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Antibacterial Activity and Fourier Transform Infrared Spectrometry of Study *Moringa oleifera* Leaf

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Authors' contributions

This work was carried out in collaboration among all authors. Authors FOO and AGO designed the study and wrote the protocol. Author AGO carried out the laboratory work, collected all data, performed the statistical analysis and wrote the article manuscript. Author KJA handled the critical revision of the manuscript. Author OAA did the literature search. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Introduction: In recent time, high incidence of resistance of microorganisms to antibiotics has increased the focus and demand for medicinal plants. *Moringa oleifera* is among such herbs with high demand that have served as sources of therapy to both man and animal.
Aim: The aim of this study was to determine the antibacterial property of *M. oleifera* and evaluate the functional groups of its bioactive responsible for its medicinal value.
Methods: The crude extracts were purified and the antibacterial screening against test isolates were determined by using standard techniques. The functional groups were recorded based on the peaks observed on FTIR *spectra*. The antibiotic sensitivity test was carried out in order to compare the susceptibility of the microorganisms to the five (5) different commercial antibiotics.
Results: In *M. oleifera* fractions, highest susceptibility was observed in *Klebsiella pneumoniae* (44.00±0.00 mm) whereas a lowest value (20.00±0.00 mm) was recorded against *Escherichia coli*. The range of zone of inhibition of the commercial antibiotics observed was from 6.80±0.20 mm to 28.67±0.67 mm with chloramphenicol and ciprofloxacin respectively. The FTIR spectrum of the

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extracts confirmed the presence of secondary alcohols, phenols, alkanes, alkenes, carboxylic acids, sulphonamide, nitro-compounds and amines.
Conclusion: These results obtained from this work justify the use of this plant in folk medicine for the treatment of various diseases in human and also revealed the herbs as novel drug sources.

Keywords: Antibacterial; Moringa oleifera; functional groups; antimicrobial.

1. INTRODUCTION

The quest for alternative antimicrobial agents to tackle the problem of resistance of bacteria to antibiotics necessitated the test for antibacterial potency of some medicinal plants popular in ethno-medicine among the people of Nigeria. Some pathogenic bacteria isolated from drinking water source were challenged with common medicinal herbs so as to provide alternatives therapy for the treatment of waterborne related infections. Since anthropogenic activities in the environment determine the quality of the water the people drink, therefore the purity of water cannot be discussed outside the condition of its immediate environment. In 2005, the World Health Organization evaluated the sanitation coverage rates in Nigeria [1] and concluded that it was amongst the lowest in the world due to limited access to adequate sanitation facilities. Therefore, people are still becoming sick as a result of consuming supposed clean water that has been contaminated from the sources. It therefore necessary to get the people armed with herbs that can alleviate ailments. *M. oleifera* is one of such useful plants with medicinal value according to Bamishaiye et al. [2]

M. oleifera tree is a pan-tropical species that belong to the family *Moringaceae*. It is widely cultivated in Nigeria and locally called "*Ewe ile"* and "*Zogeli"* among the Yoruba and Hausa speaking people respectively [2]. *Moringa* tree is a medicinal plant that has served the humanity, from the roots to the leaves.It has beneficial properties [3] such as antibacterial [4], antioxidant and antifungal activities [5], anti-inflammatory and anti-ulcerative [6]. With the trend of emergence of antibiotic resistant to bacteria, hope seems to be lying in medicinal plants as remedy to relieve people from their illnesses. Ijeomah et al. [7] reported that many phytochemicals have been found to be protective and preventive against many degenerative diseases such as coronary heart disease and even in ageing. Apart from antimicrobial properties, other pharmacological actions displayed by the medicinal plant include anti-ulcer, anti-diabetic and lipid-lowering activities [8]. Hill had earlier attributed the healing effects of the medicinal plant to the presence of bioactive compound in them [9]. The present study aims to determine the antibacterial property of purified *M. oleifera* extracts, evaluate the functional groups of their bioactive components present in the extract and thereafter compare their activities with synthetic drugs.

2. METHODOLOGY

2.1 Test Bacteria Isolates

Five already identified bacteria, previously isolated from well water samples were used. They include *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis* and *Salmonella typhi.* This test was carried out in the Postgraduate laboratory of the Department of Microbiology, the Federal University of Technology Akure, Ondo State, Nigeria.

2.2 Collection of Plants Materials and Preparation of the Plant Extracts

Leaf of *M. oleifera* was collected from a farm in Ado-Ekiti. The identification and authentication of the plant were carried out in the Herbarium of the Department of Plant Science, Ekiti State University Ado- Ekiti, Ekiti State. The leaf was collected dried in a container in the laboratory for a period of five weeks. The dried leaf material was pounded with a wooden mortar and pestle until they become powdery form. The extracts of the leaves were prepared with ethanol. About 200 g of dried powdered of the leaves were weighed into different conical flasks and was labeled and 750 mL of ethanol was added into conical flask. The extraction was allowed for a period of 3 days after which the mixture was then filtered with muslin cloth. The liquid extracted was subjected to rotary evaporator under reduce pressure. The paste extract obtained was stored in refrigerator until use.

2.3 Column Chromatography Assay: Fractionation and Identification of Bioactive Components of *M. oleifera*

The column was packed using a simple dry-pack method. A 250 mL burette was plugged with a

small piece of glass wool with the aid of applicator stick to tamp it down lightly. A weight of 60 g of column chromatography silica gel was poured into the plugged burette using a 100 mL beaker; the filled column was clamped securely to a ring stand using a small 3 pronged clamp and placed on the bench top. When properly packed, the silica gel fills the column to just below the indent on the burette. This leaves a space of 5 cm on top of the adsorbent for the addition of solvent. Petroleum ether was added to the top of silica gel to flow slowly down the column. A weight of 0.3 g of the sample to be purified was thoroughly mixed with a small amount of column chromatography silica gel, loaded onto the column fresh eluting solvent and the elution process began. Volumes of 100 mL fractions were collected in round bottom flasks and distilled. The solvent was changed with chloroform and collected fractions were distilled. Lastly, the solvent was changed with the methanol and collected fractions were distilled. More bands were observed until the bands were fully washed down with methanol [10].

2.4 Antimicrobial Assay of the Column Fractions of Leaf Extracts

Antibacterial activity of the column fractions obtained after fractionation of extracts was determined by the paper disc diffusion method [11]. The standardized bacterial isolates were grown in nutrient broth for 18 hours. Sterile Petri dishes were seeded aseptically with 1 mL of the 18 hour old broth cultures of the test organisms each while about 20 mLof sterilized Mueller Hinton agar was poured aseptically on the seeded plates. The plates were swirled carefully for even distribution and allowed to gel. Standard size, blank Whatman filter paper discs (6.00 mm in diameter) was put into bijou bottle, sterilized at 121°C for 15 minutes. The sterile paper discs were impregnated with 0.5 mL of column fractions of the extracts reconstituted in minimum amount of extracting solvent at concentration of 50 mg/ml and were applied with the aid of sterile forceps at equidistant on the seeded plates.

Before placing the discs on plates, they were air dried at room temperature to remove any residual solvent which might interfere with the determination. Filter paper discs dipped into sterile distilled water and allowed to dry were used control. The plates were then incubated at 37°C for 24 hour. Antibacterial activity was determined by measurement of zone of inhibition around each paper disc. For each extract, three replicate trials were conducted against the test organisms. Antibacterial assay of the purified fractions of *M. oleifera* extract was determined for petroleum-ether 1 (P_1), petroleum-ether 2 (P_2), petroleum-ether 4 (P_4), petroleum-ether 7 (P_7) and chloroform 1 (C_1).

2.5 Antibiotic Sensitivity Test

The antibiotic sensitivity test of the test bacteria was carried out as positive control in order to compare the sensitivity with to the different commercially available antibiotics such as ciprofloxacin, zinnacef, chloramphenicol, gentamycin and septrin were used. Therefore, sterile petri dishes were seeded aseptically with 1 ml each of the standardized broth cultures of the test organisms while about 15 mL of sterilized Mueller Hinton agar was poured aseptically on the seeded plates. The plates were swirled carefully for even distribution and allowed to gel. With the aid of sterile forceps the antibiotic discs were placed firmly on solidified plates and incubated for 24 hours at 37°C. After incubation, clear areas around the discs were measured, which represents the zones of inhibition as described by Zanpantis et al. [12] and the areas without clear zones were also observed. Unseeded agar plate with antibiotics served as the control experiment. The zones of inhibition were measured in millimeter (mm).

2.6 *Infra Red* Analysis of Column Chromatographic Fraction of Leaf Extracts of *M. oleifera*

FTIR spectroscopy analysis of the column chromatographic fraction of the leaf extracts was done with the aid of *infrared* spectrophotometer (Perkin-Elmer Spectrum bx). A drop of purified extract was placed on fused sodium Bromide (NaBr) cell. It was carefully placed on cell clamped loosely and fixed on the *infra red* beam. The *infra-red* data was compared to table of IR frequencies using the method of Mayo et al. [13].

2.7 Statistical Analysis

Data obtained were presented as mean ± SE (standard error). Significance of difference between different treatment groups was tested using one-way analysis of variance (ANOVA) and significant results were compared with

Duncan's multiple range tests using SPSS window 7 version 1.6 software. For all the tests, the significance was determined at the level of $P<0.05$.

3. RESULTS

Table 1 showed the antibacterial actions of the purified fraction of the extracts revealed that the entire test isolates were all susceptible with good zones of inhibition. With petroleum-ether 1 (P_1), highest zone of inhibition was noted against *Salmonella typhi* (42.30±0.00 mm), followed by *P. mirabilis* (40.40±0.00 mm) while *E. coli* and *K. pneumoniae*had 21.50±0.05 mm and 25.00±0.00 mm respectively. The inhibitory effects of purified fraction; petroleum-ether (P_2) also showed that *P. mirabilis* was the most sensitive with the zone of inhibition of 40.85±0.05 mm while the least value was recorded against *K. pneumoniae* (25.00±0.00 mm). However, with P_4 and P_7, 40.10±0.00 mm and 40.00±0.00 mm were marked against *S. typhi* whereas the least inhibitions recorded against the two fractions were 21.60±0.20 mm and 23.75±0.75 mm correspondingly with *P. aeruginosa* and *E. coli.* In all the antibacterial tests, *S. typhi* and *Prot. mirabilis* were the most susceptible against to the purified extracts. The test bacteria were not susceptible to chloroform 3 (C_3), methanol 1 and 2 (M_1 and M_2).

The results of the comparative inhibitive effects of purified fractions and commercial antibiotics showed interesting antimicrobial patterns (Figure 1). The range of zone of inhibition of the commercial antibiotics observed was from 6.80±0.20 mm to 28.67±0.67 mm in chloramphenicol and ciprofloxacin respectively. Ciprofloxacin recorded 28.00±0.00 mm zone of inhibition against *P. aeruginosa* and *E. coli* and 20.00±0.00 mm inhibition against *K. pneumoniae*. *S. tyhi, P. aeruginosa* and *P. aeruginosa* were resistant to zinnacef whereas *P. mirabilis* was sensitive with an inhibition of 12.00±0.00 mm and *E. coli* 8.00±0.00 mm. With chloramphicol, highest inhibition (13.33±0.67 mm) was marked against *E. coli* while least (6.80±0.20 mm) was noted against *K. pneumoniae*. Against *S. tyhi* and *P. mirabilis,* 10.00±0.00 mm inhibition was recorded when challenged with streptomycin; with the same antibiotic 25.00±0.57 mm inhibition was noted against *P. aeruginosa*. Gentamycin recorded 24.67±0.33 mm and 8.00±0.00 mm against *E. coli* and *S. tyhi* as highest and lowest inhibitions respectively.

However, the range of zone of inhibition of the purified fractions of *M. oleifera* ranged from 20.00±0.00 mm to 44.93±0.52 mm in Chloroform fraction 1 (C_1) and Petroleum-ether fraction 4 (P_4) respectively.Petroleum-ether (P1) demonstrated appreciable inhibition against all the test isolates; 30.00±0.00 mm, 20.33±2.60 mm, 26.00±0.57 mm, 40.33±0.88 mm, 45.40±0.35 mm against *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *S. typhi* and *P. mirabilis* respectively. The zones of inhibition recorded by Petroleum-ether (P2) against the test bacteria ranged from 30.00±0.00 mm to 44.93±0.52 mm correspondingly with *K. pneumoniae* and *P. mirabilis*. With P4 highest inhibition (45.10±0.38 mm) was marked against *S. typhi* while the least (26.83±3.81 mm) was noted against *P. aeruginosa*. Similarly, with Petroleum-ether (P7) highest inhibition (44.03±0.51 mm) was observed in *S. typhi* but *K. pneumoniae* had the least inhibition (23.00±2.00). *E. coli* had the least inhibition (20.00±0.00) with Chloroform fraction 1 (Ch1), *P. aeruginosa*, *K. pneumoniae* and *S. typhi* had 30.00±0.00 mm, 23.00±2.00 mm, 44.03±0.90 mm and 40.50±5.00 mm zones of inhibition respectively.

The fractions of the purified extract were analyzed with FTIR spectroscopy under IR region in the range of 400-4000 cm^{-1} to identify the functional groups of the active components using peak values they displayed. The results of spectroscopic elucidation of the purified extracts are shown in Figures 2 and 3, Tables 2 and 3 showed their spectral interpretation respectively. From purified *M. oleifera* extract (Tables 2 and 3), the wavenumber 3431cm^{-1} is indicate the presence of amine, alcohol or phenol. The band at 2970 and 2931 cm^{-1} are due to C-H asymmetric and symmetric stretching of saturated carbon. The fingerprints bands 1230 cm^{-1} and 1155 cm^{-1} wave numbers suggest amine and, or ester, alcohol, phenol, sulfonamide functional groups (Table 2). Moreover, the frequencies 3419 $cm^{-1}$and 3354 cm^{-1} which represent phenol groups while 1456 cm^{-1}, 1230 cm^{-1} and 1076 cm^{-1}, suggest C-Cstretch, C-O stretch/ Amine/ Phenol and C-O stretch of O-H (of Alcohol) respectively (Tables 3).

4. DISCUSSION

This study revealed that the purified fractions of *M. oleifera* demonstrated strong antibacterial activity against all the test isolates. This indicates that the medicinal plant possesses potent phytochemicals that can overpower the bacterial

Table 1. Antibacterial activity of column chromatographic extract of *M. oleifera*

S/N	Isolate	Zones of Inhibition (mm)							
		P_1	P_2	P_4	P_7	C_1	C_3	M_1	M_2
1	*P. aeruginosa*	20.40±0.20b	32.00±0.00e	21.60±0.20c	41.00±0.00f	30.00±0.00d	0.00±0.00a	0.00±0.00a	0.00±0.00a
2	*K. pneumonia*	25.00±0.00c	25.00±0.00c	44.00±0.00e	42.00±0.30d	23.00±0.00b	0.00±0.00a	0.00±0.00a	0.00±0.00a
3	*S. typhi*	42.30±0.00e	40.70±0.10d	40.10±0.00bc	40.00±0.00b	40.15±0.05c	0.00±0.00a	0.00±0.00a	0.00±0.00a
4	*E. coli*	21.50±0.05b	36.75±0.25c	23.75±0.75b	28.00±0.00a	20.00±0.00	0.00±0.00a	0.00±0.00a	0.00±0.00a
5	*Prot. Mirabilis*	40.40±0.00c	40.85±0.05d	40.45±0.05c	40.10±0.00b	40.45±0.00c	0.00±0.00a	0.00±0.00a	0.00±0.00a

Values with the same alphabet along the row are not significantly different P<0.05

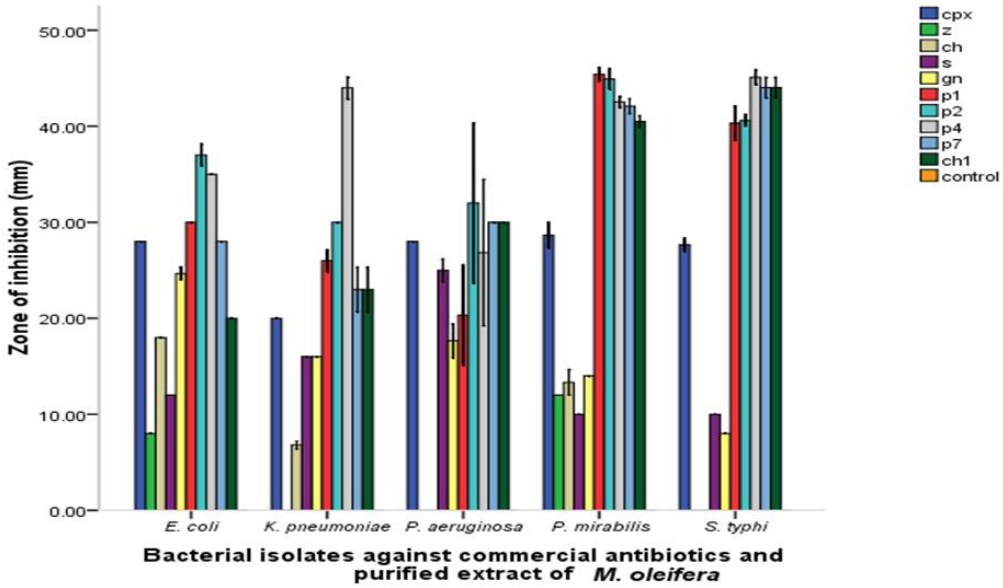

Figure 1. Comparative inhibition effect of commercial antibiotics and purified extracts of *M. oleifera*

Legend: cpx- ciprofloxacin, z- zinnacef, s- streptomycin, gn- gentamycin, p1- petroleum-ether 1, p2- petroleum- ether, p4- petroleum-ether, p7- petroleum-ether 7, ch1- chloroform 1

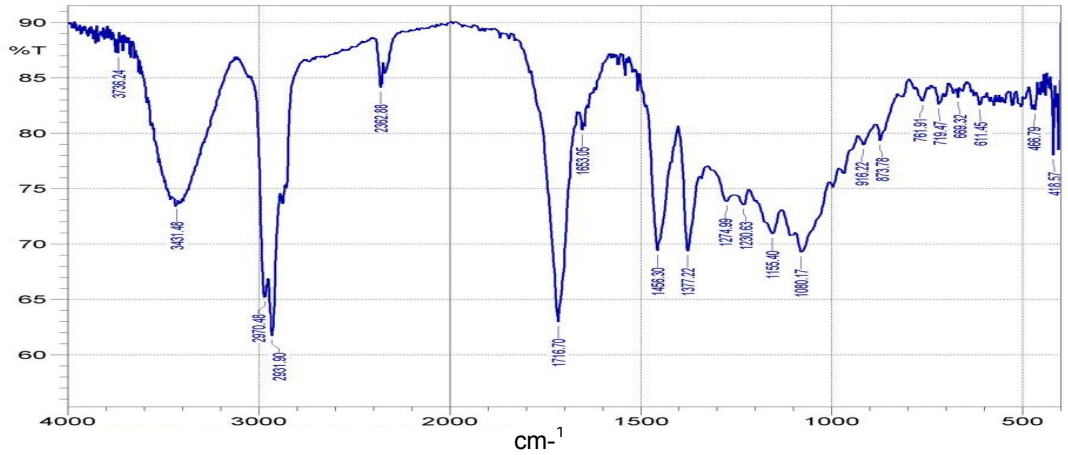

Figure 2. FTIR spectra analysis of purified extract: Petroleum-ether (P_1) of *M. oleifera* leaves

Table 2. Detected functional groups in the purified extract: Petroleum-ether (P_1) of *M. oleifera* leaves

Band	Frequency	Functional group
1	3736	Unknown
2	3431	O-$H_{stretch,}$ Phenol, Alcohol/ Amine,(Heterocyclic amine,$NH_{stretch}$)
4	2970	C-$H_{stretch}$ (Methyl, −CH_3)
5	2931	C-$H_{stretch}$ (Methylene, CH_2)
6	2362	Unknown
7	1716	C=$O_{stretch/}$ esters, saturated aliphatic
8	1653	Alkenyl C=$C_{stretch}$
9	1456	C-$H_{bending}$
10	1377	Tertiary alcohol (O-$H_{bending}$ of phenol)
11	1274	C-$O_{stretch}$of primary or secondary, OH in-plane
12	1230	C-$O_{stretch}$/ Phenol /(Aromatic ethers, aryl O stretch)
13	1155	O-$H_{stretch}$ / S=$O_{stretch}$ sulfonamide/Secondary amine, CN $_{stretch}$)
14	1080	C-$O_{stretch}$of O-H (of Alcohol)
15	916	O-$H_{bending}$

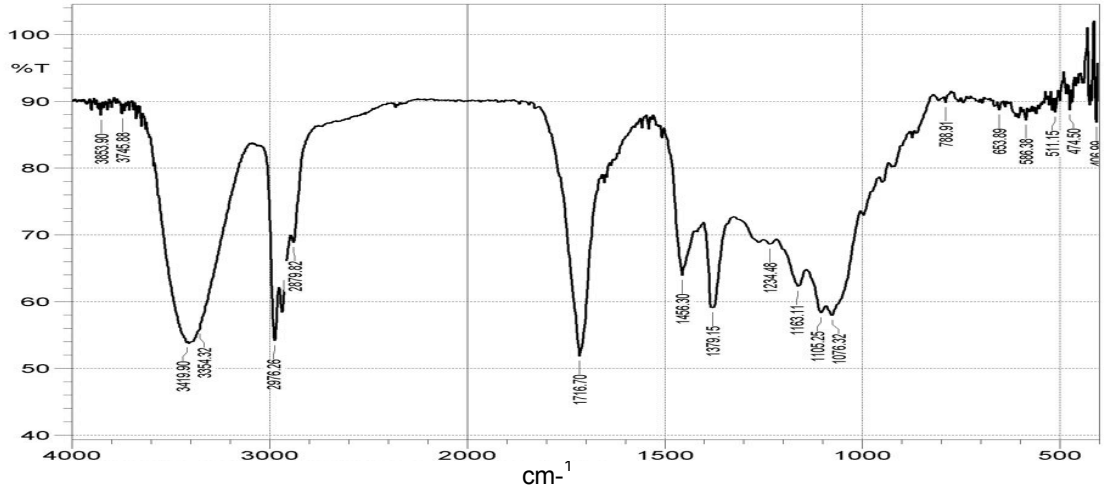

Figure 3. FTIR spectra analysis of purified extract: Chloroform (C_1) of *M. oleifera* leaves

Table 3. Detected functional groups in the purified extract: Chloroform (C_1) of *M. oleifera* leaves

Band	Frequency	Functional group
1	3853	Unknown
2	3745	Unknown
3	3419	O-$H_{stretch.}$ Phenol, Alcohol/ Amine
4	3354	Phenol, Alcohol/ Amine
3	2976	C-$H_{stretch}$
4	2879	C-$H_{stretch}$
7	1716	C=$O_{stretch}$/esters
9	1456	C-C $_{stretch}$
10	1379	O-$H_{bending}$ of phenol / (Alkane) (Aliphatic nitro compound NO_2 symmetric stretching)
11	1234	C-$O_{stretch}$
12	1230	C-$O_{stretch}$/ Amine/ Phenol
13	1163	C-$N_{stretch}$ (Aliphatic amines)
14	1105	C-$O_{stretch}$of O-H (of Alcohol)
15	1076	C-$O_{stretch}$of O-H (of Alcohol)/ Carboxylic acids
15	788	O-$H_{bending}$

pathogens. This is similar to the early work carried out by Pal et al. Jackson et al. and Oluduro [14,15,16] who observed that *M. oleifera* leaf extract possessed antibacterial property. Sofowora [17] had attributed the antimicrobial potential of medicinal herbs to bioactive compounds present in them. Moreover, comparing the potency of purified extract of *M. oleifera*with commercial antibiotics, with *M. oleifera*there was a breath-taking disparity in there zones of inhibition. Apart from chloroform 1(C1), none of the commercial antibiotics matched the purified (column chromatographic) fractions of the extract in term size of zone of inhibition.

FTIR is one of the most widely used methods to identify the functional groups of the chemical constituents and elucidate the compounds [18]. The structural elucidation of the purified extracts of the *M. oleifera* showed that phenols, alcohol, amine, ketones, carboxylic acid functional groups were detected in them. These functional groups *are* known to possess antibacterial properties according to Fiazi et al. [19]. El-Refaie et al. [20] also described hydroxyl, carboxyl and amino groups as the functional groups with high antimicrobial activity.

Phenols have been reported to be secondary metabolites secreted by the plant in response to attack from pathogenic insects, other forms of wounds and UV radiation [21], hence they are used by medicinal plants for self-defense. Some authors affirmed that phenolic compounds from natural resources do not only display antimicrobial activity [22] but also inhibit nematodes and phytophagous insects [23].

The common occurrence of amine groups in the bands could be linked to the high alkaloids content which had early been reported by Oluduro [16] to be present in *Moringa* leaf in high quantity along with phenols, flavonoid, tannins,saponin and glycosides. Amine groups can also be linked with that of proteins which represent 0.4% and 16.5% in the leaves, respectively, according to Oluduro [16]. Some authors also regard alkaloids as special case of amines. Ogunlade [24] affirmed that amine is a major functional group in alkaloids with high antimicrobial properties. Alkaloids have long-established numerous pharmacological activities among which are antibacterial, antimalarial and analgesic properties in chelerythrine, quinine and morphine respectively [25,26].

Carboxylic acid has been used in the production of pharmaceuticals and pesticides [27]. Lorand et al. [28] equally reported antibacterial activity of ketones. From the foregoing, the results obtained in this research work justify the use of *M. oleifera* in folk medicine for the treatment of various diseases in human. They can also be a novel source for the development of new drugs in the pharmaceutical industry. However, more research analyses such as Mass spectroscopy, Ultraviolet-visible spectroscopy and Nuclear Magnetic Resonance (NMR) spectroscopy need to be carried out to identify all of the chemical constituents and elucidate the compounds.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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