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Biogenic AG NPS, plant extract of *Moringa olifera* and bacteriophage effects on pathogenic bacteria isolated from Egyptian aquatic sources

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Abstract
This study obtained diverse bacterial isolates from the water station. 16s rRNA was used to identify the two dominating isolates, *Staphylococcus sciuri* and *Ralstonia insidiosa*. Substances with antibacterial activity against harmful bacteria were found in the extract of *Moringa olifera*. SEM and UV-visible analysis were used to characterize the green production of (Ag NPs) using *Moringa olifera*. The biosynthesized nanoparticles exhibited a particular peak at 420 nm, which signifies the creation of Ag NPs. Displayed a characteristic diffraction peak that showed the development of crystalline nanoparticles that matched the hexagonal and spherical shape of Ag NPs. After being isolated, the virus was described as a bacteriophage that might affect it. *Ralstonia spp*. are ubiquitous, mainly in aquatic environments, including drinking water. The action of isolated bacteriophage against tested bacteria showed its effect on G-ve bacteria (*Ralstonia insidosia)* rather than G +ve bacteria (*Staphylococcus scur*i).

Keywords: *Moringa olifera*, Ag NPS, antimicrobial activity, bacteriophage

Introduction

In today's aquatic environments, microorganisms such as bacteria, viruses, and protozoa are frequently the source of illnesses. Waterborne infections include poliomyelitis, cholera, viral hepatitis A and E, typhoid fever, and other serious human illnesses. Through sewage and fecal contamination, these pathogens get into water sources.

As a result of urbanization, industrialization, and population growth, various contaminants have contaminated our freshwater bodies. Unwanted changes in the physio-chemical and biological properties of lakes are being brought about by the disposal of household trash in these bodies of water (Singh *et al.*, 2010). Various medicinal plant species have been shown in numerous studies to have antibacterial properties against a variety of harmful bacteria (Singh *et al.*, 2005; Moon *et al.*, 2006 and Salazar *et al.*, 2006) [19, 25, 28]. Due to the extensive usage of antibiotics, resistant bacteria have become a clinical concern due to their emergence and dissemination (WHO, 2002b) [40].

Numerous phytochemicals with antibacterial and antioxidant qualities, including flavonoids, alkaloids, tannins, and terpenoids, are found in medicinal plants (Talib and Mahasneh, 2010) [32]. Many studies have been conducted on the antimicrobial properties of certain plant species. *M. oleifera* has been utilized practically and historically for many purposes, including food consumption, the manufacture of cosmetic value, and traditional therapies for various maladies (Leone *et al.*, 2015) [13]. The high content of flavonoid components, such as isoquercetin, quercetin, and kaempferol, in *M. oleifera* extract is well-known (Zhu *et al.*, 2020) [43]. These compounds are responsible for several of its pharmacological characteristics (Jaja-Chimedza *et al.*, 2017) [9].

In contrast to the current finding, the most potent natural antioxidant agent was previously found to be the aqueous and ethanolic extracts of *M. oleifera's* leaves, seeds, and roots (Nobossé *et al.*, 2018) [22]. The antioxidant and anti-inflammatory qualities acquired in Kenya have been thoroughly assessed elsewhere (Xu *et al.*, 2019) [41].

Phages are found in soil, freshwater, sewage, oceans, and other biological and environmental settings that bacterial hosts mostly inhabit. Additionally, bacteriophages have unique and highly advantageous biological activities that are used to boost output. The remarkable antibacterial activity mediated by bacteriophages is among the most significant examples (Sulakvelidze and Kutter, 2005) [30]. According to Sun, *et al.*, (2019) [31], phage therapy is the therapeutic use of phages to treat infections caused by pathogenic bacteria. According to Yu, *et al.*, (2017) [42]. The presence of appropriate lytic phages that can identify and lyse the pathogenic bacteria is the most crucial prerequisite for successful phage therapy to inactivate the germs (Lyon, 2017; Pires, *et al.*, 2017) [15, 23]. Nowadays, the two-layer plate method is widely used to isolate functional phages from soil (Lu *et al.*, 2017) [14].

Bacteriophages attach to resistant bacterial hosts, such as Salmonella (up to 5%) and Escherichia coli (up to 13%) (Bibby *et al.*, 2019) [3]. In addition to being utilized as environmental monitoring agents, bacteriophages have applications in biotechnology, ecology, health, and the environment (for the control of bacteria) (Vandamme & Mortelmans, 2019) [36]. However, phage tails that bind to host cell surface proteins or polysaccharides to mediate the recognition make bacteriophages powerful in eliminating the cell surfaces of different bacteria (Baxa *et al.*, 1996) [2]. Because different chemicals are used for stabilizing nanoparticles and because these chemicals bond to nanoparticles and alter their biological properties, practically all chemical techniques are bad for the environment (Ielo *et al.*, 2021).

In contrast, the green synthesis technique to nanoparticle production is usually regarded as safe, biocompatible, and non-toxic to the environment or organism (Lakshmeesha *et al.*, 2014). Furthermore, the green synthesis approach offers several benefits, including simplicity, reduced time

consumption, affordability, and high purity. This work intends to use bacteriophage, Ag NPs, and extract from *Moringa oleifera* as water treatment and antimicrobial agents. These substances have good biomedical applications in antibacterial and anti-inflammatory properties.

Materials and methods

Collection samples

Samples of treatment stages, net water, raw water, and processed water were taken from the Hehia station, hospital Hehia sewage, and hospital Zagazig sewage between January and December 2021.

Isolation and identification of pathogenic microorganisms from aquatic sources

The samples were handled aseptically using 500ml Duran Schott bottles that had been sterilized by the American Public Health Association's recommendations (Westbrook and Dryer, 1981) [39]. Eight hours later, the samples were assessed (Ihsanullah, 2022). To check for the formation of solitary colonies, samples were streaked on nutrient agar. To check for bacteria, culture plates were then maintained at 37 degrees for 24 to 48 hours.

Molecular characterization of the most dominant bacteria by 16S rRNA

Hi-Media Taq polymerase (500 U), Hi-Media 50mM MgCl2 (500 U), Hi-Media 10X buffer (500 U), and QIAGEN dNTPs (10 mM each) were used for the PCR. The forward primer for universal 16S rDNA was utilized. An Applied Bio-systems Veriti Thermal cycler was used to carry out PCR amplifications. Using a nucleotide BLAST search in Gen Bank, the partial 16S rRNA gene sequence of the bacteria under study was examined.

Preparation of *Moringa olifera* extract

Extraction methods

Immersing plant parts in ethanol, such as roots, leaves, bark, and seeds, yields extract that can be employed as antibacterial agents once they have been sterilely filtered. Water might dilute and weaken the concentration of the active ingredient (Hayek, Ibrahim, 2012).

Separation, Purification, and Identification of active substance(s) from the *Moringa olifera*

Paper chromatography has the benefit of allowing for rapid separations using filter paper sheets, which serve as both a substrate and a separation medium. This method can also be used to obtain highly reproducible retention factor (Rf) values on paper (2017, Martinenghi *et al.,* 2020) [16].

Separation of active components by Paper chromatography

A capillary tube was used in paper chromatography to examine the obtained samples for purity. Iodine and a UV lamp set to 254 nm were used to see the spots on paper chromatography. The chemicals that were isolated were seen at a wavelength of 254 nm. The chemicals in the fractions (60/40 hexane/chloroform, 50/50 chloroform/methanol, 70/30 chloroform/methanol, and 20/70/10 methanol/chloroform/ethyl acetate) were separated using various mobile phases. The retardation factors (RF) of the compounds must be in the range of 0.2 to 0.8 (Khan *et al.,* 2015) [12].

Measure the active component with a UV Spectrophotometer

To use ultraviolet (UV) spectroscopy to determine the concentration of a certain moringa seed. Moringa seeds often absorb near UV light with wavelengths between 200 and 400 nm (Magdalena *et al.,* 2007) [17]. The wavelength at which cloves absorb near UV light is typically between 200 and 400 nm (Mustafa *et al.,* 2016) [21].

Gas chromatography-mass spectrometry (GC–MS) analysis of the active component of Moringa seed

The primary chemicals found in the *M. olifera* extract, particularly the antibacterial agents, were identified using gas chromatography-mass spectrometry (GC-MS) analysis. (GC-MS) Thermo Fisher Scientific Corp.'s TRACE GC Ultra Gas Chromatograph and an ISQ Single Quadrupole Mass Spectrometer detector were used in the analysis. Moniruzzaman *et al.,* (2015) [18] state that a capillary column known as HP-5MS UI (cross-linked 5% methyl phenyl Silox) was used. (Sreedharan *et al.,* 2019, Martinenghi *et al.,* 2020) [16, 29]. The inert, gaseous mobile phase flows through the column, carrying the sample.

Biosynthesis silver nanoparticles from moringa seed

An aliquot (5 ml) of moringa seed extract was mixed with 50 ml of 0.01M aqueous AgNO3. To encourage the formation of nanoparticles, the reaction mixtures were exposed to direct sunlight. To determine whether nanoparticle production which is indicated by a dark brown color was taking place, the color change of the reaction mixtures was monitored.

Determine of minimum inhibitory concentration

After being incubated for 24 hours at 37°C, bacteria grow on nutrient agar media. Next, use the paper disc diffusion method to obtain varying amounts of Ag NPs and *Moringa oleifera* extract, and then incubate the plate for 24 hours at roughly 37 °C. The paper disc diffusion method was used to determine the minimum inhibitory concentration (MIC) (Schwalbe *et al.,* 2007) [27].

Isolation, purification and identification of Bacteriophage from sewage water

The wastewater samples were centrifuged for 15 minutes at room temperature at 5000 rpm. Sambrook *et al.* (2001) [26] state that sterile filters (0.45 m, Millipore) were used to filter the supernatant. Phage-containing filtrate was added to log-phase culture broth and incubated for 5 to 24 hours at 37° C in a shaking incubator set to 200 rpm to enrich the phages. The solution was again centrifuged at 5000 rpm for 15 minutes at room temperature to produce the final enhanced phage suspension.

To get rid of any last bits of cell debris, the supernatant was re-filtered (Jordan *et al.,* 2011) [10]. After thoroughly mixing 5 mL of soft agar (0.7 g agar in 100 ml of LB broth) in tubes, 1 mL of filtrate was added, and the tubes were then placed onto LB agar plates. After solidifying, the plates were placed in an incubator set to 37°C for the whole night. The incubated plates were examined for the presence of plaques every hour. The presence of phages was verified by spot testing (Jordan *et al.,* 2011) [10]. As described by Sambrook *et al.,* 2001) [26] was used to determine plaque-forming units (pfu), a measure of phage purification.

Transmission electron microscopy of bacteriophage

The phage sample was centrifuged at 45,000 rpm for two hours at 4ºC. According to Sambrook *et al.,* (2001) [26], the pellet was dissolved in SM buffer (5.8 g NaCl, 2 g MgSO4.7H2O, 50 ml/L of 1 M Tris pH 7.5, and 5 ml/L of 2% gelatin in distilled water) and kept at 4 ºC. If the phage concentration is sufficient for sample preparation, transmission electron microscopy (TEM) can be employed to evaluate morphology and component size (Ackermann, 2012).

Action bacteriophage on *Ralstonia insidosa* by TEM

Phages can also accelerate the phagocytosis of bacteria by macrophages, as demonstrated by their ability to improve bacterial phagocytosis when injected with the host bacterium (Kaur *et al*., 2014) [11]. This was connected to phages' capacity to opsonize bacteria, which entails giving the bacterium a protective layer. This opsonize may aid in the effective *in vivo* elimination of dangerous bacteria in addition to the phages' direct lytic action. According to several authors, phages contribute to the function of phagocytosis cells by continuing to lyse the phagocytosis bacteria during phagocytosis (Jo 'nczyk *et al.,* 2015). This is because, once adsorbed onto their bacterial host, phages carry on the infection process.

Application of *Moringa oleifera* extract and bacteriophage on the contaminated water source

After three days of treatment with bacteriophage and *Moringa oleifera* extract, the water station's raw water sample was examined for physical (turbidity, pH, and conductivity), chemical (alkalinity, chloride, total hardness, calcium hardness, fluoride, iron, aluminum, nitrate, and chlorine), and biological (total bacterial count, algae count, and protozoa) problems.

Statistical analysis

Results were reported as mean ± SEM (Standard Error of Mean). A one-way analysis of variance (ANOVA) by Duncan multiple tests as a post hoc test was used to assess the influence of the number of treatment groups on the different biochemical parameters. The value of $P < 0.05$ was used to indicate statistical significance. ALL Analyses and charts were done using Statistical Package for Social Sciences version 24.0 (SPSS, IBM Corp., Armonk, NY).

Results

The water station yielded various bacterial isolates from raw, processed, and net water. Hehia and two dominant isolates were identified by molecular identification using the 16S rRNA gene sequence. The two isolates were characterized morphologically by being white, Gram-negative bacteria with smooth internal surfaces and irregular margins. The first isolate was rod-shaped, while the other was cocci shaped. The tested Gram-negative bacterial isolate demonstrated a 98% similarity to the 16S ribosomal RNA sequence of *Ralstonia insidiosa* (A) with accession No. (OR616607) and *Staphylococcus sciuri* (B) with accession No. (OR166609), as illustrated in Fig. 1(a and b) using the bio-system 16S ribosomal RNA sequence.

It used the UV technique for the separation component of seed Ag/*Moringa oleifera* that appears as a separation component about wavelength (200) nm to (350) nm as shown in Fig (3). The purification of active components of *Moringa oleifera* by GC Mass. It shows different compounds, which comprise mainly hydrocarbons, fatty acids, alcohols, and esters. The UV Spectrophotometer found two compounds about 250 nm and 320 nm. These compounds are active component seeds of *Moringa olifera* that act as antibacterial activity G+ve bacteria. Table (1) shows 11 substances found in the active component of Moringa seed (Docosane (CAS), Phen.1,4.diol,2,3.dimethyl .5.trifluoromethyl, Octasiloxane, 1,1,3,3, 5,5, 7,7, 9,9, 11,11, 13,13, 15, 15-hexadecamethyl, Silicate tetramer, Nonacosane (CAS) Nonacosane (CAS), Furoscrobiculin B, Hexadecadienoic acid, methyl ester (CAS), 1-Monolinoleoylglycerol trimethylsilyl ether, Rhodopin and Phthalic acid, di- (1-hexen-5-yl) ester. Hexadecadienoic acid presents with a high percentage.

The production of silver nanoparticles using *Moringa olifera* seed extract appeared. The presence of tiny Ag-NPs causes the external surfaces of Ag NPs to become glossy in the spherical forms of the spots, and the particle sizes vary from 12.4 nm to 85.7 nm. Transmission electron microscopy was used to isolate and characterize the bacteriophage. Fig. 4 displays the virus's taxonomy, which includes the family caudovirales. Type: DNA ds the virus was: Head: 49.33 nm -54.33 nm, Tail: 164.8 nm -165.4 nm by the book Virus Taxonomy (Classification and Nomenclature of Virus), the bacteriophage is known as Ralstonia phage RSB1.

Moringa olifera seed extract in ethanol with Ag NPs demonstrated the strongest antibacterial activity against tested microorganisms, as demonstrated by the data obtained in Table (2). Since Gram-negative bacteria are more resistant to plant extract and nanoparticles, *M. olifera* extract and Ag nanoparticles showed a stronger inhibitory effect on Gram-positive bacteria due to their increased Gram reaction activity. Both *M. olifera* and silver nanoparticles do not affect *Ralstonia insidiosa*, although *S. sciuri* is significantly impacted. The inhibitory zone grew as the various concentrations were raised. The effect of different quantities of *M. olifera* extract and Ag NPs on *S. sciuri* emerged in different inhibitory zones compared with the control as indicated in Table (1).

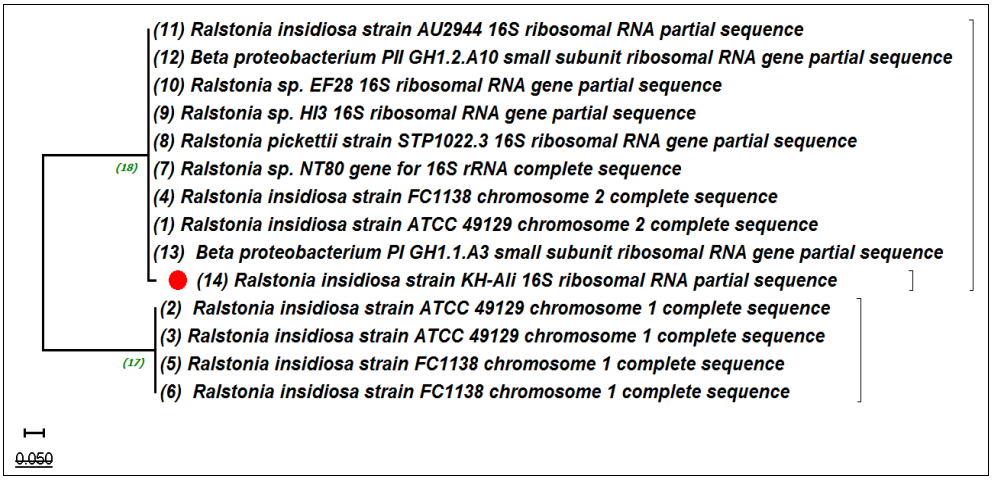
The effect on bacterial growth reduced when the concentration was increased, and for both *S. sciuri* and *R. insidiosa*, the minimum inhibitory concentration was found to be 0.001 mg/ml. As seen in Fig. (5), the mode of action of the most successful therapy by electron microscopy examined the antibacterial activity of Ag NPs and *Moringa olifera* seed extract against the strain of *Staphylococcus sciuri* (Gram-positive cocci).

The isolated bacteriophage showed action against the tested bacteria was more effective against G-ve bacteria *(Ralstonia insidosia)* than G +ve bacteria *(Staphylococcus sciuri).* The phages interact with the host bacteria, *Ralstonia insidosia*, at the cell wall using an electron microscope. *Ralstonia insidosia* is harmed by Ralstonia bacteriophage, and the pictures of the bacterium before and after exposure to the virus show various shapes Fif (5).

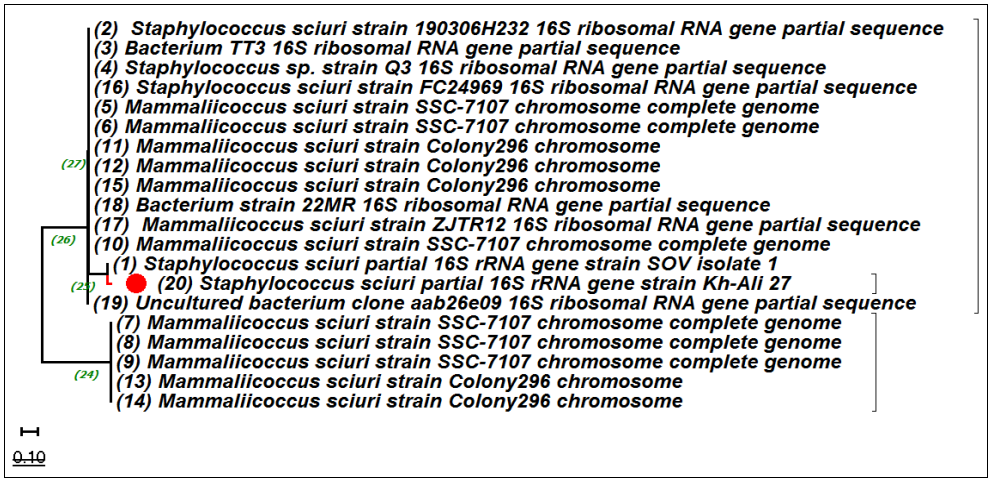
The findings in Tables 3, 4, and 5 demonstrate the usage of a large volume of water containing *Moringa olifera* and viral extract to isolate bacterial strains. These water samples' physical, chemical, and biological results before biological

treatment are compared to those obtained 24 hours, 48 hours, and 72 hours after use. The results clearly illustrated (table 3:5) the physicochemical analysis of various extracts on bulk water, which revealed the turbidity range of 24 to 453 NTU and the pH as the illustrated range of 5.69 to 7.37. These results initially appear to be high in basic, but after adding *Moringa olifera* or virus, these values decrease and gradually increase after 48 and 72 hours.

The range of conductivity values was 1043–1476 µs/cm. The values of total dissolved salts (T.D.S.) vary from 627 to 884 parts per million. The range of total hardness was 60–144 mg/l. Magnesium hardness ranges from 20 to 64 mg/l, while calcium values vary from 40 to 84 mg/l. The value of the sulfate level ranged from 21 to 56 mg/l. Chloride levels in water ranged from 10 to 81 mg/l at their greatest. Furthermore, the iron level levels were less than 0.55 mg/l.



(A)



(B)

Fig 1: Phylogenetic tree of *Ralstonia insidiosa* (A) and *Staphylococcus sciuri* (B)

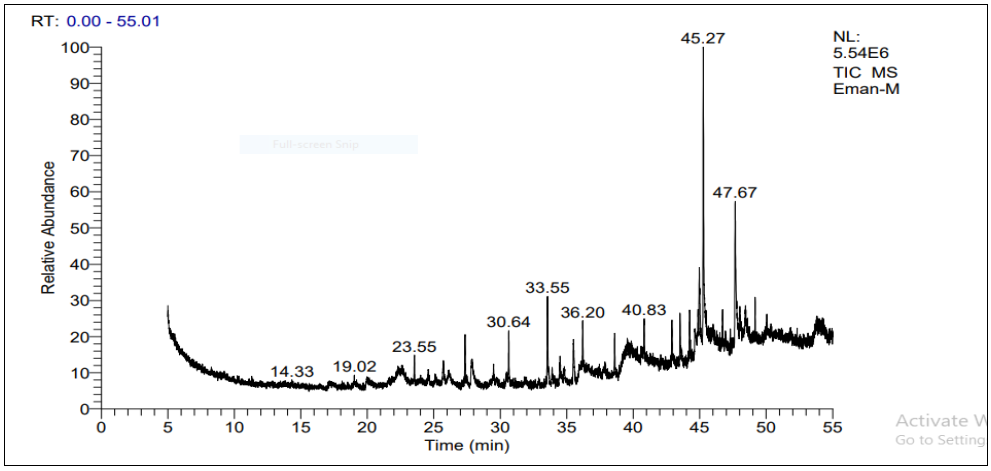


Fig 2: The chromatogram of the methanol extract of the seeds of *M. oleifera* showed two peaks

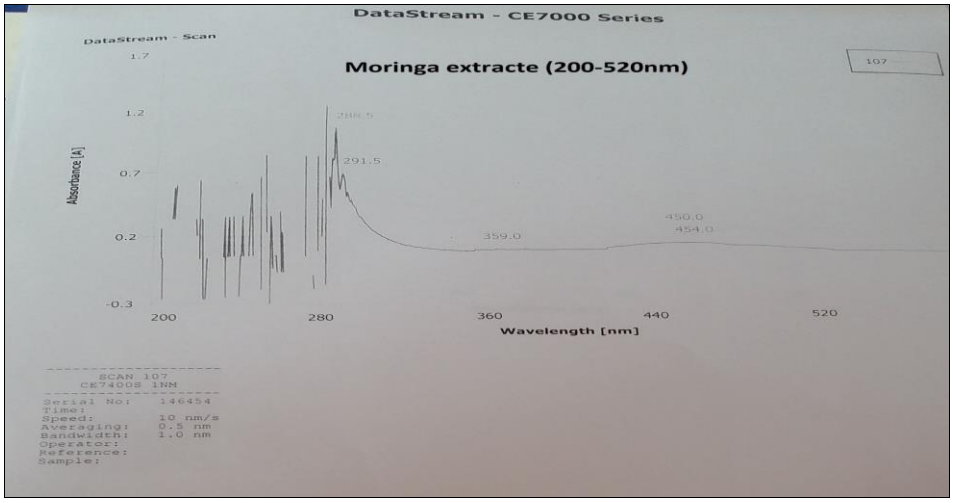


Fig 3: UV technique for separation component of seed *Ag/ Moringa oleifera*

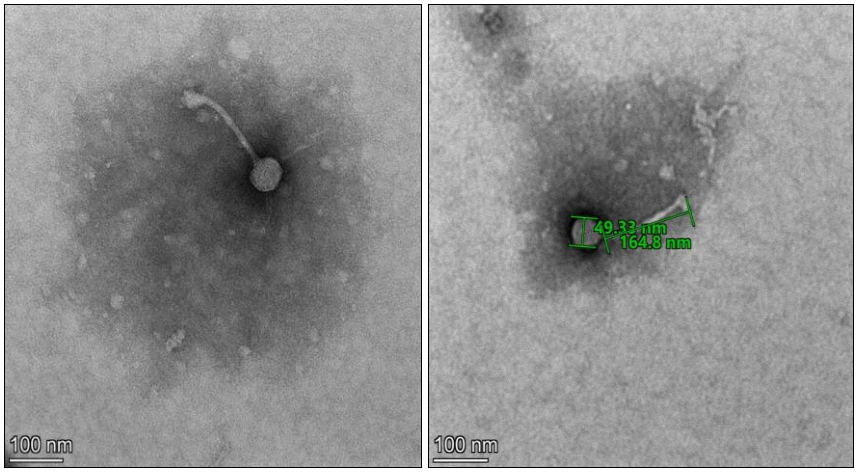


Fig 4: Bacteriophage under a transmission electron microscope.

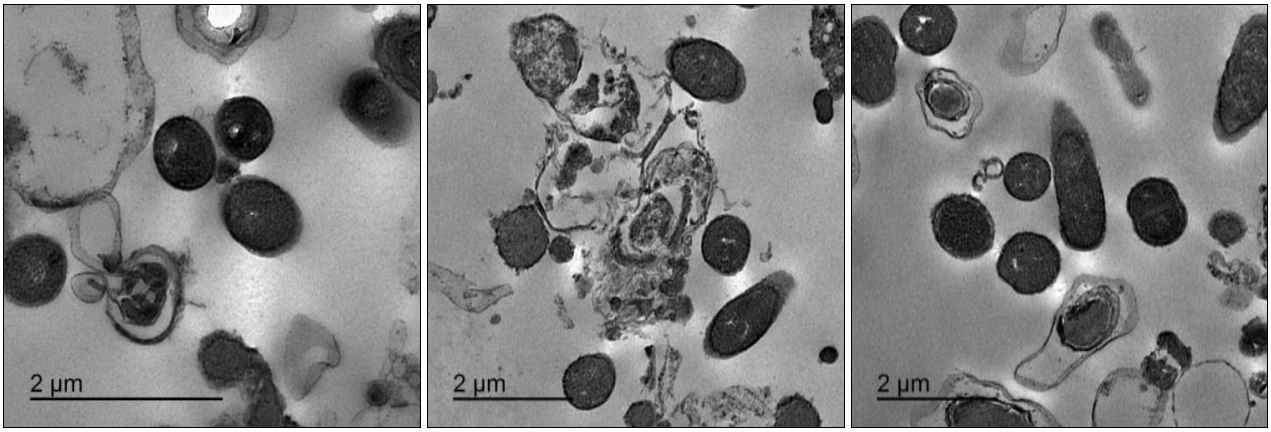


Fig 5: Mode of action bacteriophage on *Ralstonia insidosia* by Electron microscope

Table 1: Purification of active components from seed *Moringa olifera* by GC Mass

No	Compound Name
1	Docosane (CAS)
2	Phen.1,4. diol,2,3. dimethyl .5. trifluoromethyl.
3	Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl.
4	SILICATEANIONTETRAMER
5	Nonacosane (CAS)
6	Furoscrobiculin B
7	17.Pentatriacontene (CAS)
8	Hexadecadienoic acid, methyl ester (CAS)
9	1-Monolinoleoylglycerol trimethylsilyl ether
10	Rhodopin
11	Phthalic acid, di- (1-hexen-5-yl) ester

Table 2: Mode of action to *Moringa olifera* extract and Ag NPs on *Staphylococcus sciuri* and *Ralstonia insidiosa*:

Moringa olifera	Tested organisms	Control	0.1	0.01	0.001	0.0001
	Ralstonia insidosia	26±0	20±0a	17±0b	15±0b	9.25±0.75b
	Staphylococcus sciuri	33±0	27±0	21±0.58c	13±0b	12.25±0.25a

[abcde] Means within the same column carrying different superscripts are sig. different at P < 0.05 based on Duncan's multiple tests

Table 3: Effect of *Moringa olifera*, and bacteriophage on bulk from water and determine physical character

No	Tests		Basic	*M. olifera* 24	*M. olifera* 48	*M. olifera* 72	Virus 24	Virus 48	Virus 72
1	Physical Test	Color	yellow	White	White	white	Yellow	yellow	Yellow
2		Tase, odor	No accept	No accept	No accept	No accept	No accept	No accept	No accept
3		Conductivity µs	1043	1170	1169	1213	1368	1420	1476
4		pH	6.12	6.07	6.16	6.24	6.17	6.9	7.37
5		Temperature ºC	22	22.1	21.3	21.2	21.2	21.1	21
6		Turbidity NUT	26.5	53.2	172	315	24	28	31.8
7		TDS mg/l	627	701	704	819	819	852	884

Table 4: Effect of *Moringa olifera*, and bacteriophage on bulk from water and determine microbiological character

No	Tests		Basic	*M. olifera* 24	*M. olifera* 48	*M. olifera* 72	Virus 24	Virus 48	Virus 72
1	Microbiology Test	Total bacterial count (unit)	987	387	964	715	658	673	654
2		Total Chloroform count/100ml	92000	14000	35000	92000	54000	54000	54000
3		Fecal chloroform count/100	54000	14000	28000	54000	35000	35000	54000
4		Macconky agar (unit)	755	644	658	663	594	716	810
5		Protozoa	+ve	-ve	-ve	-ve	+ve	+ve	+ve
6		Algae /100ml	130	80	110	120	80	120	130

Table 5: Effect of *Moringa olifera*, and bacteriophage on bulk from water and determine chemical character

No	Tests		Basic	*M. olifera* 24	*M. olifera* 48	*M. olifera* 72	Virus 24	Virus 48	Virus 72
1	Chemical Test	Ammonia mg/l	0.35	0.25	0.25	0.37	0.35	0.40	0.41
2		F.Cl mg/l	0.01	0.0	0.0	0.0	0.0	0.0	0.0
3		Chlorides mg/l	80	50	45	10	81	45	50
4		Alkalinity mg/l	130	118	136	190	166	170	190
5		Total Hardness mg/l	120	112	114	60	124	140	144
6		Ca Hardness mg/l	80	50	56	40	96	80	80
7		Mg. Hardness mg/l	40	54	58	20	28	60	64
8		Iron mg/l	0.51	0.35	0.40	0.45	0.38	0.44	0.55
9		Residual AL mg/l	0.02	0.03	0.03	0.01	0.02	0.05	0.02
10		Sulphate mg/l	55	49	55	57	51	55	61
11		Fluoride mg/l	0.90	0.79	0.82	0.83	0.80	0.83	0.85
12		Nitrite mg/l	55	47	46	56	53	53	58

Discussion

Because of their advantageous phytochemicals, which can encourage the creation of novel medications, medicinal plant studies and uses are expanding daily. The moringa tree also contains most of these compounds. The presence of these phytochemicals is thought to be the primary cause of moringa's diverse biological characteristics and potential for disease prevention (Barakat and Ghazal, 2016) [1]. We found that the goal of our study was to find therapeutic plants that might effectively treat isolated pathogenic bacteria.

Preliminary tests were conducted using the CLSI to determine the antibacterial activity of medicinal plant extracts on specific pathogenic microorganisms of medical significance. High activity against most gram-positive bacteria was demonstrated by *M. oleifera* extract. Numerous other researchers have also noted that medicinal herbs have more antibacterial action against gram-positive bacteria than gram-negative bacteria (Bonjar, 2004) [4]. Their varying cell wall composition may be the cause of their disparities in susceptibility (Grosvenor *et al.*, 1995) [5].

When it came to gram-positive bacteria, *Moringa olifera's* MIC values demonstrated excellent antibacterial action. The extract demonstrated a wide range of activity against every pathogenic bacterium that was tested. The scanned photos demonstrated the examined microbe's significant damage, which resulted in uneven cell shape, broken cell walls, and shrinking cells. There were empty cells in some of them. Most of them also seemed to be squished together and melted. SEM image observations generally showed significant morphological change and physical damage to the investigated pathogenic microorganisms treated with *M. olifera* extract.

However, because of associated toxicities, the use of biologically hazardous chemicals and solvents, the formation of impure products, and sensitivity to environmental conditions (Wang *et al.*, 2017) [38], green approaches are thought to be essential for the synthesis of Ag NPs. The use of friendly materials in the synthesis of nanoparticles results in weak control over the product in terms of size, and therefore physicochemical properties are low (Raveendran and Wallen, 2003) [24]. Certain morphological and physicochemical characteristics, such as scale, shape, colloidal stabilization, surface corona, composition, aggregation behavior, surface coating, and surface/volume ratio, give silver its biocidal potency. If properly adjusted, these characteristics may result in a broad-spectrum inhibitory profile against several pathogenic microorganisms (Ugru *et al.*, 2018) [3].

This phage's promise as a control agent is further supported by the fact that it can infect and destroy several potentially

dangerous intestinal bacteria without the need for another phage. However, this phage would need to be able to infect its host in saline water to be utilized as a control agent in Lake Pontchartrain. The findings of this investigation indicate that the phage's ability to infect the host is suppressed in brine water. Future research on the RD 2 -2 phage will concentrate on determining its susceptibility to other Enterobacteriaceae family members. To further evaluate its potential for use in the treatment of contaminated brine water in the future, research will also concentrate on phage survivability in brackish conditions.

People are now again interested in using bacteriophages to prevent, control, and treat bacterial illnesses because of the high frequency of multidrug-resistant bacteria and the sluggish development of antibiotics. Phage treatment has demonstrated significant potential and impact in veterinary medicine, food, clinic trials, and other domains. Strong lysis capacity, the absence of a toxin gene, ease of separation and purification, good environmental stability and adaptability, and non-toxicity to the body are all desirable characteristics for a phage used in clinical trials. The immunological response, limited host spectrum, delivery dosage, and preservation conditions are some of the phage's drawbacks. There is an urgent need to fix these issues. Through the application of molecular biology technologies, we can alter the lysine, increase the host spectrum, and improve the lytic activity of the phage.

Furthermore, to expand the use of phages, global standards for their clinical application must be established as quickly as feasible. Based on current research, it is envisaged that we may overcome the current constraints and create safe, environmentally friendly, and efficient products for drug-resistant bacteria using bacteriophages. According to the information discussed here, phages can engage in several direct and indirect interactions with the mammalian immune system. However, our understanding of the extent and kind of these viruses' impact on mammalian immunity is still developing.

According to the information now available, these interactions often have an anti-inflammatory effect. It is possible that phages could affect how we interact with our commensal flora and how phage therapeutic efforts turn out if the findings of Van *et al.* (2017) [35] about the anti-inflammatory qualities of phages can be confirmed. Ralstonia species are common and mostly found in aquatic habitats, such as drinking water (Vaz *et al.*, 2017) [34]. They have also been found to be the most common species in hemodialysis water samples (Vincenti *et al.*, 2014) [37] and to be contaminants of clinical sterile solutions or materials (Boutros *et al.*, 2002).

It was postulated that gene acquisition might be the cause of the variation in gentamicin susceptibility between phylogenetically and phenotypically similar Ralstonia strains that were isolated from different aquatic habitats. A parallel study using whole genome sequencing from strains with gen bank number OR616607 revealed that only the isolate that is resistant to aminoglycosides has genes that encode lysozyme inhibitors or phage/prophages receptors, which are linked to resistance to arsenic and toxic compounds (Vaz *et al.*, 2016).

Other circumstances to investigate could be the impact of nutritional stress. *Ralstonia spp.* are a significant colonizer of extremely oligotrophic environments. Unlike hydrogen peroxide, aminoglycoside-resistant and susceptible bacteria did not differ in their ability to survive UV light or chlorine disinfection. This observation is most likely connected to the inactivation mechanisms, oxidative stress for hydrogen peroxide, DNA/RNA damage for UV, and chlorine cell metabolism (Hijnen *et al.*, 2006) [6].

These findings imply that the mechanisms underlying aminoglycoside resistance are most likely not directly related to those governing life under UV or chlorination conditions. These findings might be more consistent if studies were conducted with a larger number of isolates from various sources. The potential of *Ralstonia species* to spread or be transmitted to humans is likely determined by their ability to survive in certain environments that are subjected to disinfection processes, such as drinking water or wastewater systems. The ability of aminoglycoside-resistant strains to develop biofilm in the presence of certain environmental stressors is intriguing, as this could play a significant role in promoting the survival and dissemination of aminoglycoside-resistant *Ralstonia species* strains found in stressful situations, such as antimicrobial challenges. Ralstonia's widespread presence in aquatic environments may be attributed to these traits in addition to its oligotrophic nature.

Conflict of interest

The authors declare that they have no conflict of interest.

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