Comparing the Antibacterial Activity of Silver Nano Particles synthesised using Garlic Extract and Celery Leaf Extract and Implementing in Domestic Point use filters

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Abstract

Drinking water disinfection is the most important criterion in water treatment system and chlorination is the most commonly adopted disinfection method in India. There is a wide spread potential for human exposure to disinfection by-products. As an alternative to chlorination, Silvernanoparticles(AgNPs) which are known for their excellentant imicrobialactivity, are used as disinfecting agent in this study. The synthesis of AgNPs uses many toxic chemicals and hence a green synthesis method is used in this study. AgNPs are synthesised using SilverNitrate solution and garlic and celery leaf extract which act as a reducing agent for producing AgNPs. The synthesized AgNPs were characterized using UV-vis spectroscopy, particle size analysis, zeta potential analysis and FT-IR spectroscopy. The most effective antibacterial activity of AgNP solution was found using well diffusion method on Escherichia coli and Staphylococcus aureus and was found that, the garlic AgNPs have more antibacterial activity than celery AgNPs and the minimum effective antibacterial activity was found at the concentration of 100µL/mL on both species.The selected concentration of garlic AgNP solution was applied on point of use filter called ceramic pot filter and then tested with the water sample containing bacterial growth. The result showed nil bacterial growth and residual AgNP concentration in treated water. Finally the cost comparison of other common point use filter with that of AgNP coated ceramic pot filter was done, which showed AgNP coated ceramic pot filter is the cheapest and safest filters for domestic use.

Keywords - *Green synthesis, Silver nanoparticles, antibacterial activity, ceramic pot filter*

I. INTRODUCTION

Access to a clean, safe and sustainable source of pure water is an important need of the present century. Contamination of water with pathogenic microorganisms has detrimental effect on human health and aquatic species. According to IS 10500:2012, Drinking water specification and WHO guidelines, potable water must be free from coliform microorganisms. The common practices employing for treatment of water are neither safe nor cost effective. Most of the methods consume a significant amount of chemical agents, which often cause formation of harmful by-products raising health and environmental issues [1] – [4]. Moreover, it takes prolong time to get rid of bacterial pathogens.The use of sand filtration and chlorine disinfection marked the end of water borne epidemics in the developed world more than a century ago. However, outbreaks of water borne diseases continue to occur at unexpected high levels. According to the 2004 WHO report, at least one-sixth of the world population (1.1 billion people) lack access to safe water. The consequences are daunting: Diarrhea kills about 2.2 million people every year, mostly children under the age of 5. The importance of water disinfection and microbial control cannot be overstated.

Although disinfection methods currently used in drinking water treatment can effectively control microbial pathogens, research in the past few decades have revealed a dilemma between effective disinfection and formation of harmful disinfection by-products (DBPs). Chemical disinfectants commonly used in water treatment such as free chlorine, chloramines and ozone can react with various constituents in natural water to form DBPs, many of which are carcinogens. More than 600 DBPs have been reported [1]. Considering the mechanisms of DBP formation, it has been predicted that DBPs will be formed any time when chemical oxidants are used in water treatment [2], [3]. Furthermore, the resistance of some pathogens, such as *Cryptosporidium* and *Giardia*, to conventional chemical disinfectant requires extremely high disinfectant dosage, leading to aggravated DBP formation. Therefore, there is an urgent need to re-evaluate conventional disinfection methods and to consider innovative approaches that enhance the reliability and robustness of disinfection avoiding DBP formation.

Another important need is the lack of water treatment system in rural areas, which mainly depend on ground water (mostly from well) as main source. Many studies reported the presence of microorganisms in well and ground water source [5] – [7]. Hence disinfection of these water sources at point of use is very important. Unlike conventional chemical disinfectants, the antimicrobial nanomaterials are not strong oxidants and are relatively inert in water. Therefore, they are not exposed to produce harmful DBPs. If properly incorporated into treatment processes, they have the potential to replace or enhance conventional disinfection methods.

II. MATERIALS AND METHODS

A. Preparation of Garlic and Celery Extracts

For preparing garlic extract and celery leaf extract, approximately10gofgarlic (*Allium sativum*)was chopped(not crushed) into 2 to 3 mm thick pieces and freshcelery(*Apiumgraveolens*)leavesweighingaround10 g, were cleaned and chopped. 100mLofdeionizedwater was added to both and kept atroomtemperaturefor24hours. After 24 hours, the resulting solution was decanted, solid garlic pieces and celery leaves were removed and a pale white transparent garlic extract and pale green celery extract was obtained. Both the decanted solution was centrifuged at 2500 rpm for 10 minutes to obtain clear supernatant of garlic extract and celery extract and these clear supernatant are further used for synthesisingAgNPs.

Fig 1: Fresh garlic used for extracts preparation and chopped garlic in deionized water kept for 24 hours

Fig 2: Fresh celery leaves used for extracts preparation and chopped celery leaves in deionized water kept for 24 hours

Fig 3: Decanted garlic extract and celery leaf extract

B. Synthesis of Silver nanoparticles from the plant extracts

With the 20 mL of plant extracts obtained by the above process, equal amount of 20 mM$AgNO_3$(SilverNitrate) solution were added. After 24 hours, colour changes gradually indicating the start of the reaction. Both the solutions were centrifuged at 3500 rpm for 20 mins to obtain clear supernatant without any solid particles. After 48 hours, the celery extract and SilverNitrate solution mixture, changed its colour completely to deep brown indicating the end of the reaction and after 1 week, the garlic extract and SilverNitrate solution mixture, changed its colour fromcolourless to brown colour indicating the formation of Silver nanoparticles.

Fig 4: Celery leaf extract + 20 mMSilverNitrate solution mixture after 24 hours and 48 hours respectively

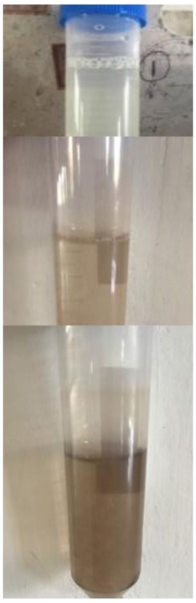

Fig 5: Garlic extract + 20 mMSilverNitrate solution mixture after 24 hours, 36 hours and 1 week respectively

C. Characterisation of synthesized Silvernanoparticles

The synthesisedAgNPs were tested for their presence, size and stability. To confrm the presence of AgNP, UV-Vis spectroscopy was done. To analyse the various size of nanoparticles formed, particle size analysis was done. To know the stability of the synthesisedAgNP solution, zeta potential analysis was performed and finally to know the various components used for the AgNP synthesis from the plant extracts, the synthesisedAgNP solution was compared with that of respective plant extract solution by Fourier Transform - Infra red (FT-IR) spectroscopy.

D. Antibacterial activity of synthesisedSilver nanoparticles

The AgNP solutions prepared was tested for their antibacterial activity by well diffusion method. The *E. coli* and *S. aureus* species were inoculated in nutrient broth and incubated at 37 ^{o} C for 24 hours. The 24 hours grown species was diluted in double distilled water, with reference to Mac Farhand solution and the diluted growth was swabbed on sterile petriplates containing Muller Hinton Agar (MHA).About 4 wells of 10 mm diameter were punched on the swabbed MHA plates. 100 µL of 100, 200, 300 and 400 µL/mLAgNP solution was poured in each well and incubated at 37 ^{o} C for 24 hours. The antibacterial activity of various concentrations of AgNP solutions was compared with each concentration and with that of one prepared from garlic and celery extracts.

E. Ceramic pot filter

The point of use filter adopted in this study is a ceramic pot filter. Ceramic pot filter is a simple pot like filter made of clay and saw dust. Saw dust is mixed along with clay to make the pot porous. 30% by weight of clay of saw dust was added in the making process. It follows a simple normal pottery making process. The filter setup which is used in this study is made of 1000 g of clay and 300g of saw dust mixture along with water. 'U' shape bottomed filter was hand moulded and kept for air dry for 3 days and then kept in firing kiln at above 800 ^{o} C for 4 to 5 hours. The dimension of the filter is 20 cm height and 10 cm diameter. The pore size of the filter varies from 0.6microns to 3 microns and the seepage rate of the filter is 1 to 2litres per hour. An earthen pot is kept at the bottom to collect the filtered water as a receptacle tank. The filter and the whole set up are shown in the fig 7 and 8 respectively. The mechanism of this filter is that, it is made of clay and saw dust mixture. When kept for firing in the kiln, the fine saw dust added to the clay will get burnt down and create pores. These pores will allow the water to seep through the filter. When the filter is coated with AgNPs, the pores will get accumulated with AgNPs and the water passing through these pores will get disinfected by the action of AgNPs on bacteria present in the water.

Fig 6: Saw dust used in making ceramic pot filter

Fig 7: Ceramic pot filter

Fig 8: Whole ceramic pot filter set up

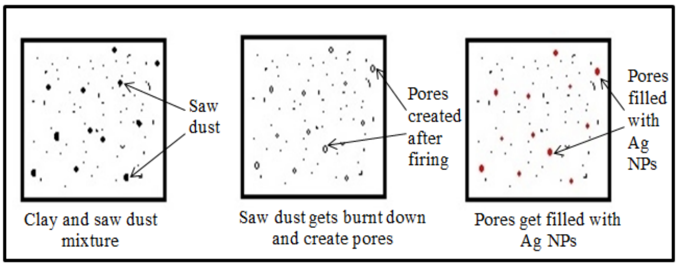

Fig 9: Mechanism of ceramic pot filter

F. Cost comparison of common household point use filter Vs AgNP coated ceramic pot filter

The cost of using common point use filtering method used in homes and AgNP coated ceramic pot filter was analysed. Commonly used filter types in home are Activated Carbon/Charcoal filter, Cation Exchange Softner, Distiller, Reverse osmosis, Ultraviolet disinfection. The cost of manufacturing, lifetime, operation and maintenance was considered while comparing the cost of various filters.

III. RESULTS AND DISCUSSION

A. UV-Vis spectroscopy analysis

To confirm the presence of AgNPs in the final brown coloured solution obtained from the mixture of 20 mMSilverNitrate solution and garlic extract and celery leaf extract respectively, UV-Vis spectroscopy analysis was carried out. The reference solution used for the test was SilverNitrate solution (20mM) and the peak absorbance of Silver (Ag) was found at the wavelength range of 400 – 450 nm. The brown coloured samples of synthesisedAgNP solutions from garlic and celery leaf extracts were analysed and the peak absorbance was obtained around the wavelength of 410 nm and 420 nm respectively indicating the presence of AgNPs. The graph obtained for both the samplesissgiven in fig 10 and 11.

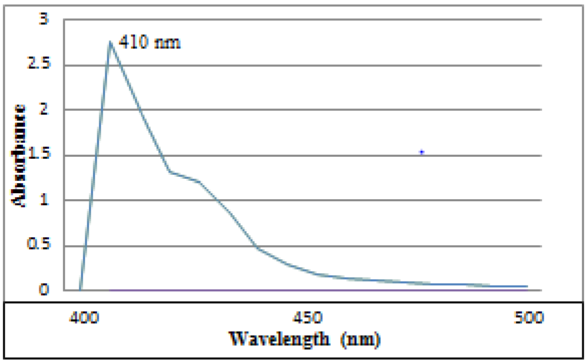

Fig 10: UV-Vis spectroscopy graph of AgNP solution synthesised from garlic extract

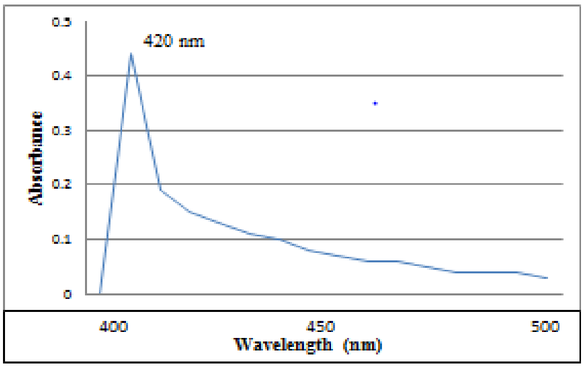

Fig 11: UV-Vis spectroscopy graph of AgNP solution synthesised from celery leaf extract

The absorbance peak 410 nm obtained for the AgNPssynthesised from garlic extract, was found to be more than 404 nm [8] and 420 nm obtained for the AgNPssynthesised from celery extract was found to be less than 450 nm [9]. The absorbance peak was observed for the AgNP solution synthesised from other plant extracts was found to be varying in the range of 380nm to 490 nm [10] – [12]. Thus the absorbance peak at 410 nm and 420 nm confirm the formation of AgNPs and the difference in absorbance peak from other's findings might be due to the difference in concentration of the extract and SilverNitrate solution taken and non-addition of any reducing agents for the synthesis process.

B. Particle size analysis

To study the various size of nanoparticles obtained, particle size analysis was done. The test was performed at the refractive index of 0.135, which is the refractive index of Silver nanoparticles. It is seen that, the particle size diameter of AgNPs formed from garlic extract are in the range between 82.33 nm to 105.10 nm and the AgNPs formed from the celery leaf extract are in the range between 72.87 nm to 246.98 nm. The particle size analysis graph for both the samples are given in fig 12 and 13 respectively. The mean particle size of AgNPs formed from garlic extract is 86.5 nm which was more than the mean particle size of 20 nm [8] and for the AgNPssynthesised from celery extract, it is 131 nm which was more than the mean particle size of 50 nm [9]. The difference in size may be due to the difference in concentration of extract and SilverNitrate solution taken for the process.

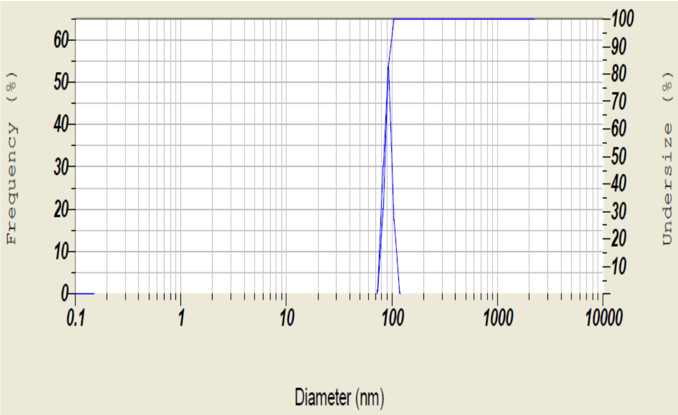

Fig 12: Particle size analysis graph of AgNP solution synthesised from garlic extract

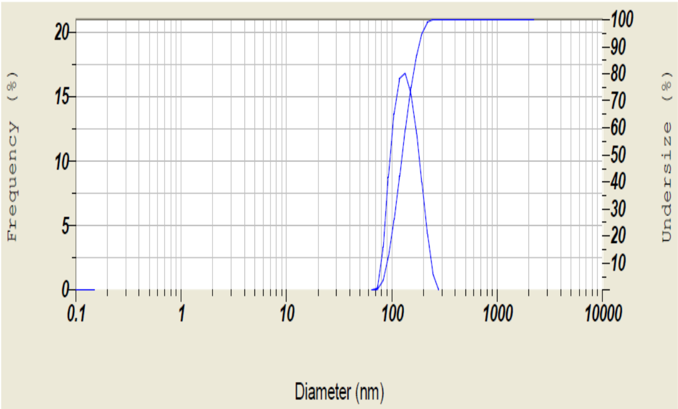

Fig 13: Particle size analysis graph of AgNP solution synthesised from celery leaf extract

C. Zeta potential analysis

To study the stability of the synthesised nanoparticles, zeta potential analysis was done. The zeta potential value for the AgNP solution synthesised from garlic extract is -14.9 mV and that of one prepared from celery leaf extract is -0.1 mV. The intensity versus zeta potential graph for both the synthesisedAgNPs are given in fig 14 and 15 respectively. Both the values are found to be more than -31 mV [8]. Even though there is difference in the values obtained, both the values are in the negative range. Hence it shows that the synthesisedAgNP solutions are stable.

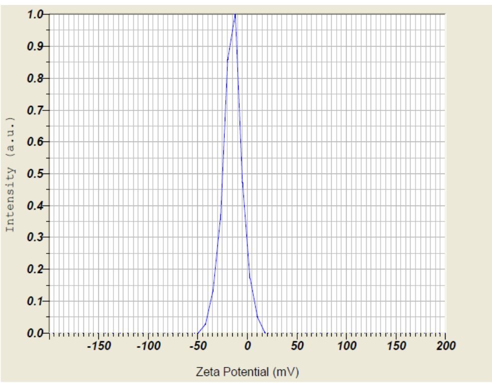

Fig 14 Zeta potential analysis graph of AgNP solution synthesised from garlic extract

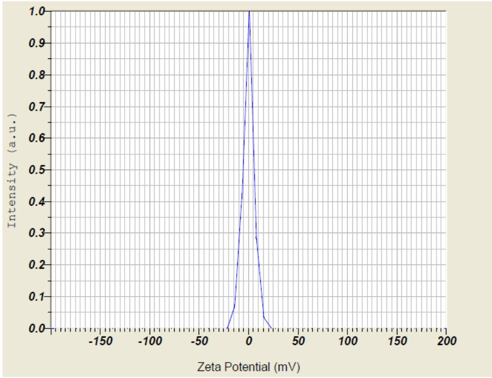

Fig 15: Zeta potential analysis graph of AgNP solution synthesised from celery leaf extract

D. Fourier transform infra - red (FT-IR) spectroscopy analysis

To know the various components used for the AgNP synthesis from the plant extracts, the synthesisedAgNP solution was compared with that of respective plant extract solution by FT-IR test. The transmittance versus wavenumber graph of synthesisedAgNP solutions and their corresponding plant extracts were given in fig 16 to 19.

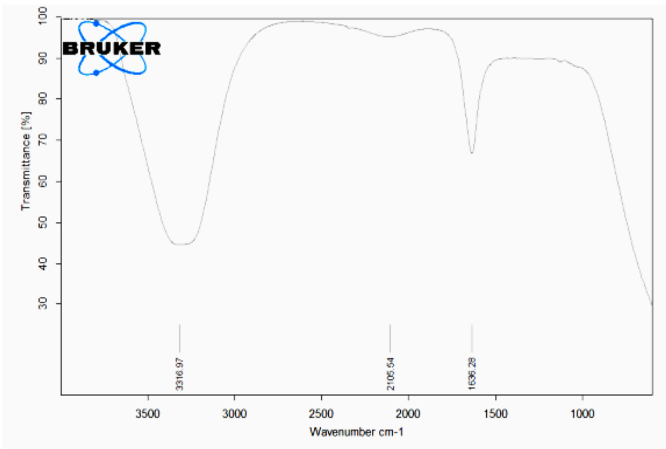

Fig 16: FT-IR analysis graph of garlic extract solution

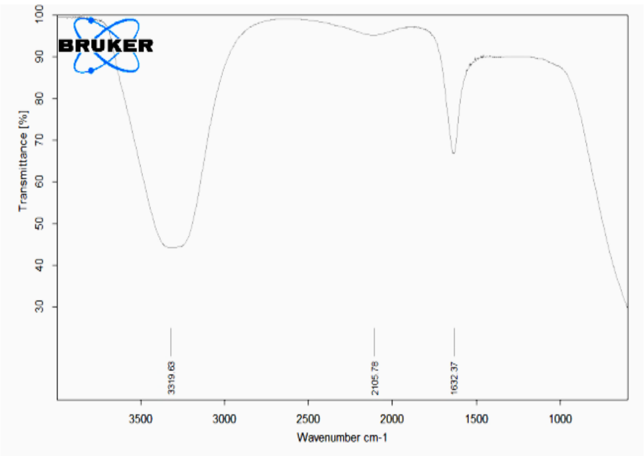

Fig 17: FT-IR analysis graph of AgNP solution synthesised from garlic extract

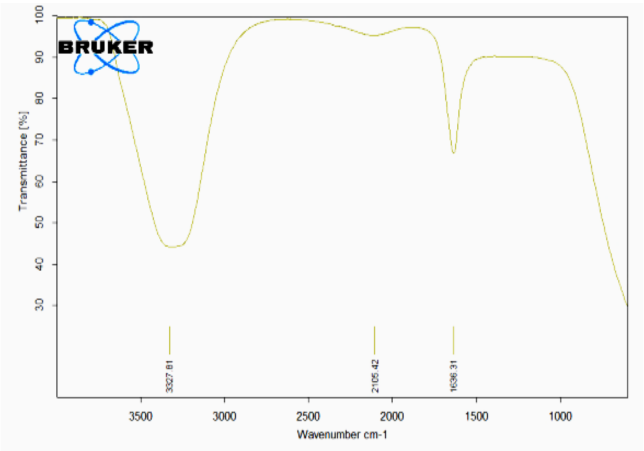

Fig 18: FT-IR analysis graph of celery leaf extract solution

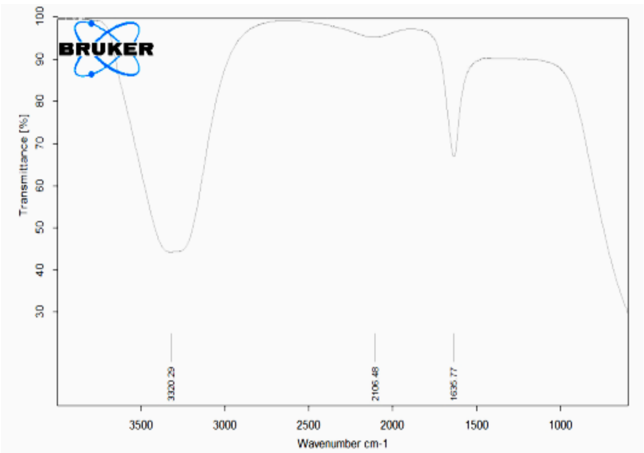

Fig 19: FT-IR analysis graph of AgNP solution synthesised from celery leaf extract

From the graphs it is seen that, the transmittance peak are observed corresponding to the wavenumber 3320 cm^{-1}, 2105 cm^{-1} and 1630 cm^{-1}. The wavenumber 3320 cm^{-1} corresponds to the -OH group [13] and 1630 cm^{-1} corresponds to N-H group and 2105 $cm^{-}1$ corresponds to alkyne group[8]and these were responsible for the formation process of AgNPs. These functional groups were present in both the plant extracts and their value seems to be lowered in the AgNP solutions indicating their involvement in AgNP production.

E. Antibacterial activity of synthesisedSilver nanoparticles

The AgNP solutions prepared was tested for their antibacterial activity by well diffusion method as stated in Section II-D. The zone of inhibition found for various concentrations of AgNP solutions is given in Table I.

Table I. Well Diffusion Method – Zone of Inhibition

Concentration of AgNP solution (µL/mL)	Zone of inhibition (mm)			
	Garlic AgNP		Celery AgNP	
	E. coli	*S. aureus*	*E. coli*	*S. aureus*
100	15	15	14	Nil
200	16	16	14	14
300	16	17	15	15
400	16	17	15	15
control	Nil	Nil	Nil	Nil

Fig 20: Zone of inhibition formed on *Escherichia coli* by garlic AgNP and celery AgNP

Fig 21: Zone of inhibition formed on *Staphylococcus aureus* by garlic AgNP and celery AgNP

Fig 22: Control plates of *Escherichia coli* and *Staphylococcus aureus*

From the results it is seen that, garlic AgNP solution shows more antibacterial activity than celery AgNPat 100µL/mLand the concentration chosen to apply on the filter is 150 µL/mL.Similar results were found in AgNP solution synthesised from other plant extracts like tea, *Cocos nucifera* and tomato [14] – [16], at the concentration of 25 µg/mL, 25 µL/mL.and 50 µg/mL respectively against various bacterial species including *Escherichia coli* and *Staphylococcus aureus.*

F. AgNP coated ceramic pot filter

The selected concentration of 150 µL/mL of garlic AgNP solution was coated on ceramic pot filter for 2 to 3 times and air dried. AgNP coated ceramic pot filter was analysed under scanning electron microscope to confirm the presence of AgNPs over the filter surface. The results are given in fig 23 and 24. It is seen that, before coating with AgNP solution, the ceramic pot was more porous and flaky in nature and after coating with AgNP, it is seen that the ceramic surface was given a coating with AgNP all over the surface and the spherical shaped AgNP were also seen, which confirms the presence of AgNP over the filter's surface.The water sample containing *Escherichia coli* and *Staphylococcus aureus*was tested for bacterial growth after treated in the AgNP coated ceramic pot filter. MPN test was conducted and it is seen that no bacterial growth were found on treated water sample. The details of the bacterial count in the water sample before and after treatment with AgNP coated ceramic pot filter is given in Table II. The fig25 and 26 shows the water filtered from ceramic pot filter and the 24 hours MacConkey broth media kept for preliminary test of treated water sample with nil bacterial growth.The treated water sample were tested for residual Silver. The water sample treated with AgNP coated ceramic pot filter for the first and second time was tested for heavy metal analysis. The test was conducted as per the IS 13428 Annex J and the results are given in Table III.The permissible level of Silver in drinking water is 0.1 mg/L as per IS 10500:2012. From the results, it is seen that the amount of Silver is below quantifiable limit which is less than 0.01 mg/L. Hence it is safe to use AgNP coated ceramic pot filter.

Table II. Bacterial Count in Sample Before and After Treatment with AgNP Coated Ceramic Pot Filter

S.No.	Name of the Species	Bacterial count before treatment	Bacterial count after treatment
1.	*Escherichia coli*	1.62 x 10^3 MPN/100 ml	Nil
2.	*Staphylococcus aureus*	2 colonies in 1mL inoculation in MSA* media	Nil

*MSA – Mannitol Salt Agar

Table III. Amount of Silver in Treated Water

S.No.	Sample name	Amount of Silver (mg/L)
1	First wash	BQL* (< 0.01)
2	Second wash	BQL* (< 0.01)

*BQL – Below quantifiable limit

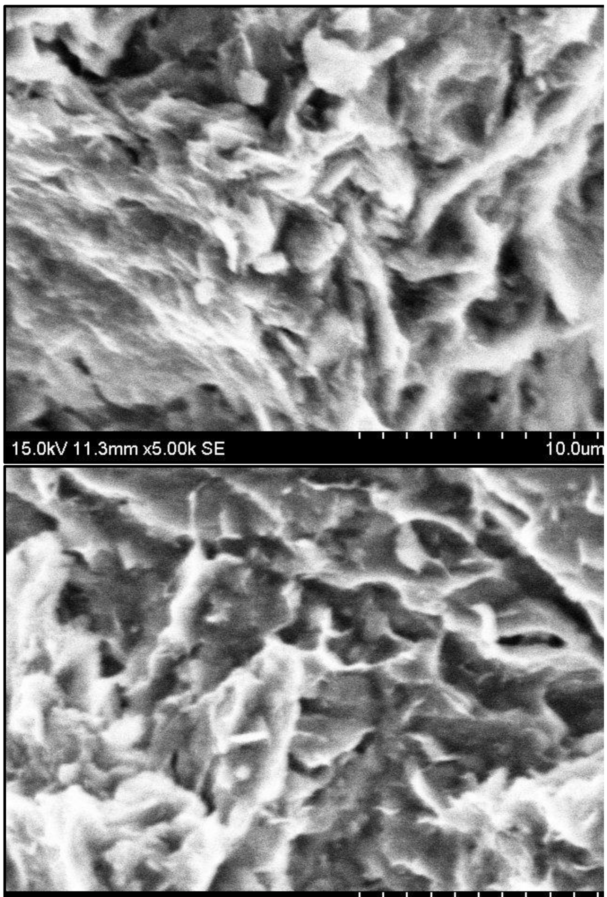

Fig 23: Ceramic pot filter before coating with AgNP solution

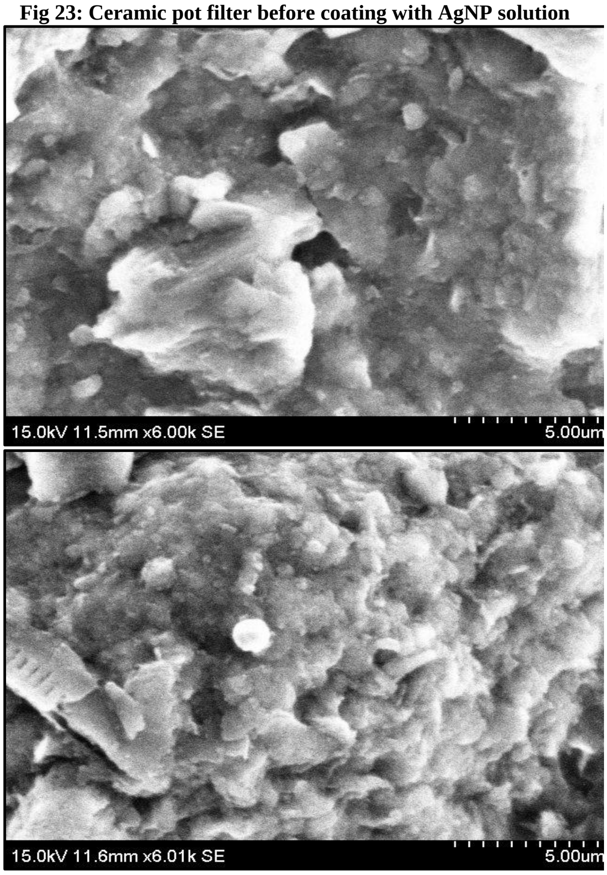

Fig 24: Ceramic pot filter before coating with AgNP solution

Fig 25: Water filtered from ceramic pot filter

Fig 26: Preliminary test of treated water sample showing nil bacterial growth

G. Cost of making AgNP coated ceramic pot filter

The filter model used in this study was 1L capacity. For making a 1L capacity AgNP coated ceramic pot filter, 150 mL of 150 µL/mL concentration of garlic AgNP solution was used that is, 22.5 mL of garlic AgNP solution was mixed in 150 mL of distilled water. Hence for making 8L capacity filter, 180 mL of garlic AgNP solution will be used, which requires 90 mL of 20 mMSilverNitrate solution and 90 mL of garlic extract.To prepare 90 mL of garlic extract, around 10 g of garlic is required and it costs around Rs. 10 and the cost of 90 mL of 20 mMSilverNitrate solution is about Rs.10. The market value of making an 8L capacity ceramic pot filter is Rs. 100. Hence the total cost of making 8L capacity of

AgNP coated ceramic pot filter is around Rs. 150 – 250.

H. Cost comparison of common household point use filters vs AgNP coated ceramic pot filter

The cost of using common point use filtering method used in homes like Activated Carbon filter, Cation Exchange Softener, Distiller, Reverse Osmosis, UV filter are compared with that of AgNP coated ceramic pot filter and analysed. The Table IV shows the details of the cost comparison of various filters. The cost of manufacturing, lifetime, operation and maintenance was considered while comparing the cost of various filters.From table III it is seen that, AgNP coated ceramic pot filter was the cheapest filter that can be afforded easily by all economic class of people. Since it was made of earthen material, it is even easy to recycle, environment friendly and it is also safe.

Table IV. Cost Comparisons of Various Household Point use Filters Vs AgNP Coated Ceramic Pot Filter

S.No	Type of filter	Mechanism used	Maintenance required	Life time (years)	Market value (Rs.)
1	Activated Carbon filter	Positively charged and highly absorbent carbon in the filter attracts and traps many impurities.	General checkup for 3 months once with replacement of cartridges 6 months once.	2-5	5000-7500
2	Cation Exchange Softner	Softens hard water by trading minerals with a strong positive charge for one with less of a charge.	General checkup for every 4 months once with a replacement of resins 3 years once.	5-7	15000-25000
3	Distiller	Boils water and condenses the purified steam.	General checkup for 3 months once with descaling of boiler 6 months once.	2-3	10000-14000
4	Reverse Osmosis	A semi permeable membrane separates impurities from water. But this filtration technique wastes a substantial amount of water during the treatment process.	General checkup for every 4 months once with a replacement of RO membrane yearly once.	3-5	10000-14000
5	Ultraviolet filtration	Ultraviolet light kills bacteria and other microorganisms.	General checkup for every 4 months once with a replacement of UV lamp yearly once.	2-5	5500-7000
6	AgNP coated Ceramic pot filter	AgNPs coated on the permeable ceramic pot filter will kills the microorganisms.	Nil. If seepage rate lowers, replace with new filter.	1-1.5	250

IV. CONCLUSION

Chlorination leaves many disinfection by-products. Hence as an alterantive, Silver nanoparticles (AgNPs) were considered as disinfecting agent. AgNPs was synthesised in environmental friendly way using garlic extract and celery leaf extract and 20 mMSilverNitrate solution. The synthesised garlic and celery AgNPs were characterised for their size, shape and stability. It was found that, AgNPs synthesised from garlic and celery extracts were stable and spherical in shape with mean diameter of 86.5 nm and 131 nm respectively. Functional groups like –OH group, N-H group and alkyne group were responsible for the formation of AgNPs from both garlic extract and celery leaf extract. The antibacterial activity of the AgNPs was tested against *E. coli* and *S. aureus*, which proved that garlic AgNPs showed more antibacterial activity than celeryAgNPs at a concentration of 100µL/mL. 150µL/mLconcentration of garlic AgNP was coated on a ceramic pot filter. The water containing coliform, treated with AgNP coated ceramic pot filter was tested for coliform growth which showed nil bacterial growth and the treated water didn't show any residual Silver concentration. Finally the cost comparison was done between various commonly used point use filters and AgNP coated ceramic pot filters. Total cost of making an 8L capacity AgNP coated ceramic pot filter is around Rs. 150 – 250 only. When compared with other common household point use filters, AgNP coated ceramic pot filter was the cheapest, easily recyclable, environmental friendly and safe filter to be used for domestic purpose.Further study may be conducted by varying the parameters such as temperature, concentration of Silver Nitrate solution, concentration of plant extracts used. The green synthesis of AgNP can also be experimented with other plant extracts and their antibacterial activity can be tested with other microorganisms. Also a better model of filter can be developed to incorporate AgNPs in water treatment system.

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