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Argentum to Become Trendy in the Nano Pharma Industry

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Abstract: The use of Nanoparticles in molecular diagnosis and drug delivery has emerged and research has increased significantly within the last decade. Silver is an oligodynamic antimicrobial compound, i.e the exposure to relatively low concentrations of metal can result in substantial reduction of viable microbial organisms. Nano silver is found to be an effective killing agent against a broad spectrum of Gram-negative and Gram-positive bacteria, including antibiotic-resistant strains. The silver nanoparticles have many applications in pathogen control, prophylaxis, diagnosis and therapeutic treatments. Using UV-Vis, SEM/TEM microscopy and Kirby Bauer method, we synthesized nano-silver both chemically as well as from herbs like Coriander, Parthenium and Artemisia and tested their antimicrobial and antifungal efficacy both as a drug and in cotton fabrics. In the present work, Nano-silver particles were found to inhibit several microbial species like Klebsiella, E.coli, Staphylococcus and Pseudomonas. The silver nanoparticles synthesized from Parthenium and Artemisia extracts showed more antibacterial activity compared to Coriander extract. Cotton fabrics coated with Nano-silver also showed highest activity against Klebsiella and the least being E.coli, which has implications in development of sterile clothes for medical applications. Recent studies on silver nanoparticles have exceptional optical properties and improved suitability for drug delivery. It has been understood that the introduction of silver Nano particles has shown to have synergistic activitywithcommon antibiotics penicillinG, ampicillin, erythromycin, clindamycin,and vancomycin against E. coli and S. aureus preventing different infectious diseases such as Pneumonia, Meningitis, sepsis, Gastroenteritis, Enteric fever, and surgical site infections. Hence, Argentum is yet to become a commercially available drug since lots of research is yet to be done to identify as to why and how they can be more effective than the present day antibiotics.

Keywords: Silver nanoparticles, herbal extract, chemical synthesis, antimicrobial activity, sterile clothes

I. INTRODUCTION

In nanotechnology, a Nano particle is defined as a small object or particle that behaves as a whole unit in terms of its transport and properties. The smaller a particle becomes, the more the proportion of surface atoms increases. As particles decrease in size the number of surface atoms becomes equal to or even exceeds the number of inner-core atoms. For a typical bulk material the surface is negligibly small in comparison to the total volume. Surface atoms are more easily rearranged than those in the center of the particle, and so the melting process, which depends on destroying the order of the crystal lattice, can get started at a lower temperature. The melting point of silver metal is1064°C. For 11-12 nm gold particles it is about 1000°C, then begins to drop dramatically to 900°C for 5 to 6 nm particles and to 700°C for 2 to 3 nm particles.

In the case of silver nanoparticles this allows them to easily interact with other particles and increases their antibacterial efficiency. This effect can be so great that one gram of silver nanoparticles is all that is required to give antibacterial properties to hundreds of square metres of substrate material. Nano silver has chemical and biological properties that are appealing to the consumer products, food technology, textiles/fabrics, and medical industries. Nano silver also has unique optical and physical properties that are not present in bulk silver, and which are claimed to have great potential for medical applications. Many chemical reduction methods have been used to synthesize silver nanoparticles from silver salts. Along with the chemical method, there are preparations of silver Nano particles using the plant extract, for example, of Neem, Parthenium, etc.

One of the most beneficial uses of silver has been as a potent antibacterial agent that is toxic to fungi, viruses and algae. Silver has long been used as a disinfectant; for example, the metal has been used in treating wounds and burns because of its broad-spectrum toxicity to bacteria as well as because of its reputation of limited toxicity to humans. Nano silver, when in contact with bacteria and fungus, adversely affects the cellular metabolism of the electron transfer systems, and the transport of substrate in the microbial cell membrane. Nano silver also inhibits multiplication and growth of those bacteria and fungi which caused infection, odor, itchiness and sores. Bacteria can easily be observed with an optical microscope, but the Nano particles require far better resolution. Atoms, nanoparticles, and nanoscale materials can actually be seen by imaging technologies such as high resolution Transmission Electron Microscopy (TEM), Scanning Probe Microscopy (SPM), also called Scanning Tunneling Microscopy (STM), Atomic Force Microscopy (AFM), Scanning Electron Microscopy (SEM) and powder x-ray diffraction (XRD).

The Nano particles show the antibacterial activity against both gram positive and gram negative bacteria. Gram-negative bacteria include genera such as Acinetobacter, Escherichia, Pseudomonas, Salmonella, and Vibrio. Based on studies that show that silver nanoparticles anchor to and penetrate the cell wall of Gram-negative bacteria (Morones et al., 2005; Sondi &Salopek-Sondi, 2004), it is reasonable to suggest that the resultant structural change in the cell membrane could cause an increase in cell permeability, leading to an uncontrolled transport through the cytoplasmic membrane, and ultimately cell death. AgNPs adhesion to microbial cells, penetration inside the cells, ROS and free radical generation, and modulation of microbial signal transduction pathways have been recognized as the most prominent modes of antimicrobial action. Recently, it has been observed that several Gram positive and Gram negative bacteria are exhibiting multidrug resistance (MDR). Among them antibiotic-resistant bacteria are penicillin-resistant Streptococcus pneumonia, macrolides resistant Streptococcus pyogenes, vancomycin-resistant Enterococcus faecium (VREF), methicillin- and vancomycin-resistant Staphylococcus aureus (MRSA and VRSA), and multidrug-resistant Listeria and Corynebacterium. The Gram-negative bacteria include members of the genera Acinetobacter, Escherichia, Klebsiella, Neisseria, Pseudomonas, Salmonella, Shigella and Vibrio. To combat MDR, AgNPs are coated with specific antibiotics so that they can be made more effective through targeted drug delivery (Dakal et al., 2016). Nanosilver is also an effective, fast-acting fungicide against a broad spectrum of common fungi including genera such as Aspergillus, Candida, and Saccharomyces. The exact mechanisms of action of silver nanoparticles against fungi are still not clear, but mechanisms similar to that of the antibacterial actions have been proposed for fungi (Wright et al., 1999).

Chemical reduction methods that have been used to synthesize silver NPs from silver nitrate vary in the choice of reducing agent, the relative quantities and concentrations of reagents, temperature, mixing rate, and duration of reaction. Silver nitrate (AgNO3) is the most widely used silver ion precursor for the production of Nano silver. This is a result of its low cost and chemical stability compared to the other available silver salts. The use of silver nitrate makes it likely that nitrate (NO3-) will be the dominant anion associated with the silver nanomaterial synthesis processes. The reducing agents can refer to any chemical agents, plant extracts, biological agents or irradiation methods that provide free electrons to reduce silver ions and form silver nanoparticles. The cotton fabrics with silver Nano particles synthesized either by chemical method or by using plant extracts may show antibacterial activity with no bacterial growth. Nanoparticles have also been incorporated in cloth which has shown promise to be sterile and thus helping in minimizing infections in hospital setups (Kumar et al., 2008).

II. METHODOLOGY

A. Preparation of Silver Nano particles

1) *Synthetic method:* A 10 ml volume of 1mM silver nitrate was added drop wise to 30ml of 2 mM chilled sodium borohydride solution(Suh et al., 1983). The reaction mixture was stirred vigorously on a magnetic stir plate. The solution turned bright yellow when the silver nitrate was added.

$$AgNO+NaBH_4 \rightarrow Ag+1/2H_2+1/2B_2H_6+NaNO_3$$

The stirring was halted and the stir bar removed. The clear yellow colloidal silver is stable at room temperature stored in a transparent vial for several weeks or months (Fang 1998). Upon aggregation the colloidal silver solution turns darker yellow, violet and grayish.

2) *Using Plant extract: Preparation of the Extract:* Extract was prepared from fresh leaves of coriander, Partheniumhysterophorus, Artemisia pallens. Leaves weighing 25g were thoroughly washed thrice in distilled water for 15min, cut into fine pieces and were boiled in a 500ml Erlenmeyer flask with 100ml distilled water up to 5 min and were filtered. 10 ml of coriander leaf extract, 50ml of the Parthenium leaf extract and 5 ml of Artemisia leaf extracts were added to 100 ml aqueous solution of 1mM and 3mM silver nitrate respectively.

B. Analysis of Silver nanoparticles

1) *UV-Vis Spectra analysis:* The reduction of pure Ag ions was monitored by measuring the UV-vis spectrum of the reaction medium at different time intervals after diluting a small aliquot of 100 micro liters of the sample with 1ml deionized water. UV-vis spectral analysis was done using a Perkin- Elmerlamda-25 spectrophotometer

2) *TEM/SEM analysis of silver nanoparticles:* Transmission Electron Microscopic or scanning electron microscopy was done. Thin film of the samples were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the TEM grid were air-dried.

C. Testing antibacterial activity of silver Nano particles – by disc method

Antimicrobial activity of silver nanoparticles obtained by chemical synthesis and from herbal extracts was measured by Kirby Bauer method against bacteria like E. coli, Pseudomonas, Salmonella, Staphylococcus and fungi like Aspergillus and Pencillum. The zone of inhibition after 24 hrs of incubation at 37˚ c was recorded. The same procedure was followed to test the antibacterial and antifungal activity of silver nanoparticles on small pieces of cotton cloth. The disc/cloth without nanoparticle was used as negative control.

III. RESULTS AND DISCUSSION

A. Chemical synthesis of silver Nano particles

Silver nanoparticles were synthesized, by using ice-cold sodium borohydride in excess, to reduce ionic silver and stabilize the nanoparticles by maintaining particle surface charge. Reaction conditions, including stirring time and relative quantities of reagents (both the absolute number of moles of each reactant as well as their relative molarities), must be carefully controlled to obtain stable yellow colloidal silver. If stirring is continued slowly, as silver nitrate is being added, aggregation begins as the yellow solution first turns a darker yellow, then violet, and eventually grayish, after which the colloid breaks down and particles settle out (Figure 1). Similar aggregation may also occur if the reaction is interrupted before all of the silver salt has been added. It was also found that the initial concentration of sodium borohydride must be twice that of silver nitrate: [NaBH4]/ [AgNO3] = 2.0. Too much sodium borohydride increases the overall ionic strength and aggregation will occur (Van Hyninget al., 1998). The aggregation can also be brought about by addition of electrolytes such as NaCl. Nanoparticles are kept in suspension by repulsive electrostatic forces between the particles, owing to adsorbed borohydride. Salt shields the charges allowing the particles to clump together to form aggregates.

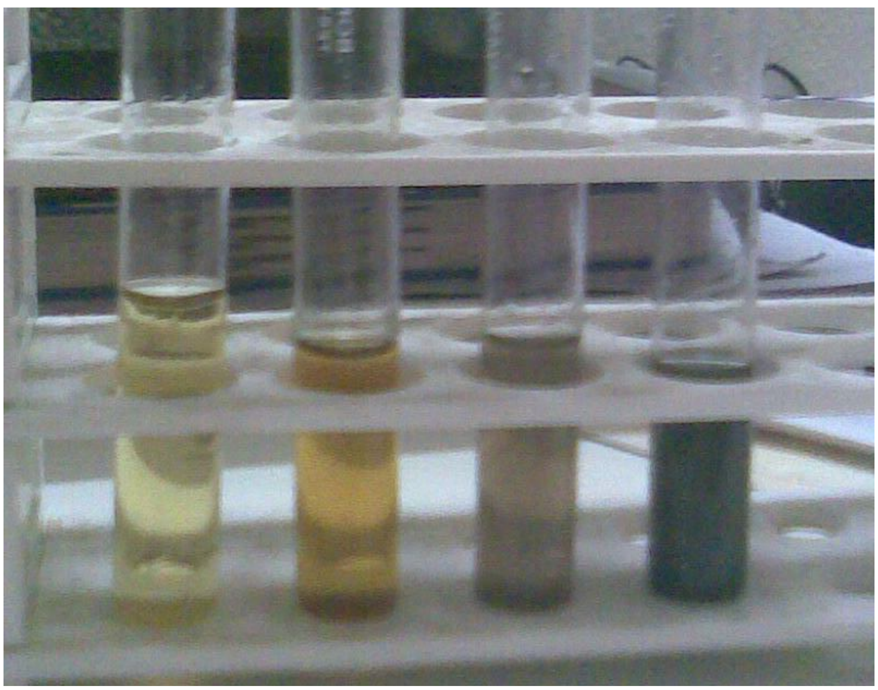


Figure 1: Change in the color of solution of Silver nano particles

B. Optical Characterization by UV-Vis Spectroscopy and Size Measurement using SEM

The distinctive colors of colloidal silver are due to a phenomenon known as plasmon absorbance. The spectrum of the clear yellow colloidal silver from the synthesis above is given in Figure 2. It has already been reported that the absorption spectrum of aqueous AgNO3 only solution exhibited λ max at about 220 nm whereas silver nanoparticles λ max at about 430 nm (Leela and

Vivekananda, 2008). The wavelength of the Plasmon absorption maximum in a given solvent can be used to indicate particle size (Table.1)

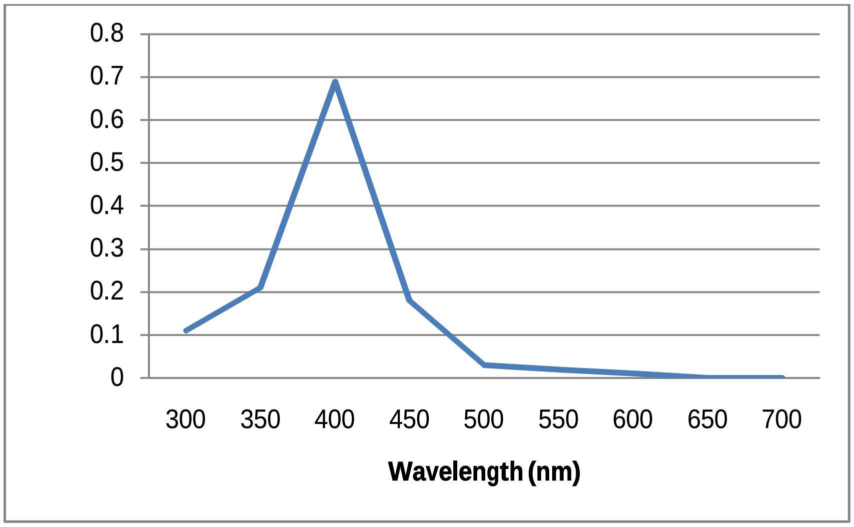


Figure 2: UV–VIS absorption spectrum of clear yellow colloidal Ag (λmax = 400 nm).

Table.1 Plasmon absorption maximum and particle size of nano particles

Particle size/nm	λ max /nm
10-14	395-405
35-50	420
60-80	438

Silver nanoparticles that produced the spectrum were examined using Scanning electron microscopy. A sample of silver nanoparticles from a freshly synthesized clear yellow sol was prepared by drying a small drop on a carbon-coated 200-mesh copper grid. The SEM image shows the silver particles are spherical with sizes of 12 ± 2 nm (Figure 3).

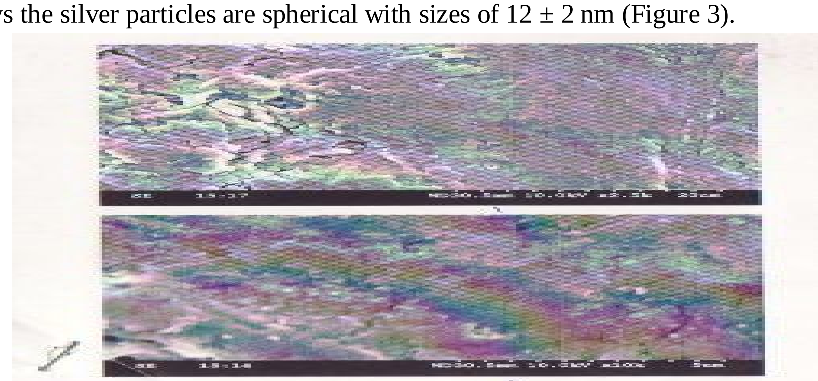


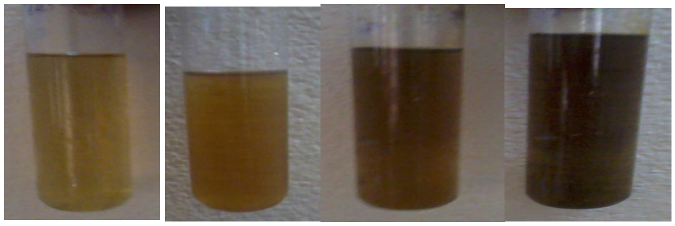
Figure 3: SEM image shows the silver particles are spherical with sizes of 12 ± 2 nm.

Colloidal silver nanoparticles were synthesizd by borohydride reduction of silver nitrate. UV – VIS spectrum confirms the appearance of plasmon absorbance near 400 nm, and SEM images show that the particle sizes are about 12 nm. The yellow colloidal silver remains stable for as long as several weeks or months.

C. Synthesis and UV-Vis/TEM analysis of silver Nanoparticle using Coriander (Coriandrum sativum) Leaf Extract

During recent times several groups have achieved success in the synthesis of Ag, Au, and Pd nanoparticles using extracts obtained from unicellular organisms like bacteria (Ahmad et al., 2003) and fungi (Mukherjee et al., 2001) as well as extracts from plant parts like Neem, Geranium, Lemon grass and others (Huang et al., 2007). Among the various bioreductants, Coriandrum sativumleaves extract was chosen for the present study since they have minerals and vitamin contents including calcium, phosphorus, iron, carotene, thiamine, riboflavin, and niacin. They also contain sodium and oxalic acid.

Extract prepared from fresh leaves of coriander was used to produce silver nanoparticles. The color change in the colloidal solution of nanoparticles reduced by coriander plant leaf extract with time is shown in Figure.4 (A-D)



A B C D

Figure 4.Colloidal solution of silver nanoparticles reduced by coriander leaf extract.

UV-Vis spectrograph of the colloidal solution of silver nanoparticles was recorded as a function of time. Absorption spectra of silver nanoparticles had absorbance peak at 400 nm (Figure 5). The reduction of silver ions and the formation of stable nanoparticles occurred rapidly within an hour of reaction, making it one of the fastest bio-reducing methods to produce Ag nanostructures reported till date (Begum *et al.*, 2009).

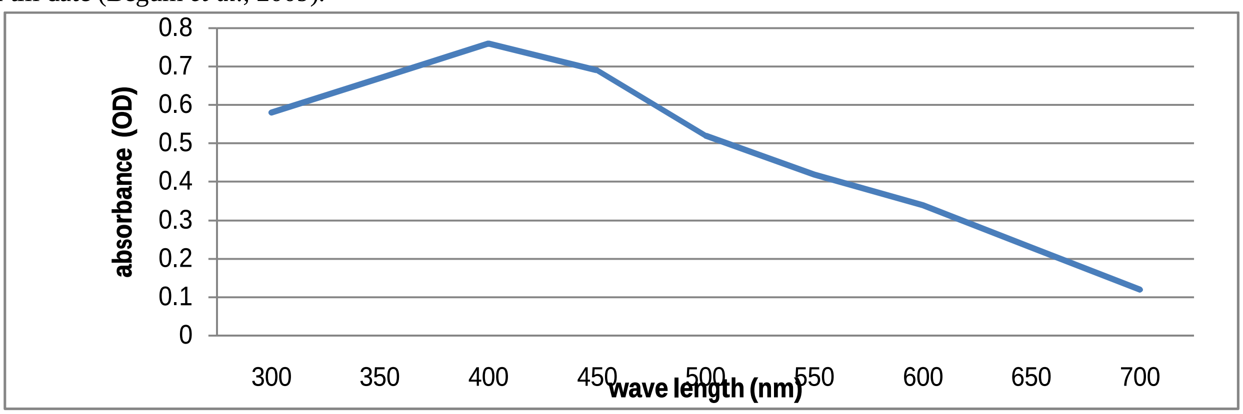


Figure 5**:**Absorption spectra of silver ions by Coriander leaf extract in the range 300 nm to 600 nm after 10 minutes reaction kinetics.

TEM showed that most of the Ag nanoparticles were spherical in shape. A few agglomerated silver nanoparticles were also observed in some places, thereby indicating possible sedimentation at a later time. Some of the silver nanoparticles synthesized using Coriander leaf extract have irregular shapes with size varing from 8nm to 75 nm and the average size estimated was 26 nm (Figure 6).

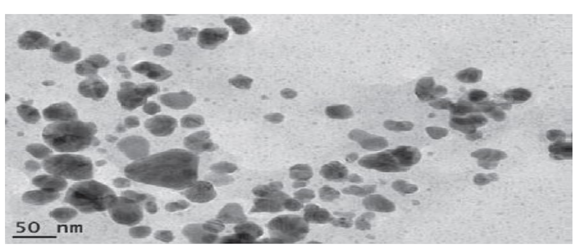


Figure 6: TEM Micrograph of the sample, showing nanoparticles of irregular shapes and sizes

D. Synthesis of silver Nanoparticle using Parthenium Leaf Extract and UV-Vis/TEM analysis

Parthenium leaves weighing 25g were thoroughly washed thrice in distilled water for 15min, cut into fine pieces and were boiled in a 500ml Erlenmeyer flask with 100ml distilled water up to 5 min and were filtered. Add 50 ml leaf extract into the aqueous solution of 1mM Silver nitrate. The color change in the colloidal solution of nanoparticles reduced by Parthenium plant leaf extract was similar to the ones synthesized by coriander leaf extract.

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Absorption spectra of silver nanoparticles, similar to the UV spectra produced by nanoparticles synthesized by coriander leaf extract, formed in the reaction media at 10 min., had absorbance peak at 450 nm, and the broadening of peak indicated that the particles were poly dispersed. TEM Micrograph (Figure.7) of the silver nanoparticles synthesized using Parthenium leaf extract had irregular shapes of 30 to 80 nm with average size 50 nm.

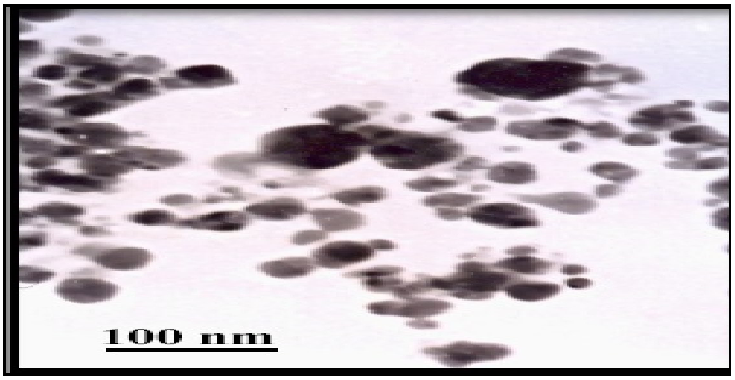


Figure 7: TEM Micrograph of the sample after the 10 minute reaction kinetics with treating leaf extract with silver ions Complex(1mM) in the ratio of 1:1

E. Synthesis of silver Nanoparticle using Davanam (Artemisia pallens) Leaf Extract and UV-Vis/TEM analysis

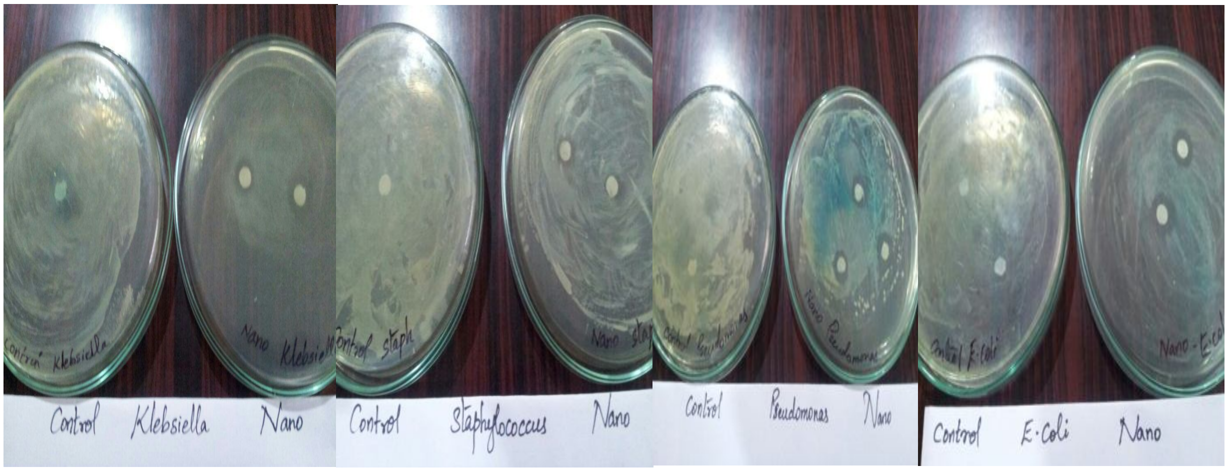
Till date, there are no reports on synthesis of nano particles from Artemisia pallens. In this report, a simple and eco- friendly method has been used to produce silver nano particles using leaf extracts of Artemisia pallens. Extract prepared from fresh leaves of Artemisia were used to produce silver nanoparticles. The color change in the colloidal solution of nanoparticles reduced by Artemisia plant leaf extract with time occurred similar to the colour change that occurred with Parthenium leaf extract. Absorption spectra of silver nanoparticles formed in the reaction media at 10 min. has absorbance peak at 400 nm, similar to that produced by coriander leaf extract.

Though numerous chemical methods are available for metal nanoparticles synthesis, copious reactants and starting materials are used in these reactions that are toxic and potentially hazardous. Increasing environmental concerns over chemical synthesis routes have resulted in attempts to develop bio-mimetic approaches. One of them is the synthesis using plant extracts eliminating the elaborate process of maintaining the microbial culture and often found to be kinetically favorable than other bioprocesses. Bio-molecules as reducing agents are found to have a significant advantage over their counterparts as protecting agents (Huang et al., 2007)As the Parthenium, Coriander and Davanam leaf extract was mixed in the aqueous solution of the silver ion complex, it started to change color from light green color to yellowish brown; colour changed due to excitation of surface Plasmon vibrations, which indicated formation of silver nanoparticle (Mulvaney P,1996). Repeated experiments were carried out by varying the amount of silver ion complex (1mM) and leaf extract; it was observed that precursors in the ratio of 1:1 gave best results of our interest. It is interesting to note that most of the particles in the TEM pictures are not in physical contact but are separated by a fairly uniform inter particle distance.

F. Antimicrobial activity of Nanoparticles Synthesized Chemically and from Herbal extracts

Antimicrobial activity of silver nano particle was measured by Kirby Bauer method against bacteria like E.coli, Pseudomonas, Klebsiella, Staphylococcus and fungi like Aspergillus and Pencillum. Nutrient Agar plates with specific cultures were incubated 24 hrs.The filter paper discs which were coated with silver nano particles 50mg/lit were placed on to the surface of agar plates. The zone of inhibition after 24 hrs incubation at 37˚ c was recorded. The disc without nano particle was used as negative control.

Our result indicated that silver nano particles synthesized from chemicals as well as herbal extract were both efficient against bacteria compared to fungi, illustrated by Figure 8.The Nano particles show antibacterial activity against both gram positive and gram negative bacteria. Comparing the zone of inhibitions (Table.2 and Table.3), it can be concluded that the silver nanoparticles synthesized from Parthenium and Artemisia extracts have more antibacterial activity compared to Coriander extract and the nanoparticles synthesized by chemical method had least antibacterial activity. However there is no antifungal activity against the two fungal species tested. Among the extracts, Parthenium showed highest activity against Klebsiella (0.8cm), whereas Artemisia showed higher activity against E.coli and Pseudomonas (1.1cm) and Coriander against Staphylococcus (0.5cm) (Table.3). The result obtained against Pseudomonas aeruginosa was found to be in line with the result obtained against the same species in the work carried out earlier (Malarkodi and Manoharan, 2013)



A B C D

Figure 8: Zone of inhibition in controls vs A. Klebsiella B. Staphylococcus C. Pseudomonas and D. E.coli

Table 2: Comparing the zone of inhibitions formed by nanoparticles synthesized by chemical method against controls

Microorganism	zone of inhibition(cm) of nanoparticles	
	Chemical synthesis	Control
Klebsiella, Sp	0.4	0.0
Staphylococcus Sp	0.3	0.0
Pseudomonas Sp	0.2	0.0
E.coliSp	0.2	0.0
AspergillusSp	0.0	0.0
PencilliumSp	0.0	0.0

Table.3: Zone of inhibitions formed by nanoparticles synthesized using herbal leaf extracts.

Microorganism	zone of inhibition(cm) of silver nanoparticles		
	Coriander Extract	Artemisia Extract	Parthenium Extract
Klebsiella, Sp	0.3	0.8	1.2
Staphylococcus Sp	0.5	0.6	0.8
Pseudomonas Sp	0.4	1.1	0.7
E.coliSp	0.2	1.1	0.5
AspergillusSp	0.0	0.0	0.0
PencilliumSp	0.0	0.0	0.0

G. Antimicrobial Properties of nanoparticles incorporated in cloth

Since the nano particles in the previous experiment did not show good response against fungal sp, the cotton cloth was analyzed only against bacterial cultures. In the cloth without silver nanoparticles (control), a significant bacterial growth was observed.

However, the cotton fabrics with silver nanoparticles synthesized by chemical method presented antibacterial activity showing no bacterial growth around it as shown representatively in Figure 9. Comparing the zone of inhibitions it was concluded that the silver nanoparticles have greatest antibacterial activity against Klebsiella and least against E.coli (Table 4).

Figure 9 Antibacterial activity of cotton fabriccoated with nanoparticles synthesized using coriander extract, against E.coli

Table 4:Measure of antimicrobial activity on different bacterial species in cloth

Microorganism	Mean width of zone of inhibition(cm)		
	Herbal extract	Chemical method	Control
KlebsiellaSp	1.5	0.6	0.0
Staphylococcus Sp	0.9	0.2	0.0
Pseudomonas Sp	0.7	0.2	0.0
E.coliSp	0.4	0.5	0.0

This study demonstrated the possibility of using silver nanoparticles by incorporating them in fabrics, thereby providing them sterile properties. Silver nanoparticles have shown promise against gram positive S. aureus. Nanoparticles have also been incorporated in cloth which has shown promise to be sterile and thus helping in minimizing infections (Kumar *et al*., 2008)

IV. CONCLUSION

This work has a novel element in that, nanoparticle synthesis from Artemisia pallens has not been reported anywhere and has proven to be a successful method of synthesis along with Coriander and Parthenium leaf extracts. Moreover, the silver nanoparticles synthesized from Parthenium and Artemisia extracts have more antibacterial activity compared to Coriander extract and the nanoparticles synthesized by chemical method had least antibacterial activity.

This study has also re-confirmed the possibility of using silver nanoparticles as antibacterial agents by targeted drug delivery which has been very recently reported (Ardhendu, 2017), and their incorporation in materials, providing them sterile properties. It has also helped us understand the application of these nanoparticles in pharmaceuticals as well as in textile industry.

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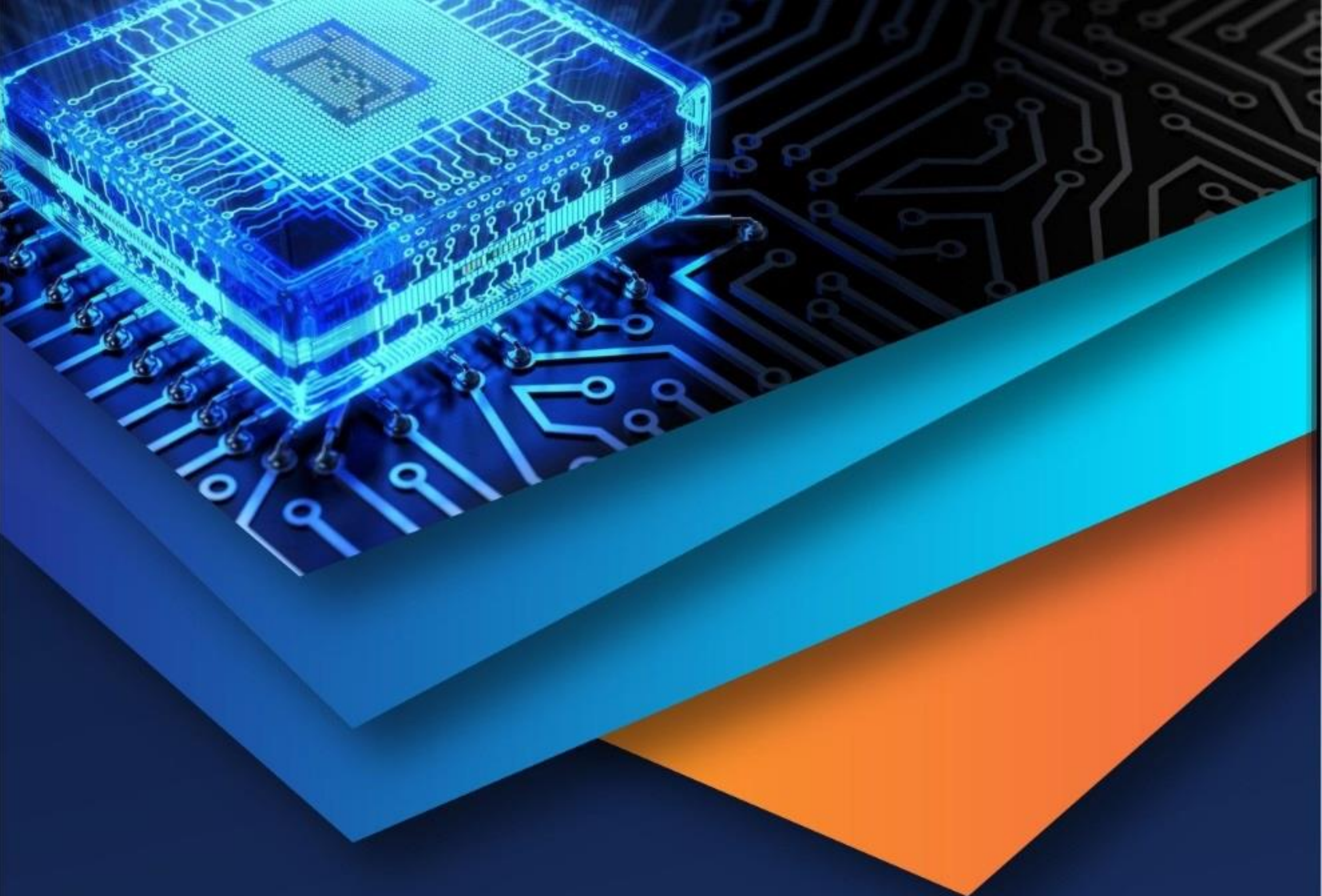

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