APPLICATION OF GREEN METHODS AS ALTERNATIVE FOR CONVENTIONAL CHEMICAL FOR WATER PURIFICATION

M. R. AL-OTHMAN[a], A. A. ALOBATHANI[a], A. R. M. A. EL-AZIZ[a*], H. ALJOHI[b]
[a]*Botany and Microbiology Department, College of Science, King Saud University, Riyadh, Saudi Arabia*
[b] *King Abdulaziz city for science and technology (KACST)*

In this study water samples were collected from three sites (wadi Namar, Hayer and sewage water) supply of Riyadh, Kingdom of Saudi Arabia and treated with powder of moringa seeds, moringa cake and gold nanoparticles (which synthesis by moringa seeds) then have been tested on water microbial, the results of wadi nemar region showed the rate of inhibition percentage ranged between (1.6 and 33.4%), (0.0 and 21.1%) and (3.6 and 35.7%) at 1%, 2% and 3% respectively. In haier region, the results showed the rate of inhibition percentage ranged between (1.4 and 9.0%), (1.8 and 24.1%) and (3.4 and 22.9%) at 1%, 2% and 3% respectively, whereas inhibition percentage in sewage samples were ranged between (0.0 and 14.7%), (8.1 and 19.1%) and (3.8 and 20.4%) at 1%, 2% and 3% respectively. Effect of treatment with moringa cake have been showed that the results inhibition percentage ranged between (0.0 and 0.25%), (0.0 and 5.8%) and (5.4 and 41.8%) for wadi nemar at 1%, 2% and 3% respectively. In haier location the results showed the rate of inhibition percentage ranged between (17.1 and 33.8%), (15.2 and 21.9%) and (5.3 and 12.4%) at 1%, 2% and 3% respectively, whereas inhibition percentage in sewag samples were ranged between (24.0 and 36.4%), (29.4 and 31.9%) and (25.1 and 26.2%) at 1%, 2% and 3% respectively.The effect of treatment with gold nanoparticles which synthesis by moringa seed at three different concentrations 5, 10 and 15% recorded that No effect was presented in inhibition percentage in wadi nemar and sewage samples at three tested concentrations at three water dilutions. Whereas in haier location, the results showed no effect were presented at 5% whereas the rate of inhibition percentage ranged between (8.5 and 16.0%) and (2.7 and 14.1%) at 10% and 15% respectively.Additionally some physico-chemical parameters like pH, total dissolved solids (TDS), fluoride (F-), chloride (Cl-), (Br-), sulphate (So4-), Lithium (Li+), sodium (Na+), potassium (K+), Magnesium (Mg+), and Calcium (Ca++) were measured and compare the standard drinking water quality values of WHO.

(Received May 26, 2019; Accepted September 3, 2019)

Keywords: *Moringa oleifera***,** Gold nanoparticles, Water purification, Water microbial, Physico-chemical parameters

1. Introduction

Moringa is commonly known as drumstick or horseradish tree, which is a small deciduous tree of 2.5–10m in height [1]. *M. oleifera* is the most widespread species; it can grow quickly, even on medium soils having comparatively low humidity [2]. *M. oleifera* is a very remarkable plant, had been known for its use and ability in water treatment application especially in the purification processes. *Moringa* seeds contain edible oil and water-soluble, in addition, *Moringa* seeds have shown interest from researcher in environmental scientific community for natural coagulant of *Moringa* seeds [3]. The powder from the seeds of *M. oleifera* has shown to be one of the most effective as a major coagulant for water purification and compared to that of aluminum. For more, the researcher discovered that the powder from *moringa* seeds has antimicrobial characteristics [4]. Besides the use of *moringa* has an added advantage over the chemical treatment of water because

*Corresponding author: aabdelaziz@ksu.edu.sa

of its biological properties and has reported as edible, and the cost of this natural coagulant would be less expensive compared to the conventional coagulant like (alum) for water purification since it is available in most rural communities [4]. Water is the most important and plentiful compound of the ecosystem. All living creature on the planet needs water to survive and grow [5]. Water played an important role in human life; it forms 50-60% of human body weight. In fact, water plays an important role in many biological processes for all microscopic and non-microscopic organisms [6]. Different water sources are often turbid and contaminated by microorganisms, which cause several diseases including guinea worm and biohazards. The level of water purity has consumed very crucial since it has a direct effect on health; however, the high cost of water process makes most people in the rural society to resort to easily available sources, which are normally of low quality exposing them to waterborne diseases [4]. Studies show that, mostly about 1.6 million people are compelled to use contaminated water, furthermore, more than million people of which two million are children died from diarrhea each year [6]. The usage for chemicals in water treatment could cause health hazards if an error happens during water treatment process, which could lead to expose to the chemicals that been used for water treatment. For example, High level of aluminum in the brain is association as a risk factor for Alzheimer's disease. In develop country, the investigation for using the non-chemicals materials mostly done before disrepute the water [4]. Water purification by natural materials as plants, for example, use *Moringa Oleifera* especially *M. oleifera* seed specifically has received a lot of attention because *Moringa oleifera* seeds minimize turbidity and pathogenic microorganisms in water treatment [7].

2. Materials and methods

Water samples collection:

The water sample was collected from wadi Namar, Hayer and sewage water supply of Riyadh, Kingdom of Saudi Arabia. The valley water was collected in a plastic tank of 200 liters and kept for at least three to five days before the using time to obtain a symmetrical quality throughout the study [8,9]. All water samples were tested before and after treatments and compared the results with the world health organization numbers.

Preparation of *M. oleifera* stock solution:

Moringa oleifera (MO) seeds which used in this study were obtained from Al-Madinah Al-munawarah, Saudi Arabia. The dry seeds were stored at room temperature until the using time. Before any experiment, the shell covering the MO seeds was manually peeled, the good quality seeds were chosen then washing several times with deionized water air-dried in a laboratory oven at 35 °C for 24 hours, after that, the seeds grinded in a grinder (Moulinex coffee grinder AR110O27) until a fine powder was obtained to achieve solubilization of active ingredients in the seed. After that we prepare three different concentrations (1, 2, 3 g of *M. oleifera* powder in 100 ml of valley water). The suspension is vigorously shaking for 1 hour using a magnetic stirrer, set on a flat surface for 1 hour, and then passed it through filter paper (Whatman No. 4). [10, 11] . We use the three dilutions of water (10^{-1}, 100^{-2} and 1000^{-3}), there are 4 replicates for each treatment and incubated at 37 ° C for 24 hours. After the incubation time, the number of colony growths on the agar is counting and recording as colony forming unit per ml.

$$(\text{cfu/ ml}) = \frac{\text{No of colonies} \times \text{dilution factor}}{\text{Volume plated}}$$

Preparation of *Moringa oleifera* seed defatted cake:

We prepared the *Moringa oleifera* Cake by using two solvents Methanol and Ethanol) by adding 12 g. of powder of *Moringa oleifera* seeds in 50 ml methanol with the seeds and 50 ml of ethanol with the other flask for 48 hours at room temperature. After that, the extracts separately filtered using sterile filter paper (Whatman No. 4), then, The solvent was evaporated to dryness in a water bath [7,9]. Then, we took (1,2,3 g.) of the cake dried powder with 100 ml of valley water (the results were recorded as colony forming mention above) [9].

Preparation of gold nanoparticles:

The Gold (III) chloride trihydrate was purchased from Sigma-Aldrich, United State of America.

Synthesis of gold nanoparticles (AuNPs):

Fresh seeds were collected and washed with deionized water, de-shelled, ground to a fine powder. After that, 20 g of the seeds were adding with 100 ml of deionized water. The seeds with the water were heated to boiled for 30 min, allowed to cool for 10 min, filtered through a Whatman No.4 filter paper; to remove the residues, 5 ml of the supernatant seeds extract then added to 45 ml of 1 ml 103 M HAuCl4 solution that we made by adding 8.5 ml of Aucl4 with 41.5 ml of deionized water at room temperature, After that, the solution stirred until we notice the color changing. We made a positive control with supernatant seeds extract sample as will a negative control sample with the 10^3 M HAuCl4 solution. After the color changing, we use the three dilutions of water (10^{-1}, 100^{-2} and 1000^{-3}), there are 4 replicates for each treatment and incubated at 37 ° C for 24 hours [1,9]).

Characterization of AuNPs:

Nano particles, with AuNPs, are mostly characterized by their size, shape, surface functional groups, and phases, and the current techniques for characterizing NPs are abbreviated in the following steps which have been done in the central laboratory in King Saud University [12].

Scanning electron microscopy (SEM):

A microscopy technique setup on a ray of electrons for the morphology of the nano particles, and it is used for morphological characterization of the NP.

Dynamic light scattering (DLS):

It is a Scattering analysis of photons which is used to determine the exterior charge and the hydrodynamic radius of the NPs.

Transmission electron microscopy (TEM):

A microscopy technique setup on a ray of electrons for the diameter of the Nano particles, and it is used for determining the diameter of NPs, A drop of hydrous gold nanoparticle sample was filled on carbon-coated copper lattice, and then allowed it to dry fully for an hour at room temperature, The TEM micrograph images were recorded on a.

Zeta sizer measurement:

Size of the nanoparticles was determined by Malvern Zetasizer ZEN 3600. This device grants the measurement of particle sized distribution in the range 2 nm^{-3} μm.

Fourier Transform Infrared Spectroscopy (FTIR):

We used it to identify variations in the total composition of Nano particles through the determination of changes in functional groups in biomolecules.

Physicochemical analysis of water samples:

The water samples were determined physicochemical by using specific methods these methods have been done by Biotechnology King Abdelaziz City for Science and Technology, which include these analyses [4,13,14].

PH measurement:

The pH of the water samples were measured using pH paper by dipping part of the paper into the solution and watching the color changing.

Determination of Turbidity

This method carried out using HACH DR/200 direct reading spectrophotometer.

Determination of Total Dissolved Solid (TDS)

It was determined by weighing a clean beaker on weighing beam balance then, the water samples are filter using the filter papers.

Electrical Conductivity (EC)

It can be measuring with the help of EC meter, which measures the resistance, offered by the water between two platinized electrodes. The instrument is standardizing with known values of conductance observed with standard KCl solution.

Sulphate ($SO4^-$), Calcium (Ca++), Magnesium (Mg++), Chloride (Cl^-), Sodium (Na+), Potassium (k+), Broline (Br^-), Fluorine (F^-), Nitrate ($No3^-$) and Lithium (Li+):

These elements were measured following the anion and cation method according to KACST: They measured the anion and the cation in water treated with MO by using the Metrohm 850 Professional IC. First, water sample treated with MO filter through 0.45um purchased from Millipore Co. (Bedford, MA). The Column used for cation was C 4-150/4.0 while the column used for anion was Metrosep A sup 16-150/4.0. The mobile phase used for cation eluent HNO_3 5m Molar and the mobile phase use for anion eluent Na_2CO_3 7.5mmol/L+ NaOH 0.25 mmol/L.

Statistical analysis:

In our study, we used the SPSS software (one way ANOVA) to perform the Statistical analysis.

3. Results

Effect of treatment with of *moringa oleifera* seed on colony forming unit per ml (as log10)

These experimentals to compare three treatment methods (moringa seeds, moringa cake and gold nanoparticles) on microbial and physical analysis of tested water samples**.** Data in Table (1) shows that effect of treatment with moringa seed at three different concentrations 1, 2 and 3% as colony forming unit per ml (as log10) at three dilutions of water sample collected from wadi nemar, haier and sewage at three water dilutions (10^{-1},$10^{-2,}$ and 10^{-3}). The results of wadi nemar region showed the rate of inhibition percentage ranged between (1.6 and 33.4%), (0.0 and 21.1%) and (3.6 and 35.7%) at 1%, 2% and 3% respectively. In haier region,the results showed the rate of inhibition percentage ranged between (1.4 and 9.0%), (1.8 and 24.1%) and (3.4 and 22.9%) at 1%, 2% and 3% respectively, whereas inhibition percentage in sewage samples were ranged between (0.0 and 14.7%), (8.1 and 19.1%) and (3.8 and 20.4%) at 1%, 2% and 3% respectively. furthermore, the results of wadi nemar samples showed moringa seed had antibacterial effect at 10^{-1} with significant differences between control and treated sample but we noticed no significant differences at $10^{-2,}$ and 10^{-3} when compared with control additionally no significant differences between three tested concentrations. Our results in haier obtained that at 10^{-1} had antibacterial effect at 2% with significant differences between control and treated sample and no significant differences at 1% and 3% whereas, no any significant differences when treated with 2% or 3% at 10^{-2} and 10^{3}. In sewage sample the results have been antibacterial effect only at 10^{-3} with significant differences between control and treated sample while no significant differences between three tested concentrations at dilution 10^{-1}.

Table 1. Effect of treatment with moringa seeds at three different concentrations 1, 2 and 3% on colony forming unit per ml (as log10) of water sample collected from three regions wadi namar, haier and sewage.

Region	Con.	Water dilution					
		10^1	Inhibition %	10^2	Inhibition %	10^3	Inhibition %
Wadi namar	0	3.198 ^{d}±0.122	0.0	3.619^{de}±0.262	0.0	4.380^{fg}±0.11	0.0
	1	2.466 ^{e}±0.196	22.9	2.412 ^{e}±1.216	33.4	4.312^{fg}±0.14	1.6
	2	2.523 ^{e}±0.396	21.1	3.608^{de}±0.019	0.3	4.551^{ef}±0.077	-
	3	2.420 ^{e}±0.083	24.3	2.327 ^{e}±1.169	35.7	4.221 ^{g}±0.175	3.6
Haier	0	4.151^{bc}±0.222	0.0	4.682^{abcd}±0.067	0.0	4.956 ^{d}±0.118	0.0
	1	3.778 ^{c}±0.059	9.0	4.365^{bcd}±0.013	6.8	4.884 ^{d}±0.103	1.4
	2	3.154 ^{d}±0.248	24.1	4.022 ^{cd}±0.105	14.1	4.867^{de}±0.06	1.8
	3	3.922 ^{c}±0.081	22.9	4.288^{bcd}±0.057	8.4	4.787^{de}±0.07	3.4
Sewage	0	5.081 ^{a}±0.048	0.0	6.037 ^{a}±0.030	0.0	6.943 ^{a}±0.013	0.0
	1	5.139 ^{a}±0.024	-	5.889 ^{ab}±0.026	2.5	5.920 ^{b}±0.026	14.7
	2	4.671^{ab}±0.212	8.1	5.151^{abcd}±0.229	14.7	5.615 ^{c}±0.102	19.1
	3	4.888 ^{a}±0.080	3.8	5.396 ^{abc}±0.125	10.6	5.527 ^{c}±0.053	20.4
LSD		0.527		1.460		0.303	

Effect of treatment with of *Moringa oleifera* cake on colony forming unit per ml (as log10)

Data in (Table 2) shows that effect of treatment with moringa cake at three different concentrations 1, 2 and 3% as colony forming unit per ml (as log10) at three dilutions of water sample collected from wadi nemar, haier and sewage at three water dilutions (10^{-1},$10^{-2,}$ and 10^{-3}). The results of wadi nemar showed the rate of inhibition percentage ranged between (0.0 and 0.25%), (0.0 and 5.8%) and (5.4 and 41.8%) at 1%, 2% and 3% respectively. In haier location The results showed the rate of inhibition percentage ranged between (17.1 and 33.8%), (15.2 and 21.9%) and (5.3 and 12.4%) at 1%, 2% and 3% respectively, whereas inhibition percentage in sewage samples were ranged between (24.0 and 36.4%), (29.4 and 31.9%) and (25.1 and 26.2%) at 1%, 2% and 3% respectively. furthermore, the results of wadi nemar sample showed moringa cake had antibacterial effect at 10^{-1} and 10^{-2} with any tested concentrations with no significant differences between control and treated sample but we noticed significant differences when the water sample was treated with 3% cake and water dilution was10^{-3}. Our results in haier obtained that at 10^{-1} and 10^{-2} had antibacterial effect at 1, 2 and 3 % with significant differences between control and treated sample and no significant differences at 1% and 2% whereas, no any significant differences at 10^{-3} with any tested concentrations. In sewage samples the results have been antibacterial effect only at $10^{-1,}$ 10^{-2} and 10^{-3} with significant differences between control and treated sample while no significant differences between 2 and 3% at 10^{-1} and 10^{-2} additionally no significant differences at three tested concentrations at dilution 10^{-2} and 10^{-3}.

Table 2. Effect of treatment with moringa cake at three different concentrations 1, 2 and 3% on colony forming unit per ml (as log10) of water sample collected from three regions Wadi namar, Haier and sewage.

Region	Conc.	Water dilution					
		10^1	Inhibition %	10^2	Inhibition %	10^3	Inhibition %
Wadi namar	0	2.807 ^{def}±0.164	0.0	3.619 ^{f}±0.262	0.0	4.380 ^{b} ±0.151	0.0
	1	2.868 ^{ef}±0.102	-	3.456 ^{f}±0.069	4.5	4.117 ^{b}±0.168	6.0
	2	2.817 ^{ef}±0.112	-	3.681 ^{ef}±0.052	-	4.143 ^{b}±0.160	5.4
	3	2.800 ^{ef}±718.957	0.25	3.409 ^{f}±0.049	5.8	2.560 ^{c}1.288	41.6
Haier	0	4.151± ^{b}0.222	0.0	4.682 ^{b}0.067	0.0	4.956 ^{b}±0.118	0.0
	1	3.443 ^{c}±0.054	17.1	3.971 ^{cde}0.030	15.2	4.693 ^{b}±0.158	5.3
	2	3.353^{c} ± 0.089	19.2	3.950 ^{de}±0.068	15.6	4.363 ^{b}±0.143	12.0
	3	2.747^{f}±0.050	33.8	3.655 ^{f}±0.105	21.9	4.339 ^{b}±0.139	12.4
Sewage	0	5.081 ^{a} ± 0.048	0.0	6.037 ^{a}±0.030	0.0	6.943 ^{a}±0.013	0.0
	1	3.860 ^{b} ± 0.046	24.0	4.263 ^{c}±0.047	29.4	5.199 ^{b}±0.029	25.1
	2	3.276 ^{cd} ± 0.077	35.5	4.114 ^{cd}±0.042	31.9	5.175 ^{b}±0.036	25.5
	3	3.233 ^{cde}±0.070	36.4	4.252 ^{c}± 0.058	29.6	5.103 ^{b}±0.088	26.5
LSD		0.415		0.276		1.138	

Characterization of the gold nanoparticles

Fig. 1 shows that addition of *M oleifera* to 10^{-3} mol/L aqueous HAuCl4 resulted in a change of the solution colour to pink, indicating AuNPs formation [15,16,17]. The change of colour is an indication of the reduction of the chloroaurate ions by the proteins present in the plant extract [18].

Fig. 1. Biosynthesis gold nanoparticles by M. oleifera seeds.

Transmission electron microscopy

The morphology and particle size of the synthesized AuNPs were investigated using TEM (Fig. 2), there was no aggregation observed in the spherical AuNPs; tiny nanoparticles. The small particle size of the AuNPs synthesized using *M. oleifera* was due to the presence of a large number of nucleation sites for AuCl4 complexation. Furthermore, *M oleifera* components can effectively prevent the aggregation of the nanoparticles. This observation is in agreement with earlier reports [19], in which AuNPs were synthesized using green tea, zimbro tea and green coconut water. Besides, AuNPs were synthesized using the flower extract of Plumeria alba [20].

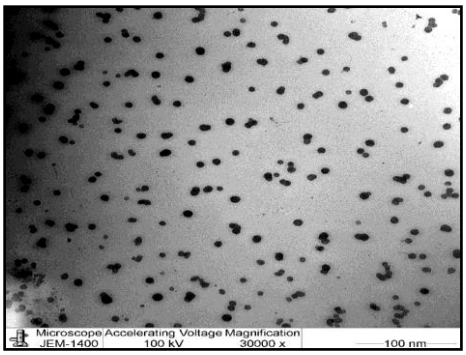

Fig. 2. TEM image of the AuNPs forms using M. oleifera seeds extract at magnification of 30,000 x.

Scanning electron microscopy

The SEM images of the synthesized AuNPs are presented in Fig. 3. At room temperature, the synthesized nanoparticles ad a small size and spherical shape.

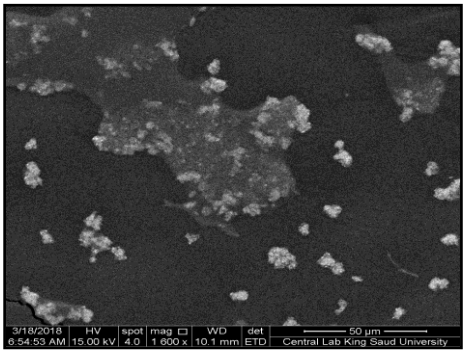

Fig. 3. SEM image of the AuNPs forms using M oleifera seeds extract at magnification of 1600x.

EDS analysis

The EDS analysis was conducted to detect the amount of AuNPs in the sample. The EDS spectrum Fig. 4 showed strong signals of AuNPs in two different places of the sample, confirming their presence, while weak signals which correspond to other elements were also present in the spectrum. These results reveal that proteins, enzymes and salts were present in the plant extract [21].

Zeta size analysis

An experiment in Fig. 5 was carried out using an advanced DLS system (Zetasizer Nano S, Malvern Instruments, UK) to measure a sample of colloidal gold. Fig. 2 shows the intensity particle size distribution obtained for the colloidal gold sample. The plot shows the relative percentage of light scattering by the particle against various size classes. The two distinct peaks at 5.89 nm and 18.17 nm indicate a bimodal distribution and imply the presence of aggregates within the samples, size distribution for the colloidal gold sample analyze with DSL reveal over 85.6% of the sample consist of small particles around 5.89 nm and indicates the presence of agglomerates with the samples around 14.4%.this result agreement with [22].

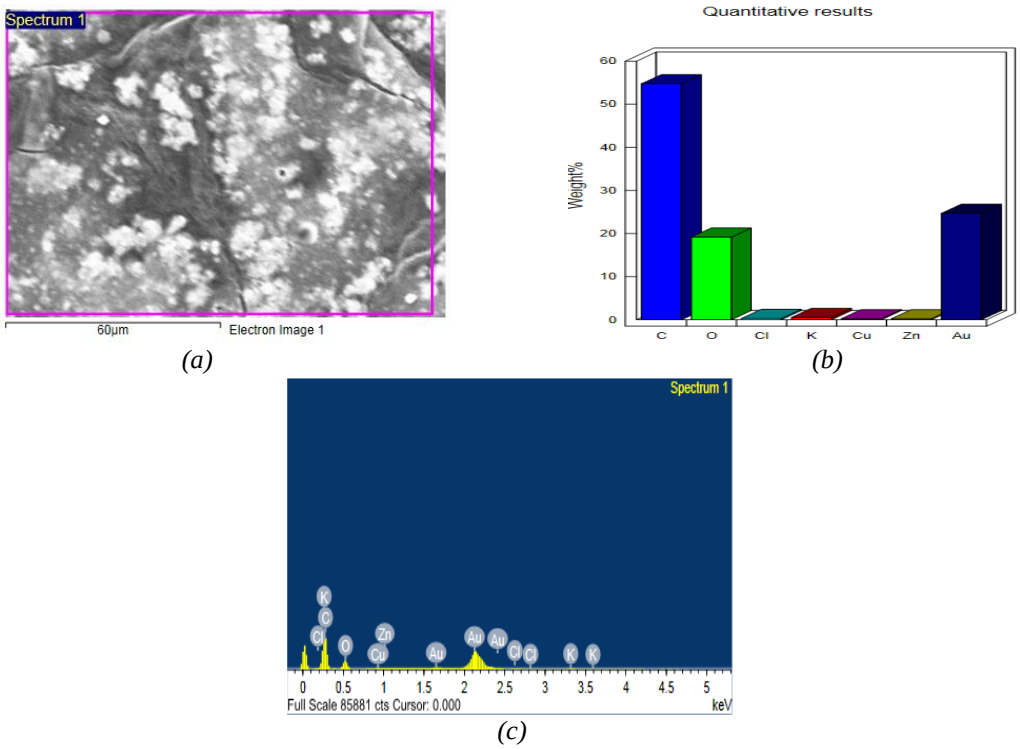

Fig. 4. SEM images of AuNPs (a), quantitive results histogram (b) and EDS spectrum of AuNPs synthesized using M oleifera seeds extract (c).

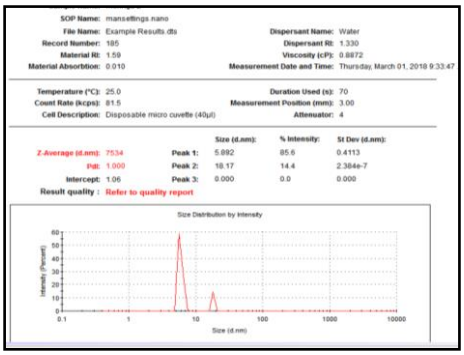

Fig. 5. Zeta potential of AuNPs synthesized using M. oleifera.

Fourier-transform infrared spectroscopy

The synthesized AuNPs were subjected to FTIR analysis to detect the bioactive compounds associated with the nanoparticles in the range of 400–4000 cm^{-1}. The FTIR spectrum of formation AuNPs by *M oleifera* is shown in Fig. 6. A number of functional bonds were related to the stability of the capped nanoparticles. The peaks at 426.21cm^{-1} and 480.47 cm^{-1} ascribed to the C–C skeleton vibrations [23]. The peak at 1639.04 cm^{-1} corresponds to the C-O bond, while the peaks at 2038.47 and 2178.88 cm^{-1} are ascribed to the C–N bond. Besides, the peak at 3295.13 cm^{-1} is ascribed to the N–H and H–O bonds, whereas the peak at 1980.81 cm^{-1} is related to the aromatic ring. Similar results have been observed in other studies [21,24,25].

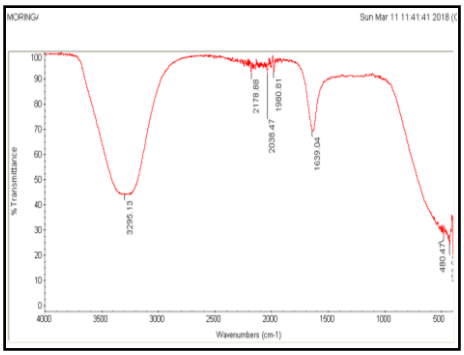

Fig. 6. FTIR spectrum of AuNPs synthesized using M oleifera seeds extract.

Effect of treatment with of *M. oleifera* AuNPs on colony forming unit per ml (as log10)

Data in Table 3 shows that effect of treatment with gold nanoparticles which synthesis by moringa seed at three different concentrations 5, 10 and 15% as colony forming unit per ml (as log10) at three dilutions of water sample collected from wadi nemar, haier and sewage at three water dilutions (10^{-1},10^{-2} and 10^{-3}). No effect was presented in inhibition percentage in wadi nemar and sewage samples were recorded at three tested concentrations at three water dilutions. Whereas in haier location, the results showed no effect were presented at 5% whereas the rate of inhibition percentage ranged between (8.5 and 16.0%) and (2.7 and 14.1%) at 10% and 15% respectively. Furthermore, our results in haier obtained that at 10^{-1} and 10^{-2} had antibacterial effect at 10% and 15 % with significant differences between control and treated sample and no significant differences at 5% whereas, no any significant differences between 5% and 10%.

Table 3. Effect of treatment with gold nanoparticles which synthesis by moringa seeds at three different concentrations 5, 10 and 15% on colony forming unit per ml (as log10) of water sample collected from three region Wadi Namar, Haier and sewage.

Region	Conc.	Water dilution					
		10^1	Inhibition %	10^2	Inhibition %	10^3	Inhibition %
Wadi namar	0	3.198^{g}±0.122	0.0	3.619^{e}±0.262	0.0	4.380^{f}±0.151	0.0
	5	3.651^{ef}±0.099	-	4.520^{bcd}±0.07	-	5.197^{de}±0.129	-
	10	3.782^{e}±0.024	-	4.238^{d}±0.085	-	5.278^{d}±0.095	-
	15	3.779^{e}±0.063	-	4.554^{bc}±0.080	-	5.195^{de}±0.039	-
Haier	0	4.151^{d}±0.222	0.0	4.682^{b}±0.067	0.0	4.956^{e}±0.118	0.0
	5	4.418^{c}±0.047	-	4.556^{bc}±0.058	-	5.574^{c}±0.011	-
	10	3.487^{f}±0.030	16.0	4.295^{cd}±0.089	8.5	5.116^{de}±0.013	-
	15	3.564^{ef}±0.079	14.1	4.555^{bc}±0.071	2.7	5.260^{d}±0.102	-
Sewage	0	5.081^{b}±0.048	0.0	6.037^{a}±0.030	0.0	6.943^{a} ±0.013	0.0
	5	5.601^{a}±0.020	-	6.288^{a}±0.046	-	7.109^{a}±0.055	-
	10	5.137^{b}±0.019	-	6.047^{a}±0.027	-	6.226^{b}±0.119	-
	15	5.165^{b}±0.042	-	6.114^{a}±0.017	-	6.903^{a} ±0.042	-
LSD		0.256		0.281		0.258	

Effect of treatment with of *M. oleifera* seeds on physicochemical analysis

The results in Table 4 shows effect of treatment with moringa seed at three different concentrations 1, 2 and 3% on physicochemical analysis of water samples collected from Wadi namar, Haier and Sewage. Our results presented that all examination samples at three tested concentrations lead to down in pH (pH stands for 'potential of Hydrogen') when compared with untreated samples and at seed concentrate 1% gave the best as compared with the World Health

Organization (6.5-8.5) where recorded 6.4, 6.9 and 6.6 in sample collected from Wadi namar, Haier and Sewage, respectively. Additionally, the reverse relationship between concentration used and pH scale. This treatment lead to increase in Total Dissolved Solid (TDS) and the reverse relationship between concentrations used compared with untreated samples and World Health Organization (WHO). For Chlorine, the treatment with seed lead to increase on compared with untreated samples while less than (WHO). Contrariwise, all seed treatment lead to decrease in flouride compared with untreated samples except in Wadi namar when treated with moringa seed at 3% but still less than standard limit in (WHO). For the Sulphate (So4) all treatment under three regions have been lead to decrease when compared with untreated samples and positive relationship between concentration used and sulfate concentration, but all treated and untreated samples have high concentration more than (WHO). For the boron all treatments under Haier and Sewage regions lead to decreased when compared with untreated samples except in Wadi namar region whereas recorded increasing after treatment with any concentration. Lithium (Li) all treatments in three regions have been leaded to increase when compared with untreated samples. Sodium (Na) and Calcium (Ca) in Wadi namar samples were recorded more increasing than (WHO) while in Haier and Sewage regions the amount of Na reached to less than standard limit. The amount of Potassium (K) was above the recommended standard limit in three regions after treatment with moringa seed at 1, 2, and 3%. Magnesium (Mg) was recorded increasing after treatment with moringa cake in Wadi namar and Haier region while variation was notes in Sewage region.

Table 4. Effect of treatment with moringa seeds at three different concentrations 1, 2 and 3% on physicochemical analysis of water sample collected from Wadi namar, Haier and sewage.

Analysis		Region 1				Region 2				Region 3			WHO
	Cont.	1%	2%	3%	Cont.	1%	2%	3%	Cont.	1%	2%	3%	
PH	7.5	6.4	4.2	4.3	7.7	4.96	6.94	6.29	6.6	6.60	4.95	3.84	6.5-8.5
TDS	2784	2828	2924	3008	1485	1818	1702	2240	968	1126	1254	1370	1500
$F^{-(ppm)}$	0.5	0.5	ND	0.8	1.7	0.8	1.4	0.9	0.8	0.8	0.5	0.0	1.5
$Cl^{-(ppm)}$	675	701	702	714	293	295	295	291	178	183	180	182	600
$Br^{-(ppm)}$	0.7	0.9	0.8	2.4	0.71	ND	0.61	ND	0.40	0.34	0.43	0.25	1-2
$So4^{-(ppm)}$	1213	1376	1528	1762	590	988	845	1134	343	529	676	806	400
$LI+^{(ppm)}$	ND	ND	ND	0.2	0.01	0.40	0.14	0.75	0.01	0.01	0.04	0.16	-
$Na+^{(ppm)}$	243	273	269	318	177	175	173	172	111	112	110	110	200
$K+^{(ppm)}$	ND	5	38	82	5	100	55	136	5	43	80	114	12
$Mg+^{(ppm)}$	48	73	78	79	41	41	34	37	23	20	7	30	150
Ca++	234	229	237	214	155	185	139	144	100	113	105	102	200

Effect of treatment with of *M. oleifera* cack on physicochemical analysis

Our results in Table 5 presented that effect of treatment with moringa cack at three different concentrations 1, 2 and 3%on Physicochemical Analysis of water sample collected from Wadi namar, Haier and Sewage, all examination samples at three tested concentrations lead to down on Ph but still in standard limit for sample collected from Wadi namar at 1% only, while less than standard limit for sample collected from Haier and Sewage compared with (WHO). Total Dissolved Solid (TDS) were increased by all treatments and compared with untreated samples and World Health Organization (WHO). Treatment with cake lead to increase on Chlorine compared with untreated samples while less than standard limit (WHO) for sample collected from Haier and Sewage whereas more than standard limit for sample collected from Wadi namar (705,715 and 715 at 1%,2% and 3% respectively.) Contrariwise, treatment with cake lead to decrease in fluoride in three tested samples additionally fluoride was disappeared in sample collected from Wadi namar and Sewage at 2 and 3%. For the boron (Br) and sodium (Na) all treatments lead to decreased in sample collected from Haier and Sewage to reach zero at 2 and 3% when compared with untreated samples while in Wadi namar region the results recorded above standard limit after treatment with any tested concentration. For sulphate (So_4) and potassium (K), the concentration of each treatment is under three regions higher than the WHO in both when compared to untreated samples

and there is a positive relationship between its concentrations and cake Concentrations were used. Lithium (Li) all treatments in three regions have been leaded to increase when compared with untreated samples. Mg and Ca were recorded increasing in amount by cake treatment and still in standard limit of (WHO) after treatment with any tested concentration except in Wadi namar samples the results recorded above standard limit in Ca++.

Table 5. Effect of treatment with Moringa cake at three different concentrations 1,2 and 3%on Physicochemical Analysis of water sample collected from Wadi Namar, Haier and Sewage.

Analysis	Region 1				Region 2				Region 3				WHO
	Cont.	1%	3%	5%	Cont.	1%	3%	5%	Cont.	1%	3%	5%	
PH	7.5	7.0	4.0	4.0	7.7	6.29	4.75	4.30	6.6	5.74	4.86	5.68	6.5-8.5
TDS	2784	2988	2982	3168	1485	1651	1824	1882	968	1292	1498	1728	1500
$F^{-(ppm)}$	0.5	0.4	ND	ND	1.7	1.4	1.1	1.3	0.8	0.7	ND	ND	1.5
$Cl^{-(ppm)}$	675	705	715	715	293	291	295	302	178	224	200	220	600
$Br^{-(ppm)}$	0.7	0.8	1.1	1.5	0.71	0.54	ND	ND	0.40	0.40	ND	ND	1-2
$So4^{-(ppm)}$	1213	1499	1578	1778	590	770	965	1078	343	534	834	764	400
$LI+^{(ppm)}$	ND	0.2	0.3	ND	0.01	0.07	0.25	0.22	0.01	0.07	0.10	0.25	-
$Na+^{(ppm)}$	243	250	321	337	177	174	173	177	111	137	124	139	200
$K+^{(ppm)}$	ND	19	76	92	5	55	93	127	5	84	129	214	12
$Mg+^{(ppm)}$	48	75	82	93	41	46	33	36	23	32	25	61	150
Ca++	234	232	236	244	155	150	157	150	100	109	101	142	200

Effect of treatment with of *M. oleifera* AuNPs on physicochemical analysis

Our results in Table 6 presented that Effect of treatment with gold nanoparticles which synthesis by Moringa seed at three different concentrations 5, 10 and 15 % on Physicochemical Analysis of water sample collected from Wadi namar, Haier and Sewage, all examination samples at 5 and 10 % in Wadi namar, and Haier lead to down on Ph but still in standard limit (WHO) whereas the best concentration is 15% in Sewage sample. Total Dissolved Solid (TDS) were decreased only in Wadi namar samples by gold nanoparticles and compared with untreated samples and World Health Organization (WHO). Treatment with gold nanoparticles lead to increase on Chlorine compared with untreated samples while less than standard limit (WHO) Contrariwise treatment with gold nanoparticles lead to decrease in potassium reach to more than standard limit in three tested samples while Mg was recorded increasing in amount and still in standard limit of (WHO) after treatment with any tested concentration. For sulphate (So_4) the results recorded the concentrations of three tested regions were higher than the WHO compared to untreated samples and there is a positive relationship between its concentrations and gold nanoparticles concentrations. For sodium (Na) and calcium (Ca) treatment with gold nanoparticles cause decreasing on (Na) and (Ca) compared with untreated samples and reach to less than standard limit (WHO) for sample collected from Haier and Sewage whereas more than standard limit for sample collected from Wadi namar. Lithium (Li) all treatments in three regions have been leaded to increase when compared with untreated samples. For the boron (Br) and florid (F) all treatments lead to decreased in sample collected from Haier and Sewage while in Wadi namar region the results recorded above standard limit after treatment with any tested concentration.

Table 6. Effect of treatment with gold nanoparticles which synthesis by moringas seed at three different concentrations 5, 10 and 15 % on physicochemical analysis of water sample collected from Wadi namar, Haier and Sewage.

Analysis	Region 1				Region 2				Region 3				WHO
	Cont	5%	10%	15%	Cont.	5%	10%	15%	Cont	5%	10%	15%	
PH	7.5	6.83	6.65	5.86	7.7	7.70	6.96	5.82	6.6	5.80	5.83	6.59	6.5-8.5
TDS	2784	2157	2080	2029	1485	1510	1466	1510	968	1036	1056	1101	1500
$F^{-(ppm)}$	0.5	1.9	2.1	1.7	1.7	1.2	1.1	1.6	0.8	1.0	0.6	0.8	1.5
$Cl^{-(ppm)}$	675	416	397	383	293	286	279	269	178	180	172	177	600
$Br^{-(ppm)}$	0.7	1.10	0.89	1.15	0.71	0.69	0.50	2.10	0.40	0.40	0.26	0.33	1-2
$So4^{-(ppm)}$	1213	980	947	935	590	620	632	608	343	417	395	479	400
$LI+^{(ppm)}$	ND	0.03	0.09	0.12	0.01	0.01	0.05	0.14	0.01	ND	0.12	ND	-
$Na+^{(ppm)}$	243	232	220	209	177	170	164	156	111	108	103	104	200
$K+^{(ppm)}$	ND	25	40	58	5	24	44	61	5	32	45	61	12
$Mg+^{(ppm)}$	48	58	65	65	41	41	45	47	23	28	27	31	150
Ca++	234	214	249	230	155	145	155	144	100	74	101	79	200

4. Discussion

In our results we noticed that there was a clear difference between the treated and untreated water samples which show, the microbial analysis of water samples revealed that there were drastic reductions in the microbial load after treatment with different concentrations of *M. oleifera* seed powder. *M. oleifera* seeds have an added benefit of having antimicrobial characteristics, these results in support to previous findings of the use of the Moringa seeds powder which was processed as a coagulant in water purification system [6,8,26]. The mode of attack of the Moringa seeds extract on the microbial cell was explained as by rupturing the cell and damaging the intercellular components, when the moringa seeds extract dips in to the cell which causes it to swell more and burst leading to death [27,28]. The seeds of *Moringa oleifera* find to use in the removal of colour and odour, reduction of turbidity, heavy metals and microorganisms, correction of pH, acidity, alkalinity and softening the water. The physical and chemical properties of the seeds play a major role in the mechanisms involved in water treatment [29].

In addition, the study considers the fact that *Moringa oleifera* coagulum can be locally produced, particularly here in Saudi Arabia by using it in water purification, and this method should be encouraged as an eco-friendly method. This is reasonable to reduce the large cost of the current water treatment systems. Several of previous studies reported the non-toxic nature of Moringa (leaves or seeds) which suggested it use *M. oleifera* as a coagulant in water purification method [30], that due to its low cost, high biodegradability, short shelf, safety to human health and environment compared to synthetic coagulants [30]. *M. oleifera* seeds act as a natural absorbent and antimicrobial agent as they contain 1% of active polyelectrolytes that neutralize the negatively charged colloid in impure waters because of all these characteristics prompted the study to use *M. oleifera* seed powder as a purifying agent in the water purification process. *Moringa oleifera* seeds contain between 30-42% of oil and contain a coagulation active component, in addition, the press cake method obtained after oil extraction has positively charged protein molecules that have coagulant properties [31]. The cake method obtained as a byproduct of the oil extraction method contains a very high level of protein. Some of these proteins (approximately 1 %) are active cationic polyelectrolytes. Our observed increase in removal with increasing dosage of seed powders is probably due to the increasing concentration of the polyelectrolytes. AuNPs is assured to have antifungal activity, which is dependent on size and shape. The high surface area of AuNPs was contribute to their antifungal effect by inhibiting the H+-ATPase activity of *Candida* species [32] or by restricting the transmembrane H+ efflux [33].

However, AuNPs have been weak or no antibacterial effect, unlike that of AgNPs [34, 35, 36,37], most gold nanoparticles or complexes containing gold have been found to be nontoxic or low toxic to the bacterial system. AuNPs have high MIC values, which are at least 120 μg/mL. At such a high concentration, the abundant AuNPs can interact directly with bacteria cells to cause

destructive effects, like penetration of cell membrane and disruption its function [38]. Another key point is that Au atom is much less reactive than Ag atom, especially against oxidation by dissolved oxygen, this means that less free ions and ROS are produced by AuNPs. To achieve the same antibacterial effect, much higher concentration of AuNPs is required. AgNPs generated superoxide and hydroxyl radicals under UV light irradiation, whereas SiNPs AuNPs and NiNPs generated only singlet oxygen. Therefore, the antibacterial activities of these NPs toward *E. coli* were AgNPs (strongest) > SiNPs > NiNPs > AuNPs [39].

Conclusions

Water samples were collected from three sites of Riyadh, Kingdom of Saudi Arabia and treated with powder of moringa seeds, moringa cake and gold nanoparticles (which synthesis by moringa seeds) the results showed the rate of microbial inhibition percentage reached to 35.7%, 41.8% and 16% respectively. Additionally some physico-chemical parameters like pH, total dissolved solids (TDS), fluoride (F-), chloride (Cl-), (Br-), sulphate (So4-), Lithium (Li+),sodium (Na+), potassium (K+), Magnesium (Mg+), and Calcium (Ca++) were measured and compare the standard drinking water quality values of WHO.

Acknowledgements

This research project was supported by a grant from the "'Research Center of the Female Scientific and Medical Colleges", Deanship of Scientific Research, King Saud University.

References

[1] K. Anand, R. Gengan, A. Phulukdaree, Journal of Industrial and Engineering Chemistry **21**, 1105 (2015).
[2] A. K. Ndabigengesere, S. Narasiah, B. G. Talbot, Water Research **29**, 703 (995).
[3] V. R. Karri, M. Samiwala, L. Kothapalli, Indian Journal of Applied Research **5**, 568 (2016).
[4] F. K. Amagloh, A. Benang. African Journal of Agricultural Research **4**, 19 (2009).
[5] P. N. Patil, D. V. Sawant, R. N. Deshmukh, International Journal of Environmental Sciences **3**, 1194 (2012).
[6] K. F. Abed, S. S. Alwakeel, Middle East J. Sci. Res. **44**, 156 (2007).
[7] A. Kabore, B. Savadogo, H. C. Otoidobiga et al., Journal of Water Resource and Protection **7**, 312 (2015).
[8] A. Ndabigesere, K. S. Narasiahm Water Research **32**, 781 (1998).
[9] F. P. Camacho, V. S. Sousa, R. Bergamasco, M. R. Teixeir, Chemical Engineering Journal **313**, 226 (2017).
[10] H. K. Bhuptawat, G. K. Folkard, S. Chaudhari, Journal of Hazardous Materials **142**, 477 (2007).
[11] M. Pritchard, T. Craven, T. Mkandawire, Physics and Chemistry of the Earth, Parts A/B/**C35**(13-14), 791 (2010).
[12] M. Teimouri, T. Maryam, F. Khosravinejad, Journal of Cleaner Production **184**, 74 (2018).
[13] A. Michel, B. Villemant, Geostandards and Geoanalytical Research **27**, 163 (2003).
[14] S. Sagar, R. P. Chavan, C. L. Patil, International Journal of Chemical Studies **3**, 24 (2015).
[15] D. Nath, P. Banerjee, Environmental Toxicology and Pharmacology **36**, 997 (2013).
[16] C. Coman, L. F. Leopold, O. D. Rugin, Journal of Nanoparticle Research **16**, 2158 (2014).
[17] T. Elavazhagan, K. D. Arunachalam, International Journal of Nanomedicine **6**, 1265 (2011).
[18] P. Kumar, P. Singh, K. Kumari. Materials Letters **65**, 595 (2011).
[19] A. N. Geraldes, A. A. da Silva, J. G. Leal, Advances in Nanoparticles **5**, 176 (2016).
[20] R. Mata, A. Bhaskaran, S. R. Sadras, Particuology **24**, 78 (2016).
[21] B. Gurunathan, P. V. Bathrinarayanan, V. K. Muthukumarasamy, Acta Metallurgica Sinica

(English Letters) **27**, 569 (2014).
[22] H. N. Verma, P. Singh, R. M. Chavan, Veterinary World **7**, 72 (2014).
[23] C. Baudot, C. M. Tan, J. C. Kong, Infrared Physics and Technol. **53**, 434 (2010).
[24] B. Ankamwar, M. Chaudhary, M. Metal-organic and Nano-metal Chemistry **35**, 19 (2005).
[25] A. R. M. Abdel-Aziz, Dig. J. Nanomater. Biostrut. **9**, 1485 (2014).
[26] A. A. Al-Anizi, M. T. Hellyer, D. Zhang Water Res. **2**, 77 (2014).
[27] M. A. Idris, A.M. Hammed, Int. J. Appl. Environ. Sci. **11**, 1469 (2016).
[28] M. H. Bichi, J. C. Agunwamba, S. A. Muyibi, J. Environ. Earth Sci. **2**, 58 (2012).
[29] A. P. Meneghel, A. C. Gonçalves, F. Rubio, Water, Air, & Soil Pollution **224**, 1383 (2013).
[30] S. A. Jahn, J. of American Water Works Association **80**, 43 (1988).
[31] K. A. Ghebremichael, K. R. Gunaratna, H. Henriksson, Water research **39**, 2338 (2005).
[32] I. A. Wani, T. Ahmad, Coll. Surf. B. **101**, 162 (2013).
[33] T. Ahmad, I. A. Wani, I. H. Lone, Mater. Res. Bull. **48**, 12 (2013).
[34] T. Chatterjee, S. Chakraborti, P. Joshi, Febs. J. **277**, 4184 (2010).
[35] X. Yang, A. P. Gondikas, S. M. Marinakos, Environ. Sci. Technol. **46**, 1119 (2011).
[36] J. Liu, C. Vipulanandan, Materials Science and Engineering C **33**, 3909 (2013).
[37] D. McShan, Y. Zhang, H. Deng, J. Environ. Sci. Health C: Environ Carcinog Ecotoxicol Revs. **33**, 369 (2015).
[38] M. R. Shah, S. Ali, M. Ateeq, New J. Chem. **38**, 5633 (2014).
[39] W. Zhang, Y. Li, J. Niu, Y. Chen, Langmuir **29**(15), 4647 (2013).