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Moringa oleifera leaf as a natural water purifier and causes decontamination of fecal-coliform bacteria

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Abstract

This study aimed to focus on the Nile's raw water purification and sanitation in the Warraq al Hadar area, Egypt, using *Moringa oleifera* leaf powder. The water turbidity, pH, and total fecal-coliforms were assessed. The water turbidity and pH were decreased on addition of *M. oleifera* leaf powder at 2, 4, 6, 8, and 10 µg\ ml; with reductions of 49.8, 68.7, 87.4, 92.0, and 93.7 % for turbidity, while the pH was reduced by 12.37, 20.0, 28.14, 38.14, and 38.14%, respectively. Using the most probable number approach (MPN), the highest decrease in the coliform bacterial count was observed at 10 µg\ ml of MOL recording 60 cfu\ 100 ml. On estimating the *in vitro* inhibitory effect of *M. oleifera* leaf powder on the fecal-coliform bacterial strains using the agar well diffusion assay, *Escherichia coli* ATCC 8739 was the most affected strain, recording an inhibition zone diameter of 37.0 mm, while *Salmonella typhi* DSM 17058 was the least inhibited strain with an IZD of 30.0 mm. The minimum inhibitory concentration (MIC) of *M. oleifera* leaf powder was 6.25 µg\ ml for *E. coli*, *Shigella sonnei* DSM 5570, and *S. typhi* DSM 17058, while it was 12.5 µg\ ml for *Enterococcus faecalis* ATCC 7080. The minimum bactericidal concentration (MBC) of the *M. oleifera* leaf powder was equivalent to 25.0 µg\ ml for *Enterococcus faecalis*, 12.50 µg\ ml for *S. typhi*, and 6.25 µg/ ml for *E. coli* and *Shigella sonnei*. Biocompatibility of the *M. oleifera* leaf powder was confirmed using the oral epithelial cell lines (OEC), which recorded an IC_{50} of 716.1 µg\ ml, whereas cell viability of 100.0, 94.1, 90.5, and 88.0 % was observed on application of the MOL powder at different doses of 25, 50, 100, and 150 µg\ ml, respectively.

Keywords: Antibacterial activity, *Moringa oleifera*, Water purification, Cytotoxicity

1. Introduction

Water is the secret of life on Earth. Water that is used by all organisms; especially for human consumption, is fresh surface water or groundwater that has undergone chemical treatment and these

chemicals have been deposited as dissolved and\or suspended elements (Rashid *et al*., 2021). Natural water exists at or near the earth's surface and comes into touch with the sedimentary and inventive rocks, thus raising metal levels into water (Liang *et al*., 2019; Hashim *et al*., 2021). Humans use water for several purposes, but the cleanliness of water they drink is crucial since it directly affects their health. According to Rodrigues and Plotkin, (2020); Chales *et al*., (2022), microbes that enter the human body through contaminated water and food account for most of the death counts in the undeveloped countries, especially in Africa.

The water treatment stations utilize inorganic chemical coagulants such as aluminum and ferric salts, as well as various charged organic polymers (Quesada *et al*., 2019). Although these coagulants are effective in reducing the particle and organic hindrances; however, they may be non-cost effective and harmful; in addition, they need special store and handling requirements (Yamaguchi *et al*., 2021). The previous study conducted by Likus *et al*., (2021) reported that the coagulants may also cause secondary water problems, such as residual iron and aluminum species, as well as harmful synthetic polymers in the treated water. All the chemicals used in water purification are costly, thus the use of native products such as plant extracts for clarification and purification of drinking water in the underdeveloped nations is an urgent need (Delelegn *et al*., 2018; Yamaguchi *et al*., 2021). One of these famous medicinal plants is the *Moringa oleifera* (MOL) that belongs to the order Brassicales, which is a drought-resistant tropical tree planted in Africa, South and Central America, and in Asia (Moyo *et al*., 2012; Boulaadjoul *et al*., 2018). *Moringa*'s nutritional and therapeutic properties that had been formerly underutilized in several African locales, have now acquired widespread attention (Leone *et al*., 2018; Delelegn *et al*., 2018).

Because of the *M. oleifera* several qualities, this plant has a variety of uses in food, pharmaceutical, and in the sectors of cosmetics (Chodur *et al*., 2018), making it one of the universe's most relevant trees to man. *Moringa*'s strong nutritional contents have piqued the curiosity of numerous people, because of its high contents of vitamin A, vitamin C, calcium, iron, potassium, and of appropriate proteins quality, which rival that of milk and eggs (de Paula *et al*., 2018). Several folklore claims about *Moringa* have been validated by scientific research's, thus encouraging the evidence-based use of medicinal plants for the basic healthcare needs (Leone *et al*., 2018; Bancessi *et al*., 2020).

Several benefits have been scientifically proven and evaluated for *Moringa* extracts properties, including antioxidant, anti-ulcer, anti-inflammatory, anti-diabetic, anti-microbial, anti-mutagenic, cytotoxic, neuroprotective, herbicidal, anti-hypertensive, cardioprotective, anti-cancer, and hormone modulation (Ajuogu *et al*., 2019; Semanka *et al*., 2022). Furthermore, *Moringa* extracts are known to contain rich secondary metabolites of chemotaxonomic importance's, such as phenolics, flavonoids, and glucosinolate (Cirmi *et al*., 2019; Chales *et al*., 2022). The plant's leaves have also been discovered to be rich sources of bioactive small proteins and peptides, some of which have anti-toxic, antioxidant, and antibacterial activities (Villase˜nor-Basulto *et al*., 2018; Shi *et al*., 2019). So, according the previous studies conducted by Ajuogu *et al*., (2019); El Bouaidi *et al*., (2022), *M. oleifera* powder can be used in water treatment to remove many contaminants, including heavy metals, pharmaceuticals, pesticides, and dyes, in addition to the contaminating microbes. Furthermore, *M. oleifera* has several advantages that enable it to be applied in raw water clarifying treatment; especially in the low-income countries, including its abundance, low cost, low by-product, biodegradability, and safety (Yamaguchi *et al*., 2021).

The drinking water quality in many African developing countries, such as the Nile River in Egypt, is worsening. As a result, the objectives of this study were to focus on water purification and on fecal bacterial growth inhibition; mainly of *E. coli*, *Enterococcus faecalis*, *S. typhi*, and *Shigella sonnei*,

using *M. oleifera* leaf powder instead of the harmful chemical treatments.

2. Materials and methods

2.1. Samples collection

About 5 l of raw Nile water samples were collected in sterilized bottles from the Nile River in Warraq al Hadar. The Nile branch of Warraq al Hadar is located in Al Jizah (Giza), Upper Egypt, at 30° 6' 3" North, 31° 12' 58" East. The water samples were tagged and then sent to the Microbiology laboratory at Ain Shams University's Faculty of Agriculture, Cairo, Egypt. The samples were kept at 4°C for further investigation.

2.2. *Moringa oleifera* leaf source and powder preparation

Moringa oleifera fresh leaves (MOL) were obtained from a Royal organic farm in Beni Suef, Egypt, which is a well-known *Moringa* distributor. The received samples were preserved in sterile plastic bags and stored at 4 °C.

According to Delelegn *et al*., (2018), *M. oleifera* leaves were air-dried under shade, crushed using a laboratory pestle, and then sieved with 2.5 mm pore sieve. The leaf powder was kept at room temperature in a sterile plastic bag in darkness until used.

2.3. Treatment of water samples with MOL

According to the method conducted by Lea, (2010), MOL powder concentrations of 2, 4, 6, 8, and 10 µg\ ml were prepared. These MOL concentrations were distributed individually into 5 Erlenmeyer flasks, each containing 1 l of Nile raw water. As a negative control, 1 liter of raw water with no MOL powder was used. The flasks were mixed for 10 min. using a magnetic stirrer to produce coagulants. These suspensions were left statically for 1 h, and then the supernatants were separated using cotton pads. The resulting supernatants were used for evaluation of the total pH, turbidity, and for the bacterial coliform count estimation.

2.3.1. Turbidity and pH measurements of the treated water samples

Following the methods of Delelegn *et al*., (2018); Semanka *et al*., (2022), turbidity of the treated water samples was measured using a turbidity meter (Lab Junction, Model: LJ-331, India) before and after treatment with various MOL powder concentrations. About 10 ml of each treated water sample was used for the turbidity estimation, against a blank sample. Results were recorded as a nephelometric turbidity unit (NTU).

The pH of the water samples before and after treatment was measured using a digital pH meter (Hanna, Model: HI2210, UK). About 10 ml of each treated water sample was placed into a flask, and then the pH was measured. The measurement was repeated twice (Delelegn *et al*., 2018).

2.3.2. Estimation of reduction of the total fecal-coliform bacteria in the treated water samples

To estimate reduction in the total fecal-coliform bacterial load in the investigated water samples, the most probable number (MPN) assay of Cochran, (1950); Woomer, (1994); American Public Health Association (APHA. 2012) was applied. The MPN is used to determine whether the water is safe for human consumption in terms of bacterial existence or not. In this assay, the samples of water were serially diluted using sterilized dist. water to prepare 10^{-1}-10^{-5} dilutions. Each dilution was then used to inoculate 5 replicate tubes of MacConkey broth medium containing inverted Durham's tubes to detect gas production. All the inoculated tubes including the control tubes (non-inoculated broth) were incubated at 37°C for 24 h. After incubation, the tubes were scored as +/- for coliform growth, according to the change in color of the medium, and according to gas formation in the Durham tube.

If a tube is scored positive, it means that at least one culturable microorganism was present in the used dilution. The positive tubes were counted for each dilution and results were recorded to obtain the MPN

number. The Cochran statistical MPN table was used to extract the MPN index number. The total coliform count in 1 ml of water sample was calculated by dividing the MPN index number over the used dilution factor multiplied by 100, in reference to Cochran, (1950).

After this presumptive test, a confirmatory test was carried out to confirm the presence of fecal-coliform bacteria. About 1 ml from each MPN positive tube was inoculated into Brilliant Green Bile Lactose (BGLB) broth medium, followed by incubation at 37°C for 18-24 h. Gas formation in the Durham tubes confirmed the presence of coliform bacteria.

2.4. Fecal-coliform bacterial strains susceptibility to MOL

Fecal-coliform disinfection by MOL was investigated in vitro using the agar well diffusion assay, according to Lopez *et al*., (2011). Presentatives fecal-coliform strains were used, including *E. coli* ATCC 8739, *Enterococcus faecalis* ATCC 7080, *S. typhi* DSM 17058, and *Shigella sonnei* DSM 5570. These strains were provided by the Department of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University, Egypt. A standard inoculum of each tested fecal-coliform bacterial strain was prepared by inoculation of 250 ml Erlenmeyer flasks containing 50.0 ml of glucose broth medium with an individual loopful of each tested strain. The inoculated flasks were incubated on rotary shaker (150 rpm) for 24 h at 30°C (Benson, 2002). An aliquot of 1 ml from the standard inoculum (5.77-6.85×10^6 cfu\ ml) of each bacterial strain was spread onto the surface of Muller Hinton agar plates using a sterilized glass spreader, and left for 30 min at room temperature. Four equidistant wells were made on each plate using an 8.0 mm diameter sterilized cork borer. Approximately, 50 µl of the prepared MOL at a concentration of 1000 µg\ ml was applied to the agar wells, while Ampicillin (1000 µg\ ml) was used as a control. All the inoculated plates were then left for 30 min. before being incubated at 37°C for 24-48 h. Formation of a clear inhibition zone around the wells indicates the bacterial strain susceptibility to the MOL (Okwori *et al*., 2006). The assay was carried out in triplicates.

2.5. Minimum inhibitory concentration of MOL against the fecal-coliform strains

In consistence with Lopez *et al*., (2011), the minimum inhibitory concentration (MIC) of MOL against the fecal coliform bacteria was determined using the agar well diffusion technique. The MOL powder solution was diluted with a series of water two-fold dilutions to make final MOL concentrations ranging from 1000 to 6.25 µg\ ml. Using a Muller Hinton (MH) agar medium, the fecal-coliform bacterial inoculum was spread on the agar's surface using a sterilized glass spreader. The various concentrations of MOL had then been added individually and aseptically in each well as previously mentioned. After 24 h of incubation at 37°C, the powder's MIC was recorded through determining the minimal concentration of MOL that inhibited the fecal-coliform growth.

2.6. Minimum bactericidal concentration of MOL against the fecal-coliform strains

The minimum bactericidal concentration (MBC) of MOL against the fecal-coliform strains was determined according to the modified method of Spencer and Spencer, (2004). MH medium that showed no growth on MIC investigation was sub-cultured again on MH agar plate and then incubated at 37°C. After incubation for 24 h, the MBC value was determined as the lowest concentration of MOL that produced no bacterial growth.

2.7. Modes of action of MOL against the fecal-coliform bacterial strains

The mode of action of the MOL as an antibacterial agent against the tested fecal-coliform bacterial strains can be recorded as the ratio of MBC\ MIC (Berche *et al*., 1988). The action was recorded as bactericidal and\ or bacteriostatic effect when the MBC\ MIC ratio was ≤ 2 or ≥ 4, respectively.

2.8. MOL biocompatibility investigation using the MTT assay

For detecting the biocompatibility and safety of MOL before application in human consumption, the cytotoxic activity of the MOL was investigated in Nawah-Scientific, Cairo, Egypt, using the Oral epithelial cell line (OEC). The cells were grown in Dulbecco's Modified Eagle medium (DMEM), containing 10 % Fetal bovine serum (FBS), penicillin (100 units/ ml), and streptomycin (100 mg/ ml). The cultures were maintained at 37°C in a humidified atmosphere with 5% CO_2. Ten concentrations of two-fold serially diluted MOL were prepared. The MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium bromide) assay was conducted in reference to Van de Loosdrecht *et al*., (1994), where confluent monolayers of OEC were cultured for 24 h in a 96 well-microtiter plate. The cells were cultured in triplicates with varying doses of the tested MOL at 37°C for 72 h under a CO_2 environment. After incubation, 20 µl of 5 mg\ ml MTT was gently added to each well, and incubated at 37°C. After 4 h, the medium was gently removed and then 150 µl MTT solvent was added. The plate was covered with tinfoil and then the cells were shaker incubated for 15 min. on an orbital shaker (Benchmark Scientific-BT 300, California). Finally, the optical density (OD) was measured at 570 nm in a micro-plate reader (BMGLABTECH®FLUOstar Omega, Germany). A dose distribution curve was constructed using the data obtained after exposing the OEC cell lines to different concentrations of MOL.

2.9. Statistical analysis

The data were analyzed statistically using the SPSS Statistics (version 19) software, according to Duncan, (1955) at a *p*-value of ≤ 0.05.

3. Results and Discussion

3.1. Turbidity and pH measurements of treated water samples

Water purification quality was determined on treatment with MOL through measuring the turbidity and pH values of the treated water samples. Turbidity, which measures the refractive index of water, is a common indication of drinking water quality. Turbidity reduction in water may be particularly advantageous, as it reduces the majority of issues related to water pollution, such as microbes, solid particles, silt, and organic and inorganic compounds (APHA. 2012).

The World Health Organization (WHO) recommended that safe drinking water should have a turbidity of less than 5 NTU (WHO. 2008). In this study, the turbidity of raw water of the Nile River in the Warraq al Hadar consortium was found to be extremely high recording 320.3 NTU. After an hour of settling, addition of MOL powder at 2 µg\ ml decreased the turbidity from 320.3 to 160.8 NTU (49.8 %). However, at 4 µg\ ml, MOL powder reduced the turbidity to 100.3 NTU (68.7 %), whereas at 6 µg\ ml the turbidity was decreased to 40.5 NTU (87.4 %). Furthermore, at 8-10 µg\ ml of MOL, the reduction reached 25.5–20.2 NTU (92.0-93.7 %), respectively. Results showed that higher concentrations of MOL powder decreased the water turbidity (Table 1 and Fig. 1a).

As reported by Moulin *et al*., (2019); El Bouaidi *et al*., (2022), MOL extracts contain numerous protein components and positively charged free radical molecules that have the ability to coagulate and suspend the different molecules in the polluted water, thus successfully reduced the turbidity in the treated water samples. In addition, Valverde *et al*., (2018) revealed that the reduction in water sample turbidity on treatment with MOL extract may be attributed to the fact that MOL powders and extracts are known to have a cationic character, which causes bridge site saturation with such contaminants molecules.

Moulin *et al*., (2019) study also showed that MOL extracts contain numerous protein components that have the ability to cause coagulation, neutralization, and sedimentation of the different suspended particles that exist in the water samples, which may therefore be influenced by the various bimolecular interactions.

Table 1: Turbidity and pH of Warraq al Hadar Nile water samples on treatment with *M. oleifera* leaf powder

MOL concentration (µg\ ml)		pH		Turbidity (NTU)	
		Mean	Reduction efficiency (%)	Mean	Reduction efficiency (%)
Before treatment	0 (Control)	9.70^{e}	0.00	320.3^{f}	0.00
After treatment	2	8.50^{d}	12.37	160.8^{c}	49.8
	4	7.76^{c}	20.00	100.3^{b}	68.7
	6	6.97^{b}	28.14	40.50^{a}	87.4
	8	6.00^{a}	38.14	25.2^{d}	92.0
	10	6.00^{a}	38.14	20.5^{e}	93.7

Where; NTU= Nephelometric turbidity units. Values in the same column followed by the same superscript letter do not significantly differ from each other at $p \leq 0.05$ (Duncan, 1955)

These coagulating active components were identified as globulin and albumin proteins (Nordmark *et al*., 2018; Arnett *et al*., 2019). Many previous studies demonstrated that MOL extracts have been proven to be particularly successful in reducing the water turbidity in low doses, with turbidity reductions ranging from 73 % to 100 % (Baptista *et al*., 2017; Camacho *et al*., 2018; Landázuri *et al*., 2018; Zaid *et al*., 2019).

The pH of drinking water is defined by the WHO as that lies between 6.5 and 8.5 (WHO. 2008). In this study, the pH of the raw untreated water samples was not within the permissible limits (pH 9.7). At 2 µg\ ml of MOL, the pH dropped significantly to 8.5 (12.4 %) and to 7.76 (20.0 %), at 2-4 µg\ ml. The pH decreased to 6.97 (28.1 %) at MOL of 6 µg\ ml; however, at 8-10 µg\ ml of MOL the pH was decreased to 6.0 with 38.14 % reduction (Table 1 and Fig. 1b).

According to several previous studies, the active components of MOL that are responsible for pH reduction in the treated water samples are the water-soluble and thermo-resistant proteins, and the free fatty acids that exist in the *Moringa* powders (Baptista *et al*., 2017; Semanka *et al*., 2022). In addition, Santos *et al*., (2016); Nordmark *et al*., (2018) previously proposed that MOL proteins have a considerable adsorption affinity for destabilizing the particulate dispersions, which lowers the pH level.

Moreover, the decrease in pH of water observed with increasing the concentration of *Moringa* powder could also be attributed to the possible addition of excess positively charged protons (H^{+}) into water from the *Moringa* powder. When crushed *Moringa* seed powder was mixed with water, it yielded water-soluble proteins that possess a net positive charge (Chales *et al*., 2022). As a result, the *M. oleifera* leaf powder greatly lowers and/or elevates the pH of water depending on the used concentration. In agreement with the current findings, Moulin *et al*., (2019); Chales *et al*., (2022); Semanka *et al*., (2022) reported that the *Moringa* powder had decreased the pH of raw water. On contrary to the current results, previous studies conducted by Pritchard *et al*., (2009); Delelegn *et al*., (2018) highlighted the MOL powder did cause any detectable pH alterations in the treated raw water.

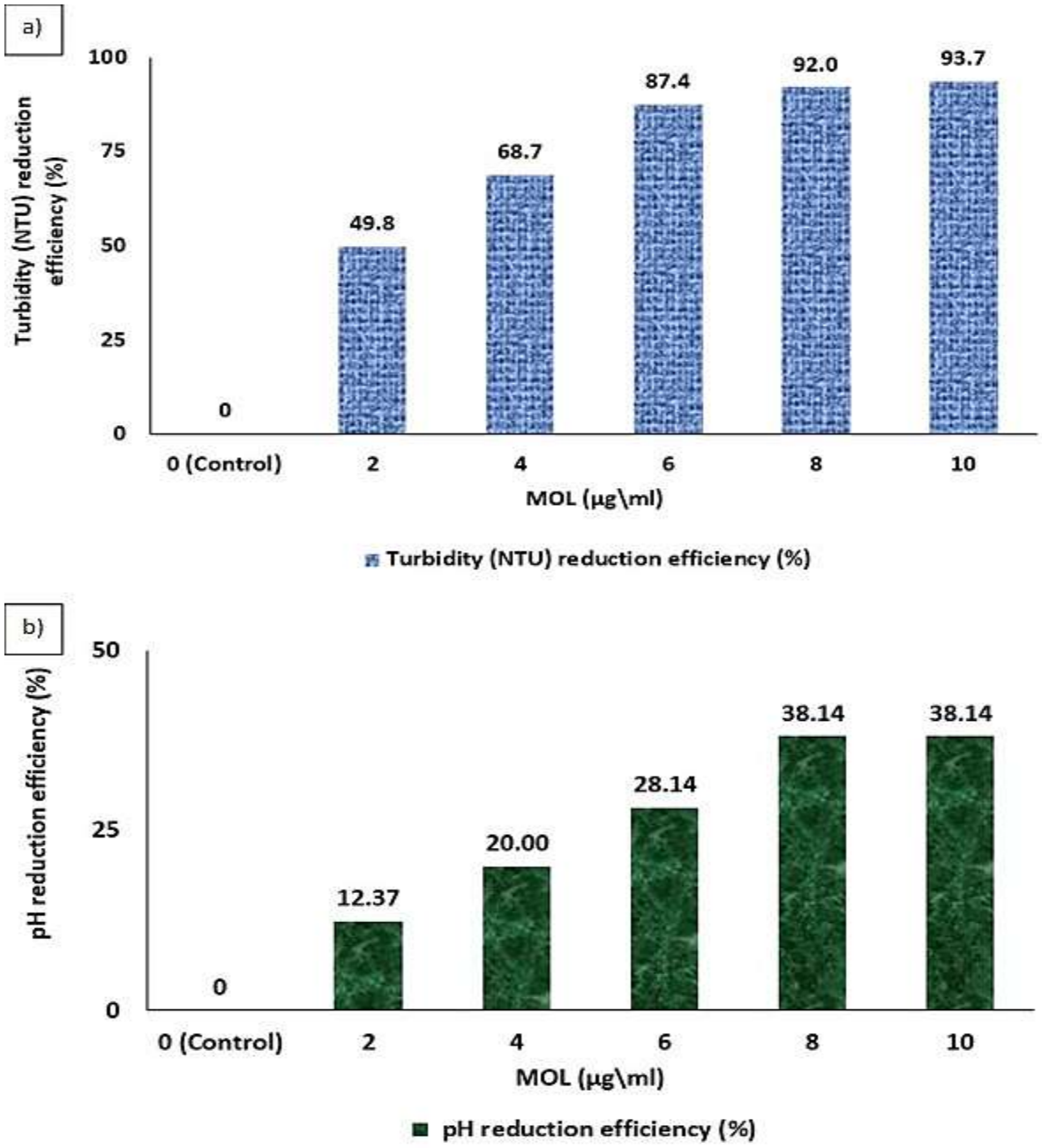

Fig. 1: Percentage (%) decrease in, a) Turbidity, and b) pH of Warraq al Hadar Nile water samples on treatment with varied MOL concentrations**.** Results are averages of 2 replicates

3.2. Estimation of reduction of total fecal-coliform load in the treated water samples

The data presented in Table (2) and Fig. (2) display the fecal-coliform count for both raw and treated water samples applying the most probable number (MPN) technique. The coliform counts differed significantly among the treated and untreated water samples. The MOL treated water samples had much lower coliform numbers, while the untreated samples contained more than 2600 coliforms\ 100 ml. The total coliform count was recorded to be 1400 coliforms\ 100 ml for the lowest dosage of MOL (2 µg\ ml), and 150 coliforms\ 100 ml for the concentration of 4 µg\ ml. However, at higher concentrations (6-8 µg\ ml), the total coliform count was 100-80 coliforms\ 100 ml, while it was 60 coliforms\ 100 ml for the highest concentration of 10 µg\ ml. The coliform number reductions were 46.2 %, 94.2 %, 96.2 %, 96.9 %, and 97.7 % for 2, 4, 6, 8, and 10 µg\ ml of MOL, respectively. Furthermore, gas production in the brilliant green lactose bile (BGLB) medium confirmed the presence of fecal-coliform bacteria in the examined water samples.

Table 2: Fecal-coliform total counts of Warraq al Hadar Nile water samples treated with *M. oleifera* leaf powder at various concentrations

MOL concentration (µg\ ml)		Coliform counts\ 100 ml	Reduction (%)
Before treatment	0 (Control)	2600^{f}	0.0
After treatment	2	1400^{e}	46.2
	4	150^{d}	94.2
	6	100^{c}	96.2
	8	80^{b}	96.9
	10	60^{a}	97.7

Where; values in the same column followed by the same superscript letter do not significantly differ from each other at $p \leq 0.05$ (Duncan, 1955). Results are averages of 5 replicates

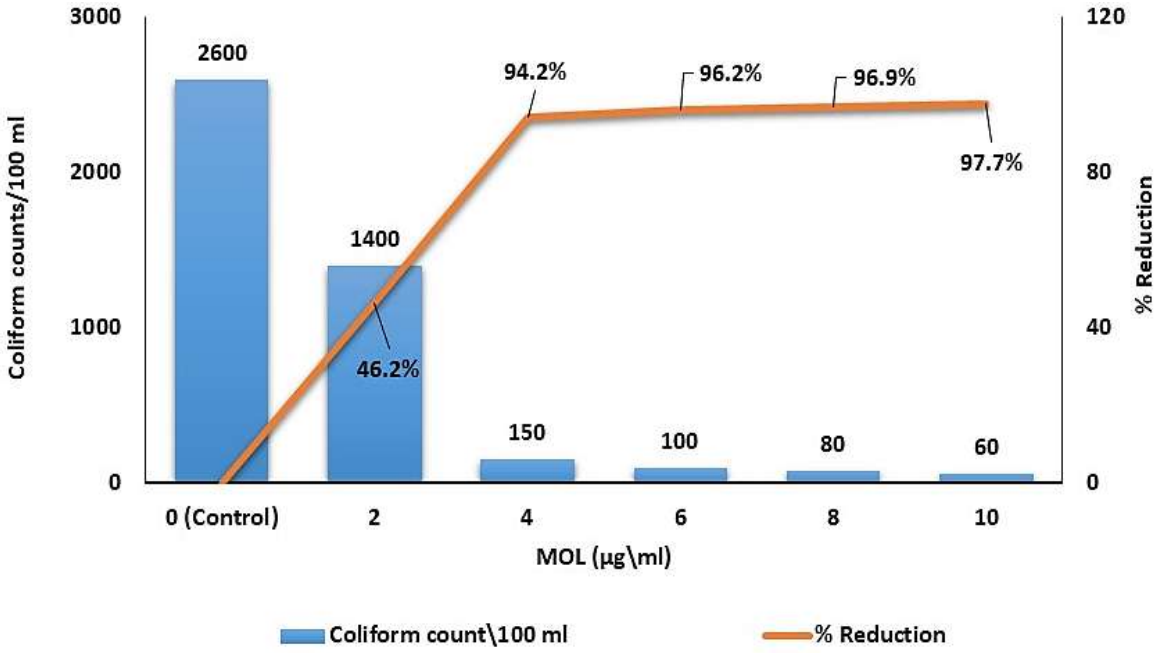

Fig. 2: Reduction of count and percentage (%) of coliform counts detected in Warraq al Hadar Nile water samples treated with various concentrations of *M. oleifera* leaf powder. Results are averages of 5 replicates

Similarly, Delelegn *et al*., (2018) reported that raw water samples collected from the Angereb and Shinta Rivers (i.e., one of the Nile River sources in Ethiopia and Eastern Sudan) had more than 2400 coliforms\ 100 ml, which were reduced to 500-900 coliforms\ 100 ml and 60-70 coliforms\ 100 ml on treatment with MOL at concentrations of 8.0 and 16.0 µg\ ml, respectively. The antibacterial potential of MOL that was responsible for coliform count reduction was attributed to its phytochemical ingredients, including flavonoids, glucosinolate, phenolic glycosides isothiocyanates, and phenolic acids, in addition to Vincosamide that belongs to indole alkaloids and glycosides of pyrrole alkaloids (Fahey *et al*., 2018; Cheng *et al*., 2019). On treatment of raw water with MOL, the proteins produced positive charges that served as magnets; attracting and swiftly sticking to clay, silt, bacteria, and other particles, which are mainly negatively charged particles that exist in the contaminated and turbid water (Panda and Ansari, 2013).

3.3. Fecal-coliform antibacterial susceptibility to MOL

According to the current results, all the fecal bacterial strains of *E. coli* ATCC 8739, *Enterococcus faecalis* ATCC 7080, *S. typhi* DSM 17058, and *Shigella sonnei* DSM 5570 were substantially sensitive to MOL powder ($p \leq 0.05$). The recorded IZ diameter of *E. coli*, *Enterococcus faecalis*, *S. typhi*, and *Shigella sonnei*, on treatment with MOL (1000 µg\ ml) were 35.0, 33.0, 37.0, and 30.0 mm, respectively. However, Ampicillin displayed the highest inhibitory effectiveness against all the tested bacterial strains, recording IZ diameter of 40.0, 35.0, 33.0, and 35.0 mm for *E. coli* ATCC 8739, *Enterococcus faecalis* ATCC 7080, *S. typhi* DSM 17058, and *Shigella sonnei* DSM 5570, respectively (Table 3).

Table 3: Inhibition zone diameter (mm) of coliform-fecal bacterial strains treated with MOL, compared to the ampicillin antibiotic

Fecal-coliform pathogenic strains	Inhibition zone diameter (mm)	
	Ampicillin (1000 µg\ ml)	MOL (1000 µg\ ml)
E. coli ATCC 8739	40.0± 0.1^{a}	37.0± 0.1^{bc}
Enterococcus faecalis ATCC 7080	35.0± 0.1^{b}	33.0± 0.5^{e}
S. typhi DSM 17058	35.0± 0.3^{b}	30.0± 0.7^{bc}
Shigella sonnei DSM 5570	35.0± 0.6^{b}	35.0± 0.5^{e}

Where, (±) = standard error (SE). Results are averages of 3 replicates. Values in the same column followed by the same superscript letter do not significantly differ from each other at $p \leq 0.05$ (Duncan, 1955). Results are average of 3 replicates

It was reported that tannins and polyphenols in *M. oleifera* have antibacterial properties (Khosravi and Behzadi, 2006; Manguro and Lemmen, 2007). In addition, in the previous works conducted by Ghebremichael, (2007); Santos *et al*., (2016), the coagulant proteins in MOL extracts diminished the count of *E. coli*. Williams *et al*., (2017) study confirmed that there were cationic polypeptide compounds in the MOL extracts that inhibited the fecal bacteria. Furthermore, the active compounds that exist in MOL are responsible for the flocculation mechanism of bridge formation, which occurs when positively charged macromolecules bind to the surfaces of negatively charged particles, resulting in surface charge neutralization, electrostatic repulsion reduction, and suspended particle agglomeration (Bancessi *et al*., 2020). In accordance with results of this study, Oluduro and Aderiye, (2007) highlighted that treatment of surface water with *M. oleifera* extract reduced the total coliforms counts by 97.5 %. Nkurunziza *et al*., (2009) revealed that treatment of raw contaminated water with *M. oleifera* powder resulted in 96 % removal of *E. coli*. In addition Xiong *et al*., (2018) investigated the use of MOL as beds to disinfect the raw water from *E. coli*, which exhibited a high removal efficiency of 99.0 %, with a total coliform count of 3-4 cfu\ 100 ml. In the same line, Chandrashekar *et al*., (2020); Begum *et al*., (2021) indicated that a crude extract from *Moringa* contained a coagulant protein that exhibited cell aggregation and growth inhibition of *E. coli* and *S. paratyphi* B coliform bacteria, which was attributed to the presence of peptides with antibacterial properties in the *Moringa* extract. In a recent study conducted by Semanka *et al*., (2022), it was observed that reduction of coliform count recorded more than 65 % of the total coliform.

From all these recorded results, it is clear that the *Moringa* leaf powder had an antibacterial effect that was concentration dependent. This observation is in consistent with Semanka *et al*., (2022), who stated that the antibacterial efficacy of *M. oleifera* powder is dependent on the concentration of the seed powder; as the concentration increases the antibacterial efficacy also increases. On the contrary, the previous study of

Adejumo *et al*., (2012) documented that no significant difference in the coliform count was observed after treatment of the water samples with varying concentrations of *M. oleifera* leaf powder.

3.4. MIC and MBC of MOL against the Fecal-coliform bacterial strains

Mangifera oleifera leaf powder recorded MIC values that ranged from 6.25 to 1000 µg\ ml against the tested fecal-coliform bacterial strains. The MIC value for *E. coli*, *Shigella sonnei* and *S. typhi* was 6.25 µg\ ml. Meanwhile, *Enterococcus faecalis* was inhibited at 12.5 µg\ ml (Table 4). Results illustrated on Table (4) show that the antibacterial potential of MOL at the tested concentrations that ranged from 25.0 to 1000 µg\ ml had 100 % inhibition; however, at concentrations of 12.5 µg\ ml and 6.25 µg\ ml, the MOL recorded 75 %, 50 % inhibition, respectively. In consistence with the current results, the previous study of Delelegn *et al*., (2018) revealed that the aqueous extract of *M. oleifera* had an MIC value of 6.25 µg\ ml against *E. coli* ATCC 2592, *Shigella dysenteriae*, and *S. typhi*. On the other hand, the ethanolic extract of *M. oleifera* had antibacterial potential against *E. coli* ATCC 2592, *Shigella dysenteriae*, and *S. typhi*, recording MIC value of 12.5 µg\ ml, while the recorded MIC value of the methanolic extract of *M. oleifera* against *E. coli* ATCC 2592, *Shigella dysenteriae*, *S. typhi* was 25.0 µg\ ml.

Using the MOL powder, the maximum obtained MBC value for *E. coli* and *Shigella sonnei* was 6.25 µg\ ml, and for *S. typhi* it was 12.5 µg\ ml, while the minimum MBC value for *Enterococcus faecalis* was 25.0 µg\ ml (Table 5). Results represented in Table (5) show that the antibacterial potency of MOL at the tested concentrations that range from 50.0 to 1000 µg\ ml was 100 %, while at 25.0 µg\ ml the activity was 75.0 %. The concentration of MOL that ranged from 12.5–6.25 µg\ ml displayed 50-25 % antibacterial activity against the tested strains. It was also revealed by Delelegn *et al*., (2018) that the aqueous extract of *M. oleifera* had the highest and most comparable MBC (25.0 µg\ ml) against *Shigella dysenteriae* and *S. typhi*, whereas the MBC against *E. coli* ATCC 2592 was the lowest; recording 12.5 µg\ ml. This finding is in the same line to the previous work conducted by Moyo *et al*., (2012), which showed a higher MIC and MBC values of 5.0 µg\ ml against E. coli ATCC 25922 treated with *M. oleifera* aqueous leaf extract, compared to the methanolic extract that recorded MIC and MBC of 10.0 µg\ ml against the same strain.

The observed mode of action of MOL against the fecal-coliform bacterial strains is presented in Table (6). Results indicated that the MOL had a bactericidal effect against all the tested bacterial strains with MBC\ MIC $\leq$ 2. The bactericidal action of MOL was attributed by Suarez *et al*., (2005) to its active substances that caused hydrophobic layers disruption in the bacterial cell membrane, thus inhibited biofilm formation. Similarly, another investigation conducted by Shebek *et al*., (2015) confirmed that the bactericidal action of the cationic proteins extracted from MOL powder affected the bacterial cell membranes via membrane fusion. Moreover, albumin; globulin, prolamin, and glutelin in the MOL extract expressed antibacterial potency through interacting with the bacterial membrane phospholipids, leading to membrane disruption and reorganization (Ullah *et al*., 2015).

3.5. Cytotoxicity of MOL against the OEC cell lines

Results of OEC cell viability treated with MOL are demonstrated in the dose distribution curve (Fig. 3). The recorded cell viabilities were 100, 94.1, 90.5, and 88.0 % for MOL applied at varying doses of 25, 50, 100, and 150 µg\ ml; respectively, with IC_{50} value of 716.1 µg\ ml. Accordingly, it is observed that MOL powder can be used at higher concentrations for water purification and sanitation through acting as a fecal antibacterial agent. In accordance, in the previous study of Khor *et al*., (2020), the MOL extract displayed a strong inhibitory potential against the Kasumi-1 cell line at a concentration > 400 µg\ ml. Moreover, biosafety of *Moringa* at high doses was confirmed in several previous studies conducted by Stohs and Hartman, (2015); Adebayo *et al*., (2017).

Table 4: *Mangifera oleifera* leaf powder MIC tested against the fecal pathogenic bacterial strains

Fecal pathogenic strains	MIC of MOL (µg\ ml)								
	1000 (control)	500	250	125	75	50	25	12.5	6.25
E. coli ATCC 8739	–	–	–	–	–	–	–	–	–
Enterococcus faecalis ATCC 7080	–	–	–	–	–	–	–	+	+
S. typhi DSM 17058	–	–	–	–	–	–	–	–	+
Shigella sonnei DSM 5570	–	–	–	–	–	–	–	–	–
Spectrum activity (%)	4/4	4/4	4/4	4/4	4/4	4/4	4/4	3/4	2/4
	100	100	100	100	100	100	100	75	50

Where; - = No growth, + = growth. Results are averages of 3 replicates

Table 5: *Mangifera oleifera* leaf powder MBC tested against the fecal pathogenic bacterial strains

Fecal pathogenic strains	MBC of MOL (µg\ ml)								
	1000 (control)	500	250	125	75	50	25	12.5	6.25
E. coli ATCC 8739	–	–	–	–	–	–	–	–	–
Enterococcus faecalis ATCC 7080	–	–	–	–	–	–	+	+	+
S. typhi DSM 17058	–	–	–	–	–	–	–	+	+
Shigella sonnei DSM 5570	–	–	–	–	–	–	–	–	+
Spectrum activity (%)	4/4	4/4	4/4	4/4	4/4	4/4	3/4	2/4	1/4
	100	100	100	100	100	100	75	50	25

Where; - = No growth, + = growth. Results are averages of 3 replicates

Table 6: *Mangifera oleifera* leaf powder MIC and MBC values on the tested fecal pathogenic bacterial strains

Fecal bacterial strains	MIC (MOL µg\ ml)	MBC (MOL µg\ ml)	MBC\ MIC Ratio	Effect
E. coli ATCC 8739	6.25	6.25	1.0	Bactericidal
Enterococcus faecalis ATCC 7080	12.5	25.0	2.0	Bactericidal
S. typhi DSM 17058	6.25	12.5	2.0	Bactericidal
Shigella sonnei DSM 5570	6.25	6.25	1.0	Bactericidal

Where; The bactericidal effect is recorded on MBC\ MIC = ≤ 2, while the bacteriostatic effect is recorded on MBC\ MIC = ≥ 4

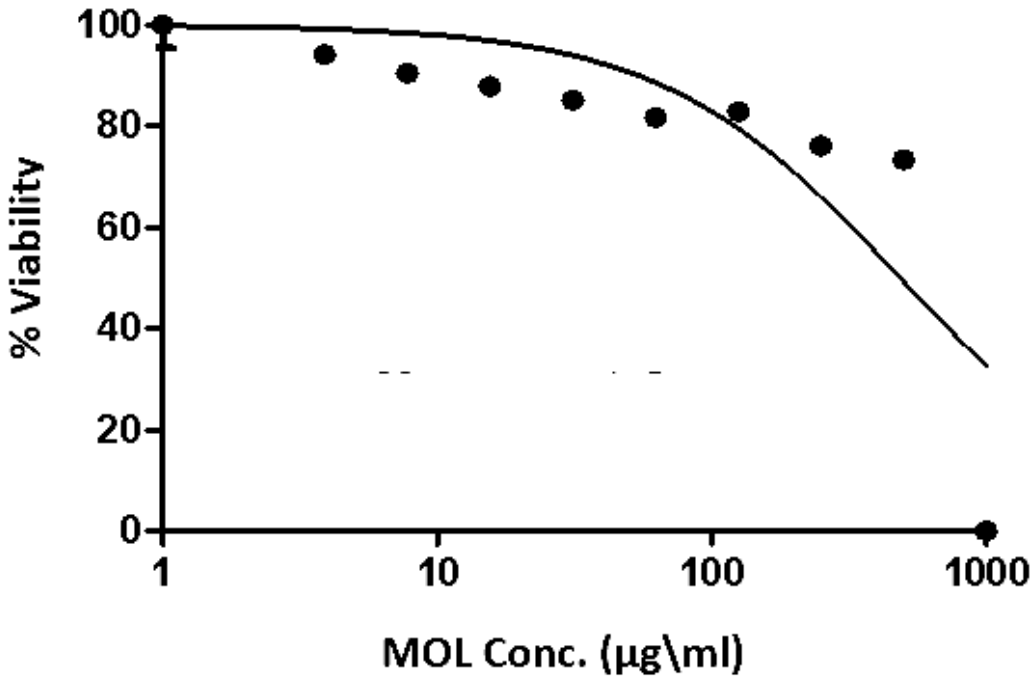

Fig. 3: Dose distribution curve of OEC cell lines treated with various concentrations of MOL powder. Results are averages of 3 replicates

Conclusion

Plants are rich in many metabolites that were used to treat a variety of diseases and infections. One of the famed traditional plants is *Moringa*. *M. oleifera* leaves were found to be effective in removing turbidity and lowering the water pH. The agar well diffusion was used to assess the *M. oleifera* antibacterial properties. All the tested fecal bacterial strains were shown to be inhibited by the *M. oleifera* leaf powder with variable degrees, depending on its concentration. These findings point to a novel route for using *M. oleifera* as a powerful antibacterial agent against the fecal pathogenic bacteria. The cytotoxicity assessment results indicated the biocompatible usage of *M. oleifera* for human consumption and for any other field of application.

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Conflict of interest

The author declares that there is no conflict of interests regarding publication of this manuscript.

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Ethical approval

None applicable.

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