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ASSESSMENT OF ANTIBACTERIAL ACTIVITY OF *Moringa oleifera* LEAF EXTRACT AGAINST BACTERIA ISOLATED FROM SOME DRINKING WATER SOURCES IN KATSINA METROPOLIS

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ABSTRACT
Continuous consumption of sachet and tap water in Nigeria is of public health significance as the potential bacterial water contaminants could develop resistance to commonly used antimicrobials. This study was carried out to assess the bacteriological quality and TLC-Bio autographic profile of water samples using M. oleifera leaf extract. One hundred samples comprising 50 sachets and 50 tap water were collected from five different locations in Katsina metropolis. Most probable number (MPN) was used to identify indicator bacteria and characterized using biochemical tests followed by 16S rRNA gene sequencing identification. The results showed that the pH and temperature level of all the samples were significantly different. Turbidity (0.2-0.47NTU), dissolved oxygen (1.34-2.03mg/L and 0.85-2.45mg/L) and biological oxygen demand (0.49-1.09mg/L and 0.19-0.59mg/L) were below the minimum permissible limits set by WHO and NAFDAC (≤5). Total coliform counts exceeded limits of 2 MPN/100ml and Escherichia coli was detected from tap water of Kofar Marusa, Kofar Durbi and Kofar Kaura as well as the sachet water of Kofar Marusa and Kofar Keke. However, there was no statistically significant difference (P≤0.05) between the total coliform counts of both sachet and tap water. The 16S rRNA gene identification showed the presence of Escherichia marmotae and Enterobacter kobei in both sachet and tap water. The GC-MS demonstrated that M. oleifera leaf extract contained 9,12,15-Octadecatrienoic acid, ethyl ester, Octadec-9-enoic acid and Hexadecanoic acid, The TLC-bioautography result indicate antibacterial activity, with inhibition zones ranging from 15±0.00 to 19.5±4.95mm (E. coli) to 23.5±6.36mm (E. faecalis) and 20±0.00 to 22±1.41mm (E. faecium). Waterborne faecal bacterial contaminants were found associated with the sachet and tap water analyzed. M. oleifera leaf has bioactive principles with nobility to be used as candidate for drug development.
Keywords: Coliforms; Water quality; TLC –Bioautography, Moringa oleifera

INTRODUCTION

A large number of plants are claimed to possess antibiotic properties in the traditional medicine and are used comprehensively by different tribes' the world over (Dhanavade *et al*., 2011). Many Plants have been known to relieve various diseases through the biological activities Bioactive compounds present in medicinal plants are always of great interest as they usually serve as auxiliary to chemotherapy due to microbial *Moringa oleifera* belonged to one of the 14 species of the family Moringaceae, which is native to India, Africa, Arabia, South Asia, South America and the pacific and Caribbean Islands (Coppin, 2008). *Moringa oleifera* is used as an extremely nutritive vegetable in Nigeria and resistance commonly experienced in antibiotics (Akinjogunla *et al*., 2019).

The world health organization (WHO) estimates that ~80% of the global inhabitants depend on traditional medicines as the first line of solution for primary health care (Akinjogunla *et al*., 2019). Plants have long made the basis of sophisticated traditional medicine systems, and purportedly provide excellent leads for new drug development (Akinjogunla *et al*., 2019).

other African Countries, such as Ghana, Ethopia and Malawi (Anthonia, 2012).The leaves, flowers, seeds and tender pods are commonly consumed and reported to have medicinal potentials (AbdulRazis *et al.*, 2014).

The quality and quantity of pipe borne water for drinking is deteriorating in Katsina Metropolis due to inadequacy of treatment plants and inefficient management of piped water distribution system (Ayotunde *et al.*, 2011; Aderibigbe *et al.*, 2008).This study aimed at evaluating Bacteriological Quality of some water samples from Katsina Metropolis and the TLC-Bioautographic Profile of the isolates against *Moringa oleifera* leaf extract.

MATERIALS AND METHODS

Sample collection and handling

A total of 50 water samples, 10 each from sachet and tap water samples were collected using simple random sampling in five (5) different areas within Katsina Metropolis in accordance with the protocols of American Public Health Association (APHA, 2004). The sampling areas include Kofar Kaura, Kofar Kwaya, Kofar Marusa, Kofar Durbi and Kofar Keke area of Katsina Metropolis. The physicochemical analysis was carried out to determine the quality of the water samples accordance to the methods of APHA, (2004). Parameters analyzed include temperature, pH, Dissolved oxygen (DO) and Biological oxygen demand (BOD). The *M. oleifera* leaf was obtained from irrigation farm at Tudun katsira of Kofar Marusa area within Katsina Metropolis in a clean polythene bag and transported to Microbiology Laboratory, Umaru Musa Yaradua University Katsina for analysis.

Coliform examinations

Bacteriological analysis was carried out for indicator organisms; total and fecal coliform (*E. coli*) using MPN technique (APHA, 2004). This was carried out according to the three-stage process of presumptive test, confirmed test and completed test. Bacteria present in the samples were characterized using the catalase, citrate, indole, methyl red, Voges-Proskauer, motility and oxidase biochemical tests.

Extraction of *M. oleifera* leaf

Fifty (50) grams of fresh leaves of *M. oleifera* were shade dried at 28^{o}C for 5 days. The dried leaves were ground into powder using a mortar and pestle. Twenty-five grams (25g) of the powdered leaves were dissolved in 50ml of 60% petroleum ether. The conical flasks were plugged with rubber corks, and then shaken at 120 rpm for 30 minutes thereby allowed to stay at 28^{o}C for 5 days with occasional manual agitation of the flask using a sterile glass rod at every 24 hours. The preparation was filtered using Whatman No.1 filter paper. The resulting filtrate was concentrated in a rotatory evaporator at 50^{o}C (Anthonia, 2012).

Gas Chromatography Mass Spectrometry technique was used to identify the phytocomponents present in the *Moringa oleifera* leaf extract (Martinez *et al.*, 2020).The plant extract was analyzed using the Thermo Scientific GC-MS (SHIMADZU QP2010) gas with software GCMS solution ver. 2.53. The gas chromatograph was interfaced to a mass spectrometer equipped with Elite-1 fused silica capillary column of length: 30.0m, diameter: 0.25mm, film thickness: 0.25 and composed of 100% Dimethyl poly siloxane. The column oven temperature was maintained at 70^{o}C and injector temperature at 240^{o}C (Salisu *et al.*, 2017).

Bacteria identification

The identity of the isolated bacterial species was carried out through the amplification of the conserved region using genus specific primer (Danbing, *et al.*, 1999).Pure colonies of the bacterial isolates were cultured overnight in Luria-Bertani (LB) broth medium.The overnight culture was centrifuged for 2 minutes at 16000g.Bacterial cells were re-suspended in 200µl sterile distilled water and DNA extraction was carried out using Zymoresearch^{TM} Genomic DNA extraction kit (Irvin, CA. USA) in accordance with the manufacturer's protocols (Ganiyu *et al.*, 2017).The DNA extracts were subjected to polymerase chain reaction (PCR) for the detection and amplification of 16S rRNA genes. Primers used were obtained commercially using the conditions of the initial denaturation at 94^{o}C for 5 minutes, denaturation at 94^{o}C for 30 seconds, annealing at 5^{o}C for 45 seconds and extension at 72^{o}C for 1 minute (Hamid *et al.*, 2020).

Antibacterial activity using TLC-Bioautographic profile

Susceptibility testing was carried out using bio-autography alternative method (Saik *et al.*, 2014).

Mueller-Hinton agar plates were used and 0.2ml of *M. oleifera* leaf extract was loaded onto the TLC plates in a narrow band and elucidated using the suitable mobile solvents (n-hexane, ethyl acetate and dichloromethane). The chromatogram layer was faced down onto the inoculated agar layer for 10 minutes to enable the diffusion. Chromatogram was removed and the agar layer was incubated at 37^{o}C for 24 hours. Plates were observed for clear zone of inhibition for the diameter measured in mm, recorded and compared with a known antibiotic (Ciprofloxacin).

RESULTS AND DISCUSSION

Physiochemical parameters of the water

The mean physicochemical parameters of both sachet and tap water are shown in Table 1 and Table 2. The pH of both sachet and tap water samples were within the range of 4.5-6.5. The pH for tap water is not statistically significant ($p = 0.784$),but for sachet water there was a significant difference ($p = 0.012$).The temperature of both sachet and tap water sample were found to be within the range of 26-29^{o}C, and were significantly different ($p = 0.027$). The turbidity of both sachet and tap water samples were found to be within the range of 0.2-3.5NTU, but there was no significant difference.in turbidity for sachet and tap water. With regards to dissolved oxygen, it was found to be within the range of 0.2-2.4mg/L, and there was no significant difference between sachet and tap water. BOD of the water samples was found to be within the range of 1.2-6.0 and 1.2-3.6mg/L.

The values of these parameters in all the samples were within the range of WHO and NAFDAC permissible limits (SON, 2007; WHO, 2021). Previous studies tend to show lower temperatures, which can be due to storage of the water samples, or differences in climate. Dirican *et al*. (2009), in their study, reported average water temperatures of 15.80±3.95 and 14.10±2.40^{o}C, from underground water. The variance in temperature between sachet and tap water can be due to the exposure of the sachet water to environmental conditions (rainfall, sunlight and human activities) and the tap water is transported via underground pipes which show variation in temperature with regards to time of sample collection.

In the current study, the pH values obtained were all below 6.5. According to the WHO (2010), consuming water of pH less than 6.5 units may be harmful to humans, and a pH range 6.5-8.5 units would be suitable for the protection of human and aquatic animals (Egemen *et al.*, 2011). Dissolved Oxygen of all the tap water samples fall outside the permissible limits of WHO and NAFDAC (5-8mg/L). However, since the values are below, not above the threshold limits, this is not an issue of concern. However, some previous studies had reported dissolved oxygen in waters above 5mg/L, which was reported to be adequate for human and aquatic life (Egemen *et al.*, 2011).

The turbidity of both tap and sachet water samples were not significantly different to the WHO and NAFDAC permissible limit of tap water (5) and sachet water (≤5) respectively. This showed that relatively adequate purification techniques were used for the removal of suspended solids from the waters used for the study. Summarily, sachet water is more physicochemically qualitative than tap water.

Table 1: Mean Values of Physicochemical Parameters of sachet water obtained from five locations within Katsina Metropolis

Parameters	Profiles of sachet water from five sampling sites						WHO	NAFDAC
	SDU	SKR	SKE	SKW	SKM	P-value		
pH	7.02±0.00^{a}	5.72±0.00^{b}	4.58±0.00^{d}	5.26±0.00^{cd}	4.85±0.00^{c}	0.012*	6.5 – 9.2	6.5 – 8.5
Temperature (^{o}C)	26.70±0.00^{bc}	27.50±0.40^{a}	26.90±0.00^{ab}	26.60±0.00^{bc}	26.20±0.00^{c}	0.027*	20 – 30	20 – 27
Turbidity (NTU)	0.20±0.00^{a}	0.20±0.00^{a}	0.35±0.05^{b}	0.40±0.00^{ab}	0.47±0.01^{a}	0.001**	5	≤5
DO (mg/l)	1.44±0.07^{bc}	1.34±0.06^{c}	1.63±0.06^{b}	2.03±0.07^{a}	1.55±0.06^{bc}	0.000**	5 – 8	5 – 8
BOD (mg/l)	0.79±0.00^{b}	0.19±0.00^{c}	0.59±0.00^{c}	1.09±0.01^{a}	0.49±0.00^{d}	0.000**	1.2 – 6.1	1.2 – 6.1

Legends: Means having different superscripts across the row are significantly different (P≤ 0.05). NTU = Nephelometric Turbidity Unit, DO=Dissolved Oxygen, BOD=Biological Oxygen Demand. * Significant at (P≤0.05) ** Significant at (P≤0.01).
SDU= Kofar Durbi, SKW= Kofar Kwaya, SKR= Kofar Kaura, SKE= Kofar Keke , SKM= Kofar Marusa.

Table 2: Mean Values of Physicochemical Parameters of tap water obtained from five locations within Katsina Metropolis

Parameters	Profiles of tap water from five sampling sites						WHO	NAFDAC
	TKR	TKW	TKM	TDU	TKE	P-value		
pH	5.91±0.18^{a}	5.93±0.19 ^{a}	5.82±0.18 ^{a}	5.90±0.13 ^{a}	6.11±0.12 ^{a}	0.784$_{ns}$	6.5 – 9.2	6.5 – 8.5
Temperature (^{o}C)	29.72±0.32^{a}	27.81±0.57^{a}	28.59±0.5^{a}	28.14±0.4^{a}	28.86±0.29 ^{a}	0.430$_{ns}$	20 – 30	20– 27
Turbidity (NTU)	1.58±0.02^{b}	3.58±0.03^{a}	0.71±0.02^{c}	0.45±0.02^{d}	0.67±0.02^{c}	0.000**	5	≤5
DO (mg/l)	1.85±0.06^{b}	1.45±0.06^{c}	2.45±0.06^{a}	1.87±0.07^{b}	0.85±0.06^{d}	0.000**	4 – 6	4 – 6
BOD (mg/l)	0.19±0.000^{d}	0.39±0.00^{c}	1.19±0.00^{a}	0.59±0.01^{b}	0.19±0.00^{d}	0.000**	1.2 – 3.6	1.2 – 3.6

Legends: Means having different superscripts across the row are significantly different (P≤ 0.05). NTU=Nephelometric Turbidity Unit, DO=Dissolved Oxygen, BOD=Biological Oxygen Demand. * Significant at (P≤0.05) ** Significant at (P≤0.01), ns=not significant.
TDU= Kofar Durbi, TKW= Kofar Kwaya, TKR= Kofar Kaura, TKE= Kofar Keke, TKM= Kofar Marusa.

Microbiological analyses (MPN technique)

Higher MPN values were obtained in tap water than in sachet water. The highest counts of 3 were obtained for sachet water of Kofar Kwaya and tap water of Kofar Keke (Table 3).Three samples were found to be not-potable: sachet water from Kofar Kwaya and tap water from Kofar Marusa and Kofar Keke. The rest of the samples all have MPN<1, and hence are potable (Table 3). Therefore, the MPN results from the study indicated that the bacterial count of sachet water consumed in Katsina Metropolis is lower, compared to the tap water. Thus, the results from this study indicate that microbiologically, sachet water is a better source of drinking water than tap water (Oyedeji *et al*., 2010).

Table 3 Coliform count for the number of positive tubes from the water samples

SAMPLES ID	No. of tubes showing positive reaction	MPN/100 ml
SKWa	1-1	3
SKWb	1-0	2
SKWc	1-0	2
SKWe	1-1	3
SKWf	1-1	3
SKWg	0-0	0
SKWi	1-0	2
SKRa	1-0	2
SKRh	1-0	2
TDUh	1-0	2
TKEc	1-1	3
TKEd	1-0	2
TKEf	1-1	3
TKEh	1-1	3
TKEj	1-0	2
TKMa	1-0	2
TKMi	1-0	2

Legend: T = tap water, S = Sachet water, a,b,c,d,e,f,g,h,i = sample collection points, KM= Kofar Marusa, KD=Kofar Durbi, KKR=Kofar Kaura, KKE-Kofar Keke, KKW=Kofar Kwaya.

Isolation and Identification of Bacteria

On the basis of biochemical test reactions, colonial morphology and gram's reaction, 17 bacterial isolates were identified in this research. *E. coli* and *E. faecalis* were the most frequently identified isolates from the research (6/17, or 35.29%, each) followed by *E. faecium* (5/17, or 29.41%) (Table 4).

Thus, based on the biochemical characterization, the predominant bacterium identified from the water sample was *Escherichia coli*, and this is in agreement with previous findings of Ibemesim (2014) who isolated *Escherichia coli* from sachet and tap water. Moreover, the isolation of *Escherichia coli* and *Enterococcus faecalis* from the waters is in conformity with previous studies, such as Svagzdiene *et al*. (2010).

Table 4: Biochemical test identification of the isolated bacteria

Isolate No.	GS	Cat	Cit	Ind	L	M	Man	MR	Mot	TSIA			VP	Suspected Organism
										L	S	G		
SKWa	-	+	-	+	-	-	-	+	+	-	+	+	-	Ec
SKWb	-	+	-	+	-	-	-	+	+	-	+	+	-	Ec
SKWc	-	+	-	+	-	-	-	+	+	-	+	+	-	Ec
SKWe	-	+	-	+	-	-	-	+	+	-	+	+	-	Ec
SKWf	+	-	-	-	+	+	+	-	-	+	-	-	+	Efl
SKWg	+	-	-	-	+	+	-	-	-	+	+	+	+	Efc
SKWi	+	-	-	-	+	+	-	-	-	+	+	+	+	Efc
SKRa	+	-	-	-	+	+	-	-	-	+	+	+	+	Efc
SKRh	-	+	-	+	-	-	-	+	+	-	+	+	-	Ec
TDUh	+	-	-	-	+	+	+	-	-	+	-	-	+	Efl
TKEc	+	-	-	-	+	+	+	-	-	+	-	-	+	Efl
TKEd	+	-	-	-	+	+	-	-	-	+	+	+	+	Efc
TKEf	+	-	-	-	+	+	-	-	-	+	+	+	+	Efc
TKEh	+	-	-	-	+	+	+	-	-	+	-	-	+	Efc
TKEj	-	+	-	+	-	-	-	+	+	-	+	+	-	Ec
TKMa	+	-	-	-	+	+	+	-	-	+	-	-	+	Efl
TKMi	+	-	-	-	+	+	+	-	-	+	-	-	+	Efl

Legends:

Cat= Catalase, Cit = Citrate, Ind= Indole, L= Lactose, M = Maltose, Man= Mannitol, MR= Methyl red Mot= Motility TSIA= Triple sugar iron agar, VP= Voges-Proskauer, Ec = *E. coli,* Efl = *E. faecalis,* Efc = *E. faecium,* S = Sucrose, G = Glucose

Molecular (16S rRNA) identification of the bacteria

The commonest bacteria isolated from the water samples (six isolates) were further subjected to molecular identification. The gel electrophoresis result of the PCR amplification of the 16S rRNA gene were shown in figure 1. Two isolates had their 16S rRNA gene sequences successfully amplified, and their sequences were successfully obtained. The first of these isolates was as found to share the closest similarity to *Enterobacter kobei* strain CIP 105566 (97.86% similarity) and hence was assigned the name *Enterobacter kobei* AA01 (figure 2, table 5).On the other hand, the second isolate was found to share the highest similarity with *Escherichia marmotae* strain HT073016(79.53%) and hence was assigned the name *Escherichia marmotae* AN01 (figure 3, table 5). However, this percentage similarity is rather low, and hence, further studies, such as DNA-DNA hybridization studies, are necessary to definitively confirm the identity.

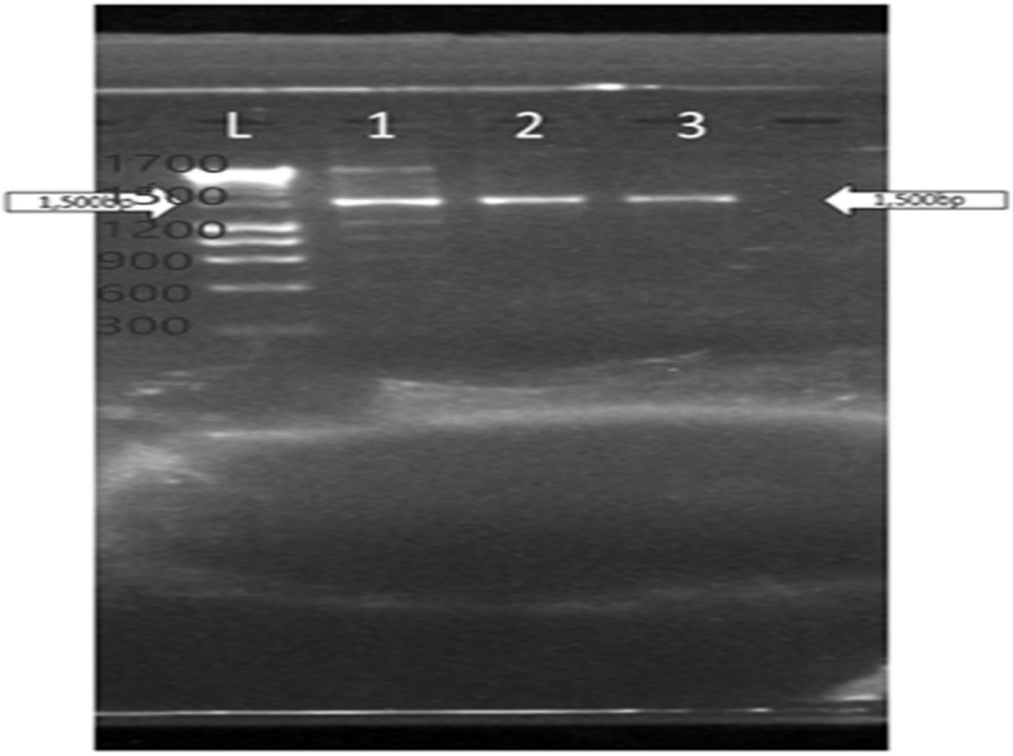

Figure 1: The Agrose gel electrophoresis image of the PCR products of fragment 16 small sub-unit ribosomal RNA (16S rRNA) from bacterial isolates. Lane 1 represent X-sample, 2 for Y-samples and 3 represent Z-sample. L represent hyperladder 1kb [bioline, biosystem (200bp to 10, 000kb)].

Generally, 16S rRNA analysis is regarded as a gold standard in identification of microbes from water sources (Enaigbe *et al*., 2019; Olowe *et al*., 2017; Ghatak *et al*., 2013). The bacteria identified in this study had been reported before, in some few literatures. The first mention of *Enterobacter kobei* was by Kosako *et al*. (1996), where they described as Gram-negative, motile rods of the family *Enterobacteriaceae* isolate from clinical samples. *Enterobacter kobei* isolated from the samples represents a novel water contaminant. The bacterium is grouped into the *Enterobacter cloacae* complex, and has been isolated from filtered water (Kamper *et al*., 2015).

As for *Escherichia marmatae*, it was first described as a novel species in 2015 by Liu *et al.* (2015), as a Gram negative, non-sporulating, non-motile, short rods (0.5–161–2.5 mm), isolated from a rodent in the Himalayas, having close similarity with *Escherichia fergusonii* ATCC35469T(99.3%), *Escherichia coli* ATCC11775T(99.2%) and *Escherichia albertii* LMG20976T(98.9%). This shows the link between this bacterium and contamination of water with faeces of human/animal origin (Liu *et al.,* 2015). To the best of our knowledge, this study is amongst the first to report the presence of *Escherichia marmatae* as a potential faecal contaminant of water.

Table 5. BLASTN sequence of *Enterobacter* and *Escherichia* species

S/N	Name of the bacterial specie	Max Score	Total Score	Query Cover	E value	% Identity
1	***Enterobacter kobei* AA01***	**-**	**-**	**-**	**0.0**	**-**
2	*Enterobacter cloacae* subsp. *dissolvens* ATCC 23373	1291	1291	71%	0.0	97.99%
3	*Enterobacter ludwigii* EN-199	1291	1291	71%	0.0	97.99%
4	*Enterobacter kobei* CIP 105566	1288	1288	71%	0.0	97.86%
5	*Enterobacter bugandensis* strain 247BMC	1242	1242	70%	0.0	97.15%
6	****Escherichia marmotae* AN01**	**-**	**-**	**-**	**0.0**	**-**
7	*Escherichia marmotae* HT073016	436	436	99%	0.0	79.53%
8	*Escherichia ncoli* NBRC 102203	442	442	99%	0.0	79.66%
9	*Escherichia albertii* Albert 19982	422	422	93%	0.0	79.83%
10	*Escherichia fergusonii* ATCC 35469	442	442	99%	0.0	79.69%

Key: * = The sequence of the isolated bacterium designated *Enterobacter kobei* strain AA01; ** = The sequence of the isolated bacterium, designated *Escherichia coli* AN01. = Not applicable, E value = Error/random background noise.

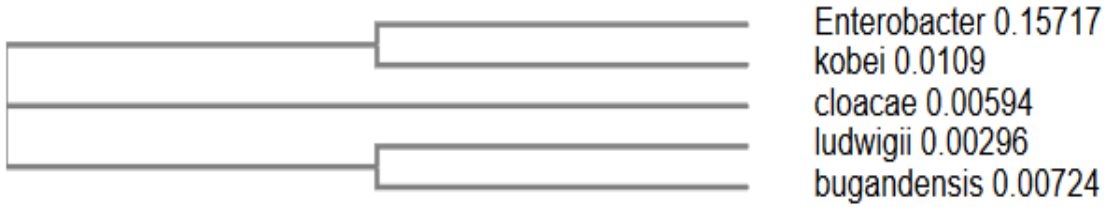

Figure 2. Phylogenetic clustering of *Enterobacter* species nearest to the isolated organism's sequence. Enterobacter represents the bacterium's sequence (designated *Enterobacterkobei* AA01); kobei represents *Enterobacter kobei* strain CIP 105566, which shares the homology with the sequence of the isolated bacterium; cloacae represents *Enterobacter cloacae* subsp. *dissolvens* strain ATCC 23373; ludwigii represents *Enterobacter ludwigii* strain EN-199 and bugandensis represents *Enterobacter bugandensis* strain 247BMC.

Figure 3. Phylogenetic clustering of *Escherichia* species nearest to the isolated organism's sequence. Escherichia represents the bacterium's sequence (designated *Escherichia coli* AN01); marmotae represents *Escherichia marmotae* strain HT073016, which shares the homology with the sequence of the isolated bacterium; coli represents*Escherichia coli* strain NBRC 102203; albertiii represents *Escherichia albertii* strain Albert 19982 and fergusonii represents *Escherichia fergusonii* strain ATCC 35469.

GC-MSanalysis of the *M. oleifera* leaf extract

The total ion chromatogram (TIC) showed the fingerprint of fifteen compounds identified in the *M. oleifera* leaf by GC-MS analyses. The chromatogram showed fifteen peaks which indicated the presence of fifteen bioactive constituents. The main abundant compounds were (9, 12, 15-Octadecatrienoic acid, ethyl ester-with peak area 25.62%) and 32.6352 RT, followed by (Octadec-9-enoic acid with peak area 12.53%) and 32.5456 RT, then (Hexadecanoic acid, ethyl ester with peak area 11.11%) and 31.3169 RT. The least constituent was (9-Octadecenoic acid with peak area 1.74%) and 32.733 RT (Table 6).

Table 6.Most abundant compounds in the Gas-Chromatography Mass Spectrometry Chromatogram of *M. oleifera*

S/N	Retention time	Peak area	Name of the compound	Usage (in previous literature)	Reference
1	32.6352	25.6168	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)- $C_{18}H_{30}O_2$	This compound belongs to the class of molecules called surfactants, used in agriculture, as surface active agents, and in the pharmaceutical industry. It is classed as safe/of low concern by the US Environmental Protection Agency.	[NCBI, 2021]
2	32.3443	0.7187	Octadec-9-enoic acid $C_{18}H_{34}O_2$	It is used in the manufacture of soap, as an excipient in pharmaceuticals, as an emulsifier/solubiliser in aerosols and in the synthesis of nanoparticles.	[Yin *et al.*, 2017]
3	34.3841	9.9346	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester ($C_{21}H_{38}O_4$)	Known as 'the magic lipid', this compound is considered as one of the most widely used substances in drug delivery, emulsion stabilization and protein crystallization. This compound is also used pharmaceutically to offer protection to drug active components against degradation.	[Kulkarni *etal.*, 2011]
4	32.1773	6.4201	7,10,13-Hexadecatrienoic acid, methyl estrer	This compound is considered as one of the leading phytochemicals expected to spearhead novel drug development, and had been shown to be antimicrobial to multidrug resistant gram positive bacteria, and also selected strains of gram negative bacteria. It is also used as an adjuvant in agriculture,	[Kurkehar *etal.*, 2020]

These compounds identified from the petroleum ether fraction of *M. oleifera* are medicinally valuable and possess various pharmaceutical applications (Visveshwari *et al.*, 2017). Moroever, 9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)- $C_{18}H_{30}O_2$ belongs to surfactants, or surface active agents, and is commonly used in the pharmaceutical industry. It is classed as safe/of low concern by the US Environmental Protection Agency (NCBI, 2021).Octadec-9-enoic acid $C_{18}H_{34}O_2$is used as an excipient in pharmaceuticals, as an emulsifier/solubiliser in aerosols and in the synthesis of nanoparticles, which can have pharmaceutical applications (Yin *et al.*, 2017). Furthermore, -Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester ($C_{21}H_{38}O_4$) is known as the the magic lipid', this compound is considered as one of the most widely used substances in drug delivery, and is also used pharmaceutically to offer protection to drug active components against degradation (Ganem *et al.,* 2000; Kulkarni *et al.*, 2011); while 7,10,13-Hexadecatrienoic acid, methyl ester is considered as one of the leading phytochemicals expected to spearhead novel drug development, and had been shown to be antimicrobial to multidrug resistant gram positive bacteria, and also against selected strains of gram negative bacteria (Kurkehar, 2020).Furthermore, most of these identified compounds have a great impact in human body as well as human health in particular because some are used as food products; while others are used in the production of medical drugs/antibiotics (Aja *et al.*, 2014). Moreover, previous studies had identified other medicinally active phytochemicals in *M. oleifera*. Bukar *et al.* (2010), reported that the presence of a short polypeptide, 4 (ȧ- L - rhamnosyloxy) benzyl- isothiocyanate in *M. oleifera* act directly against microorganisms and result in growth inhibition by disrupting cell membrane synthesis or synthesis of essential enzymes.

The present study results confirmed the traditional uses of *M. oleifera* as an antimicrobial and in water treatment (Delelgn *et al*., 2018). This is in agreement with the study of Enas *et al*. (2014), where *M. oleifera* leaves methanol extract seem to possess medicinal properties. This implies that *M. oleifera* leaves can be used in water treatment, since the extract possesses antibacterial activity against common bacterial contaminants found in water. This result also corroborates the use of *M. oleifera* leaves in water purification, as described before (Ayotunde *et al*., 2011).

Antimicrobial activity of the *M. oleifera* leaf extract using TLC-bioautography

In this study, the isolates identified using biochemical characterization (table 4 above) are from three species, namely: *E. coli, E. faecalis* and *E. faecium.* Therefore, pure cultures of these bacteria were obtained and used for the TLC-bioautography experiments.

The results, presented in table7 below, showed that the petroleum ether extract of *M. oleifera* leaf exhibit a significant antibacterial profile of against the isolated bacteria. All the bacterial isolates were tested against various concentration of the *M. oleifera* leaf (100mg/ml, 300mg/ml and 500mg/ml). The isolated *E. coli* were susceptible at 500mg/ml which is the highest concentration of the extract that inhibited bacterial growth resulting in visually clear zone overnight incubation, followed by TLC plate with range from 15±0.00 to 19.5±4.95 mm. There is a significant difference ($p<0.05$) among the zones elicited against the bacterial isolates at 500mg/ml.

Similarly, the isolated *E. faecalis* were sensitive at 500mg/ml of *M. oleifera* leaf extract. The TLC plate also revealed promising activity ranging from 15±7.07 to 23.5±6.36 mm. There is also significant difference ($p<0.05$) among the zones elicited against the bacterial isolates at 500mg/ml. Moreover, the isolated *E. faecium* were also sensitive at 500mg/ml, followed by TLC plate with range from 20±0.00 to 22±1.41 mm. The result was found to be significantly higher ($p<0.05$). Hence, there is significant difference among the bacterial isolates at 500mg/ml (table 7).

Table 7: TLC-Bioautographic profile of *M. oleifera* leaf extract against the isolated bacteria

Sampling sites/Isolated Bacteria	***Moringa oleifera* extract**				**Ciprofloxacin**		
	Zone of inhibition produced (mm ± standard deviation)						
E. coli	**100mg/ml**	**300mg/ml**	**500mg/ml**	**TLC**	**100mg/ml**	**300mg/ml**	**500mg/ml**
SKWa	11.5±0.71	12.5±0.71	16±1.41	16.5±4.95	16±1.41	21±1.41	24.5±0.71
SKWb	11±2.83	11±1.41	16±0.00	15±0.00	22.5±3.54	19±4.24	22.5±3.54
SKWc	12.5±2.12	13.5±2.12	15±2.12	15±0.00	21±0.00	21.5±4.95	20±0.00
SKWe	12.5±3.54	15±1.41	17±1.41	13.5±2.12	18±1.41	20±0.00	21±1.41
SKRh	10.5±0.71	15±0.00	17±0.00	17±1.41	18±2.83	22.5±3.54	25±0.00
TKEj	11.5±0.71	11±1.71	15±0.00	17.5±3.54	19.5±4.95	20.5±0.00	24±0.00
E. faecalis							
SKWf	9.5±0.71	13±1.41	16.5±2.12	15±7.07	22.5±3.54	21±1.41	25±0.00
TDUh	10±0.00	13±0.00	18±1.41	17.5±3.54	17.5±3.54	19.5±0.71	21±1.41
TKEc	12±1.41	14.5±0.71	16.5±2.12	22.5±3.54	20.5±0.71	22±4.24	24.5±0.71
TKEh	13±1.41	15±0.00	18±0.00	23.5±6.36	21.5±2.12	22±0.00	23±2.83
TKMa	11±1.41	16±0.00	19±0.00	16±5.66	22.5±3.54	21±4.24	26.5±2.12
TKMi	11.5±0.71	11±1.71	16±1.41	17.5±3.54	20.5±4.95	20.5±0.00	25±0.00
E. faecium							
SKWg	12±1.41	14.5±0.71	18.5±0.71	20±0.00	19.5±0.71	22.5±3.54	24.5±6.36
SKWi	13±0.00	17±1.41	19.5±0.71	20.5±0.71	20.5±2.12	21.5±0.71	21±1.41
SKRa	12.5±2.12	17±2.83	19±1.41	22±1.41	19±1.41	21.5±2.12	22.5±3.54
TKEd	12.5±3.54	15.5±0.71	19.5±0.71	20±1.41	20±0.00	24±2.83	24.5±4.95
TKEf	13±1.41	15±1.41	19±0.00	21±1.41	22±0.00	22±4.24	24±2.83

Legends:SKW= Sachet water from KofarKwaya;TDU= Tap water from KofarDurbi;TKE= Tap water from Kofar Keke; TKM= Tap water from Kofar Marusa; SKR = Sachet water of Kofar Kaura, a,b,c,d,e,f,g,h,i,j = sampling locations.

The TLC bioautography showed promising potential of the extract against all the tested bacteria, even though ciprofloxacin showed higher activities. Zone of inhibition values are helpful in estimating the potential of antibacterial activities by comparing with the respective control. Adeyinka and Famurewa (2011), reported that plant extracts containing chemicals with antibacterial properties have been useful in treating bacterial and fungal infections.

The TLC bioautography showed promising potential of the extract against all the tested bacteria, even though ciprofloxacin showed higher activities. The activities of zone of inhibition values are helpful in estimating the potential of antibacterial activities by comparing their respective control. Adeyinka and Famurewa (2011), reported that plant extracts containing chemicals with antibacterial properties have been useful in treating bacterial and fungal infections. The strong activities suggest that *M. oleifera* may be used for treatment of infections due to *E. coli*, *E. faecalis* and *E. faecium*. Moreover, previous studies had reported the antibacterial activity of methanol extracts of *M. oleifera* against a wide variety of pathogenic microbes, in particular *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus faecalis*, *Klebsiella pneumonia* and *E. coli* (Zubair, 2020).

CONCLUSION

The physicochemical parameters of sachet and tap water consumed within Katsina Metropolis are within the range of different from WHO, FAO and NAFDAC standards, however, the microbiological quality of some water samples exceeds WHO allowable limits, indicating tendency of inadequate processing. *Escherichia coli, Enterobacter faecalis* and *Enterobacterfaecium* were identified from the water sample. Based on 16S rRNA analyses, *Enterobacter kobei* and *Escherichia marmotae* were found as uncommon bacterial species that could contaminate sachet and tap water in the study area. Fifteen (15) bioactive compounds, (including: 9,12,15-Octadecatrienoic acid, ethyl ester; 9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester; Octadec-9-enoic acid; and7,10,13-Hexadecatrienoic acid, methyl ester) found in *M. oleifera* leaf could be responsible for its antibacterial effect against the bacterial isolates. Government and Regulatory agencies should encourage domestic and industrial sewage treatment in order to avoid point source contamination of sachet and tap water. However, further studies should be carried out on water purification using *Moringa oleifera* seed.

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