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Phytochemical Screening, GCMS Analysis and Antibacterial Activity of *Moringa oleifera* Ethanolic and aqueous Leaf Extracts against some Clinical Isolates

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Abstract

In response to the escalating concerns surrounding antibiotic resistance and associated side effects, interest in plant extracts and bioactive compounds derived from medicinal herbs has been resurgent. This study investigates the Phytochemical Screening, Gas Chromatography-Mass Spectrometry (GCMS) Analysis, and Antibacterial Activity of Moringa oleifera Leaf Extracts against clinical isolates. Utilizing aqueous and ethanolic extractions, the study determined the yield percentages as 16.25% and 7.14%, respectively. Phytochemical analysis revealed the presence of alkaloids, tannins, flavonoids, glycosides, steroids, terpenoids, and saponins in both extracts, with the absence of phenol. The antibacterial activity was assessed using the agar well diffusion method, showing inhibitory effects against the tested isolates. The ethanolic extract exhibited superior antibacterial activity, with a maximum zone of inhibition (17mm) against Pseudomonas aeruginosa at 800mg/ml. The aqueous extract demonstrated a maximum zone of inhibition (12mm) against the same bacterium at the same concentration. Comparative analysis with standard antibiotics revealed competitive inhibitory effects, especially against Staphylococcus aureus and Pseudomonas aeruginosa. Furthermore, GCMS analysis identified sixteen phytochemical compounds in the ethanolic extract and eleven in the aqueous extract. The findings underscore the significant antibacterial potential of Moringa oleifera extracts, particularly against Staphylococcus aureus and Pseudomonas aeruginosa. The GC-MS results provide crucial insights into the bioactive chemical profile, supporting the potential therapeutic applications of Moringa oleifera in combating various infections. This study contributes valuable knowledge to exploring alternative treatments amid growing antibiotic resistance concerns.

***Key**: phytochemical Screening, Moringa oleifera, Antibacterial Activity, Ethanolic Extract, and Antibiotic Resistance.*

INTRODUCTION

Humans have turned to traditional medicine for millennia, and in the last decade, interest in this field has skyrocketed worldwide (Amabye & Tadesse, 2016). This is especially true with herbal medicines based on medicinal plants. According to the World Health Organization (2002), herbal medicine is used by over 80% of the world's population (Dogara *et al.*, 2022). More and more people are looking to medicinal plants as a safe and effective alternative to conventional antibiotics to combat the rising tide of antibiotic-resistant bacteria (Bagheri*et al.*, 2020). Many problems with antibiotic resistance have prompted modern medicine to focus on plants, having a history of success in traditional medicine. Several popular pharmaceuticals were originally derived from plants because of their traditional therapeutic usage or the extraction of unique active compounds (Abdel-Aty *et al.*, 2019). Secondary metabolites are naturally occurring substances or chemicals produced by plants. They play crucial roles in plant defense, pollination, and adaptability. Many areas of a man's daily existence use the secondary metabolites produced by the plant's parts. These chemical products, treated or unprocessed, are recognized to have numerous biological applications (Abdulrahman *et al.*, 2019). Traditional medical systems in developing and developed countries have long used plants and plant parts to treat various illnesses and conditions (Abdulrahman *et al.*, 2019).

When bacteria, viruses, fungi, and parasites, among other microbes, can adapt and flourish in the presence of drugs that earlier negatively affected them, this phenomenon is known as antimicrobial resistance (AMR).AMR is regarded as a danger to the public health systems worldwide, not just in underdeveloped nations world (Enerijiofi *et al.*, 2021). The fact that antibiotics can no longer be used to treat infectious infections shows an uncertain healthcare future (Olorundare, 2015). AMR infection results in severe illnesses, extended hospital stays, rising healthcare costs, and increased failures in therapy and the price of second-line medications. Taking Europe as an example, according to estimates, antimicrobial resistance has a connection to more than 9 trillion euros annually. In addition, the Centers for Disease Control and Prevention Antimicrobial resistance costs the Centers for Disease Control and Prevention (CDC) $20 billion annually (Olorundare, 2015). A human immune system's ability to combat infectious diseases is compromised by antibiotic resistance, which increases the risk of complications for vulnerable individuals undergoing chemotherapy, dialysis, surgery, and joint replacement (Enerijiofi *et al.*, 2021).
The emergence of germs that can survive in the presence of multiple antimicrobials is a major cause for concern. More and more research suggests that medicinal plants could be viable for treating milder forms of infectious diseases. In addition, some research establishes scientific grounds for the widespread use of plants against infectious diseases, and they could potentially serve as a source of novel, affordable antibiotics to which pathogenic strains are not resistant (Van *et al.*, 2022).
Moringa oleifera is a tropical tree that grows naturally in the foothills of the Himalayas. It has since spread to other tropical zones, including Africa, Asia, and South America (Enerijiofi *et al.*, 2021). The trees belong to the family Moringaceae, genus Moringa, order Brassicales, and have gained the nickname "miracle tree" for their many uses (van den Berg & Kuipers, 2022).
Moringa oleifera is a useful crop because of its medicinal and nutritional properties. It also has a minimal demand for soil nutrients and can even be grown on a stack of granite stones (Anzano *et al.*, 2022). *Moringa oleifera* has been called a "wonder plant" for its many therapeutic applications (Olorundare, 2015). *Moringa oleifera* is a widely used medicinal herb originating in Africa, Asia, and the Americas. Eastern Nigeria, Africa, uses this vegetable frequently. In addition to "Mother's Best Frie," it is also known as the Horseradish tree, the Drumstick tree, the Ben oil tree, and the Miracle tree (Enerijiofi *et al.*, 2021). It has a long history of traditional use as an herbal treatment for a wide variety of both infectious and noninfectious medical disorders, and it has recently been proposed as a possible source of a novel antibacterial agent (Olorundare, 2015).
As the number of infections caused by pathogenic bacteria has increased, these bacteria's resistance to antimicrobials has improved (Tenover, 2006). Drug-resistant strains of pathogenic microorganisms are becoming increasingly common due to the widespread use of many medications to combat them in the human body (Abdulrahman *et al.*, 2019; Olorundare, 2015).
Different parts like seeds, roots, stems, bark, leaves, flower, and plant fruits have their phytochemical compositions and potential medicinal properties. Moringa has various species across the globes which are known for their variety of usages few examples of Moringa species are *Moringa longituba, Moringa drouhardii, Moringa ovalifolia,* etc. (Leone *et al.*, 2015). *Moringa Oleifera* is one of the magical plants considered in India due to its high medicinal properties. However, there is still a lot to unleash the potential of *Moringa Oleifera* by understanding their photo components and variation in extraction due to solvents, understanding their potential properties, and establishing their applications in various fields. This study aims to determine the Phytochemical Screening, GCMS Analysis, and Antibacterial Activity of *Moringa oleifera* Leaf Extract against some clinical isolates

MATERIALS AND METHODS

Sample Site

The plant samples *Moringa oleifera* (leaves) were collected from a home garden at Bakori local government, Katsina state.

Sample collection

The sample was collected by hand picking in a polythene bag and brought to the Microbiology laboratory at Federal University Dutsinma for analysis.

Taxonomic Identification of the Leaves Sample

Herbarium specimens of *Moringa oleifera* were collected for taxonomic identification and confirmation, the specimen was deposited at the FUDMA Herbarium Department of Plant Science and Biotechnology, and the remaining sample was transported to the Microbiology Department for further analysis.

Sample Extraction

Extraction using Ethanol Solvent

Tap water cleaned the plant's lower leaves of dirt, stains, and latex. After washing, leaves are put in their proper places and covered with tissue paper to dry at room temperature. A motor and pestle were used to pulverize dried leaf samples (Santhi & Sengottuvel, 2016). The leaf samples were ground into a powder and then weighed (500 g). The plant samples were macerated to remove the ethanolic components. To filter the extracted material, Whatman No. 2 filter paper was utilized (Santhi & Sengottuvel, 2016). Ethanol crude extract was obtained using evaporation

Extraction using Aqueous

Tap water cleaned the plant's lower leaves of dirt, stains, and latex. After washing, leaves were put in their proper places and covered with tissue paper to dry at room temperature. A motor and pestle were used to pulverize dried leaf samples (Santhi & Sengottuvel, 2016). The leaves samples were ground into a powder and then weighed (500g) (Abdulrahman *et al.*, 2019). The plant samples were macerated to remove the aqueous components. To filter the extracted material, Whatman No. 2 filter paper was utilized (Santhi & Sengottuvel, 2016). Aqueous crude extract was obtained using evaporation equipment.

Phytochemical Screening

The extract underwent preliminary phytochemical analysis using the procedures provided by Brain and Turner (Santhi & Sengottuvel, 2016).

Alkaloids Detection

The filtrate was subjected to the Mayer reagent test. The presence of alkaloids is indicated by the formation of a yellow-cream precipitate (Santhi & Sengottuvel, 2016).

Flavonoids Detection

A few drops of lead acetate solution were used to test the extracts. Flavonoids are a yellow precipitate forming (Santhi & Sengottuvel, 2016).

Steroids Detection

Each extract weighing 5 mg was mixed with 2 ml of H_2SO_4 and 2 ml of acetic anhydride. When steroids are present, the sample's color will shift from violet to blue or green.

Terpenoids Detection

The leaf extract (five milligrams) was combined with two milliliters of chloroform, and three milliliters of concentrated H_2SO_4 were added in a layer. The presence of terpenoids was indicated by the emergence of a reddish-brown color on the inner face.

Phenols Detection

A few drops of ferric chloride solution were used to evaluate extracts of 10 mg. The presence of phenol can be seen by the formation of a bluish-black color

Saponins Detection

100 mg extract was added to 2 mL 25% H_2SO_4, then autoclave 120 minutes 100 ° C. Extracted with ether and dried. 1 mL aquadest is added, then vortex for 5 minutes (Santhi & Sengottuvel, 2016). 50 µl Anisaldehyde added, shaken out, then let stand for 10 minutes. Added 2 ml of 50% H_2SO_4, then heated in a water bath for 10 minutes at 60ºC (Santhi & Sengottuvel, 2016). Aquadest added up to 10 ml. Diluted 10 times, read absorption at λ 435 nm. The results obtained are plotted against the standard Quillaja bark curve. The total saponin is expressed as mg of Quillaja bark equivalent/g extract.

Glycosides Detection

Extract solution dissolved in pyridine then added sodium nitroprusside solution and made alkaline. The brick red color indicated the presence of glycosides.

Tannin Detection

According to the Gelatin test, 100 mg of crude extract was dissolved in 5mL distilled water + 1% gelatin solution + 10% NaCl. A white precipitate indicates the presence of tannin.

Test Isolates

Clinical isolates were collected from the laboratory department of Microbiology Federal Universit Dutsinma.

Standardization of Inoculums

A 1% v/v solution of sulfuric acid, specifically barium sulfate, was made by combining 1 ml of concentrated H_2SO_4 with 99 ml of distilled water. A solution of barium chloride with a concentration of 1% weight per volume was made by dissolving 0.5g of dehydrated barium chloride. The solution was mixed with 99.4 mL of a sulphuric acid solution to produce a suspension of barium sulfate with a concentration of 1.0% w/v (Hassen *et al.*, 2022). A volume of 0.1ml from each overnight broth culture of *Staphylococcus aureus* and *Escherichia coli* was distributed into individual test tubes containing a sterile solution. This serves as the standard inoculate (Hassen *et al.*, 2022).

Preparation of Plant Concentration

The ethanolic and aqueous leaf extracts were diluted to 0.2 g/mL, 0.4g/mL, 0.6g/ml, and 0.8 g/mL in DMSO (Abdulrahman *et al.*, 2019; Olorundare, 2015).

Streaking Method of Inoculation

A sterilized loop topick an isolated colony from the agar plate culture and spread it over the plate containing selective media for respective isolates. (Olorundare, 2015).

Confirmation of Isolates

Gram staining

Using a sterile wire loop, a loopful of colony of the bacteria was collected, fixed on a sterile glass slide, smeared, and then allowed to air dry. It was then gently flooded with crystal violet, tilted the slide, allowed to stand for 6o seconds, rinsed with water, and blot dry (Hassen *et al.*, 2022). Gently flood the smear with grams of iodine and allow for 6o seconds again, then rinse with water. Decolorize using acetone, tilting the slide slightly, and immediately flush with water. Finally, it was flooded with safranin, allowed to stand for 45 seconds, then rinsed. Then, it was blot dry and viewed under the light microscope under oil immersion (Hassen *et al.*, 2022).

Biochemical Test

Catalase test 2mL of hydrogen peroxide solution was poured into a test tube, and bacteria colonies were immersed in the hydrogen peroxide solution. Observe for immediate bubbling (Hassen *et al.*, 2022).

Indole test: Sterilized test tubes containing 4 mL of tryptophan broth. Inoculate the tube aseptically by taking the growth from 18 to 24 hours of culture. The tube incubates at 37°C for 24-28 hours. 0.5 mL of Kovac's reagent was added to the broth culture. The presence or absence of a ring was observed.

Methyl red test: pure culture of the bacterial were inoculated into the MRVP (Methyl Red and Voges-Proskaue) broth and incubated at 35 -37 °C respectively, for a minimum of 48 hours in ambient air, 5 to 6 drops of methyl red reagent per 5mL of broth were added and color change was observed in the broth medium

Antibacterial activity

Ager well Diffusion Method 200 L of microbial loads of 1.106 (CFU mL^{-1}) were plated onto Mueller Hinton agar. A sterile cork borer was used to create a well of 6 mm depth and divide the plates into five equal quadrants. Each well had 100 L of a 200, 400, 600, and 800 mg/mL solution of the crude extract (ethanolic and aqueous solutions were used) (Abdulrahman *et al.*, 2019).

Gas Chromatography-Mass Spectrometry (GCMS) Analysis

The mass spectrometer is linked with an Agilent GC/MS. HP-5MS 30 m x 0.25 mm, 0.25 mm film thickness was used to separate the compound at a programmed temperature of 59 °C for 9 minutes, followed by a programmed temperature of 230 °C for 1 minute at 3 °C per minute with a one-minute hold. The injector temperature was 245 degrees Celsius, and the carrier helium gas flow rate was 1 milliliter per minute. The ion source and analyzer temperature for the MS will be 260 °C at 70 eV. The compounds were identified after comparing the spectral configurations obtained with the available mass spectral database (NIST and WILEY libraries).

RESULTS

It was discovered that the aqueous extract produced a greater yield than the ethanolic extract. *Moringa oleifera* leaves extracted with ethanol produced the highest yield (16.25 %), followed by leaves extracted with ethanol (7.14%) (Tabla 1). The secondary metabolites responsible for the leaves' various biological activities were found in the plant's ethanolic and aqueous extracts, according to a phytochemical analysis. All the extract was found to contain alkaloids, tannins, flavonoids, glycosides, steroids, terpenoids, and saponins, except phenol was absent in both extracts (Table 2)

All of the extracts showed some activity against the tested bacterial species. Although the activity improved with increasing concentrations of both the ethanolic and aqueous extracts

Table 3 shows the result of the antibacterial activities of *Moringa oleifera* ethanolic extract against test isolates at various concentrations with different diameters of zones of inhibition. The result shows that the crud extract of different extracts exhibited antibacterial activity at different concentrations, with *Pseudomonas aeruginosa* having the maximum activity with the zone of inhibition of 17mm at 800mg/ml well *Klebsiella pneumoniae* possessing the least zone of inhibition of 8mm at the concentration of 200mg/ml, and the control Levofloxacin zone range between 20mm to 27mm in *Klebsiella pneumoniae, Staphylococcus aureus* and *Pseudomonas aeruginosa, Proteus mirabilis, Escherichia coli Respectively*.

In Table 4, *Pseudomonas aeruginosa* has the highest activity with a zone of inhibition of 12mm at 800mg/ml well, *Escherichia coli* and *Klebsiella pneumoniae* possess the least zones of inhibition of 6mm at the concentration of 200mg/ml, and Levofloxacin that was used as the control show zone of 24mm in *Klebsiella pneumoniae* and *Escherichia coli*, 23mm *in Pseudomonas aeruginosa* and 22mm in *Staphylococcus aureus* and, *Proteus mirabilis, Respectively*.

Table 5 shows the result of the Phytocomponent identified from the ethanolic crude extract of Moringa oleifera by GCMS Analysis. The retention time (RT), molecular formula, molecular weight (MW), area %, and compound name were presented.

The GC-MS analysis revealed a total number of sixteen (16) compounds as follows: Heptane, 2-hexanone, Phthalic acid, Decane, UnDecane, n-hexadecanoic acid, Undec-10-ynoic acid, Octane, 9-Octadecynoic acid, Octadecanoic acid, 9-Octadecynoic acid, Z,Z,Z-1,4,6,9-Nonadecatetraene, 9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-, Bicyclo (4.1.0) heptane, Tetra contane and Squalene. The chromatogram is also shown in Figure 1

Table 6 shows the result of the Phytocomponent identified from the ethanolic crude extract of *Moringa oleifera* by GCMS Analysis. The retention time (RT), molecular formula, molecular weight (MW), area %, and compound name were presented. The GC-MS analysis revealed total number of Eleven (11) compounds as follows: Carbamic acid, 4-Benzyloxy-2-methoxymethoxy-phenol, Oxirane, Hexadecanoic acid, Dodecyl-, Hexadecanoic acid, Heptadecanone, Nonanoic acid, Cis-11-Hexadecenal, 13-octadecadienol and Z-10-Tetradecen-1 ol acetate. The chromatogram is also shown in Figure 1.

Table 1: Physical properties of the crude extracts of *Moringa oleifera*

Physical Parameters	Aqueous extract	Ethanolic extract
Weight of plant leaves (g)	80g	80g
Yield of the extract recovered (g)	13g	5.71g
Percentage Yield (%)	16.25%	7.14%
Color	Dark green	Dark green
Texture	Gummy	Gummy

Table 2: Phytochemical Properties of the crude extracts of *Moringa oleifera*

Phytochemical Test	Ethanol	Aqueous
Alkaloids	+	+
Flavonoids	+	+
Steroids	+	+
Terpenoids	+	+
Phenols	-	-
Glycosides	+	+
Tannins	+	+
Saponins	+	+

Key

Present = +
Absent =

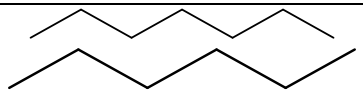
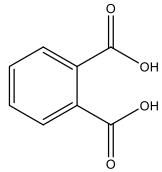
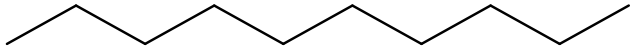
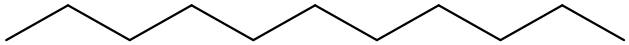
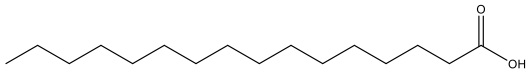
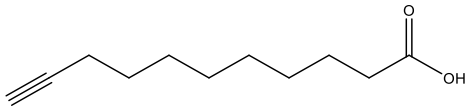
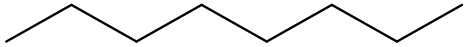
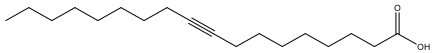
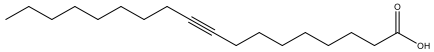
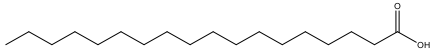
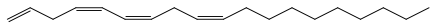
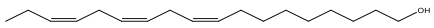
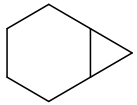
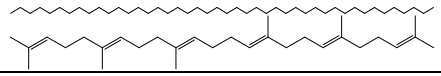
Table 3: Antibacterial activities of Ethanol Extract of *Moringa oleifera* leaves

Test organisms	Zones of inhibitions (mm)				
	Concentrations (mg/mL)				
	200	400	600	800	Control
Escherichia coli	9	9	10	11	27
Proteus mirabilis	10	10	11	12	27
Pseudomonas aeruginosa	9	12	12	17	27
Staphylococcus aureus	9	12	13	16	20
Klebsiela pneumonia	8	9	9	10	20

Table 4: Antibacterial activities of Aqueous Extract of *Moringa oleifera* leaves

Test organisms	Zones of inhibitions (mm)				
	Concentrations (mg/mL)				
	200	400	600	800	Control (µm)
Escherichia coli	6	8	9	10	24
Proteus mirabilis	7	8	8	10	22
Pseudomonas aeruginosa	6	11	11	12	23
Staphylococcus aureus	9	8	9	11	22
Klebsiela pneumonia	9	9	9	9	24

Table 5: Phytochemical Components identified from Ethanolic Leaf extract of *Moringa oleifera* by GC-MS Analysis

S/N	Retention Time	Molecular Structure	Molecular Formula	Molecular weight	Molecular Name	Area %
1	7.429		C_9H_{20}	128	Heptane	0.12
2	10.178		C_8H_{18}	114	Hexane	0.16
3	11.778		$C_{20}H_{26}O_4$	330	Phthalic acid	1.06
4	12.661		$C_{12}H_{26}$	170	Decane	0.47
5	15.390		$C_{13}H_{28}$	184	Undecane	0.54
6	17.033		$C_{16}H_{32}O_2$	256	n-Hexadecanoic acid	21.85
7	18.849		$C_{11}H_{18}O_2$	182	Undec-10-ynoic acid	0.15
8	19.182		$C_{11}H_{24}$	156	Octane	0.61
9	19.842		$C_{18}H_{32}O_2$	280	9-Octadecynoic acid	0.84
10	20.070		$C_{18}H_{32}O_2$	280	9-Octadecynoic acid	27.46
11	20.361		$C_{18}H_{36}O_2$	284	Octadecanoic acid	25.64
12	21.902		$C_{19}H_{32}$	260	Z,Z,Z-1,4,6,9-Nonadecatetraene	1.50
13	22.164		$C_{18}H_{32}O$	264	9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	13.45
14	23.187		$C_{11}H_{18}O$	166	Bicyclo[4.1.0]heptane	0.66
15	26.219		$C_{44}H_{90}$	618	Tetratetracontane	4.71
16	26.514		$C_{30}H_{50}$	410	Squalene	0.80

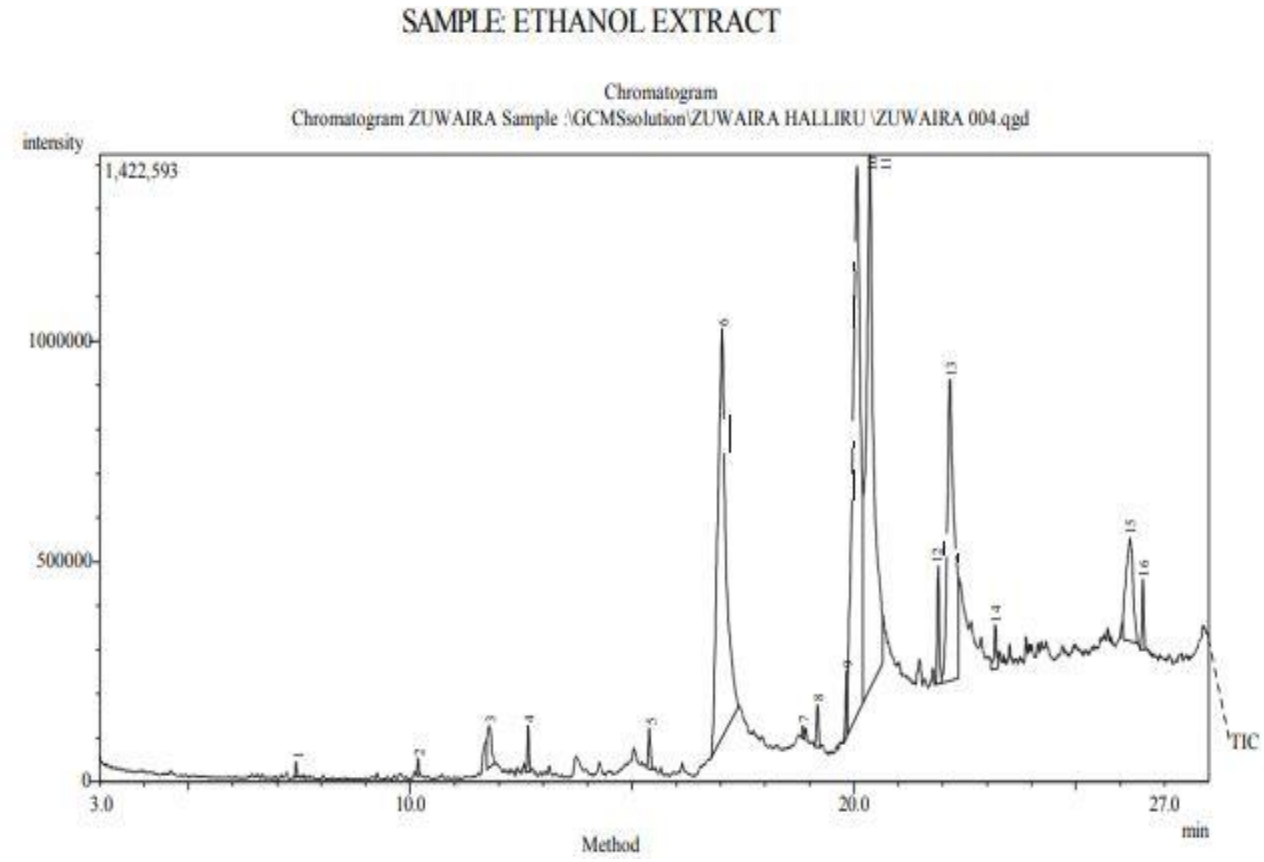


Figure 1 Chromatogram of the *Moringa oleifera Ethanolic* Leaf extract

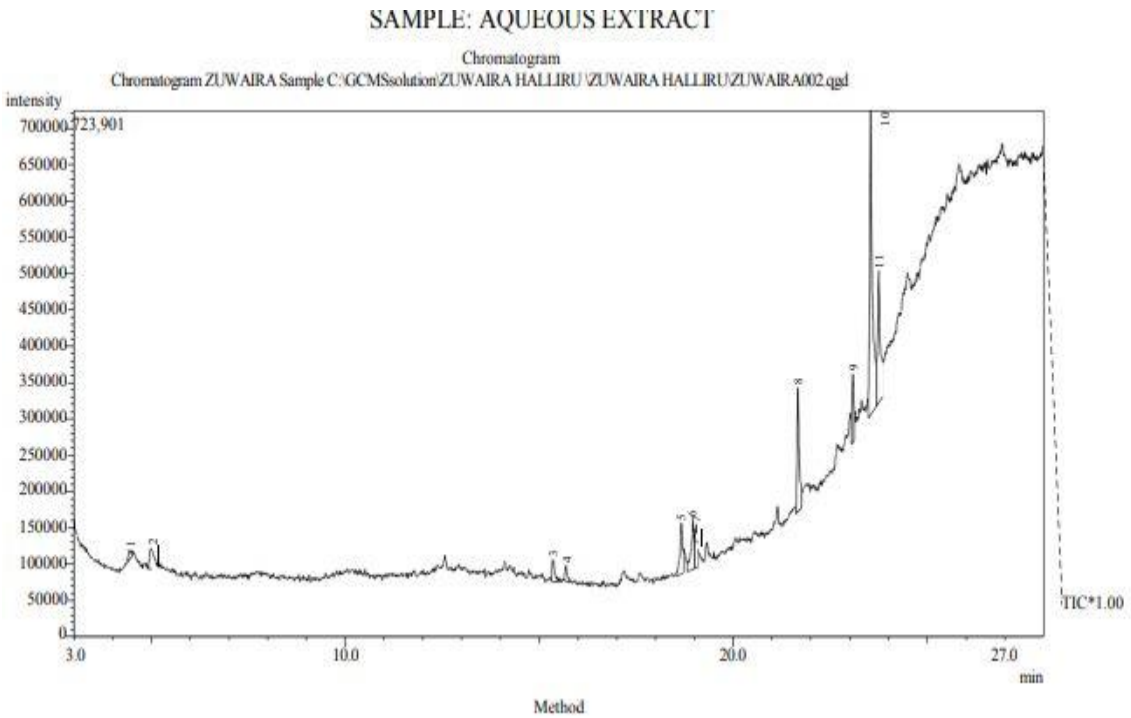
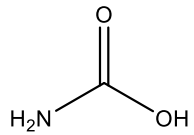
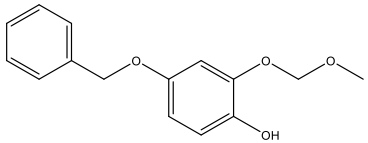
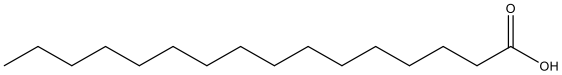
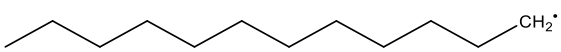
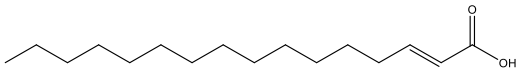
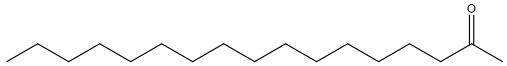
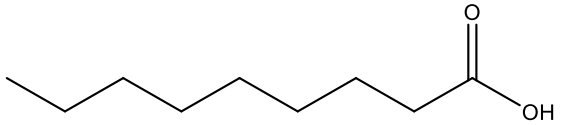
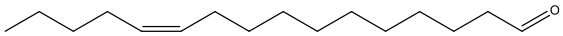
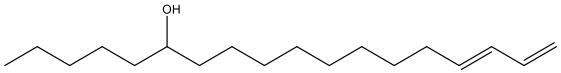
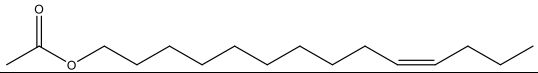


Figure 2 Chromatogram of the *Moringa oleifera Aqueous* Leaf extract

Table 6: **Phytochemical Components identified from Aqueous Leaf Extract of *Moringa oleifera* by GC-MS Analysis**

S/N	Retention Time	Molecular structure	Compound formula	Molecular weight	Molecular name	Area %
1	4.434		$C_7H_7NO_2$	137	Carbamic acid	1.24
2	4.992		$C_{15}H_{16}O_4$	260	4-Benzyloxy-2-methoxy ethoxy-phenol	1.88
3	15.350		$C_6H_{12}O$	100	Oxirane	2.81
4	15.690		$C_{18}H_{36}O_2$	284	Hexadecanoic acid	1.69
5	18.662		$C_{14}H_{28}O$	212	Dodecyl-	6.59
6	18.959		$C_{17}H_{32}O_2$	268	Hexadecanoic acid	6.84
7	19.040		$C_{17}H_{34}O$	254	Heptadecanone	4.85
8	21.666		$C_9H_{18}O_2$	158	Nonanoic acid	12.33
9	23.082		$C_{16}H_{30}O$	238	Cis-11-Hexadecenal	6.05
10	23.540		$C_{19}H_{36}O$	280	13-octadecadienol	38.21
11	23.752		$C_{16}H_{30}O_2$	254	Z-10-Tetradecen-1-ol acetate	17.52

DISCUSSION

The yield from the aqueos extract was found to be higher than that of the ethanolic extract. The maximum yield (16.25%) was obtained from aqueous-extracted *Moringa oleifera* leaves, followed by ethanolic-extracted leaves (7.14.%). This research also correlates with the findings of Javadi et al., (2014), who found a much lower yield percentage in the ethanolic extracts than in the aqueous extraction method. The finding also disagrees with Enerijiof *et al.*, (2021) reported that ethanol extract gave more yield than the aqueous extract of the *Moringa oleifera* plant. The finding of Abdulrahman (2022) agreed with the current research, which reported a higher yield in aqueous extract of leaves then the ethanolic. The success of the solvent in evaporating the compounds of interest from the samples may account for the high yields observed for Aqueos extracts. The extraction method, extraction solvent, chemicals present, and polarity of metabolites all play major roles in the variation in extract yield from medicinal plant parts, as reported by (Ramli *et al.*, 2017).

The phytochemical analyses in ethanolic and aqueous plant extracts were responsible for the leaves' varied biological functions. With the exception of phenol, all the extract reveals alkaloids, tannins, flavonoids, glycosides, steroids, terpenoids, and saponins in both extracts. As such, phytochemicals such as flavonoids, saponins, and tannins were earlier reported as antibacterial agents (Adetitun *et al.* 2013; Unuigbe *et al.* 2014; Oyama *et al.* 2019). In particular, the flavonoids were reportedly responsible for antimicrobial activity associated with some ethno-medicinal plants (Singh and Bhat, 2003). The presence of different phytochemical components in the plant extract was due to the extraction solvent, which was reported by (Ramli *et al.*, 2017).

This study reported that both leaf extracts exhibit the highest inhibition against antibacterial activity on all the isolates. However, ethanol extracts exhibit better antibacterial activity than aqueous extracts. This implied that the antibacterial components were more inherent in the alcohol concentrations than aqueous (Ajayi and Fadeyi 2015; Singa *et al.* 2021). Also, the extracts' antibacterial efficacy, particularly ethanol, was observed to increase as the concentrations of the plant extract increased. Also, Bukar *et al.* (2010) reported that the ethanol extracts of Moringa oleifera leaf had the broadest activity on the test bacterial isolates. The high activity observed in the ethanolic extracts may be due to the solvent's capacity to extract more chemicals from the samples. This result agreed with the Earlier studies by Enerijiof and Isola (2019) and Singa *et al.* (2021) reported that ethanol extracts had better antibacterial activity than aqueous plant extracts. The enhanced efficacy of many extracts is thought to be due to the synergistic effects of a number of bioactive chemicals and their metabolites found in plant extracts reported by Dogara, 2023. The antimicrobial activities of *Moringa oleifera* have been corroborated by (Broin *et al.*, 2002 and Pliego *et al.*, 2007), which agreed with the findings of this research work. The activity exhibited by the extracts may be related to the presence of saponins tannins, in addition to flavonoids that are reported to be responsible for the antimicrobial properties of some ethnomedicinal plants, as reported by Singh and Bhat (2003).

Gas chromatography-mass spectrometry (GC-MS) has proven to be a valuable tool for the dependable identification of bioactive components in plant studies (Balamurugan *et al.*, 2015). However, some of the identified compounds were similar to the ones earlier documented by Azwanida (2015): butanoic acid, 1,5-heptane, 3,3, dimethyl-(E) and 2-propanoic acid, 2 propanyl ester in leaf and Squalene, 1-hexanol, 2-ethyl2-propyl, hexanedioic acid, heptane, heptanoic acid, from the bark of Moringa concanensis. These organic compounds identified could be accountable for the antimicrobial, anti-cancer, analgesic, hepatoprotective, and anti-inflammatory properties which support their wide use as health aid by tradomedical practitioners (Farooq *et al.*, 2012; Vongsak *et al.*, 2013; Husni *et al.*, 2021). In this study, the higher number of phytochemical components is in ethanolic extract (Table 5) of *Moringa oleifera* in comparison to aqueous (Table 6) with eleven (11) could be due to difference in their polarity, which could have led to the difference in the extraction of phytochemical. This recent study is similar to the work of Ijeoma and Chikwendu (2023), who identified seventy-eight bioactive compounds from the aqueous extract of *Moringa oleifera* by GC-MS analysis. Similarly, N-hexadecanoic acid, Hexadecanoic acid, 9, 12 - Octadecadienoic acid, and Squalene were identified in the ethanol leaf extract of Aloe Vera (Arunkumar and Muthuselvam, 2009) and Vitex negundo (Praveen *et al.*, 2010). Squalene is used in cosmetics as a natural moisturizer. Devi *et al.* (2009) reported that Euphorbia longan leaves mainly contained n hexadecanoic acid and 9, 12-Octadecadienoic acid. These reports are in accordance with the results of this study.

CONCLUSION

The study revealed that *Moringa oleifera* leaves exhibit antibacterial activity and that ethanol extract was a better extraction solvent and showed better inhibitory activity against the bacterial isolates. The GC-MS analysis results revealed the bioactive chemical profile of the extracts, which offer fundamental knowledge that tends to support the possible use of *M. oleifera* as a therapeutic agent in treating a variety of disorders and suggest that it may be a potential natural remedy for infections and could be useful in identifying the best extraction methods for specific compounds.

Recommendation

Further studies are recommended to isolate, identify and purify each of the bioactive constituents present in the leaf extracts of *Moringa oleifera*.

REFERENCE

Abdel-Aty, A. M., Hamed, M. B., Salama, W. H., Ali, M. M., Fahmy, A. S., & Mohamed, S. A. (2019). Ficus carica, Ficus sycomorus and Euphorbia tirucalli latex extracts: Phytochemical screening, antioxidant and cytotoxic properties. *Biocatalysis and Agricultural Biotechnology*, 3(2), 130-134.

Ajayi AO, Fadeyi TE (2015) Antimicrobial activities and phytochemical analysis of Moringa oleifera leaves on Staphylococcus aureus and Streptococcus species. *Am J Phytomed Clin Therap* 3(10):643- 653.

Arunkumar S and Muthuselvam M: 2009. Analysis of Phytochemical constituents and antimicrobial activities of Aloe vera L. against clinical pathogens. *World J. Agricultural Sci.*, 5(5): 572-576.

Abdulrahman, M. D. (2021). Standardization of Jatropha curcas. L. *Journal by Innovative Scientific Information & Services Network*, 18(2), 1585-1589.

Abdulrahman, M. D. (2022). Review of Ethnopharmacology, Morpho-Anatomy, Biological Evaluation and Chemical Composition of Syzygium polyanthum (Wight) Walp. *Plant Science Today*, 9(1), 167-177.

Abdulrahman, M. D., Bradosty, S. W., Hamad, S. W., Ibrahim, M. T., Lema, A. A., Sunusi, N., Wada, N. (2022). Traditional Methods for Treatment and Management of Measles in Northern Nigeria: Medicinal plants and their molecular docking. *Ethnobotany Research and Applications*, 23,1-18.

Abdulrahman, M. D., Hasan Nudin, N., Khandaker, M. M., Ali, A. M., & Mat, N. (2019). In vitro biological investigations on Syzygium polyanthum cultivars. *International Journal of Agriculture and Biology*, 22(6), 1399-1406.

Abdulrahman, M. D., Zakariya, A. M., Hama, H. A., Hamad, S. W., Al-Rawi, S. S., Bradosty, S. W., & Ibrahim, A. H. (2022). Ethno pharmacology, biological evaluation, and chemical composition of Ziziphus spina-christi (L.) Desf.: A Review. *Advances in pharmacological and pharmaceutical sciences*, 4(1), 140-134.

Amabye, T. G., & Tadesse, F. M. (2016). Phytochemical and antibacterial activity of Moringa oleifera available in the market of Mekelle. *Journal of Analytical & Pharmaceutical Research*, 2(1),1-4.

Adetitun DO, Araoye HK, Akinyanju JA, Anibijuwon II (2013). The antimicrobial effects of the leaf extracts of Moringa oleifera on selected clinical bacterial isolates. *Agrosearch* 13(1):95-113.

Anzano, A., de Falco, B., Ammar, M., Ricciardelli, A., Grauso, L., Sabbah, M., Lanzotti, V. (2022). Chemical analysis and antimicrobial activity of Moringa oleifera lam. Leaves and Seeds. *Molecules*, 27(24), 89-120.

Azwanida NN (2015) A review on the extraction methods use in medicinal plants, principle, strength and limitation. *Med Aromatic Plants* 4:19.

Balamurugan V, Balakrishnan V, Sundaresan A (2015) GC-MS analysis of leaf and Bark Extract of Moringa concanensis Nimmo, a siddha medicinal plant of South India. *Eur J Biotechnol Biosci* 3(12):57-61

Bukar A, Uba A, Oyeyi TI (2010) Antimicrobial profle of Moringa oleifera lam. Extracts against some food-borne microorganisms. *Bayero J Pure A ppl Sci* 3(1): 43-48

Bagheri, G., Martorell, M., Ramírez-Alarcón, K., Salehi, B., & Sharifi-Rad, J. (2020). Phytochemical screening of Moringa oleifera leaf extracts and their antimicrobial activities. *Cellular and Molecular Biology*, 66(1), 20-26.

Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of pharmaceutical analysis*, 6(2), 71-79.

Bhatia, A., & Zahoor, S. (2007). *Staphylococcus aureus* enteroto xins: a review. *J Clin Diagn Res,1(3), 188-197.*

Broin M., Santaella C., Cuine S., Kokou K., Peltier G., and Joet T. (2002) Flocculent activity of a recombinant protein from Moringa oleifera Lam seeds. *Applied Microbiology and Biotechnology* 60(1):114-119.

Dogara, A. M. (2023). A systematic review on the biological evaluation of Calotropis procera (Aiton) Dry and Future *Journal of Pharmaceutical Sciences*, 9(1), 16-23.

Dogara, A. M., Hamad, S. W., Hama, H. A., Bradosty, S. W., Kayfi, S., Al-Rawi, S. S., & Lema, A. A. (2022). Biological evaluation of Garcinia kola Heckel. Advances in pharmacological and pharmaceutical sciences, 5(1), 130-134.

Devi P, Nagarajan M, Christina AJM, Meera R and Merlin NJ: (2009) GC-MS analysis of Euphorbia longan leaves. *Int. J. of Pharmaceutical Res and Development*; 8: 1-4.

Enerijiofi, K. E., Akapo, F. H., & Erhabor, J. O. (2021). G.C.-M.S. analysis and antibacterial activities of Moringa oleifera leaf extracts on selected clinical bacterial isolates. *Bulletin of the national research centre*, 45,1-10.

Ezema, B., Omeje, K., Ozioko, J., Urama, D., Omeje, H., Nnawelezi, A., & Ejim, A. (2021). Cholinesterase inhibition, biological activity and characterization of Chrysophyllum albidum leaf and stem-bark chloroform extract using GC-MS: An in vitro study. *Trop. J. Nat. Prod. Res*, 5(1), 130-134.

Enerijiof KE, Isola OB (2019) Preliminary Phytochemical screening and in-vitro antibacterial activities of aqueous and ethanol extracts of Ageratum conyzoides L. Leaf, Stem, Flower and Root on some Bacterial isolates associated with Diarrhoea. *Niger J Pure Appl Sci* 32(2): 3480-3489. Doi:

Foster, T. J. (2002). Staphylococcus aureus. Molecular Medical Microbiology, 839-888.

Farooq F, Rai M, Tiwari A, Farooq S, Khan A (2012) Medicinal properties of Moringa oleifera: an overview of promising healer. *J Med Plants Res* 6(27):4368-4374.

Giske, C. (2016). Escherichia coli: an old friend with new tidings. *FEMS microbiology reviews*, 40(4), 437-463.

Gomes, T. A., Elias, W. P., Scaletsky, I. C., Guth, B. E., Rodrigues, J. F., Piazza, R. M., Martinez, M. B. (2016). Diarrheagenic Escherichia coli *Brazilian journal of microbiology*, 47, 3-30.

Hassen, N. I., Badaluddin, N. A., Mustapha, Z., & Zawawi, D. D. (2022). Identification and prevention of microbial contaminants in musa paradisiaca tissue culture. *Malaysian Applied Biology*,51(5),129-143.

Husni E, Badriyya E, Putri L, Aldi Y (2021) The effect of ethanol extracts of Moringa leaf (Moringa oleifera Lam) against the activity and capacity of phagocytosis of macrofag cells and the percentage of leukosit cells of white mice *Pharmacognosis J* 13(3): 706- 712.

Ifeoma I. A, Chikwendu C. I, Ekperechi S. A, Nwogwugwu N, Ursulla and Ihenetu F. C (2023) Aqueous Extract of Moringa oleifera Leaves using GC-MS Analysis. *Journal of Advances in Microbiology* Volume 23, Issue 6, Page 8 -18, 2023.

Jang, J., Hur, H. G., Sadowsky, M. J., Byappanahalli, M., Yan, T., & Ishii, S. (2017). Environmental Escherichia coli: ecology and public health implications-a review. *Journal of applied microbiology*,123(3),570-581.

Javadi, N., Abas, F., Hamid, A. A., Simoh, S., Shaari, K., Ismail, I. S., . . . Khatib, A. (2014). GC-MS-based metabolite profiling of Cosmos caudatus leaves possessing alpha-glucosidase inhibitory activity. *Journal of Food Science*, 79(6), 1130-1136.

Lowy, F. D. (2000). Is Staphylococcus aureus an intracellular pathogen? *Trends in microbiology*, 8(8),341-343.

Leone A., Spada A., Battezzati A., Schiraldi A., Aristil J., Bertoli S. (2015) Cultivation, genetic, ethnopharmacology, phytochemistry and pharmacology of Moringa oleifera leaves: an overview. *Int.J. Mol. Sci.*;16:12791-12835.

Miller, L. G., & Kaplan, S. L. (2009). Staphylococcus aureus: a community pathogen. *Infectious diseaseclinics of North America*, 23(1), 35-52.

Ohtera, A., Miyamae, Y., Nakai, N., Kawachi, A., Kawada, K., Han, J., Maejima, K. (2013). Identification of 6-octadecynoic acid from a methanol extract of Marrubium vulgare L. as a peroxisome proliferatoractivated receptor γ agonist. *Biochemical and biophysical research communications*, 440(2), 204-209.

Oyama MO, Egbebi AO, Akharaiyi FC (2019) Phytochemical analysis and antibacterial activities of some plant extracts on Staphylococcus aureus isolates from patients receiving hospital treatments in Ekiti State, Nigeria. *J Herbal Med Pharmacol* 8(1):14 20

Olorundare, O. (2015). Antibacterial activity of the crude extracts of *Moringa oleifera* leaf Lam (Moringaceae). *Nigerian Journal of Pharmaceutical Sciences,* 14(1). 23-45.

Parvekar, P., Palaskar, J., Metgud, S., Maria, R., & Dutta, S. (2020). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of silver nanoparticles against Staphylococcus aureus. *Biomaterial investigations in dentistry*, 7(1), 105-109.

Pliego M. P.C. (2007) Effect of natural antimicrobials against Salmonella, *Escherichia coli* 0157:H7 and *Listeria monocytogenes*. Unpublished Msc Thesis submitted to the office of Graduate studies of Texas A & M University.

Ramli, S., Radu, S., Shaari, K., & Rukayadi, Y. (2017). Antibacterial activity of ethanolic extract of Syzygium polyanthum L.(Salam) leaves against foodborne pathogens and application as food sanitizer. *Bio.Med research international*, 2(2), 30-44.

Praveen kumar P, Kumaravel S and Lalitha C: (2010)Screening of antioxidant activity, total phenolics and GC-MS study of Vitex negundo. *Afr. J. Biochemistry Res;* 4 (7): 191-195.

Sánchez-Machado, D. I., Núñez-Gastélum, J. A., Reyes-Moreno, C., Ramírez-Wong, B., & LópezCervantes, J. (2010). Nutritional quality of edible parts of Moringa oleifera. *Food analytical methods*,3, 175-180.

Singa NI, Hanaf M, Yantih N (2021) Identifcation of chemical compounds and antibacterial activity of 96% ethanol extract from Moringa oleifera LAM leaves against methicillin-resistant Staphylococcus aureus. *Int. J Appl Pharmaceutics* 13(2):111114

Singh, B and Bhat, T.K. (2003): Potential therapeutic application of some antinutritional plant secondary metabolites. *Journal. Agric. Food Chm.* 51:5579-5597.

Santhi, K., & Sengottuvel, R. (2016). Qualitative and quantitative Phytochemical analysis of Moringa concanensis Nimmo. *International Journal of Current Microbiology and Applied Sciences*,5(1),633-640.

Tenover, F. C. (2006). Mechanisms of antimicrobial resistance in bacteria. *The American journal of medicine*, 119(6), S3-S10.

Usman, M., Abdulrahman, M. D., Hamad, S., Hama, H. A., & Lema, A. A. (2022). Antioxidants, Antiinflammation, Anti-hyperglycemia and Chemical Evaluation of the whole plant extracts of Anisopus mannii N.E. Br. *Zanco Journal of Pure and Applied Sciences*, 34(5), 114-122.

Unuigbe CA, Okeri HA, Erharuyi O, Oghenero EE, Obamedo DA (2014) Phytochemical and antioxidant evaluation of Moringa oleifera (Moringaceae) leaf and seed. *J Pharmacol Bioresour* 11(2):51-57

Vongsak B, Sithisarn P, Mangmool S, Thongpraditchote S (2013) Maximizing total phenolics, total favonoids contents and antioxidant activity of Moringa oleifera leaf extract by the appropriate extraction method. *Ind Crops Prod J* 44:566571

van den Berg, J., & Kuipers, S. (2022). The antibacterial action of Moringa oleifera: A systematic review. *South African journal of botany*, 151, 224233.

Yadav, V., Ahmad, S., & Zahra, K. (2019). Assessment of the protective effects of *Moringa oleifera* leaf extract against Neem-Oil induced toxicity in zebra fish, Danio rerio. *Journal of Pharmacognosy and* phytochemistry, 8(3), 4263-4270

Zhang, M., Hettiarachchy, N. S., Horax, R., Kannan, A., Praisoody, M. A., Muhundan, A., & Mallangi,C. R. (2011). Phytochemical, antioxidant and antimicrobial activity of Hibiscus sabdariffa, Centella asiatica, Moringa oleifera and Murraya koenigii leaves. *J. Med. Plants Res*, 5(30),6672-6680.