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Comparative Analyses of the Antimicrobial, Antioxidant, and Phytochemical Composition of Two Species of Moringa in Ghana

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Moringa oleifera and *M. stenopetala* have been used to cure a variety of ailments in around the world. The phytochemical profile, antioxidant and antibacterial activity of the leaves, roots and stem bark were investigated. Elements were analysed and bioactive components were characterised using FTIR and GC/MS methods. Total phenolics, tannins, and flavonoids were assessed while

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radical scavenging activity was evaluated by the DPPH assay. Some organic compounds like phenols and aromatics were present, along with iron, zinc, and copper. The total phenolic and tannin concentrations varied from 98.73 to 255.57 mg GAE/100 g, and from 29.45 to 243.27 mg GAE/100 g, respectively. The total flavonoid content ranged from 717.90 to 73052.62 g QE/100 g. The methanolic extract of *M. oleifera* roots had the best DPPH scavenging efficacy. The extracts showed some antibacterial and antifungal activity. Therefore, *M. oleifera* and *M. stenopetala* contain phytochemicals and may be beneficial to health.

Keywords: Moringa; antimicrobial; antioxidant; Minimum Inhibitory Concentration (MIC).

1. INTRODUCTION

Moringa oleifera is a Moringaceae family plant that grows quickly and is commonly accessible in the tropics and subtropics for therapeutic purposes. It has been more popular in recent years due to its natural efficiency in water filtration and lack of negative side effects [1]. The nutraceutical and medicinal advantages of Moringaceae plant components have varied pharmacological activities and toxicity profiles that are yet to be fully elucidated [2].

Moringa species' leaves have traditionally been used for a range of biological purposes, including anticancer, antioxidant, anti-inflammatory, diuretic, antihepatotoxic, hypotensive, hypocholesterolemic, and hypoglycemic properties [3]. The roots, flowers, gums, and seeds of this plant are widely employed as anti-diabetic drugs and in the treatment of cardiovascular inflammation, haematological, hepatic, and renal illnesses [4].Moringa species' leaves, fruits, and seeds are high in protein, vital minerals (calcium, magnesium, potassium, and iron), and vitamins A, C, and E. [5,6] . According to WHO, herbal or medicinal plants are the finest source of any type of medication [7] Because of their outstanding nutritional and therapeutic capabilities, Moringa species are well-documented plant herbs. *Moringa oleifera* and *M. stenopetala* are the most extensively farmed Moringaceae species [8] Because it is exclusively found in Ethiopia and northern Kenya, *M. stenopetala* is sometimes known as the African Moringa Tree. It also has the most luscious green foliage and grows even during very extended dry seasons. It grows into a spherical shrub-like tree and has been planted as an ornamental in private gardens in Kenya, reaching a height of 10-12 m and a trunk diameter at least 2-3 times that of M. oleifera in Sudan [9].

M. oleifera is known as a "miracle tree" or a "wonder tree" [10], and its nutritional, pharmacological [11,10], and industrial uses make it a valuable socio-economic resource [12,13]. This plant's leaves contain a variety of trace elements and are an excellent source of proteins, vitamins, beta-carotene, amino acids, and different phenolic compounds [14]. *M. oleifera* is a major food product with a great lot of attention as the 'natural nutrition of the tropics'. This tree's leaves, fruit, blossoms, and immature pods are consumed as a highly nutritious vegetable in many nations, including India, Pakistan, the Philippines, Hawaii, and many parts of Africa [15].It has been demonstrated that the phytochemical constituents of plant materials are modified by species and environmental conditions. The current study sought to evaluate the phytochemical, antioxidant, and antibacterial activities of various solvent extracts of two Moringa species, *M. oleifera* and *M. stenopetala*.

2. MATERIALS AND METHODS

2.1 Sample Collection, Identification, and Authentication

The plant materials were obtained from the Kwame Nkrumah University of Science and Technology's (KNUST; MCMP+27 Kumasi) botanic garden and taxonomically identified at the Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, with a voucher specimen deposited in the herbarium unit for reference purposes (Table 1).

2.1.1 Preparation of plant extract

For 24 hours,100 g of each sample was macerated in 500 mL of 50% ethanol, methanol, and hot distilled water with periodic shaking. The mixture was then filtered via cheesecloth, and the filtrates were concentrated separately in a 60 ℃ water bath. The extracted materials were weighed, the yield was estimated, and the extracts were kept in the refrigerator for future use.

Table 1. Plant Taxonomy and voucher number

Scientific name	Part	Voucher number
M. oleifera	Stem bark	KNUST/HMI/2019/SB019
	Root	KNUST/HMI/2019/R007
	Leaf	KNUST/HMI/2019/LO14
M. stenopetala	Stem bark	KNUST/HMI/2019/LO13
	Root	KNUST/HMI/2019/R008
	Leaf	KNUST/HMI/2019/SB020

2.2 Qualitative Phytochemical Evaluation

The extracts were subjected to the following chemical tests for the identification of various natural products such as tannin, flavonoid, saponin, coumarin, triterpenoid, steroid, alkaloid, and glycoside using standard methods previously described by Bruhn and Bohlin [16], Harborne [17], Kuete [18].

2.3 Dpph Radical Scavenging Assay

The DPPH scavenging activity was accessed using standard methods previously described by Oliveira et al. [19] and used by Donkor et al. [20] with slight modification agaist gallic acid standard. The UV-Visible spectrophotometer was used to measure the absorption of all samples at 517 nm. The same procedure was carried out using extracts at the same quantities as gallic acid. The assay was performed in triplicates.

2.4 Determination of Total Phenolic Content (TPC)

The Folin-Ciocalteu technique was used to determine the total phenolic content of all samples [21] against standard gallic acid. Briefly, 10 mg/mL standard gallic acid solution was prepared and standard curve generated by producing solutions of 0.1, 0.5, 1.0, 2.5, and 5 mg/mL in methanol from a stock solution. Each of these serial dilutions was mixed with 500 L of water, followed by 100 L of Folin-Ciocalteu reagent, and left to stand for 6 minutes. The reaction mixture was then treated with 1mL of 7% sodium carbonate and 500 L of distilled water. After 90 minutes, the absorbance was measured at 750 nm. The extract's total phenolic content was determined as gallic acid equivalents (mg GAE/g). All tests were carried out in triplicate.

2.5 Determination of Total Flavonoid Content (TFC)

Total Flavonoids Content (TFC) was determined using an aluminium chloride colorimetric assay [22] against standard gallic acid. Briefly, 10 mg/mL standard quercetin solution was prepared. 25 µL aliquots of each sample were combined with 75 µL of 95% ethanol (v/v). Following that, 5 µL of 10% $AlCl_3$ and 5 µL of 1 M potassium acetate were added, followed by 140 µL of deionized water. The samples were vortexed and left for 30 minutes at room temperature. Following that, the absorbance of clear supernatants was measured at 415 nm against deionized water. All assays were done in triplicate, and the findings were represented as mg quercetin equivalent (QE) per gram of material.

2.6 Determination of Total Tannins Content (TTC)

The tannin content of plant extracts was determined using the Folin-Ciocalteu technique with slight modifications [23]. A total of 100 L of sample extract and fractions were combined with 5 mL of distilled water, 500 L of Folin-Ciocalteu reagent, and 1 mL of 35% Na_2CO_3 solution. The mixture was well mixed and kept at room temperature for 30 minutes. The same method was used to generate a series of reference standard solutions for gallic acid (0.2, 0.4, 0.6, 0.8, and 1 mg/mL). At 725 nm, the absorbances of test and standard solutions were measured against a blank. The calibration curve was used to measure the total tannin content, which was given in GAE/g mg.

2.7 Determination of Heavy Metal Concentration

All glasswares were immersed overnight in 10% (v/v) nitric acid before being washed with 10 % (v/v) hydrochloric acid, rinsed with double-distilled water, and dried to remove impurities. 1 g of each extract was precisely weighed into a 50 mL digestion tube. For 20 minutes, each sample was wet-digested with 1 mL H_2O, 2 mL HCl, 5 mL 1:3 HNO_3: $HClO_4$, and 2mL H_2SO_4. In a digestion block, the mixture was cooked to 150 °C. After cooling, the digested

sample was diluted with 50 mL of distilled water. The heavy metal levels in each extract were determined by analysing the digests. Heavy metals such as lead, copper, nickel, zinc, and iron were evaluated with an atomic absorption spectrometer (Varian AA 240FS) equipped with a long route air acetylene burner and cathode lamp.

2.8 Analysis

2.8.1 FTIR spectroscopic analyses

To make transparent sample discs, 10 mg of dried extracts were encapsulated in 100 mg of KBr pellet. Each extract was powdered and fed into an FTIR spectrophotometer (UATR Spectrum 2, PerkinElmer) with a scan range of 400 to 4000 cm^{-1} and a resolution of 4 cm^{-1}. The FTIR peak values were recorded and translated to functional groups using standard tables.

2.8.2 GC-MS Analyses

The PerkinElmer GC Clarus 580 Gas Chromatograph was interfaced with the PerkinElmer Mass Spectrometer (Clarus SQ 8 S) with ZB-5HTMS (5 percent diphenyl/95 percent dimethylpolysiloxane) fused with the capillary column (300.25 m ID 0.25 m DF) to perform GC-MS analyses on methanolic extracts of plant parts. The oven temperature was designed to rise from 100 °C (isothermal for 2 minutes) to 200 °C, then 5 °C/min to 280 °C and kept at 280 °C for 22 minutes. For GC-MS detection, the electron ionisation device was operated in electron impact mode with an ionisation energy of 70 eV. As a carrier gas, helium gas (99.9999 percent purity) was employed at a constant flow rate of 1 mL/min and an injection volume of 1l. The injector temperature was kept at 250 °C, while the ion-source temperature was kept at 220 °C. Mass spectra were collected at 70 °C with scan intervals of 1s and pieces ranging from 50 to 500 Da. The solvent delay ranged from 0 to 3 minutes, and the overall GC/MS time was 50 minutes. Turbo-Mass was the mass detector utilised in this work, and Turbo-Mass Ver-6.1.0 was the software used to analyse mass spectra and chromatograms. The GC-MS mass-spectrum was interpreted using the National Institute of Standards and Technology (NIST) database, which contains over 62,000 patterns.

2.8.3 Determination of Minimum Inhibitory Concentration (MIC)

The isolates, *Staphylococcus aureus* ATCC 25923 (Gram-positive), *Escherichia coli* ATCC 25922 (Gram-negative), *Salmonella typhi* ATCC 6539 (Gram-negative), *Klebsiella pneumonia* ATCC 13883 (Gram-negative), and *Candida albicans* ATCC 10231 (Fungi) were obtained from the Centre for Plant Medicine Research (CPMR), Akuapim Mampong, Ghana. The test organisms were activated and purified by sub-culturing on selective media; mannitol salt agar and brilliant E. coli^{R} were used for the purification and identification of *S. aureus* and *E. coli*.

The test employed nutrient agar (Oxoid, CM0003, England), bacteriological peptone (Sigma-Aldrich, P0556, Germany), and Mueller-Hinton agar (Oxoid, CM0337, Oxoid Ltd, England) and was prepared according to the manufacturer's instructions.

Test organisms were inoculated into sterile 9 mL tubes of Oxoid CM0001 nutritional broth and cultured at 37 ^{o}C for 16 hours until MacFarland's level of 0.5 was about 1105 CFU/mL of inoculum stock. Before usage, stock solutions were kept chilled.

The MIC values of the extract fractions were determined using a 96-well microplate dilution method similar to that published by Eloff [24] with minor modifications. Each fraction's stock extract concentration was 300 mg/mL, and an aliquot of 200 µL was put to the beginning well and labelled correspondingly. The transfer of 100 L of stock extract fraction to successive wells containing 100 L of sterile bacteriological peptone (1 percent), followed by subsequent dilutions at the necessary concentration, resulted in serial dilutions at the sixth power (9.375 mg/mL) of each fraction. As a positive control, chloramphenicol (100 g/mL) was serially diluted in another column of the microplate.Aliquots of 100 µL of active-growing test species (0.5 McFarland) were applied to each dilution, except for negative controls, and the microplates were covered and incubated at 37 ^{o}C for 24 hours. After the incubation, 40 µL of 0.2 mg/mL of Indonitrotetrazolium chloride (INT) was added to each well. The microplates were then analyzed after an additional 30 to 120 minutes of incubation. Bacterial growth was indicated by the red colour (conversion of INT to formazan) and the lowest concentration at which the red color was invisible relative to the next dilution was considered to be the MIC value.

2.8.4 Statistical analysis

Numerical data were expressed as mean±SD and analyzes using one-way analyses of variance followed by student's t-test using the Graph Pad Prism 8.0. P< 0.05 was considered significant.

3. RESULTS

3.1 Yield

Table 2 shows the yield of solvent extracts of the different parts of *Moringa oleifera* and *Moringa stenopetala* expressed in percentages. Hydroethanol gave the maximum yield for the *M. stenopetala* leaf (21.47%) while methanol gave the minimum yield for *M. stenopetala* stem at 3.34%.

3.2 Phytochemical Constituent of Moringa Extracts

Table 3 shows the phytochemical composition of the Moringa methanolic extracts (MME), ethanolic extracts (MEE), and aqueous extracts (MAE) of the two species of Moringa. The samples contained tannins, flavonoids, saponins, triterpenoids, steroids, glycoside, alkaloids, and coumarins. The polar solvent water extracted glycosides, flavonoids, and saponins. Methanol, which is also slightly polar extracted saponins and flavonoids. The hydroethanolic solvent extracted alkaloids, glycosides, and coumarins. The least present compound was the flavonoid.

3.3 DPPH (2,2-Diphenyl-1-picrylhydrazyl) Scavenging Activity

The IC50 values for *M. oleifera* and *M. stenopetala* extracts are shown in Table 4. When compared to normal gallic acid, the IC50 values obtained demonstrated excellent activity for the extracts examined. The % inhibition of *M. stenopetala* leaf aqueous extract was lower than that of *M. oleifera*. For methanolic extracts, the percentage inhibition of the stem was lower in *M. oleifera* than in *M. stenopetala*, as was the percentage inhibition of the root in *M. stenopetala*. In all cases, the methanolic extract of *Moringa oleifera* had the highest percent inhibition, whereas the aqueous stem extract of *Moringa stenopetala* had the lowest.

3.4 Total Phenol Content

The total phenolic content was calculated using the gallic acid standard curve (Fig. 1; $y = 0.4149x - 0.0483$; $R^2=0.9331$), and the results are shown in Table 5. The aqueous extract of *M. oleifera* had the greatest TPC in the roots, while the hydroethanolic extract of *M. stenopetala* had the lowest. The TPC of the stem was lowest in *M. stenopetala* hydroethanolic extracts and greatest in *M. oleifera* aqueous extracts. The maximum TPC was found in *M. oleifera* hydroethanolic extracts, whereas the lowest was found in *M. stenopetala* methanol aqueous extracts.

Table 2. Yield of Solvent Extracts of *Moringa oleifera* and *Moringa stenopetala* parts

	Parts	Extract	Percentage yield
M. oleifera	Root	Aqueous	7.23
		Methanol	5.68
		Hydroethanolic	5.69
	Stem	Aqueous	9.30
		Methanol	3.88
		Hydroethanolic	6.43
	Leaf	Aqueous	5.07
		Methanol	7.51
		Hydroethanolic	15.61
M. stenopetala	Root	Aqueous	7.35
		Methanol	4.55
		Hydroethanolic	9.72
	Stem	Aqueous	8.91
		Methanol	3.34
		Hydroethanolic	9.92
	Leaf	Aqueous	19.83
		Methanol	7.3
		Hydroethanolic	21.47

Table 3. Phytochemical composition of serial extracts of *M. oleifera* and *M. stenopetala*

Plant	Parts	Extract	Tannin	Flavonoid	Saponin	Coumarin	Triterpenoid	Steroid	Alkaloid	Glycoside
M. oleifera	Root	Aqueous	+	+	+	+	-	+	-	+
		Methanol	+	-	+	+	+	+	+	+
		Hydroethanolic	+	-	+	+	+	+	+	+
	Stem	Aqueous	+	-	-	+	+	-	+	+
		Methanol	+	+	+	+	+	+	-	+
		Hydroethanolic	+	-	+	+	+	-	+	+
	Leaf	Aqueous	+	-	+	+	+	+	-	+
		Methanol	+	-	+	-	+	+	+	+
		Hydroethanolic	+	+	+	+	+	+	-	+
M. stenopetala	Root	Aqueous	-	-	-	+	+	+	-	+
		Methanol	+	-	+	+	+	+	-	-
		Hydroethanolic	+	-	+	+	+	+	+	+
	Stem	Aqueous	+	-	+	+	+	+	-	+
		Methanol	+	+	+	-	+	+	+	+
		Hydroethanolic	+	-	+	+	+	+	-	-
	Leaf	Aqueous	+	-	+	-	+	+	+	-
		Methanol	+	-	+	-	+	-	+	+
		Hydroethanolic	+	+	+	+	+	+	+	+

Table 4. DPPH scavenging of different extracts of *Moringa oleifera* and *M. Stenopetala*

Plant	Parts	Extract	IC_{50} (mg/mL)	Conc of Max Inhibition (mg/mL)	% Inhibition
Gallic acid			0.04	5.00	94.15
M. oleifera	Root	Aqueous	0.06	0.16	72.13
		Methanol	0.52	2.50	84.73
		Hydroethanolic	4.28	5.00	50.70
	Stem	Aqueous	3.98	5.00	59.44
		Methanol	3.98	5.00	59.44
		Hydroethanolic	0.75	1.25	58.08
	Leaf	Aqueous	>5.0	1.25	35.73
		Methanol	1.1	2.50	77.40
		Hydroethanolic	0.78	1.25	53.28
M. stenopetala	Root	Aqueous	>5.0	5.00	40.04
		Methanol	1.88	5.00	71.47
		Hydroethanolic	>5.0	5.00	42.04
	Stem	Aqueous	>5.0	0.16	11.02
		Methanol	2.4	5.00	75.67
		Hydroethanolic	>5.0	5.00	38.75
	Leaf	Aqueous	1.48	5.00	61.50
		Methanol	0.92	2.50	82.52
		Hydroethanolic	>5.0	1.25	46.67

Table 5. Total phenolic content on Moringa extracts

	Total Phenolic Content (GAE/mg)			
	Hydroethanolic	**Methanol**	**Aqueous**	**P value**
Moringa oleifera				
Root	164.70±0.60	129.02±2.89	255.57±6.49	<0.0001
Stem	171.90±8.35	158.11±3.46	172.20±0.90	0.0912
Leaves	157.51±3.83	144.91±5.23	131.11±1.67	0.0015
Moringa stenopetala				
Root	122.42±4.17	157.51±0.60	159.90±1.67	<0.0001
Stem	102.33±5.12	102.63±5.70	98.73±3.65	0.7880
Leaves	129.02±5.50	125.42±0.52	177.00±5.75	<0.0001

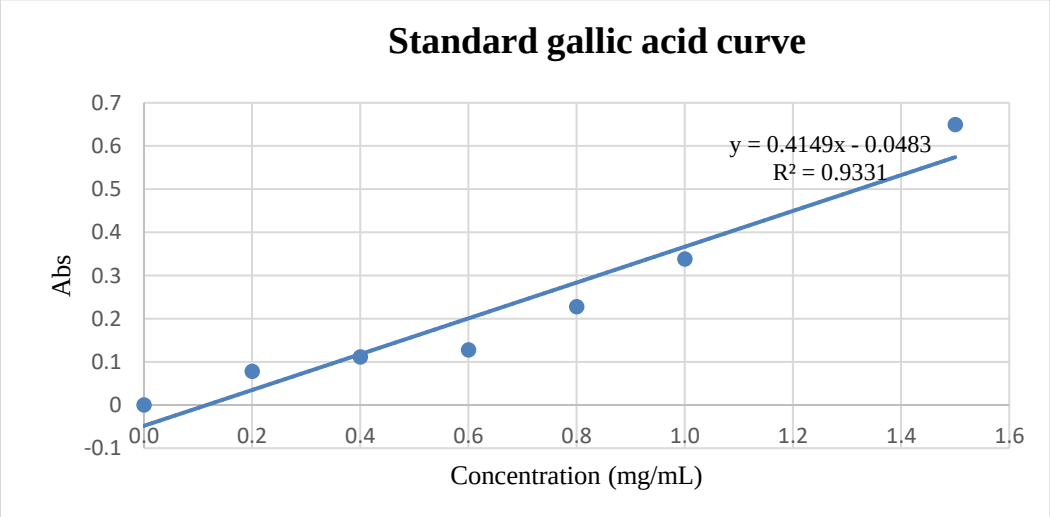

Fig. 1. Standard gallic acid curve for estimation of total phenol

3.5 Total Flavonoid Content

The TFCs of the different crude extracts were represented as quercetin equivalency (QE) (Table 6) using the quercetin standard curve (Fig. 2; y=232.09x-,0.752, R2=0.9985). The most flavonoid was identified in the methanolic leaf extracts (87,892.02 QE/mg), while the least was found in the methanolic roots extract (2,961.44) for *M. oleifera*. Methanolic leaves (60,210.31) were the most abundant in *M. stenopetala*, whereas methanolic root extract (717.90) was the least abundant. In terms of flavonoids, *M. oleifera* was more powerful than *M. stenopetala*.

3.6 Total Tannins Content

Based on the standard gallic acid curve (Fig. 3; y=3.3071x-0.0312, R2=0.9715), the TTCs of the various crude extracts are represented in terms of GAE and are reported in Table 7. For *M. oleifera*, the maximum tannin content was found in aqueous root extracts (377.92 GAE/mg), while the lowest was found in aqueous leaves extract (91.19 GAE/mg). For *M. stenopetala*, hydroethanolic leaves extract (89.33 GAE/mg) had the greatest concentration, while aqueous roots (29.45 GAE/mg) had the lowest. In general, *M. oleifera* contained more tannins than *M. stenopetala*.

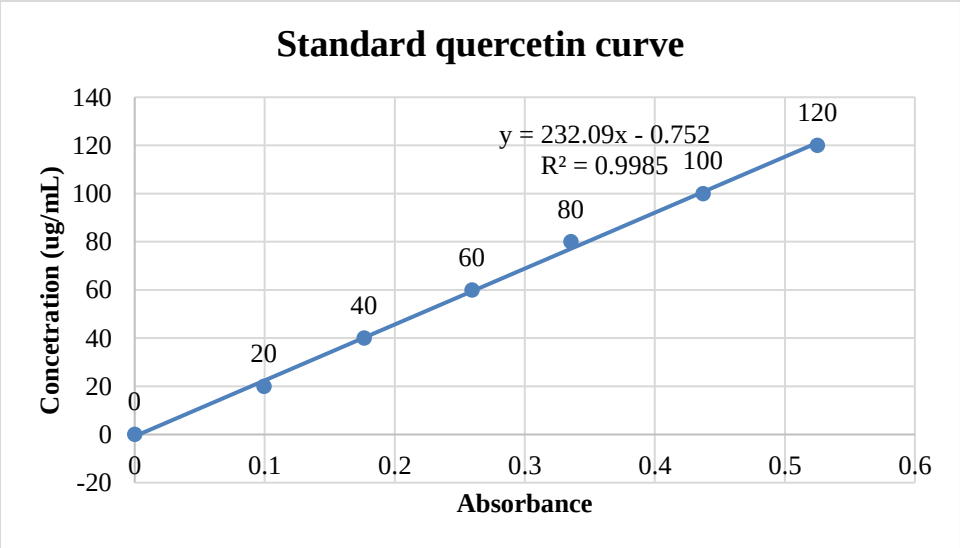

Fig. 2. Standard quercetin curve for estimation of total flavonoids

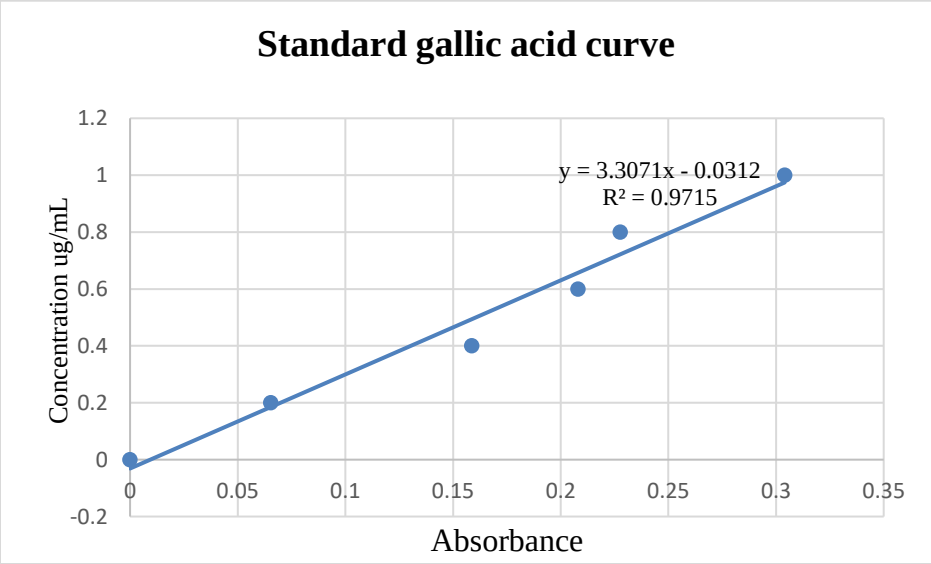

Fig. 3. Standard gallic acid curve for estimation of total tannins (y=3.3071x-0.0312, R^2=0.9715)

Table 6. Total phenol content in extracts of *M. oleifera* and *M. stenopetala*

	Total Flavonoid Content (QE/mg)			
	Hydroethanolic	**Methanol**	**Aqueous**	**P value**
Moringa oleifera				
Root	73052.62±3139.66	2961.44±709.05	4353.98±232.09	<0.0001
Stem	640.54±134.00	42107.29±2758.15	63691.66±8005.51	<0.0001
Leaves	57657.32±3675.37	87829.02±6654.60	27640.34±3197.27	<0.0001
Moringa stenopetala				
Root	18202.02±2011.45	717.90±77.36	3193.53±746.06	0.0715
Stem	48760.53±1598.63	10233.59±278.94	17196.29±2843.56	0.0002
Leaves	6984.33±299.81	60210.31±4202.02	1104.72±134.00	<0.0001

Table 7. Total tannin content in extracts of *M. oleifera* and *M. Stenopetala*

	Total Tannin Content (GAE/mg)			
	Hydroethanolic	**Methanol**	**Aqueous**	**P value**
Moringa oleifera				
Root	236.65±1.59	172.72±4.25	377.32±9.18	<0.0001
Stem	243.27±11.74	218.14±4.67	149.35±0.88	<0.0001
Leaves	227.40±8.52	111.87±8.05	91.19±2.20	<0.0001
Moringa stenopetala				
Root	80.52±6.53	44.84±1.76	29.45±2.75	<0.0001
Stem	48.33±6.17	56.31±10.61	41.36±5.77	0.2449
Leaves	89.33±9.55	85.85±4.41	46.21±3.77	0.0004

3.7 Heavy Metal Composition of Moringa Extracts

Table 8 demonstrates the heavy metal content in the extracts of the two Moringa varieties' components and extracts. Lead (Pb), iron (Fe), zinc (Zn), nickel (Ni), and copper (Cu) were found in the samples. The Ni and Cu concentration of several *M. oleifera* and *M. stenopetala* extracts was below the detection limit. Copper concentration was higher in *M. oleifera* root extracts than in *M. stenopetala* root extracts. The concentration of lead (Pb) in *M. stenopetala* extracts was greater than in *M. oleifera* extracts. The Pb concentration of *M. stenopetala* leaves was greater on average than that of the roots and stems. The concentration of iron (Fe) in M. stenopetala extracts was greater than in *M. oleifera* extracts.

3.8 Fourier Transform Infrared Spectroscopic analysis

Fig. 4 a,b,c and 5 a,b,c are representative FTIR spectra of methanol extracts from plant parts of *M. oleifeira* and *M. stenopetala*. The predominant groups were alcohols, amines, aromatics, nitrochemicals and phenols. There were also unassigned groups.

3.9 GC-MS Analyses and Compounds of Extracts of *M. oleifera* and *M. Stenopetala*

GC-MS investigation revealed many chemicals in *M. oleifera* and *M. stenopetala* methanolic extracts. It found 31, 32, and 32 distinct phytocompounds in *Moringa oleifera* leaves, stem bark, and root bark, respectively, and 30, 33, and 30 different compounds in *Moringa stenopetala* leaves, stem bark, and root bark. *M. oleifera* leaves contained a high concentration of components such as Astaxanthin, Hexadecanoic acid, Oleic acid, Megestrol acetate, and others, but *M. stenopetala* leaves included Octadecadienoic acid, Rhodopin, and Milbemycin. Propanoic acid, cis-Vaccenic acid, and Benzyl cinnamate were found in *M. oleifera* stems, but *M. stenopetala* stems contained 7,8-Epoxylanostan-11-ol,3-acetoxy-Octadecane,1,1'-[1,3-propanediylbis(oxy)] bis-Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl), and psi-carotenoic acid are all examples of carotenoids. Phytocompounds found in the root of *M. oleifera* included 7,8-Epoxylanostan-11-ol, 3-acetoxy-, Oleic Acid, and 5H-Cyclopropa[3,4]. benz[1,2-e] Azulen-5-one, 3,9,9a-tris, azulen-5-one, azulen-5-one (acetyloxy) -3-[(acetyloxy)methyl] While the roots of *M*

stenopetala contained Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl, and 9-Octadecenoic acid (Z)-, 2-(9-octadecenyloxy)ethyl ester (Z). Propanoic acid and psi-carotenoic acid, on the other hand, were found in both leaves and stem bark and root, indicating that some components are uniformly distributed throughout the plant. Phytochemical screening and GC-MS analysis of moringa extracts indicated the existence of secondary metabolites with antibacterial and antioxidant properties, indicating a potential source of industrial use.

3.10 Antimicrobial Activity

The results of the incubation cycle showed that all extracts had detectable antibacterial activity at a concentration of 300 mg/mL, but the activity varied with subsequent dilutions in the two plant species studied, with variations in the activity of the different parts (root, stem bark, and leaves) as well as the different extracts (aqueous, hydroethanolic and methanol). The results for *M. stenopetala* indicated some antibacterial activity in all extracts, but a comparative analysis revealed that stem extract had the lowest antimicrobial activity with a mean MIC value of 300 0.87 mg/mL, root extract was second with a mean MIC of 150 1.14 mg/mL, and leaf extract had the highest antimicrobial activity. *M. oleifera* leaf extracts showed the highest antibacterial activity, with a mean MIC of 37.5 mg/mL, followed by the root extract, which had a MIC of 75 mg/mL, and the stem extract, which had the lowest activity, with a MIC of 150 mg/mL.

4. DISCUSSION

Plants have a plethora of phytochemical elements, many of which are recognised to be physiologically active chemicals with a wide range of pharmacological effects [25]. Phytochemicals are secondary metabolites found in plants that accumulate in large quantities but have little or no function in plant growth and development. Throughout history, however, people have used phytochemicals as medicine to heal and protect against many ailments. Moringa methanolic, hydroethanolic, and aqueous extracts contained tannins, flavonoids, saponins, coumarins, triterpenoids, steroids, alkaloids, and glycosides, according to this study. Moringa contains phytochemicals that can be used to cure a variety of diseases. Tannins may be useful in avoiding ulcer formation due to their protein precipitating and vasoconstriction effects [26]. Tannins, as an astringent, may precipitate microproteins at the ulcer site, generating an impermeable protective pellicle over the lining to prevent poisonous chemicals and resist assault by a proteolytic enzyme [27]. They are capable of tanning leather or precipitating gelatine from solution [28], preventing local cancers [29], inactivating, and killing bacteria [29], and inactivating and killing microorganisms [30].

Flavonoids have been found to provide some protection against ulcer formation by enhancing capillary resistance and boosting microcirculation, making the cells less susceptible to precipitating stimuli [11]. They also help to lower high blood pressure [31]. Because of their antioxidant and anti-inflammatory qualities, flavonoids have been shown to have significant anti-carcinogenic and anti-mutagenic capabilities [30]. Saponins operate as antimicrobial agents, particularly in cold-blooded species, but their toxicity to mammals is modest [32]. Saponins are employed in the treatment of hypercholesterolemia, hyperglycemia, antioxidant, anticancer, anti-inflammatory, and weight reduction [33].

Some of these plant secondary metabolites are an essential source of natural antioxidants, which are favoured over synthetic antioxidants due to safety concerns [34]. The use of DPPH scavenging activity to determine the antioxidant activity of herbal extracts and phytochemicals has become commonplace. The quantity of sample required to reduce the initial DPPH concentration by 50% is a commonly used criterion to assess antioxidant activity. Table 4 shows the DPPH radical scavenging properties of extracts of the two Moringa species' leaves, stems, and roots. DPPH radicals were considerably decreased by *M. oleifera* leaf extract. Gallic acid, as a positive control, with an IC50 of 0.038. The extract's DPPH scavenging performance may be related to its hydrogen donating ability, and mature leaf extract demonstrated considerable scavenging activity. The discrepancies in IC50 values can be related to different extraction techniques, extraction solvents, and extraction times. It was previously reported that the IC50 value for the radical scavenging activity of *M. stenopetala* leaves powder was 59.5 g/mL after two weeks of extraction with 100% methanol [35]. For the aqueous decoction of *M. stenopetala* leaves powder, [36] found an IC50 value of 41.5 g/mL. *M. olifiera* had an IC50 of 62.94 g/mL when extracted with 70% ethanol, 122 g/mL when

extracted with 50% methanol for three days, and 78.15 g/mL when extracted with aqueous decoction for five minutes [37]. The IC50 of the entire sample revealed in this study was lower than the value reported by Saikia and Upadhyaya [38].

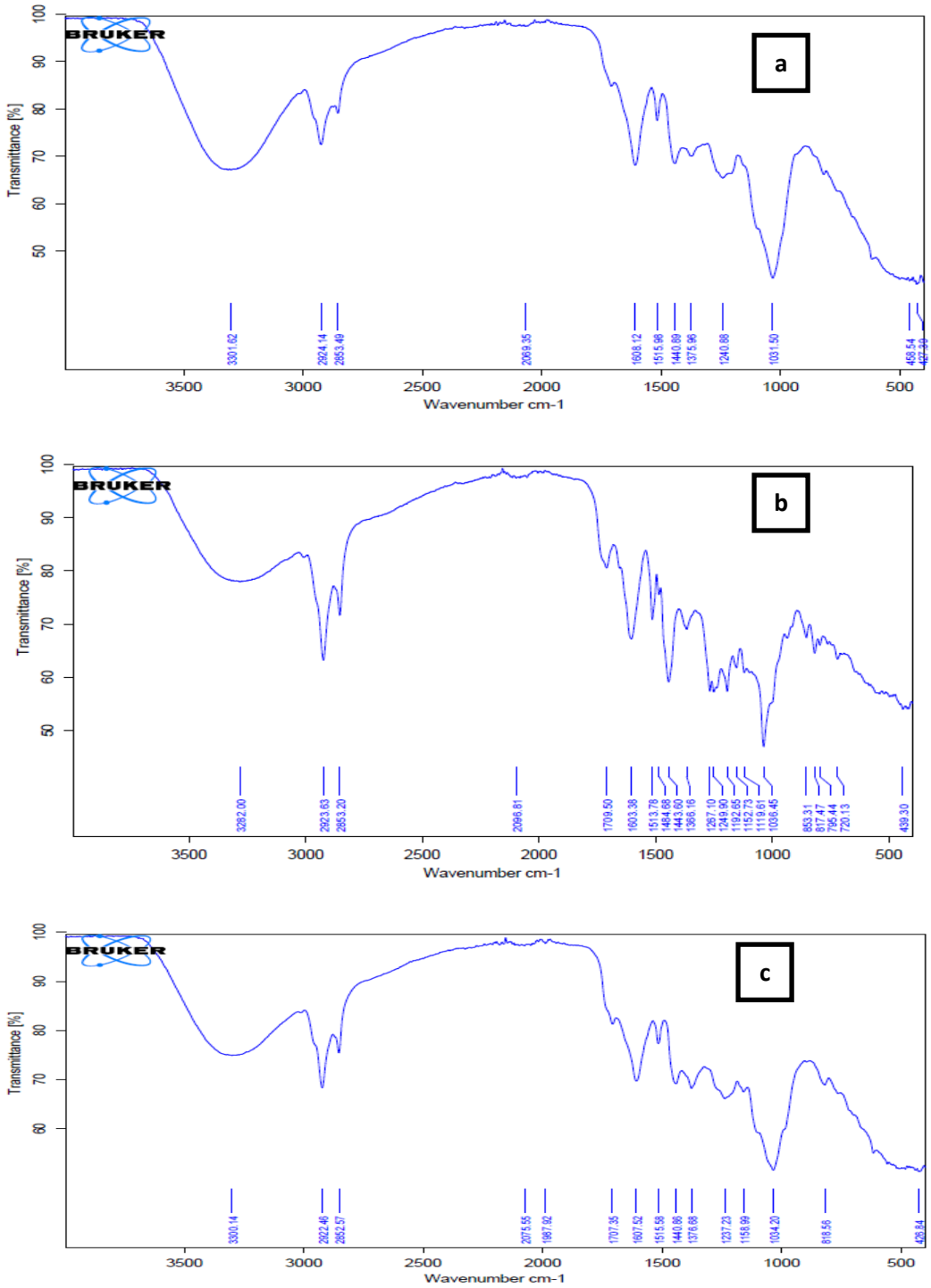

Fig. 4. Representative FT-IR Spectra of *M. oleifera* methanol extract of (a) Root; (b) Stem and (c) Leaves

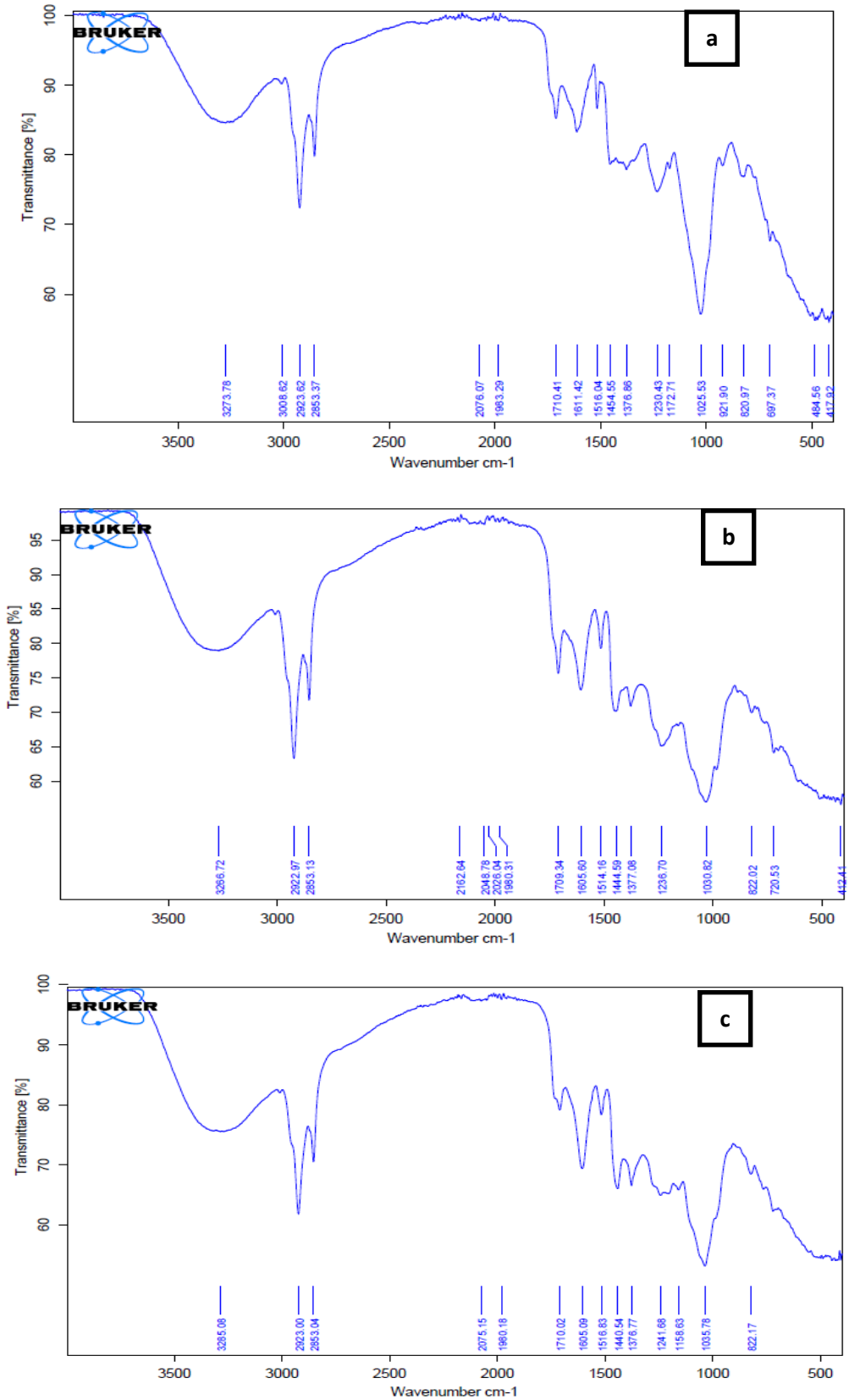

Fig. 5. Representative FT-IR Spectra of *M. oleiferra* methanol extract of (a) Root; (b) Stem and (c) Leaves

Table 8. Metal concentration in M. oleifera and M. stenopetala extracts

Part	Solution	*M. oleifera* Concentration, mg/L					*M. stenopetala* Concentration, mg/L				
		Pb	**Fe**	**Zn**	**Ni**	**Cu**	**Pb**	**Fe**	**Zn**	**Ni**	**Cu**
Root	Aqueous	0.0677	4.251	1.626	B D L	0.2063	0.1755	4.220	0.5265	B D L	B D L
	Methanol	0.0825	0.4156	1.221	B D L	0.0648	0.2045	0.7943	0.8109	B D L	0.0308
	Hydroethanolic	0.0835	1.671	1.553	B D L	0.2269	0.2103	1.285	1.061	B D L	0.1088
Stem	Aqueous	0.0939	3.627	1.436	B D L	B D L	0.2283	6.845	0.6698	B D L	B D L
	Methanol	0.1081	0.3143	0.0945	B D L	B D L	0.2343	0.4140	0.1345	B D L	B D L
	Hydroethanolic	0.1296	0.3941	0.5525	B D L	B D L	0.2807	0.4208	0.5451	B D L	B D L
Leaf	Aqueous	0.1569	1.784	0.3745	B D L	B D L	0.2799	2.020	0.6852	B D L	B D L
	Methanol	0.1373	0.9003	0.2780	B D L	0.0483	0.2927	0.9467	0.1417	B D L	B D L
	Hydroethanolic	0.1641	0.7789	1.402	B D L	0.0098	0.3051	1.049	0.4378	B D L	B D L

Table 9. MIC of extracts of M. stenopetala and M. oleifera against test organisms

Extract	Test organism	*M. oleifera*			*M. stenopetala*		
		Root (mg/mL)	Stem (mg/mL)	Leaf (mg/mL)	Root (mg/mL)	Stem (mg/mL)	Leaf (mg/mL)
Aqueous	*Staphylococcus aureus*	75	150	75	300	300	18.75
Hydroethanolic		75	37.5	18.75	75	300	9.37
Methanol		75	37.5	37.5	150	300	9.37
Aqueous	*Escherichia coli*	150	150	150	300	300	75
Hydroethanolic		150	150	37.5	150	300	18.75
Methanol		150	150	75	150	300	75
Aqueous	*Salmonella typhi*	75	150	75	300	150	150
Hydroethanolic		75	75	75	75	75	75
Methanol		150	75	75	150	75	75
Aqueous	*Klebsiella pneumoniae*	150	150	150	300	150	150
Hydroethanolic		37.5	150	75	300	150	150
Methanol		75	150	75	300	150	150
Aqueous	*Candida albicans*	150	75	75	300	300	75
Hydroethanolic		37.5	75	37.5	150	300	18.75
Methanol		150	75	75	150	300	75

The maximum phenolic concentration was found in an aqueous extract of *M. oleifera* roots (255.576.49 GAE/mg). An examination of the results in Table 5 demonstrates that the measured antioxidant activity of Moringa extracts corresponds with their phenolic content. Because polyphenols are responsible for antioxidant activity, the quantity of total polyphenols found in the extract suggested that the extract had a significant antioxidant activity. Phenolic antioxidants are crucial plant elements because they serve as free radical terminators due to hydroxyl groups. According to Packer and Witt [39], oxygen is significant in the degradation of phenolic content and will reduce the quantity of phenolic content in oil owing to structural breakdown. According to Thoo et al. [40], excessive extraction time reduces phenolic and antioxidant yields. This is because antioxidants can degrade if exposed to ambient conditions for an extended period of time. The leaves of *M. stenopetala* have a high phenolic content, and it was shown that phenolic compounds in plant extract are frequently connected with other molecules such as chlorophyll, proteins, polysaccharides, terpenes, and other inorganic compounds [41]. Contrary to popular belief, immature leaves have a higher phenolic content than developed leaves [3].

Flavonoids are antioxidants that are commonly found in plants [42]. Phenolics and flavonoids include at least one hydroxyl group replaced with an aromatic ring and can form chelate complexes with metal ions, allowing them to be quickly oxidised and giving electrons to scavenge free radicals [43]. Total flavonoid concentration was detected in significant proportions in both Moringa species; the discrepancies may be related to seasonal impacts [44]. Flavonols have been shown to be antioxidants, radical scavengers, and metal chelators [45,46]. Other major biological functions of flavonoids include skin protection from UV light exposure, DNA protection, capillary strengthening, antiinflammatory effect and radiation protection, moistening, softening, calming, antimicrobial, and others. Flavonoids can be employed as ingredients in cosmetics and medicinal goods due to their characteristics [47]. Flavonoids are major bioactive compounds known for their potential health benefits that have been used against many chronic diseases such as cancer, viral infection, inflammation, cardiovascular and neurodegenerative disorders; it is widely assumed that active dietary constituents are antioxidant nutrients found in fruits and vegetables [48].

AAS was used to determine the amounts of five trace elements (Fe, Zn, Ni, Pb, and Cu) in Moringa digested and diluted solutions. The limit of detection (LOD) of each analyte obtained by AAS is determined: for Cu 0.034 mg/ L, for Ni 0.305 mg/L, for Pb 0.290 mg/L,for Fe is 0.890 mg/L and for Zn 0.994 mg/L. Their levels indicated that the plant was a source of nutrients. Table 8 shows the metal concentrations determined during the study. Fe was detected in the greatest quantity compared to the others, with a concentration of 4.25 mg/L, and Ni was the least been below the detectable limit (BDL) of

Ni 0.305 mg/L . The quantities of metals measured in Moringa samples were grouped in ascending order of concentration, Ni information indicated it contained some chemical compounds that are prone to have neurotoxic effects [49]. According to studies, *M. oleifera* is good in removing heavy metals such as lead from water [50]. [51] discovered that *M. stenopetala* is a superior water clarifier than *M. oleifera*, implying that the same agents, the polyelectrolytes, are involved in both water clarity and lead removal. The leaf had various quantities of copper. Copper is a micronutrient that is required for the formation, development, and maintenance of bone, connective tissue, the brain, the heart, and many other bodily organs. Copper boosts the immune system's ability to fight infections, restore damaged tissues, and promote healing [52]. Zinc was found in variable amounts in all plants. Zinc's involvement in diabetes has been researched [53,54], and zinc supplements have been advised for diabetic patients. Zinc is required by about 200 enzymes involved in digestion, metabolism, reproduction, and wound healing [55]. The heavy metal concentrations found in the two Moringa species were below the acceptable maximum values of elements in plants.

FTIR spectroscopy data analyses aid in understanding the chemical functionality of the substance in the plant sample, and when performed in the IR region of 400-4000 cm-1, the peaks in all of the plant samples varied. Based on the peak values in the infrared radiation band, the FTIR spectrum was utilised to determine the identification of the functional groups included in the extract. The presence of alcohols, phenols, alkanes, aldehydes, ketones, aromatics, aliphatic amines, aromatic amines, amides, carboxylic acids, esters, nitro compounds, alkynes, primary and secondary amines, and alkyl halides were shown by FTIR investigations (Figs. 4 a,b,c and 5 a,b,c). FTIR spectroscopy examination showed the existence of the following groups: C-Br, O-H, C-H, C=C, C=O, C-C, N-H, C-H, C-N, C=O. The presence of certain functional groups in the Moringa plant may be responsible for a variety of therapeutic effects.

Moringa included several bioactive phytoconstituents belonging to diverse groups, according to the findings of GC-MS investigations, such as tannins, glycosides, alkaloids, flavonoids, steroids, and so on. Using GC-MS, [56] studied different phytochemicals found in the leaves, seeds, and flowers of an ethanolic extract of *M. oleifera*. Hexadecanoic acid, Ethyl palmitate, Palmitic acid ethyl ester, 2, 6-Dimethyl-1, 7-octadiene-3-ol, 4-Hexadecen-6-yne, 2-hexanone, 3-cyclohexyliden-4- ethyl - E2-Dodecenylacetate, 2-hexanone, 3-cyclohexyliden-4- The main chemicals extracted from the seeds were Roridin E, Veridiflorol, and 9-Octadecenoic acid. 9- Octadecen-1-ol, cis-9-Octadecen-1-ol, Oleol, Satol, Ocenol, Sipo, Decanoic acid, and Dodecanal were discovered as significant components in the flowers.

M. oleifera extract demonstrated broad spectrum action on the test organisms based on antibacterial and minimum inhibitory concentration (MIC) values. Except for *Klebsiella pneumonia*, where the root was more powerful than the leaves and stem, the stem extract of *M. stenpetala* was more effective than the root and leaves extracts. Moringa roots have antibacterial properties because they are high in antimicrobial compounds [57]. *M. oleifera* stem extracts were effective against *K. pneumonia* and *C. albicans*. The bark extract is antifungal, whilst the juice from the stem bark is antibacterial against *S. aureus* [58]. The fresh leaf juice was found to limit the development of harmful germs (*P. aeruginosa and S. aureus*) [11]. The extract of the leaves has only little antibacterial action against *E. coli*. This suggests that at the dose utilised, *M. oleifera* leaf had no effect on these species. The aqueous and methanolic extracts, on the other hand, demonstrated significant antibacterial activity against the five test species. According to Napolean et al.[56], ethanol leaf extract was sensitive to *S. aureus and E. coli*. [59] discovered antibacterial activity of *M. oleifera* leaf extract against *S. aureus*.

5. CONCLUSION

A phytochemical research of the Moringa plant was carried out in the current study in order to determine its effectiveness in traditional medicine. Preliminary chemical tests indicated the existence of numerous classes of bioactive chemical components with varying antioxidant and antibacterial activity in methanolic, hydroethanolic, and aqueous extracts of *M. oleifera* and *M. stenopetala*. As a result of the phytochemical ingredients present, *M. oleifera* and *M. stenopetala* exhibit therapeutic effects (antioxidant and antibacterial), adding to the previous research on Moringa's numerous potential advantages.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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