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Formulation of silver Nanoparticle from a methanolic extract of Onosma bracteatum and evaluation of their antibacterial properties

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ABSTRACT

This study focuses on the green synthesis of silver nanoparticles (AgNPs) using the methanolic extract of *Onosma bracteatum*, a medicinal plant known for its antimicrobial properties. The synthesized AgNPs were characterized using UV-Vis spectroscopy, FTIR, SEM, and DLS techniques. The antimicrobial activity was evaluated against selected bacterial and fungal strains using the agar well diffusion method. Results indicated significant antimicrobial activity of the formulated AgNPs, suggesting their potential in pharmaceutical and biomedical applications. Antibacterial evaluation demonstrated that both the methanolic extract and the biosynthesized AgNPs exhibited significant activity against various bacterial strains, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus capitis*. These findings suggest that the plant-derived AgNPs could serve as effective natural alternatives to conventional antibiotics. Overall, this eco-friendly synthesis approach represents a promising alternative to traditional physical and chemical nanoparticle production methods and holds potential for application in antimicrobial therapies and other biological systems

Keywords: Onosma bracteatum, Silver Nanoparticles, Green Synthesis, Methanolic Extract, Antimicrobial Activity, Phytoconstituents, Characterization

1. INTRODUCTION

Anti-microbial resistance (AMR) is a growing global health concern due to the development of drug-resistant bacteria as a result of the widespread use of antibiotics. This has resulted in an urgent need for the development of new and effective antimicrobial agents. Medicinal plants have long been used in traditional medicine for the treatment of various ailments, including bacterial infections. Numerous studies have reported the antibacterial activity of plant extracts against a wide range of bacteria [1].

These plant-based antimicrobial agents offer several advantages over synthetic antibiotics, including a lower toxicity profile, fewer side effects, and a diverse range of bioactive compounds that can target different aspects of bacterial growth and survival, making it difficult for bacteria to develop resistance. Plant extracts are known to contain various bioactive compounds, such as alkaloids, flavonoids, tannins, and phenolic compounds, which have been shown to possess antimicrobial activity [2]. However, the efficacy of plant-based antibiotics is limited due to factors such as poor solubility, bioavailability, and stability. To address these limitations, researchers have turned to the use of nanoparticles, which possess unique physical and chemical properties that make them promising candidates for antimicrobial applications [3]. Silver nanoparticles, in particular, have been extensively studied for their broad-spectrum antimicrobial activity against both gram positive and gram-negative bacteria [4].

In this study we have taken the plant Onosma L. (Boraginaceae) .The name onosma for this genus was introduced into modern botanical nomenclature by Linnaeus, which is derived from a Latin word “osma” originated from a Greek word, “osma” means smell. All species grow in dry or moist and sunny habitats usually in rock crevices and popularly known as rock garden plants. Traditionally, genus Onosma L. plants are used as a stimulant in rheumatism, bladder pain, kidney irritation, palpitation of heart and roots for their diuretic, cooling, astringent and demulcent action. While in India, it is used in the treatment of hypertension, fever and nervous conditions [5]. The aim of this research work is to evaluate the bioactive constituents like phenols, flavonoid and study of silver nanoparticles as well as set the pharmacognostic and antimicrobial specifications of selected medicinal plant

Material and methods

Isolation and identification of pathogens from water bodies:

COLLECTION OF SAMPLES

This phase involved collection of water samples from selective water bodies across the Bhopal, districts of Madhya Pradesh, India. Water bodies which are in close proximity of human population and people come in direct or indirect contact with it, were selected for the sampling purpose. Twelve water samples (three replicates) were collected according to standard methods for examination of water and waste water **(APHA, 2005)** from different four water bodies during winter 2020 in range of water temperature from 19℃ to 26℃. [6] The four water bodies selected for the study were:

Hatayekheda lake, Bhopal, MP

Upper Lake, Bhhopal, MP

Lower Lake, Jahangirabad, Bhopal, MP

Kaliasot Dam, Bhopal, MP

The water samples were collected in sterile 100 ml plastic containers (Tarson) aseptically from below the surface of water. At any selected water-body, all the samples were collected on the same day from different locations. The samples were carried to laboratory with minimum disturbance in cooling boxes to avoid direct sunlight and were processed within 24 hrs. Depending upon the size and accessibility of each water body, different numbers of water samples were collected.

2. MICROORGANISMS

Water samples were cultured on nutrient and MacConkey agar medium (HiMedia Laboratories Ltd., India) and incubated for 24 hours at 37±1℃. Gram stain for different bacterial isolates was done. Using conventional methods Gram-negative bacterial isolates were identified by testing reactions of bacterial isolates according to Bergey's Manual of Determinative Bacteriology, 8th Edition [7]. The tested organisms were selected from identified bacterial genera included seven common Gram-negative bacteria: Escherichia coli, Pseudomonas aeruginosa, Citrbacterfreundii, Proteus penerri, P. vulgaris, Proteus sp. and Aeromonas janadaei.

Screening of samples

This phase deals with the screening of all the samples collected from selected water bodies. All samples were subjected to the following:

I. pH Determination

II. Most Probable Number (MPN)

III. Isolation of pathogenic bacteria

I. pH Determination

pH is defined as the reciprocal of the common logarithm of hydrogen ion activity, which is the product of hydrogen ion concentration and the activity of coefficient. Conventionally it is used as a scale of hydrogen ion concentration of a sample solution The pH value of water is an indicator of its corrosivity or possible toxicity on aquatic bodies. It can also control the efficiency of water treatment. The pH can be measured in all types of water including drinking water, mineral water, rainwater, bathing water, surface or ground water, industrial and waste water [8].

The analysis of samples was carried out under standard conditions using pH meter. The pH standard buffer solution of pH - 4, pH - 7 and pH - 9.2 were prepared and used for standardization of the pH Meter [9].

All the pH values were noted and maintained.

Most Probable Number (MPN)

The technique of enumerating coliforms by means of multiple-tube fermentation (MTF) has been used for over 80 years as a water quality monitoring method. The method consists of inoculating a series of tubes with appropriate decimal dilutions of the water sample [10].

III. Isolation of pathogenic bacteria

The collected water samples were processed by direct streaking on various selective and differential media using the T-streak method. The plates were incubated at 37˚C for 24 hrs and observed for their colony characteristics which were noted down. The isolated organisms were then sub cultured on nutrient agar medium [11].

Media used:

The media used for sample processing and isolation of water borne pathogens were:

- Sterile Thiosulphate citrate bile salts sucrose agar - for *Vibrio cholerae*
- Sterile MacConkey's agar - for *Escherichia coli*
- Sterile Salmonella Shigella agar - for Shigella spps and Salmonella spps

PHASE III – IDENTIFICATION OF ISOLATES

The identification of the isolates was carried out on the basis of Gram's character, colony characters and biochemical properties according to Bergey's Manual of Determinative Bacteriology, 8th Edition.

I. Grams Character Determination

Culture suspensions of the isolates were prepared from their 24 hrs old cultures using sterile saline. A loopful of the culture was smeared on a clean, dry and grease free slide. The smear was heat fixed and allowed to dry and staining was carried out using Gram's Method. After drying, the smear was observed under oil immersion lens and the Gram's character and morphology of the isolates was determined and noted down.

II. Biochemical Tests

The following biochemical tests were carried out with the isolates obtained as per the Bergey's Manual of Determinative Bacteriology, 8th edition [6].

 Sugar fermentation using sterile peptone water base with Andrade's indicator with inverted Durham's tube and 1% of the following sugars

a. Glucose

b. Lactose

c. Maltose

d. Mannitol

e. Xylose

f. Sucrose

 Indole test using sterile 1% Tryptone broth

 Methyl Red test using sterile Glucose Phosphate broth

 Voges Proskaeur test using sterile Glucose Phosphate broth

 Citrate Utilization test using sterile Simmon's Citrate agar slant

 Nitrate Reduction test using sterile Peptone Nitrate broth

 Urease test using sterile Christenson's Urea agar slant

 Triple Sugar Iron test using sterile TSI agar slant and butt

 Gelatinase test using sterile Frazier's Gelatin agar plate

 Casien hydrolysis using sterile Milk agar plate

 Starch hydrolysis using sterile Starch agar plate

 Lysine decarboxylase test

Special tests carried out were:

 Catalase test using Hydrogen peroxide

 Oxidase test using Oxidase reagent

 Cholera red test using H_2SO_4

Comparative testing of sensitivity of the bacterial isolates against antibiotic disc:

This phase deals with the antibiotic sensitivity testing of all the isolates and selection of multi drug resistant isolates. The antibiotic sensitivity testing of the isolates was carried out by the Agar Disc Diffusion Method**.** The method allows the susceptibility testing of the isolates against a variety of antimicrobial agents as sensitive, resistant or intermediate. For the testing, commercially prepared filter paper discs, impregnated with specific amounts of antibiotics were used. Nine different antibiotics were used for the antibiotic sensitivity testing, based on their use in treatment by clinicians [12].

3. PRINCIPLE

The filter paper discs impregnated with known concentrations of antibiotics were placed on the surface of the agar medium inoculated with the test organism and incubated at 37˚C for 24 hrs. The drug on the disc diffuses through the medium. As the distance from the disc increases, the concentration of the drug decreases logarithmically, creating a concentration gradient of the drug in the medium around each disc. In the areas where the concentration of the drug is inhibitory, there is no growth,

creating a clear zone (zone of inhibition) around the disc. No clear zone indicates no inhibition or resistance.

4. REQUIREMENTS

1. Test Organism

The isolates obtained from pathological samples were the test organisms. A suspension of 24hr old culture of each isolate was prepared and its optical density was adjusted to match the first tube in the Brown's Opacity standard.

2. Antibiotic discs used

- Ampicillin (10 mcg)
- Cephalothin (30 mcg)
- Chloramphenical (30 mcg)
- Clindamycin (2 mcg)
- Erythromycin (15 mcg)
- Gentamicin (10 mcg)
- Oxacillin (1 mcg)
- Vancomycin (30 mcg)
- Tetracyclin(30 mcg)
- Ciprofloxacin (5 mcg)
- Metranidazole(30 mcg)
- Doxycycline (30 mcg)

Standard antibiotic discs were obtained from Hi Media.

3. Medium

The medium used to perform the test was sterile Mueller Hinton Agar plate.

Procedure

1) Sterile Mueller Hinton agar plates were poured and allowed to dry. After 10 minutes, the plates were turned over to avoid moisture accumulation.

2) The culture suspensions were streaked on the dry Mueller Hinton agar plates using sterile cotton swabs aseptically. The swabs were streaked thoroughly on the surface of the medium.

3) The antibiotic discs were then placed on the streaked plates using sterile forceps under aseptic conditions and were pressed gently to ensure uniform contact. The discs were evenly placed with at least 24 mm (centre to centre) gap between them.

4) The plates were incubated at 37˚C for 24 hrs.

The plates were observed for zone of inhibition after 24 hrs. The diameter of the zone of inhibition for every isolate against every antibiotic used was noted down.

6) The zone diameters measured around each disc were interpreted using the Kirby – Bauer's Chart. On the basis of these results, the isolates were reported as Sensitive, Resistant or Intermediate.

Fabrication of nanoparticles:

Preparation of the Extract

Weighing 25 g of *Onosma bracteatum* was thoroughly washed in distilled water, dried, cut intofine pieces and was smashed into 100 ml sterile distilled water and filtered through WhatmanNo.1 filter paper (pore size 0.45 µm) and was further filtered through 0.22 µm sized filters. The extract was stored at 4 ^{0}C for further experiments.

Synthesis of Silver nanoparticles from *Onosma bracteatum* extract

The aqueous solution of 1mM silver nitrate (AgNO3) was prepared and used for the synthesis of silver nanoparticles. 10 ml of *Onosma bracteatum* extract was added into 90 ml of aqueous solution of 1 mM silver nitrate for reduction into Ag+ ions and kept for incubation period of 15 h at room temperature. Here the filtrate acts as reducing and stabilizing agent for 1mM of AgNO3.[13]

Evaluation of nanoparticles:

UV-Vis Spectroscopy[14]

The silver nanoparticles were characterized in a Systronic 2202 PC-based UV visible spectrophotometer to know the kinetic behavior of ZnO and Ag nanoparticles. The scanning range for the samples was 200-800 nm at a scan speed of 480 nm/min. The spectrophotometer was equipped with "UV Win lab" software to record and analyze data. Baseline correction of the spectrophotometer was carried out by using a blank reference. The UV-Vis absorption spectra of all the samples were recorded and numerical data were plotted.

X-RAY Diffraction method

The phase evolution of calcined powder as well as that of sintered samples was studied by X-ray diffractometer (Tokyo, Japan) by Rigaku Smart Lab SE, after using CuKα radiation (40 kV and 40 mA). The generator voltage and current was set at 35 KV and 25 mA respectively. The samples were scanned at the rate of 15 ^{O}/min with step size 0.01^{O}.The samples were scanned in the 2θ ranges 15 to 70°C range in continuous scan mode. Phases present in the sample has been identified with the search match facility available with software [15].

Particle size & surface morphology:

It provides crucial information on the control of the preparation procedure and can be used to improve the process. The size, zeta potential, and shape of vesicular dispersion were determined using a Malvern zeta sizer and field emission scanning electron microscopy (FESEM). FESEM was used to examine the surface morphology of an optimized formulation of transferosomes, ethosomes, and niosomes. For the analysis, 2 drops of material were deposited on carbon support film, dried, and then negative stained using a 1 percent solution of Urenyl acetate [16].

Zeta Potential (ZP):

Determination of the ZP of the prepared vesicles was carried out using the Malvern zeta sizer (Malvern Instrument Ltd., Worcestershire, UK). ZP measuring was done by utilizing the equipment to notice the particles' electrophoretic movement in the electric field. Each sample was measured for three times and the results represent the average value ± SD [17].

Determining the MIC of Biosynthesized silver nanoparticles:

Silver nanoparticles were synthesized by using leaves extract. Two-fold dilutions of silver nanoparticles were prepared i.e 10,20,40,80,160 µg/ml and utilized in this study. 200µl bacterial suspension was innocated in each test tube containing different concentrations of silver nanoparticles and similar amount of Muller Hinton Broth (MHB) and incubated at 37°C for 24 hrs. A positive control (tube contain only bacterial suspension and nutrient media without nanoparticles) and a negative control(tube containing nanoparticles and nutrient medium without bacterial suspension) was also involved in methodology.[18]

5. RESULTS

Isolation and identification of pathogens from water bodies:

A total of 12 water samples were collected from 04 water bodies across across the Bhopal, districts of Madhya Pradesh, India. The sampling was done for all winter seasons. All the samples were screened within 24 hours of their collection.

Table1, gives the details of sampling carried out across the 04 locations. Photographic data of sampling is presented in Figure 1.

Table 1: Water bodies across the Bhopal region

Sr. No.	Water Bodies	No. of Sample collected in winter season	Total samples
1	Hatayekheda lake, Bhopal, MP	03	03
2	Upper Lake, Bhhopal, MP	03	03
3	Lower Lake, Jahangirabad, Bhopal, MP	03	03
4	Kaliasot Dam, Bhopal, MP	03	03
Total Samples			12

Figure 1: Collections of water sample from different water bodies

PHASE II – SCREENING OF SAMPLES

All the samples collected from selected water bodies were subjected to the following:

I. pH Determination

The pH values for all the 12 water samples was monitored and noted. The monitoring was done under standard operating conditions.

The Central Pollution Control Board (CPCB) Guidelines for Water Quality Monitoring suggest a range pH 6.5 to pH 8.5 for drinking water source without conventional treatment but with chlorination. Similarly for drinking water source with conventional treatment the recommended pH range is pH 6 to pH 7. The pH value of most of the water samples collected remained within the prescribed range.

Table 2: temperature, pH and turbidity of water samples at different water bodies

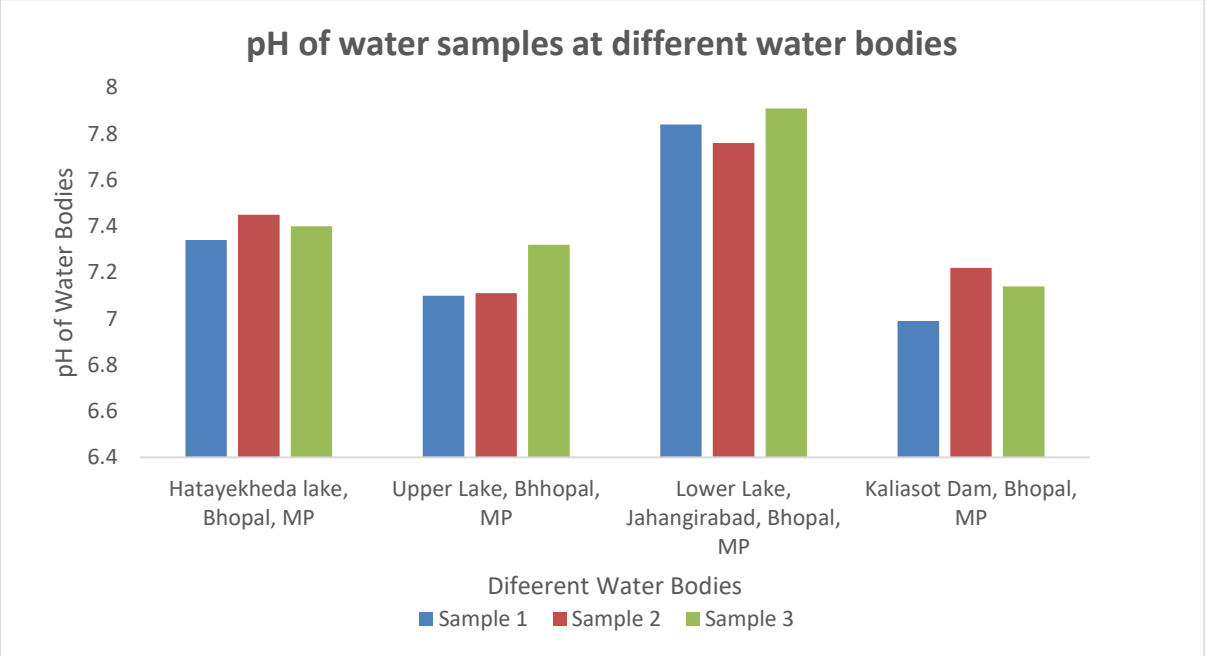
Water Bodies	Sample	Temperature ℃	pH	Turbidity/NTU
Hatayekheda lake, Bhopal, MP	HS1	20.8	7.34	5.68
	HS2	21.4	7.45	6.66

	HS3	21.2	7.40	5.98
Upper Lake, Bhhopal, MP	US1	22.3	7.10	4.52
	US2	23.1	7.11	4.73
	US3	22.9	7.32	5.01
Lower Lake, Jahangirabad, Bhopal, MP	LS1	19.8	7.84	7.81
	LS2	21.1	7.76	7.62
	LS3	20.7	7.91	7.93
Kaliasot Dam, Bhopal, MP	KS1	23.8	6.99	4.38
	KS2	24.5	7.22	4.29
	KS3	24.8	7.14	4.51

NTU= nephelometric turbidity units, Turbidity < 40.00 NTU for class 1 and < 100 NTU for class 2 and 3.

Physicochemical parameters such as pH, Temperature and Turbidity have key influence on the growth of bacterial population. For example, pH values ranging from 3 to 10.5 could support growth of indicator and pathogenic bacteria (Aydin, 2006). At extreme pH (8.2) cells die-off can be expected but generally, as in our case, the pH range for the most drinking water sources was close to 7 not inhibiting the growth of bacteria (Million, 2008). However, pH of drinking water less than 7.0 causes corrosion of water pipes so metal thus releases into the drinking water causing metal contamination and deteriorating the water quality although the pH of drinking water usually ranges from 5.5 to 9. Moreover, turbidity and temperature may also affect the microbial population of drinking water. The higher values of turbidity are often associated with higher population of microorganisms as well as the suspended particles . As NTU of all water bodies is less than 40, so all samples can be considered as in accordance with the quality standard for this parameter.

Graph 1 gives us information about the variations in the pH value of water samples within a season and also across the different location.



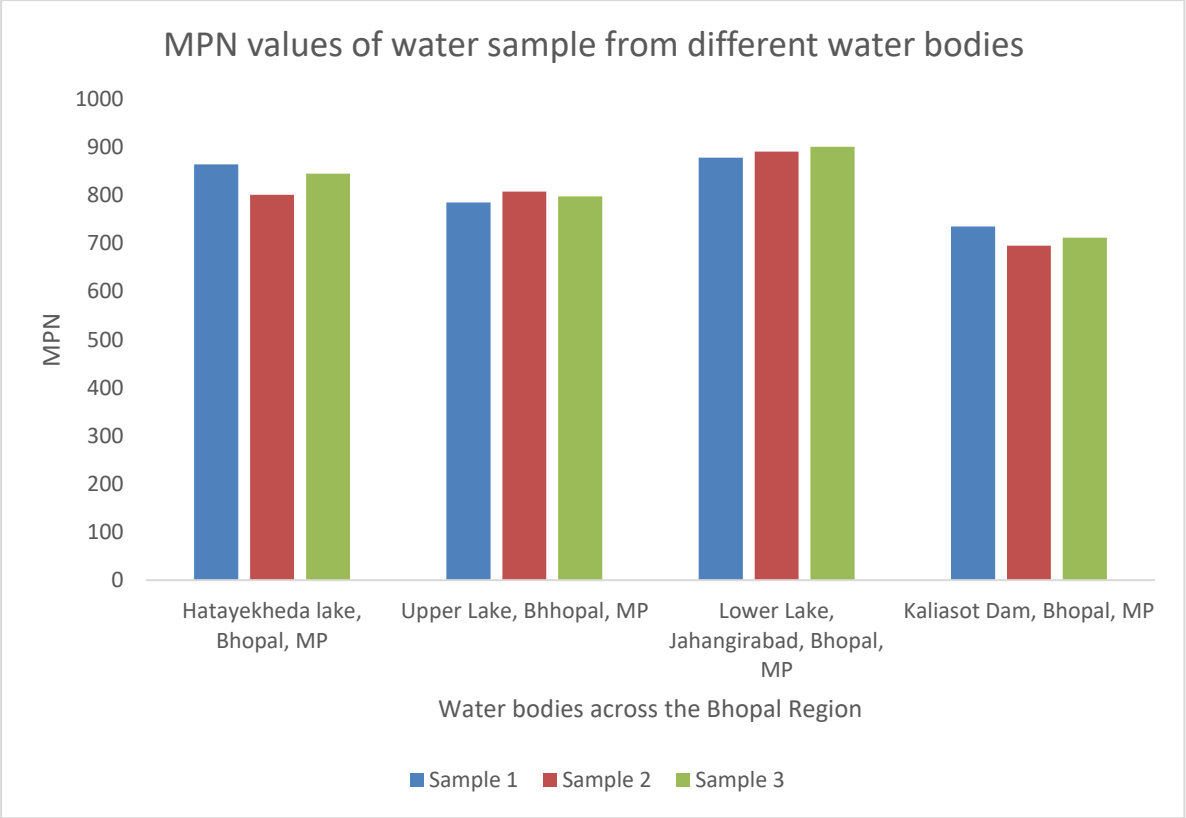
Graph 1: pH of water samples at different water bodies

Regarding the turbidity, a water of natural source usually ranges from 3.00 to 500.00 nephelometric turbidity units (NTU), while in drinking water, the turbidity should be lower than 1.00 NTU. The turbidity of the samples ranged from 0.17 to 93.20 NTU. Since the classification that the rivers are class 2, all the samples can be considered as in accordance with the quality standards for this parameter

II. Most Probable Number (MPN)

The MPN values for all the 12 water samples were determined using standard methods. Figure 5 gives us information about the variations in the MPN value of water samples within a season and also across the three seasons.

The Central Pollution Control Board (CPCB) Guidelines for Water Quality Monitoring recommends Most Probable Number (MPN) count to be below 500 for drinking water source without conventional treatment but with chlorination. Similarly for drinking water source with conventional treatment the suggested limits are below 5000.



Graph 2: MPN values of water sample from different water bodies

Table 3: MPN of water samples from water bodies across the Bhopal region

Water Bodies	Sample	MPN
Hatayekheda lake, Bhopal, MP	HS1	864
	HS2	801
	HS3	845
	US1	785

Upper Lake, Bhhopal, MP	US2	808
	US3	798
Lower Lake, Jahangirabad, Bhopal, MP	LS1	878
	LS2	891
	LS3	901
Kaliasot Dam, Bhopal, MP	KS1	735
	KS2	695
	KS3	712

All the results obtained from present studies are shown in the Figure 5.As shown in the Table 4, result of the experiment in 24 hours comes positive in 8 samples and in rest 4 samples results were negative. But after 48 hours all 12 samples ofwater gives positive results of MPN Test.Graph were also developed to show the resultsobtained from the experiment. The MPN values for Kaliasot Dam, Bhopalwater samples stay reasonably low i.e. 695-735.In contrast, the MPN values for Lower Lake, Jahangirabad, Bhopalsamples are highest i.e. 878-901.

From all the results obtained by applying MPN test it is clear that the water from different water bodies is highly polluted due to sewage discharge. Results of MPN tests are positive which confirms the presence of coliform bacteria in waters. According to the parameters given by WHO presenceof coliforms in any water sample indicates that the water is polluted and is not of potable quality, because coliforms may cause various diseases like cholera, which are highly destructive.

Isolation of Pathogenic Bacteria

The collected water samples were processed by direct streaking on various selective and differential media using the T-streak method.

Media used:

The media used for sample processing and isolation of water borne pathogens were:

- Sterile Thiosulphate citrate bile salts sucrose agar - for *Vibrio cholerae*
- Sterile MacConkey's agar - for *Escherichia coli*
- Sterile Salmonella Shigella agar - for Shigella spps and Salmonella spps

Fig. 2, 3 & 4 gives information about the numbers of colonies obtained on the selective media plates used for isolation of pathogens. Salmonella Shigella agar plates show more number of colonies because of the wide range of bacteria that can grow on it. MacConkey's agar and TCBS agar show comparatively lesser colonies. The colony count mostly goes higher during winter season. The maximum number of colonies was obtained during sampling of Hatayekheda lake, Bhopaland Lower Lake, Jahangirabad, Bhopal. Similarly, least number of colonies were collected duringsampling of Kaliasot Dam, Bhopal, MP. A total of 373 colonies were obtained during the entire process.

Figure 2: Sterile Thiosulphate citrate bile salts sucrose agar

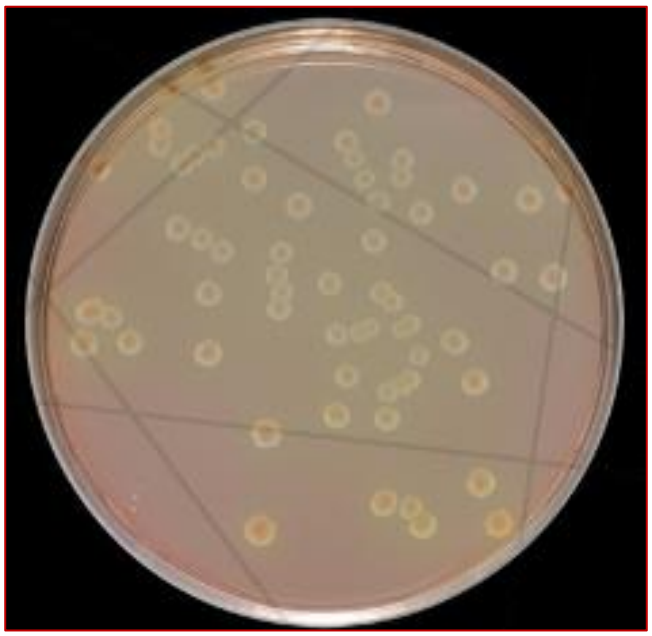


Figure 3: Colonies obtained on MacConkey's Agar plates

Figure 4: Sterile Salmonella Shigella agar

Graph 3: Representation of the distribution of colonies obtained on selective plates

Identification of isolates

The identification of the isolates was carried out on the basis of Gram's character, colony characters and biochemical properties according to Bergey's Manual of Determinative Bacteriology, 8th Edition.

I. Grams Character Determination

The Gram staining of all the 373 isolates was carried out using standard methods. The results of Gram staining are presented in Table 4. All the isolates showed Gram Negative characteristics. Most of the isolates showed rod shaped morphology. Few of the isolates showed short rod or curved morphology. It was observed that different bacterial colonies were isolated from the waterof these four lakes. Colonies varied from circuar, irregularmargins as well as rhizoidal and filamentous in shape. Differentcolonies obtained were subjected to gram staining and thus cocci,bacilli and coccobacilli forms were identified. It was found thatmaximum strains when subjected to microscopic examinationrevealed that gram negative bacilli were predominant followedby gram positive cocci and gram positive bacilli. The results aretabulated in Table 4.

Table 4: Morphological observations of the isolates from Hatayekheda lake (HL)

Sample	Strain	Form	Surface	Color	Margin	Eelvation	motility
Sample 1	HS11	Circular	Smooth	Cream	Convex	Raised	-
	HS12	Circular	Smooth	Yellow	Entire	Flat	+
	HS13	Circular	Smooth	Whitish	Entire	Convex	+
Sample 2	HS21	Filamentous	Glistening	White	Lobate	Umbonate	+
	HS22	Circular	Smooth	Whitish	Entire	Convex	+
	HS23	Irregular	Rough	Creamy	Undulate	Flat	+
Sample 3	HS31	Smooth	Shiny	White	Entire	Convex	+

	HS32	Circular	Smooth	Colourless	Lobate	Slighlty raised	-
	HS33	Circular	Smooth	Whitish	Entire	Convex	+
	HS34	Filamentous	Glistening	White	Lobate	Umbonate	+

Table 5: Morphological observations of the isolates from Upper Lake (UL), Bhopal

Sample	Strain	Form	Surface	Color	Margin	Eelvation	motility
Sample 1	US11	Circular	Smooth	Whitish	Entire	Convex	+
	US12	Circular	Smooth	Yellow	Entire	Flat	+
	US13	Irregular	Rough	Creamy	Undulate	Flat	+
Sample 2	US21	Circular	Smooth	Whitish	Entire	Convex	+
	US22	Irregular	Rough	Creamy	Undulate	Flat	+
Sample 3	US21	Smooth	Shiny	White	Entire	Convex	-
	US32	Circular	Smooth	Colourless	Lobate	Slightly raised	-
	US33	Irregular	Glistening	Cream	Entire	Raised	-
	US34	Circular	Smooth	Whitish	Entire	Convex	+

Table 6: Morphological observations of the isolates from Lower Lake, Jahangirabad

Samples	Strain	Form	Surface	Color	Margin	Eelvation	motility
Sample 1	LS11	Circular	Glistening	Cream	Entire	Raised	+
	LS12	Circular	Smooth	Whitish	Entire	Convex	+
	LS13	Circular	Smooth	Yellow	Entire	Flat	+
Sample 2	LS21	Irregular	Glistening	Cream	Entire	Raised	-
	LS22	Circular	Smooth	Whitish	Entire	Convex	+
	LS23	Irregular	Rough	Creamy	Undulate	Flat	+
Sample 3	LS31	Smooth	Shiny	White	Entire	Convex	+
	LS32	Irregular	Rough	Creamy	Undulate	Flat	+
	LS33	Circular	Smooth	Whitish	Entire	Convex	+

Table 7: Morphological observations of the isolates from KaliasotDam(KD), Bhopal

Samples	Strain	Form	Surface	Color	Margin	Eelvation	motility
Sample 1	KS11	Filamentous	Glistening	White	Lobate	Umbonate	+
	KS12	Irregular	Rough	Creamy	Undulate	Flat	+
	KS13	Circular	Smooth	Whitish	Entire	Convex	+
Sample 2	KS21	Filamentous	Glistening	White	Lobate	Umbonate	+
	KS22	Circular	Smooth	Whitish	Entire	Convex	+
	KS23	Irregular	Rough	Creamy	Undulate	Flat	+
	KS24	Circular	Smooth	Cream	Convex	Raised	-
Sample 3	KS31	Filamentous	Glistening	White	Lobate	Umbonate	+
	KS32	Circular	Smooth	Yellow	Entire	Flat	+
	KS33	Irregular	Rough	Creamy	Undulate	Flat	+

Biochemical Tests

After determination of the Gram's character for all the isolates, the isolates were subjected to biochemical tests for identification. A number of biochemical tests were performed for the identification of bacterial isolates with the help of Bergey's Manual. The principal tests used for this purpose are Indole Test (IND), Methyl Red Test (MR), Voges-Proskauer Test (VP), Citrate Utilization Test (CUT), Urease Test (UT), Nitrate Reduction Test (NRT), Oxidase Test (OXI), Catalase Test (CAT), etc.

After considering the results of Gram's character and primary biochemical tests, further biochemical tests were selected.

Table 8: Biochemical Test observations of the isolates from Hatayekheda lake

Sample	Strain	IND	MR	VP	CUT	UT	NRT	OT	CT	Microbe Identification
Sample 1	HS11	-	+	+	+	-	+	-	-	Enterococus faecalis
	HS12	+	+	-	+	-	+	-	+	Salmonella sp
	HS13	-	+	-	+	-	+	+	+	Pseudomonas sp
Sample 2	HS21	-	+	-	+	-	+	+	+	Pseudomonas sp
	HS22	+	+	-	-	-	+	-	+	Escherichia coli
	HS23	-	-	+	+	-	+	+	+	Bacillus subtilis
Sample 3	HS31	-	-	+	+	+	+	-	+	Enterobacter sp.
	HS32	+	+	-	+	-	+	-	+	Salmonella sp
	HS33	+	+	-	-	-	+	-	+	Escherichia coli
	HS34	-	+	-	+	-	+	+	+	Pseudomonas sp

Table 9: Biochemical Test observations of the isolates from Upper Lake, Bhopal

Sample	Strain	IND	MR	VP	CUT	UT	NRT	OT	CT	Microbe Identification
Sample 1	US11	-	+	-	+	-	+	+	+	Pseudomonas sp
	US12	+	+	-	+	-	+	-	+	Salmonella sp
	US13	-	-	+	+	-	+	+	+	Bacillus subtilis
Sample 2	US21	+	+	-	-	-	+	-	+	Escherichia coli
	US22	-	-	+	+	-	+	+	+	Bacillus subtilis
Sample 3	US21	-	-	+	+	+	+	-	+	Enterobacter sp.
	US32	+	+	-	+	-	+	-	+	Salmonella sp
	US33	-	-	+	+	+	+	_	+	Klebsella pneumonia
	US34	-	+	-	+	-	+	+	+	Pseudomonas sp

Table 10: Biochemical Test observations of the isolates from Lower Lake, Jahangirabad

Sample	Strain	IND	MR	VP	CUT	UT	NRT	OT	CT	Microbe Identification
Sample 1	LS11	-	+	+	-	+	+	-	+	Proteus mirabilis
	LS12	-	+	-	+	-	+	+	+	Pseudomonas sp
	LS13	+	+	-	+	-	+	-	+	Salmonella sp
Sample 2	LS21	-	-	+	+	+	+	_	+	Klebsella pneumonia
	LS22	+	+	-	-	-	+	-	+	Escherichia coli
	LS23	-	-	+	+	-	+	+	+	Bacillus subtilis
Sample 3	LS31	-	-	+	+	+	+	-	+	Enterobacter sp.
	LS32	-	-	+	+	-	+	+	+	Bacillus subtilis
	LS33	-	+	-	+	-	+	+	+	Pseudomonas sp

Table 11: Biochemical Test observations of the isolates from Kaliasot Dam, Bhopal, MP

Sample	Strain	IND	MR	VP	CUT	UT	NRT	OT	CT	Microbe Identification
Sample 1	KS11	-	+	-	+	-	+	+	+	Pseudomonas sp
	KS12	-	-	+	+	-	+	+	+	Bacillus subtilis
	KS13	+	+	-	-	-	+	-	+	Escherichia coli
Sample 2	KS21	-	+	-	+	-	+	+	+	Pseudomonas sp
	KS22	+	+	-	-	-	+	-	+	Escherichia coli
	KS23	-	-	+	+	-	+	+	+	Bacillus subtilis

	KS24	-	+	+	+	-	+	-	-	Enterococus faecalis
Sample 3	KS31	-	+	-	+	-	+	+	+	Pseudomonas sp
	KS32	+	+	-	+	-	+	-	+	Salmonella sp
	KS33	-	-	+	+	-	+	+	+	Bacillus subtilis

Table 12. Bacterial occurrence in different drinking water samples

Microbes Identified	No. of Isolates	Samples Contaminated
Pseudomonas sp	10	HS13, HS21, HS34, US11 US34, LS12, LS33 KS11, KS21, KS31
Bacillus sp	8	HS23, US13, US22, LS23, LS32, KS12, KS23, KS33
Salmonella sp	6	HS12, HS32, US12, US32, LS13, KS32
Escherichia Coli	6	HS22, HS33, US21, LS22, KS13, KS22
Enterobacter sp	3	HS31, US21, LS31
Klebsiella sp	2	US33, LS21,
Enterococcus feacalis	2	HS11, KS24
Klebsella pneumonia	2	US33, LS21,
Proteus mirabilis	1	LS11

Comparative testing of sensitivity of the bacterial isolates against antibiotic disc.

The antibiotic sensitivity testing of the target isolates was carried out by the **Agar Disc Diffusion Method.** From the 40 total isolates, 9 isolates were identified as the target organisms. All these 9 isolates were screened against 10 antibiotic agents. The isolates were surface spread on Sterile Mueller Hinton Agar plates and filter papers discs impregnated with antibiotics of known concentration were placed on the plates. The plates were observed for inhibition around the discs after 24 hrs of incubation.

The results for all the 9 isolates are represented as..

Isolates were subjected to an antibiotic susceptibility test using 10 different antibiotics from which their antibiotic resistance profiles and multiple antibiotic resistance phenotypes were compiled.. The results revealed that a large proportion of the environmental isolates were resistant to erythromycin, followed by trimethoprim and amoxicillin. None of the isolates were resistant to ciprofloxacin and only very few isolates from dam were resistant to streptomycin and neomycin.

Result showed from Table 8 that all organisms isolated, were resistant, intermediate or susceptible to all the antibiotics. Escherichia coli was shown to be resistant to erythromycin, Pseudomonas aeroginosa showed resistance to teracyclin, chloramphenicol, amoxycillin and nitrofurantoin. Staphylococcus aureus shows resistance to erythromycin, chloramphenicol, nalidixic acid and cotrimozazole. Also, Klebsiella pneumoniae shows resistance to chloramphenicol, gentamycin and amoxycillin. Streptococus faecalis was only resistant to erythromycin. Entrobacter aerogenes was resistant to chloramphenicol and nalidixic acid. Bacillus subtilis was only resistant to erythromycin. All the microorganisms isolated were susceptible to ofloxacin and augmentin.

The antibiotic sensitivity tests showed that ofloxacin and augumentin proved most effective against the bacterial isolates. The prevalence of antibiotics resistant bacteria isolates is of greater importance .Some isolates exhibit multiple resistances while others showed single resistance pattern.

6. CNCLUSION

In the current study, phytochemical analysis of the medicinal plant selected plant *Onosma bracteatum* revealed that it contains several phytochemical with significant antioxidant potential. The current work also illustrated a green and environmentally acceptable nanosynthetic mechanism for the phytoproduction of durable and spherical silvernano particles.

During the phyto-manufacturing of silver nanoparticles (AgNPs) from silver nitrate, extract of the medicinal plant Onosma bracteatum was used as a capping and reducing agents. The findings of physical-chemical characterization showed that the produced AgNPs had a spherical form and the optimal size range. Furthermore, the results of antibacterial analysis suggests that the methanolic extract and silver nanoparticles could be used as natural alternatives to synthetic antibiotics for the treatment of bacterial infections, particularly against E. coli, P. aeruginosa, B. subtilis and S. capitis. Overall, this environmentally friendly technology may be a competitive and alternative to existing physical or chemical processes for production of nanoparticles and may be employed as inhibitors in a range of biological applications.

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