Biotechnol Res 2018; Vol 4(3):88-96
eISSN 2395-6763

ORIGINAL RESEARCH

Biosynthesis of Silver Nanoparticles and their *in vitro* Action against *Aeromonas* sp. and in Water Purification

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• *Received: 10 April 2018 • Revised: 05 June 2018 • Accepted: 17 July 2018 • Published: 04 September 2018* •

ABSTRACT
Silver nanoparticles are known to be highly toxic to microorganism showing strong biocidal effect and are nontoxic to human body at low concentration. The aim of this work was to determine the potential of biosynthesized silver nanoparticle's (BioAgNP's) antimicrobial activity against food borne pathogen *Aeromonas* sp. and in water purification. The *Aeromonas* sp. was isolated from decayed fish. *Psidium gaujava* plant extract showed high antibacterial activity against the isolated organism. The chemical constituents of the leaf extract were analyzed by Fourier Transform Infrared Spectroscopy (FTIR). Silver nanoparticles were biosynthesized using the *P. gaujava* plant extract. The characterization of BioAgNP was done by UV–Vis spectroscopy and Scanning Electron Microscopy (SEM). The biosynthesized silver nanoparticle was then adsorbed on Granular Activated Charcoal (GAC) and their presence was confirmed using FTIR. GAC can be used as a bacterial filter for treating contaminated water. The antibacterial action of silver nanoparticle was studied using Colony Forming Unit (CFU) and Most Probable Number (MPN) test. Enumeration of coliforms and percentage of reduction was studied which showed that effectiveness increases with increase in dose of adsorbed charcoal and treatment time. The obtained result showed that the silver adsorbed activated charcoal can be used as excellent antibacterial media and would have several applications in water treatment system.

KEY WORDS: *BioAgNP, FTIR, SEM, UV–Vis, GAC*

INTRODUCTION

Silver is a nontoxic, safe and inorganic antibacterial agent which itself have an antibacterial property and has been used for centuries, it is even capable of killing about 650 types of pathogenic diseases. Silver has also been used as a water purifier since 1900 or so; since the 1930s, silver has been used to impregnate water filters to kill germs in the water or which might grow in the filter medium (Monisha*et al*., 2014). Highly reactive metal oxide nanoparticles exhibit excellent biocidal action against Gram positive and Gram negative bacteria. Thus, the synthesis, characterization, functionalization of nanosized particles opens the possibility of formulation of a new generation of bactericidal material (Kim *et al*., 2008).

Green synthesis provides advancement over chemical and physical method as it is cost effective, environment friendly, easily scaled up for large scale synthesis and in this method there is no need to use high pressure, energy, temperature and toxic chemicals (Elumalai*et al*., 2010).In this study silver nanoparticles were successfully synthesized using *Psidiumgaujava*(Guava leaf). The present study represents a clean, non toxic as well as eco friendly procedure for synthesizing silver nanoparticles(AgNPs). This technique

gives us a simple and efficient way for the synthesis of nanoparticles with tunable optical properties governed by particle size. From the nanotechnology point, this is a noteworthy development for synthesizing AgNPs economically (Cynthia Mason *et al*., 2012). The fixed ratio of plant extracts and silver ions were mixed and kept at room temperature for reduction. The color change from yellow to reddish brown confirms the formation of nanoparticles. Further, the confirmation of synthesized nanoparticles were characterized by UV–Vis Spectroscopy, SEM, Transmission Electron Microscopy (TEM) and FTIR data. The antimicrobial activity of synthesized nanoparticles has also been examined in Gram positive and Gram negative bacteria and encouraging results are in hand (Kim *et al*., 2005).

Plant extracts play an important role in remediation of toxic metals through reduction in the metal ions. Silver nanoparticles exhibit new optical properties, which are observed neither in molecules nor in the bulk metals. The surface plasmon resonance (SPR) and large effective scattering cross section of individual silver nanoparticles make them ideal candidates for molecular labelling, where phenomena such as Surface Enhance Raman Scattering can be exploited. In addition, silver nanoparticles have recently been shown to be a promising antimicrobial material (Ravindra *et al*., 2010).

Aeromonas sp.belongs to the family Aeromonadaceae; they are gram negative, facultative anaerobic motile rods with rounded ends (bacilli to coccibacilli shape). *Aeromonas* infections arise mostly during environmental changes, stresses, in contaminated environments, and when an organism is already infected with a virus or another bacterium. Two major diseases associated with *Aeromonas* in humans are gastroenteritis which occurs through ingestion of contaminated water or food and wound infections with or without bacteremia that result from exposure to contaminated water (Agger *et al*., 1985; Ananthan and Alavandi, 2003; Vila *et al*., 2003).

The bacterial contamination of drinking water is a worldwide environmental problem. As water is the medium which can support the growth of variety of microorganisms and the presence of such disease causing microbes in water is unhealthy and can even turn to be fatal. Nanotechnology is one of the emerging technologies which can be applied to overcome this issue as silver nanoparticles are highly toxic to microorganisms, showing strong biocidal effect and are non-toxic to human beings at a very low concentration (Harikumar *et al*., 2011).

Activated carbon filtration (AC) is effective in reducing certain organic chemicals and chlorine in water. It can also reduce the quantity of lead in water although most lead-reducing systems use another filter medium in addition to carbon. Water is passed through granular or block carbon material to reduce toxic compounds as well as harmless taste- and odour-producing chemicals (Lemley *et al*., 1995). The length of the contact time between the water and the carbon – which is determined by the rate of water flow – also affect the rate of contaminant adsorption (Dvorak and Skipton, 2008). The activated charcoal when it is applied for water treatment it does not have any biological activity so it is reinforced on to biosynthesized silver nanoparticles for increasing the effectiveness in removing organic contaminants in water.

The characteristics of the carbon material (particle and pore size, surface area, surface chemistry, density, and hardness) influence the efficiency of adsorption. Compounds that are less water soluble (hydrophobic) are more likely to be adsorbed to a solid. If several compounds are present in the water, strong adsorbers will attach to the carbon in greater quantity than those with weak adsorbing ability. These combined factors enable the activated carbon material to draw the molecule out of the water (Lemley *et al*., 1995).

By using MPN and CFU methods the efficiency of water purification by activated charcoal treatment can be determined. In MPN method, the acid and gas production on the tubes was observed. The number of total coliforms is determined by counting the number of tubes giving positive reaction (i.e. both colour change and gas production) and comparing the pattern of positive results with standard statistical tables (Bartram and Pedley, 1996). In CFU method, the sample is spread or poured uniformly on a surface of an agar plate and then incubated at some suitable temperature for a number of days. The colonies that form are counted (Harikumar *et al*., 2011).

Several studies have shown that silver adsorbed GAC can be used as an excellent antimicrobial media and have various application in water treatment system. A filter with GAC is mostly preferred as it is a proven option to remove chemical and organic contaminants in the water, activated charcoal itself doesn't have antimicrobial activity. When the filter with GAC is adsorbed with Biosynthesized AgNPs , the silver nanoparticles increases the efficiency in killing the microorganisms in the water.This work proves that biosynthesized silver nanoparticles adsorbed on GAC are efficient and economical in water purification; it can be effectively used as a filter for the treatment of drinking water.

RESULTS AND DISCUSSION

Isolation and identification of organism

Pink colored rod shaped bacteria was observed on Gram staining. The organism showed positive results to Oxidase, Voges Proskauer, Glucose Fermentation, Mannitol motility and TSI tests. True motility of the organism was observed on hanging drop test. The organism *Aeromonas* sp. grew as creamy white colonies over the Aeromonas Differential agar

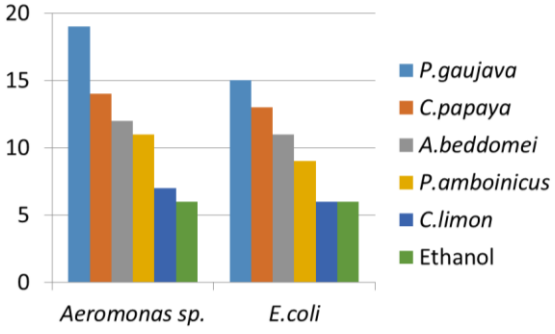

Figure 1: Antibacterial activity of plant extracts against *Aeromonas* sp. and *E. coli*

Fourier Transform Infrared Spectroscopy

The chemical constituents of the leaf extract of *P. gaujava* were analyzed by FTIR spectroscopy. The various IR bands Peaks were centred at 2750 cm^{-1}, 2500 cm^{-1}, 1500 cm^{-1} and 1250 cm^{-1} which corresponds to functional groups such as amines, azo compounds, aldehydes or ketones and aromatic compounds respectively. The First region ranging from 4000 to 2500 the highest peak is shown in the region of 2750 IR spectra corresponding to N–H, C–H and O–H single bonds which show the presence of hydrogen bonded primary amines. The IR absorption peak range from 2500 to 2000; the characteristic peak is shown in the range of 2500, the peak corresponds to the absorption caused by triple bonds indicating the presence of N=N stretching of the azo compounds. The third region ranging from 2000 to 1500, the characteristic peak shown in region of 1500 are the C=O bond indicating the presence of aldehyde or ketone and aromatic compounds (Begum *et al.,* 2013)

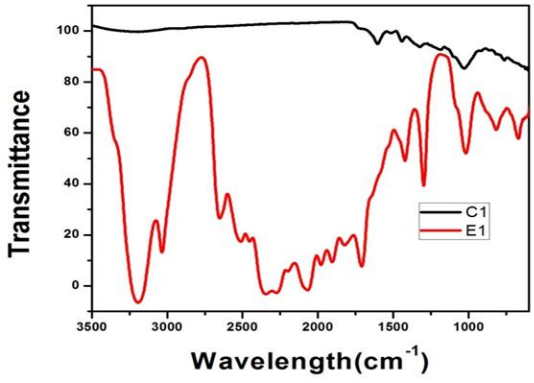

Figure 2: FTIR spectra of plant extract

UV–VIS spectral analysis

The addition of guava leaf extract to silver nitrate ($AgNO_3$) solution resulted in colour change of the solution from transparent to brown due to the production of silver nanoparticles. The colour changes arise from the excitation of surface plasmon vibrations with the silver nanoparticles (Singh *et al*.,2010). Absorbance of AgNPs from wavelength ranging 300 to 700 nm was read. It was observed that the absorbance peak was centred near 450 nm. The UV–Vis absorption spectra of the AgNP were shown in Figure 3

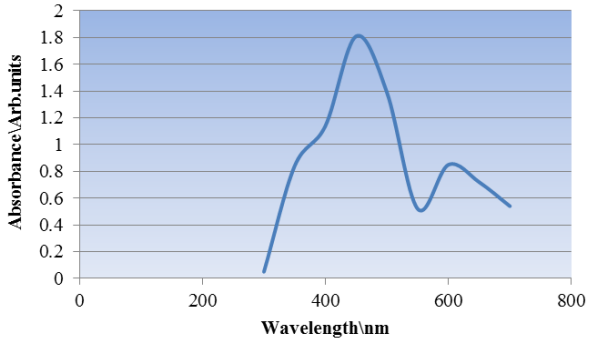

Figure 3: UV–VIS spectra of silver nanoparticles

Scanning Electron Microscopy (SEM) analysis

The SEM image of silver nanoparticles synthesized by "Green synthesis" process by using 10% of plant extract and 1 mM $AgNO_3$ concentration. It gave a clear image of highly dense silver nanoparticles. The silver nanoparticles were spherical in shape with particle size ranging from 93 to 106 nm. The larger silver particles may be due to the aggregation of the smaller ones. The SEM image showing silver nanoparticles synthesized using *P. gaujava* extract confirmed the development of silver nanostructures (Vishnudas *et al.*, 2012) (Figure 4).

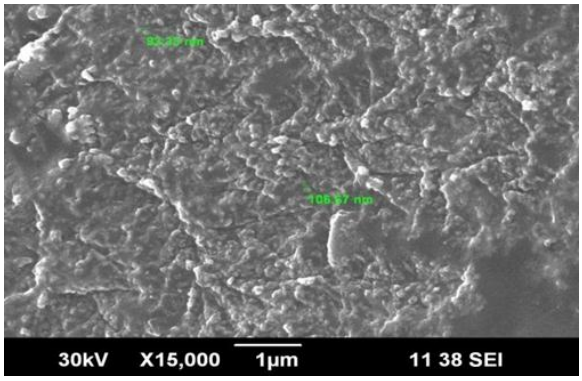

Figure 4: SEM image of silver nanoparticles

Antimicrobial assay of plant extract and BIOAgNP against *Aeromonas* sp. and *E. coli*

The *P. gaujava* silver nanoparticles showed good zone of inhibition against *Aeromonas* compared to the control kept. The green synthesized silver nanoparticles hence found to be having high antibacterial activity against the fish pathogen, *Aeromonas* sp. (Figure 5, 6)

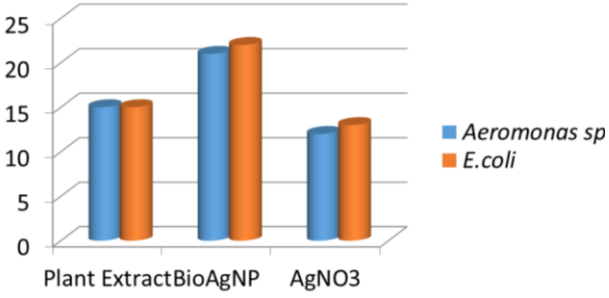

Figure 5: Antimicrobial assay of plant extract and BIOAgNP against *Aeromonas* sp. and *E. coli*.

Figure 6: Antimicrobial assay of plant extract and BIOAgNP against *Aeromonas* sp. and *E. coli*.

The physical appearance of the reaction mixture turning from yellow to brown is due to the SPR of the silver nanoparticles, which is considered to be the primary signature of nanoparticle formation (Singh *et al*.,2010) (Figure 7)

Figure 7: Plant extract (before incubation) and BIOAgNP (after incubation)

Fourier Transform Infrared Spectroscopy of BIOAgNP adsorbed charcoal

The surface chemistry of BioAgNPs adsorbed charcoal via *P. gaujava* is revealed by the appearance in the FTIR spectra of IR bands. The various IR bands peaks centred at 2600, 2400, 1600 and 1500 absorption spectra which correspond to various groups. The *P. gaujava* BioAgNPs adsorbed charcoal FTIR absorption spectra indicates the presence of N–H stretching hydrogen bonded primary amine 2600 cm^{-1}, C–H stretching hydrogen bond 2600 cm^{-1}, C=O stretching hydrogen bond 1600 cm^{-1}. The absorption peak situated around 1600 are the C=O characteristic peak

indicating the presence of aldehyde or ketone and aromatic compounds. The peak at 2400 cm^{-1} indicates the presence of N=N stretching of the azo compounds.

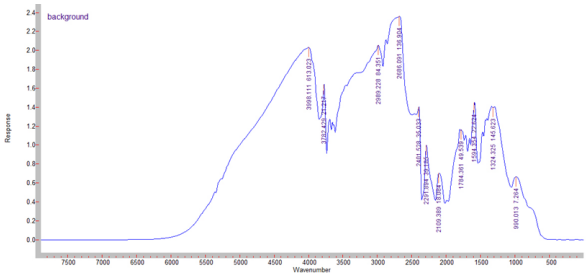

Figure 8: FTIR image of BIOAgNP adsorbed charcoal powder

The FTIR analysis of BioAgNPs adsorbed charcoal via *P. gaujava* confirms the capping of synthesized nanoparticles by *P. gaujava* biomolecules. Many plant phytochemical such as terpenoids, flavonoids, tannins, terpene alcohol and minor constituents including alcohol, aldehyde, esters, terpene esters and terpene oxides are known as a capping and stabilizing agents of AgNPs. Therefore, we conclude that the aldehyde/ketone, aromatic, azo and nitro compounds of *P. gaujava* extract participate in the bio reduction and stabilization of AgNPs by coating them, thereby hindering agglomeration (Begum *et al*., 2013) (Figure 8).

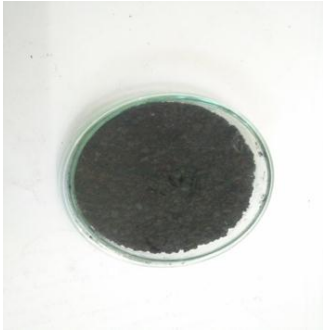

Figure 9: BIOAgNP adsorbed charcoal powder

Utilization of BIOAgNP in Water Purification

From the MPN and CFU methods, when the bacteriologically contaminated water was treated with 2 g adsorbed charcoal, 99.26% efficiency was noted. Similarly upon addition of 0.5 g adsorbed charcoal with 10 ml plant extract, 96.71% efficiency was observed. Correspondingly, when treated with 1 g adsorbed charcoal, 97.51% efficiency was recorded. 1.09% of reduction was shown when the sample was treated with 2 g activated charcoal, which indicates that activated charcoal itself does not have antimicrobial activity.(Table 1,2) The percentage of reduction of coliforms was studied which revealed that the efficiency is directly depended upon the dose of adsorbed charcoal and treatment time.

CONCLUSION

Nanotechnology has grown to be an important research field in all areas. For several years, scientists have constantly explored different synthetic methods to synthesize nanoparticles. On the contrary, the green method of synthesis of nanoparticles is easy, efficient and eco-friendly in comparison to chemical-mediated or microbe-mediated synthesis. This study was conducted for the Green Synthesis of silver nanoparticles from Indian medicinal plant and their action against food borne pathogen *Aeromonas* sp. and in water purification. The organism was isolated from the food sample and confirmed by microscopic and biochemical evaluation. The *P. gaujava* (Guava) plant extract was employed for the synthesis of silver nanoparticles. The chemical constituent of the *P. gaujava* plant extract was analyzed by FTIR (Fig 1). Characterization of the synthesized silver nanoparticle was done using UV–Vis spectrometer (Fig 2) and SEM (Fig 3). Antimicrobial assay of synthesized silver nanoparticles was carried out against the *Aeromonas* sp. and *E. coli* (Fig 4). The biosynthesized silver nanoparticle was adsorbed onto GAC and their presence was confirmed by FTIR(Fig 6). The efficiency of BioAgNP adsorbed charcoal in drinking water purification was determined by MPN (Fig 8) and CFU (Fig 9). The obtained results showed that the increase in concentration of the BioAgNP adsorbed charcoal and treatment time will enhance its efficiency in water treatment. Hence, the study signifies the activity of BioAgNP adsorbed charcoal for the treatment of drinking water purification. Thus the major objective of the work was met.

MATERIALS AND METHODS

Isolation of *Aeromonas* sp. from food sample

Decayed fish sample (sardine) was taken in a petri plate. The intestine was separated and crushed using the mortar and pestle. 1 g of the crushed sample was serially diluted up to 10^{-7} dilution. 0.1 ml

Table 1: MPN chart of water samples

SAMPLE	DOUBLE STRENGTH 10 ml			SINGLE STRENGTH 1 ml			SINGLE STRENGTH 0.1 ml			MPN VALUE	MPN INDEX
	Tube 1	Tube 2	Tube 3	Tube 1	Tube 2	Tube 3	Tube 1	Tube 2	Tube 3		
Bacteriologically contaminated water	A/G	A/G	A/G	A/G	A/G	A/G	A/G	A/G	A/G	3-3-3	>1100
Bacteriologically contaminated water with 2 g adsorbed charcoal	-	-	-	-	-	-	-	-	-	0-0-0	<3
Bacteriologically contaminated water with 0.5 g adsorbed charcoal and 10 ml plant extract	-	-	-	-	-	-	-	-	-	0-0-0	<3
Bacteriologically contaminated water with 1 g adsorbed charcoal	-	A/G	-	-	A/G	-	-	-	-	1-1-0	7
Bacteriologically contaminated water with 2 g activated charcoal	A/G	A/G	A/G	A/G	A/G	A/G	A/G	A/G	A/G	3-3-3	>1100

of the sample from 10^{-6} dilution was plated (Spread plate) on to *Aeromonas* differential agar plates. The plates were incubated at 37°C for 48 hours. After incubation well isolated typical colonies were picked up, transferred to nutrient broth, and incubated at 37°C for 48 hours.

The isolates were identified using Gram staining and various biochemical identification tests such as Oxidase test, Voges Proskauer test, Glucose fermentation test, Mannitol motility test, Triple sugar iron test and Hanging Drop test was done to determine its true motility.

Antimicrobial screening of plant extracts

Five plant samples was taken such as *Psidium gaujava*(Guava), *Carica papaya* (Papaya), *Citrus limon*(Lemon),*Adathoda beddomei*(Adalodakam) and *Plectronthus amboinicus* (Panikoorka) for screening on the basis of their antimicrobial activity. The solvent which was used for the extraction of these leaves was 75% ethanol.

Table 2: Enumeration of coliforms and percentage of reduction after treatment

	Bacteriologically contaminated water	**Bacteriologically contaminated water with 2 g adsorbed charcoal**	**Bacteriologically contaminated water with 0.5 g adsorbed charcoal and 10 ml plant extract**	**Bacteriologically contaminated water with 1 g adsorbed charcoal**	**Bacteriologically contaminated water with 2 g activated charcoal**
No. of colonies	1368(TNTC)	10	45	34	1353(TNTC)
Percentage of reduction (%) after 3 hours	-	99.26%	96.71%	97.51%	1.09%

Preparation of plant extract

Each of the plant leaves taken was sliced into small pieces and kept in a petri plate and oven dried. 1 g of each of the dried plant leaves were crushed with 5 ml of 75% ethanol in pestle and mortar. The extract was then transferred to Eppendorf tubes and the kept for overnight incubation centrifuged at 10,000 rpm for 10 minutes and supernatant was then transferred to a labeled Eppendorf tube. The extract was later stored at 4^{o}C for further analysis (Ravindranath *et al*., 2016).

Antimicrobial activity of plant extract

The antimicrobial activity of the plant extract was studied by Standard Well diffusion method on Muller Hinton Agar (Albew Bereket *et al*., 2014). The media was autoclaved, cooled and poured into the petri plates and kept for half an hour to solidify. The bacterial cultures *Aeromonas* sp. and *E. coli* was swabbed on the Muller Hinton Agar plates and the wells were prepared on the agar using sterile well puncture (5 mm in diameter). The plant extracts (100 µl) each was transferred to the wells. The culture plates were then incubated at 37^{o}C for 24 hours without disturbing and the zone of inhibition was measured and recorded.

Selection of leaf extract and synthesis of silver nanoparticles from leaf extract

The plant extract (*P. gaujava*) which showed highest antimicrobial activity against the cultured bacteria (*Aeromonas* sp.) based on the zone of inhibition was selected.

Preparation of the extract

The leaf sample *P. gaujava*(Guava leaf) was washed thoroughly in distilled water and cut the leaves into fine pieces and smashed into 100 ml of distilled water. It is then boiled for 10 minutes. Later it was filtered through Whatmann No. 1 filter paper and the extract was stored at 4^{o}C for further experiments. The chemical constituents of the plant extract was characterized using FTIR.

Synthesis of silver nanoparticles from leaf extract

For the synthesis of silver nanoparticles aqueous solution of 1 mM silver nitrate ($AgNO_3$) was prepared. 20 ml of 1 mM silver nitrate ($AgNO_3$) was prepared and 2 ml of the leaf extract was added to it and kept for incubation under dark condition for 3–5 hours. Primary formation of silver nanoparticle was investigated (Choudhary R S *et al*., 2014).

Adsorption of silver nanoparticles into GAC

For this preparation, 25 ml of the biosynthesized silver nanoparticles was added to 10 g GAC and allowed to absorb by keeping it in an orbital shaker for 3 days. After that the activated charcoal was

filtered using Whatmann No. 1 filter paper, the filtrate was then transferred into sterile petri plate and oven dried at 80°C(Harikumar *et al*., 2011).

Antimicrobial assay of synthesized silver nanoparticles

Standard well diffusion method was followed to perform the antimicrobial assay on Muller Hinton agar plates. The media was autoclaved, cooled and poured in to the petri plates and kept for 30 minutes to solidify. The bacterial cultures, *Aeromonas* and *E. coli* were swabbed on the Muller Hinton agar plates. Wells were prepared on the Muller Hinton agar using sterile well puncture (5 mm diameter). 100 µl of green synthesized silver nanoparticle in aqueous solution was transferred to one of the wells made on the Muller Hinton agar. 100 µl of silver nitrate solution was kept as control in another well. The cultured plates were incubated at 37°C for 24 hours without disturbing and the zone of inhibition was measured and recorded. The silver nanoparticles thus synthesized were characterized using UV–Vis Spectroscopy, SEM, and FTIR.

Activity of biosynthesized silver nanoparticle adsorbed charcoal in water purification

Antibacterial action of charcoal adsorbed silver nanoparticle was studied using CFU and MPN. Bacteriologically contaminated (*E. coli*) five water samples were taken. The first sample was bacteriologically contaminated water. Correspondingly, the second sample was added with 2 g AgNP Adsorbed charcoal. The third sample was fed with 0.5 g AgNP Adsorbed charcoal and 10 ml of plant extract, and parallely the fourth sample was treated with 1 g AgNP Adsorbed charcoal. The fifth sample was added with 2G activated charcoal. These samples were collectively named as Sample A, Sample B, Sample C, Sample D and Sample E, respectively. In CFU detection method the samples were spread on nutrient agar plates and after incubation at 37°C for 24 hours, the number of colonies was counted. In MPN method 10 ml, 1 ml, 0.1 ml of the samples were transferred to MacConkey fermentation tubes, incubated at 37°C and after 48 hours, recorded the presence or absence of growth. Production of both acid and gas constitutes a positive presumptive test. The number of positive tubes for each dilution was noted and compared with the MPN index chart to get the MPN of total coliforms.

REFERENCES

Lemley, A., Wagenet, L. and Kneen, B.(1995).Activated Carbon Treatment of Drinking Water.*Cornell Cooperative Extension*.

Agger, W.A., McCornick, J.D. and Gurwith, M.J. (1985). Clinical and microbiological features of *Aeromonas hydrophila* associated diarrhoea. *Journal of Clinical Microbiology*21:909–913.

Ananthan, S.and Alavandi, S.V.(2003).Biochemical characteristics, serogroups, and virulence factors of *Aeromonas* species isolated from cases of diarrhoea and domestic water samples in Chennai. *Indian Journal of Medical Microbiology* 21(4):233–238.

AbewBereket, Samuel Sahile and FelekMoges(2014).Invitro antibacterial activity of leaf extracts of *Zehneriascabra* and *Ricinuscommunis* against *Escherichia coli* and methicillin resistance *Staphylococcus aureus*. *Asian Pacific Journal of Tropical Biomedicine* 4(10):816-820.

Dvorak, B.I. andSkipton, S.O. (2008). Drinking Water Treatment: Activated Carbon Filtration. *NebGuide.*

Cynthia Mason, SingaraveluVivekanandhan, ManjusriMisra, Amar Kumar Mohanty(2012). Switchgrass(*Panicumvirgatum)* Extract Mediated Green Synthesis of Silver Nanoparticles. *World Journal of Nanoscience and Engineering* 2:47-52.

Choudhary R.S, N.B. Bhamare,B.VMahure(2014).Bioreduction of Silver Nanoparticles using Different Plant Extracts and its Bioactivity against *E.coli* and *A.niger*.*IOSR Journal of Agriculture and Veterinary Science* 7(7):7-11.

Elumalai, E.K., Prasad, T.N.V.K.V., Kambala, V., Nagajyothi P.C. and David, E. (2010). Green synthesis of silver nanoparticle using *Euphorbia hirta* L and their antifungal activities. *Archives of Applied Science Research* 2(6):76–81.

Harikumar, P.S., Joseph, L. and Manjusha, C.M.(2011).Biosynthesis of silver nanoparticles and its application in microbial treatment of drinking water.*Journal of Nanoscience and Nanotechnology* 5(1):23–27.

Bartram, J. and Pedley, S. Microbiological Analyses(1996).*Water Quality Monitoring-A Practical Guide to the Design and Implementation of Fresh water Quality Studies and Monitoring Programmes*.

Monisha, J., Tseten, T., Ambalagan, N., Mathew, B.B., andGowda, K.N.B. (2014).Toxicity, mechanism and health effects of some heavy metals.*Journal of Interdisciplinary Toxicology* 7(2):60–72.

Kim, K.J., Sung, W.S., Moon, S.K., Choi, J.S., Kim, J.G. and Lee, D.G. (2008). Antifungal effect of silver nanoparticles on dermatophytes.*Journal of Microbiology and Biotechnology* 18:1482–1484.

Kim, S., Yoo, B., Chun, K., Kang, W., Choo, J., Gong, M. and Joo, S. (2005). Catalytic effect of laser ablated Ni nanoparticles in the oxidative addition reaction for a coupling reagent of benzylchloride and bromoacetonitrile. *Journal of Molecular Catalysis A: Chemical* 226:231–234.

Ravindra, S., Mohan, Y.M., Reddy, N.N. and Raju, K.M. (2010). Fabrication of antibacterial cotton fibres loaded with silver nanoparticles via “Green Approach”. *Colloids and Surfaces A: Physicochemical Engineering Aspects* 367:31–40.

Devika Ravindranath,Nayana O.V and Liji Cebil(2016).Antimicrobial Plant extract incorporated gelatin beads for potential application in pharmaceutical industry.*Biotechnological Research* 2(1):11-14.

Begum, S., Ayub, A.,Zehra, S.Q. and Siddique, B.S.(2013).ChemicalConstituentsofTheAerialParts of *Lantanacamara*.*Chemistry of Natural Compounds* 49(3):566–567.

Singh, A., Jain, D., Upadhyay, M.K., Khandelwal, N. and Verma, H.N. (2010).Green Synthesis of Silver Nanoparticles Using *Argemonemexicana* Leaf Extract and Evaluation of Their Antimicrobial Activities.*Digest Journal of Nano Materials andBiostructures* 5:483–489.

Vila, J., Ruiz, J., Gallardo, F., Vargas, M., Soler, L., Figueras, M.J. and Gascon, J.(2003).*Aeromonas* spp. and Traveller's Diarrhea:Clinical Features and Antimicrobial Resistance. *Emergency Infectious Diseases* 9(5).

Vishnudas D, Mitra, B., Sant, S.B. and Annamalai, A.(2012). Green Synthesis and Characterisation of Silver Nanoparticles by aqueous leaf extracts of *Cardiospermum helicabum*. *Drug Invention Today* 4(2):340–344.