved in distilled water in a 100 ml volumetric flask and the solution was made up to 100 ml. as Mayer's Reagent. For the test for Alkaloids, 1.0 ml of 1% HCL was added to 3ml of each of the extracts in a test tube. The mixture was heated for 20mins with continuous shaking in a water bath cooled and filtered and the process repeated for other extract samples. 1ml of each filtrate was added to 0.5ml of Mayer's reagent. 1.0 ml of each filtrate was added to 0.5 ml of Wagner's reagent. For the test for Saponins, the Frothing test was conducted according to Iwu, *et al*., (2018). 3.0 ml of each extract and dilute with 2ml of distilled water was added in a test tube. The mixture was shaken vigorously. The Emulsion Test was also according to Iwu, *et al*., (2018). 3.0 ml of each extract was added to 5 drop of Olive oil in a test tube and the content was vigorously shaken. The Flavonoids test was also conducted according to 3ml of each extract was added to 5 drop of Olive oil in a test tube and the content was vigorously shaken. 3.0 ml of each extract was added to 10ml of distilled water the solution was shaken. 1.0 ml of 10% NaOH solution was added to the mixture. The test for Steroids followed the Salkowski method reported by Iwu, *et al*., (2018): 5.0 drops of concentrated $H_2SO_4$ were added to 1ml of each extract in a separate test tube. The test for Tannins was according to Iwu, *et al*., (2018). 2.0 ml of each extract in a separate test tube were boiled gently for 2min and allowed to cool. 3 drop of ferric chloride solution was added to each extract. The test for Glycosides was according to Iwu, *et al*., (2018). 1.0 ml of aqueous extract was mixed with 1ml of 20% solution of 3,5dinitrosalic acid in methanol and 1ml of a 5% aqueous NaOH was added. GC-MS analysis was carried out on GC-MS-QP2010 Shimadzu system comprising a gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column VF-5MS fussed silica capillary column (30.0 m x 0.25 mm x 0.25 µm, composed of 5% phenyl/95% dimethylpolysiloxane), operating in electron impact mode at 70 ev; helium (99.999%) was used as carrier gas at a constant flow of 1. ml/min and an injection volume of 0.5 µl was employed (split ratio of 10:1) injector temperature 240 $^{0}$C ion-source temperature 200 $^{0}$ C. The oven temperature was programmed from 70 $^{0}$C (isothermal for 3 min), with an increase of 10 $^{0}$C/min, to 240 $^{0}$C, ending with a 9min isothermal at 280 $^{0}$ C. Mass spectra were taken at 70ev; a scan interval of 0.5 seconds and fragments from 40 to 440 Da. Total GC running time was 40 min. For the antimicrobial analysis the microorganisms; Staphylococcus *aureus*, Streptococcus *spp*., Pseudomonas *aeruginosa*, Candida *albicans*, E.


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*coli*. and salmonella *typhi* were used for the analysis. They were clinical isolates of human pathogens obtained from the Federal Medical Centre Owerri and were brought to the laboratory and resuscitated in buffered peptone broth (Secharian chemie) and thereafter into nutrient agar medium and incubated at $37^0$C for 24 hrs. The test solution of each extract was prepared by dissolving 0.1 g of the plant extract separately in 1.0 $cm^3$of dimethyl sulphoxide (DMSO) to get a concentration of 100mg/$cm^3$. The antibacterial activity was performed by filter paper disc diffusion technique. Filter paper disc (Whatman No 1.6 mm diameter) were placed in glass Petri dishes and sterilized in hot air over. The media (10 g nutrient Agar in 200 $cm^3$ distilled water, autoclaved at 115 $^o$C for 30 minutes) was cooled to 50 $^o$C. The sterile nutrient Agar media were poured into the sterile petri dish and allowed to solidify. The bacteria were swabbed with a sterile wire loop. Each disc was impregnated with 0.2 $cm^3$of plant extract. .The discs were used after drying them in an incubator at 40 $^o$C to remove any trace of solvent. Discs were introduced into the surface of the medium and incubated at 37 $^o$C for 24 hours to obtain zones of inhibition. The minimum inhibitory concentration of the extract was determined by incorporating constant volume 0.2$cm^3$ of each diluents of the extract into the perforated disc on a seeded nutrient agar plate as described in the anti-microbial susceptibility test section.0.1g of each extract was dissolved in 1.0 $cm^3$ of DMSO to obtain 100mg/$cm^3$. This concentration of DMSO was then doubled to obtain 50.0 mg/$cm^3$ then doubled again to obtain 12.5 mg/$cm^3$ and again to obtain6.25mg/$cm^3$. Each concentration was then used in the method earlier described to obtain zone of inhibition. The least concentration that showed inhibitory zones was taken as the Minimum Inhibitory Concentration (MIC).

## RESULTS AND DISCUSSION

The analysis of the leaf of Moringa *oleifera* was carried out using Gas Chromatography - Mass Spectroscopy (GC-MS) and the results of preliminary qualitative phytochemical analysis

**Table-1: Phytochemical screening of *Moringa oleifera* leaves**

| Alkaloids | Flavonoids | Steroid | Tannins |
|---|---|---|---|
| + | + | + | + |
| Saponins | Anthraquinon | Terpenoid | Cardiac glycoside |
| – | – | + | + |

Key: (+), represent presence and ( - ) represents absence.

The results of preliminary phytochemical screening of plant extracts showed the presence of alkaloids, flavonoids, steroids, tannins and terpenoids and cardiac glycoside. Interpretation on mass spectrum of GC-MS was done using the database of National Institute of standard and Technology (NIST) with more than 62,000 patterns. The results of GC-MS led to the identification of number of compounds from the methanol extract of *Moringa Oleifera* plant. GC-MS chromatogram showed 16 peaks, indicating the presence of 16 compounds and many


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of these components reported in this plant for the 1st time like Dodecanoic acid ($C_{12}H_{24}O_2$), Tetradecanoic acid, ($C_{14}H_{28}0_2$), Hexadecanoic acid, ($C_{17}H_{34}O_2$), n-Hexadecanoic acid, ($C_{16}H_{32}0_2$), 9-Octadecanoic acid ($C_{19}H_{36}O_2$), cis-Vaccinic acid, ($C_{18}H_{34}O_2$), Oleic acid, ($C_{18}H_{34}O_2$), Eicosanoic acid, ($C_{21}H_{42}O_2$), Oleic acid, 3-hydroxylpropyl ester ($C_{21}H_{40}O_3$), Oleic acid, ($C_{18}H_{34}O_2$), Docosanoic acid, Methyl ester, ($C_{23}H_{46}O_2$), Vitamin E ($C_{29}H_{50}O_2$), Trioxa-5-phosphaheptacos-18-en-1-aminium,4-hydroxyl-N,N,N-trimethyl-10-oxo-{(1-oxo-9-ocl)}, ($C_{44}H_{84}NO_8P$), Oleic acid, 3-(octadecyloxy)propyl ester, ($C_{39}H_{76}O_3$), Oleic acid, 3-(octadecyloxy)propyl ester, ($C_{39}H_{76}O_3$), 9-Octadecanoic acid [Z]., 2-hydroxyl-1,3-propanediylester,($C_{39}H_{72}O_5$).

Fig. 1 GC-MS spectra of M. *oleifera* extract.

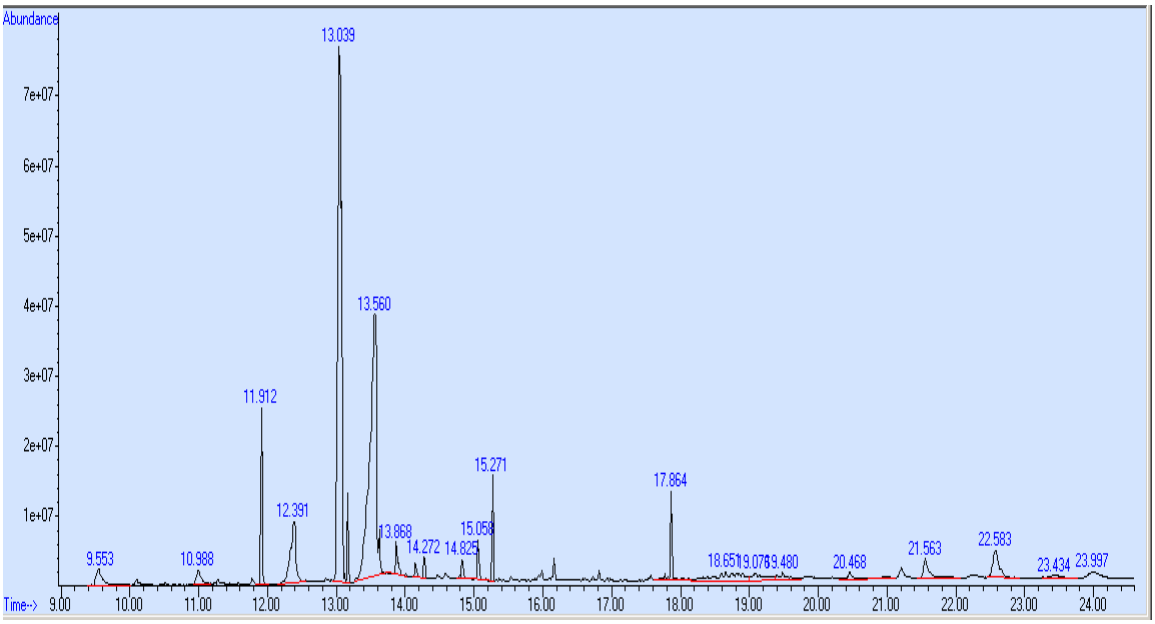
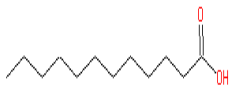
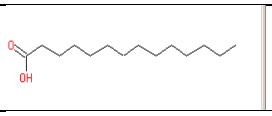
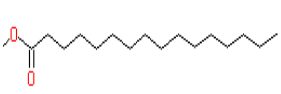
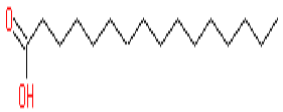



**Table 2: Phytocomponents identified in the methanolic extracts of *Moringa Oleifera***

| Peak | Compound Name | Molecular formular | Molecular weight (g) | Retention time (s) | Structure |
|---|---|---|---|---|---|
| 1 | Dodecanoic acid | $C_{12}H_{24}O_2$ | 200 | 9.553 |  |
| **2** | Tetradecanoic acid | $C_{14}H_{28}O_2$ | 228 | 10.988 |  |
| **3** | Hexadecanoic acid, Methyl ester | $C_{17}H_{34}O_2$ | 270 | 11.912 |  |
| **4** | N-Hexadecanoic acid | $C_{16}H_{32}O_2$ | 256 | 12.391 |  |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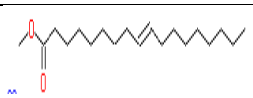
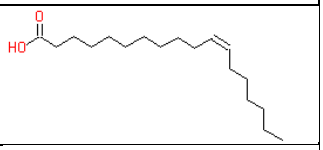
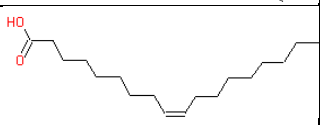
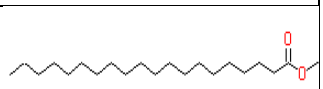
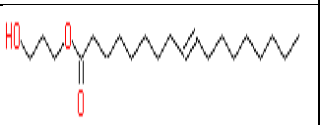
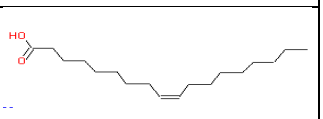
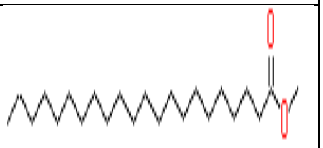
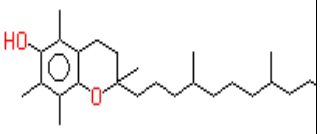
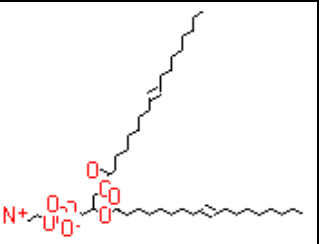
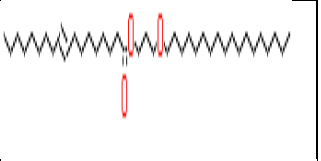
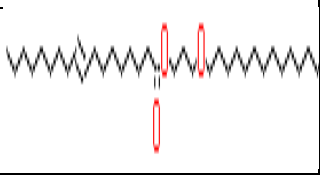
|---|---|---|---|---|---|
| **5** | 9-Octadecenoic acid, methyl ester, [E] | $C_{19}H_{36}O_2$ | 296 | 13.039 |  |
| **6** | Cis-Vaccenic acid | $C_{18}H_{34}O_2$ | 282 | 13.560 |  |
| **7** | Oleic acid | $C_{18}H_{34}O_2$ | 282 | 13.868 |  |
| **8** | Eicosanoic acid, methyl ester | $C_{21}H_{42}0_2$ | 326 | 14.272 |  |
| **9** | 9-Octadecenoic acid, methyl ester, [E] | $C_{19}H_{36}O_2$ | 296 | 13.039 |  |
| 10 | Oleic acid | $C_{18}H_{34}O_2$ | 282 | 15.058 |  |
| 11 | Docosanoic acid,methyl ester | $C_{23}H_{46}0_2$ | 354 | 15.271 |  |
| 12 | Vitamin E | $C_{29}H_{50}O_2$ | 430 | 17.864 |  |
| 13 | 3,49-Trioxa-5-phosphaheptacos-18-en-1-aminium,4-hyroxyl-N,N,N-trimethyl-10 oxo-7-[{1-oxo-9-ocl}] | $C_{44}H_{84}NO_8P$ | 785 | 20.468 |  |
| 14 | Oleic acid, 3-[octadecyloxy] propyl ester] | $C_{39}H_{76}O_3$ | 592 | 21.563 |  |
| 15 | Oleic acid, 3-[octadecyloxy] propyl ester] | $C_{39}H_{76}O_3$ | 592 | 22.583 |  |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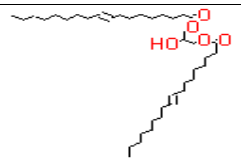
|---|---|---|---|---|---|
| 16 | 9-Octadecenoic acid [Z]-,2-hydroxyl-1,3-propanediyl ester | $C_{39}H_{72}O_5$ | 620 | 23.997 |  |

**Table 3: result of antimicrobial analysis of M. *oleifera* leaf**

| Microorganism | Diameter of inhibition (mm) | MIC (mg/g) |
|---|---|---|
| Staphylococcus aureus | 3.8 | 50 |
| Streptococcus. spp | 5 | 50 |
| Escherichia. coli | 20 | 25 |
| Salmonela. typhi | 3 | 50 |
| p. aureginosa | 3 | 50 |

## DISCUSSION

The result obtained from Gas chromatography mass spectroscopy (GC-MS) revealed about 16 bio-active compound which include Dedecanoic acid also known as Lauric acid ($C_{12}H_{24}O_2$). Lauric acid has been proven to be used for the treatment of viral infections incuding influenza (The flu); swine flu; avian flu; the common cold; fever bluster, cold sores, and genital herpes caused by herpes simplex virus (HSV); genital warts caused by human papillomavirus (HPV); and HIV/AIDs. It is also used for the preventing the transmission of (HIV) from mother to children. The GC-MS of *M.oleifera* leave also show Vitamin E ($C_{29}H_{50}O_2$).Vitamin E which function mainly as an antioxidant, which means it helps protect cells from damage caused by unstable molecules called free radicals. It protect cell from damage and it will aid in lowering the risk of a variety of health problem, from heart diseases to cancer, and possibly end demania. The antimicrobial analysis of *M.oleifera* plant extract had activities against six selected human pathogens; such as staphlococcus aureaus with diameter of inhibition 3:8 and Minimum inhibition concentration (MIC) of 50ml/g. The plant extract counter the bacteria at diameter of inhibition 3.8 mm.

## CONCLUSION

*Moringa oleifera* leaf contains phytochemicals like Flavonoid, Alkaloids, Tannin, Phenol, Glycosides, Saponin, and steroids indicative of high medicinal properties. In the present study, methanolic extract of the Moringa *oleifera* cultivated in Nigeria was analyzed and the mass spectrum compared with the NIST database library which gave more than 90% match as well as a confirmatory compound structure match. This work help to identify the compounds, which are active against human pathogens such as Staphylococcus aureus, Streptococcus spp Pseudomonas *aeruginosa*, Candida *albicans*, E. *coli*, salmonella *typhi* and may be used in further body products, drugs, pharmaceutical and therapeutic value since many components isolated from this plant reported for the first time, also the present study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results were confirmed the traditional uses of this plant as an antioxidant, anti-inflammatory , antispasmodic, diuretic, antiulcer, flavour agent, antimicrobial, antifungal, pesticide.

## REFERENCE

Akerele, O., 1993. Summary of WHO guidelines for the assessment of herbal medicines. HerbalGram, 28:13-19

Anwar. F, Latifs, Ashraf M, Gilani AH. Moringa Oleifera: a food plant with multiple medicinal use. Phytotherapy Research. 2007;21:17-25.

Anwar. F. and Rashid, U. (2007). Physiochemical characteristics of *Moringa Oleifera* seeds and seed oil from a wide provenance.

Bharadi R, Tabassum J, Azad MRH, 2003. Chemomodulatory effect of moringa Oleifera, Lam. On hepatic carcinogen metabolizing enzymes, antioxidant parameters and skin papillomagenesis in mice. Asian pacific Journal of cancer prevention, 4:131-139

B S Rathi, S L Bodhanker & A M Baheti. (2006). Evaluation of aqueous leaves extract of Moringa Oleifera linn for wound healing in albino rats pp. 898-901

Caceres A., O Cabrera, O Morales, P Mollinedo, and P Mendia. (1991). Pharmaoclogical properties of moringa Oleifera. 1: preliminary screening for antimicrobial activity. J Ethnopharmacol 33:213-216

Caceres A., A Saravia, S Rizzo, L Zabala, E De Leon, and F Nave. (1992). Pharmaoclogical properties of moringa. 2: Screening for antispasmodic, anti-inflammation and diuretic activity. J Ethnopharmacol 36:233-247

Iwu. I. C, Chijioke-okere. M, Onu. U. L, Uchegbu. R, (2018). GC-MS, Phytochemical and Antimicrobial Analysis of the Leaf of Newboudia laevis P. Benth. *International Journal of Innovative Research and Development* 7 (7 ) pp. 242- 250

Hammer, K.A., Carson, C. F., Riley, T. V. (1996). Susceptibility of transient and commensal skin flora to the essential oil of melalenca alternifolia (tea tree oil). American Journal of infection control, 24,186 189.

Nadkami, K. M. Indian Materia Medica with Ayuvedic. Vol. 2. 1954

Sulaiman, MN Somchit, DA Israt, Z Ahmad, and S moin. (2004). Antinociceptive effect of Moringa of melastoma malabathricum ethanolic extract in mice. Fitoterapia 75:667-672

Verma, S.P., Diaz-Gonzalaz, L., Gonzalez-Remirez, R., (2009), Relative efficiency of single-outlier discordancy data on reference materials and application to Geostandards and Geoanalytical Research, 33(1), 29-49.


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