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Research Article

EFFECT OF PHYSICO-CHEMICAL FACTORS ON PHYTOFABRICATION OF SILVER NANOPARTICLES BY RHIZOME EXTRACT OF MARANTA ARUNDINACEA L. ITS ANTI-BACTERIAL EFFICACY

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A B S T R A C T

Background: Green nanotechnology is gaining importance due to the elimination of harmful chemicals and provides a safe, environmental friendly and economic method for synthesis of nanoparticles.
Methods: In the present investigation rhizome extract of Maranta arundinacea L. was used for synthesis of silver nanoparticles which was confirmed by changing the colour of reaction mixture from colourless to brown. Synthesized nanoparticles were characterized by UV-vis, FTIR, XRD, EDX, AFM, HR-TEM and testing the antibacterial activity against E. coli and S. aureus.
Results: Silver nanoparticles shows characteristic UV-Vis absorption peak at 410 nm. The effect of physico-chemical parameters – pH, reaction temperature, rhizome extract and silver nitrate concentration were studied and optimized nanoparticles of diverse sizes. Synthesized silver nanoparticles were further characterized by FTIR, EDX, AFM and HR-TEM. The size of silver nanoparticles ranges from 30 to 70 nm and are spherical in shape. Synthesized silver nanoparticles were crystalline in nature. Silver nanoparticles were highly toxic to E. coli than the S. aureus.
Conclusion: Physico-chemical conditions of reaction mixture have been standardized to obtain sharp intense absorption peaks which indicate that formation of small sized monodispered silver nanoparticles. Silver nanoparticles show good antibacterial activity against E. coli and S. aureus.

INTRODUCTION

Synthesis of nanoparticles is an emerging area of nanoscience and nanotechnology. They are present at a higher surface-to-volume ratio with decreasing size of nanoparticles. Nanoparticles possess unique optical and electronic properties compared to bulk material. Nanoparticles possess wide range of application due to their small size in the field of catalysis [1], antimicrobial [2], optoelectronic [3], food industry [4], Antidiabetic [5,6], Antiviral [7], anticancer [8], Anti-inflammatory [9] and antiplasmodial. [10] Nanoparticles are synthesized by different physical and chemical methods but these methods have certain disadvantages due to involvement of toxic chemicals. A large amount of toxic chemicals are produced during the synthesis of nanomaterials and these chemicals pose a serious threat to environment. Thus, there is a need for safe, clean, nontoxic and environment-friendly method for the synthesis of nanoparticles.

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Researchers in the field of nanoparticles have laid emphasis on biological system for synthesis of nanoparticles by biomimetic approach. The various plants and plant parts such as leaf, fruit, flower, tuber and rhizome extract were used for the synthesis of silver and gold nanoparticles, further testing the efficacy of nanoparticles for antimicrobial activity Viz. *Garcinia mangostana* [11], *Olea europea* leaves [12], *Tribulus terrestris* [13], *Artemisia nilagirica* [14], *Hibiscus cannabinus* [15], *Phyllanthus maderaspatensis* [16], *Calendula officinalis* [17], *Lawsonia inermis* [18], *Linum usitatissimum* L.[19], *Lavandula intermedia* [20], *Leea indica* [21,22] *Allophylus serratus* [23], *Bauhinia acuminate* and *Biophytum sensitivum* [24] and *Polygonum glabrum*. [25] Silver nanoparticles were synthesized by using plant extracts from different origin *Cucurbita maxima* (petals), *Moringa oleifera* (leaves) and *Acorus calamus* (Rhizome) and their anticancer activity against epidermoid A431 carcinoma. [26] *Maranta arundinacea*, L. also known as arrowroot *Maranta*, it is belonging to Marantaceae. Arrowroot is a perennial plant. It is rich in medicinal properties such as antidysentric, antidote, aphrodisiac, antipyretic, depurative, hypocholesterolemic etc. Its leaves are lanceolate. The edible part of the plant is the rhizome. The

Maranta arundinacea L. possess various chemical components such as alkaloids, carbohydrate, cardiac glycosides, proteins, amino acids, phenolic compounds, terpenoids, saponins, flavones and gum. The present investigation was undertaken to study phytosynthesis of silver nanoparticles, factors governing the synthesis of nanoparticles and antibacterial activity of synthesized silver nanoparticles.

Fig 1 (A) Habit of *M. arundinacea* (B) Flower (C) Rhizome

MATERIAL AND METHODS

Silver nitrate was obtained from Sigma-Aldrich chemicals. All glassware's were washed with distilled water and dried in oven. Fresh rhizome of *M. arundinacea* was collected from Siddapur, Uttarkannad, Karnataka, India

Preparation of rhizome extract

Ten gram of rhizome was thoroughly washed with de-ionized water and was cut into small pieces and boiled in 100 ml of deionized water for 20 min. Extract was filtered with Whatman 41 filter paper and store at 40^0C and used for further experiments.

Synthesis of silver nanoparticles

Aqueous solution (1 mM) of silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. 5 ml of *M. arundinacea* rhizome extract was added into 95 ml of aqueous solution of 1 mM silver nitrate for reduction of Ag^+ ions to silver nanoparticles. The colour of solution changes from colourless to brown, indicates the formation of silver nanoparticles and further confirmed by characteristic absorption spectra by UV-Vis spectrometer.

Different parameters for investigation

The effect of various parameter of reaction mixture on end product was measured by UV-Vis spectrophotometrically.

Hydrogen ion concentration (pH)

The pH of reaction mixture was maintained at4, 5, 6, 7, 8, 9, 10 and 11 respectively. The pH was adjusted by using either 0.1 N HCl or 0.1 N NaOH.

Reaction Temperature

The reaction temperature was maintained at 0, 10, 37, 40, 50, 60, 70, 80 and 90 ^{0}C respectively, using water bath.

Concentration of leaf extract

The varying concentration (1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 ml) of fruit extract was used for treating 1mM silver nitrate solution.

Concentration of silver nitrate solution

The reaction was monitored by using different concentration of silver nitrate (1, 2, 3, 4 and 5 mM) solution for the synthesis of nanoparticles.

Characterization of silver nanoparticles

The UV–VIS spectrum of the sample was measured on a UV-2450 (Shimadzu) spectrophotometer operated at a resolution of 1 nm. The bio-reduction of silver ions in aqueous solution was monitored by UV–Vis spectrum of the solution between the ranges of 300–800 nm. Further characterization was done by FT-IR (F-7000FL) spectrometer. In order to remove any free biomass residue, the residual solution after reaction was centrifuged at 4000 rpm for 20 min and the resulting suspension was redispersed in 10 mL sterile distilled water. The centrifuging and redispersing processes were repeated for three times. Finally, the dried samples were palletized with KBr and analyzed using FT-IR. For EDX analysis, the reduced silver was dried on a carbon tape placed on a copper stub and performed on a HITACHI SU6600. Dried sample was collected for the determination crystalline structure of Ag nanoparticles by X' Pert pro X-ray diffractometer operated at an voltage of 40 kv and a current of 30mA with Cu Kα radiation. Morphological characterization of the sample was done by HR-TEM (JEOL JSM6701–F), a pinch of dried sample was coated on a carbon tape. It was again coated with platinum in an auto fine coater and then the material was subjected to analysis.

Antibacterial activity

Antimicrobial activity was analyzed with synthesized silver nanoparticles by well diffusion method against Gram positive bacteria Viz. *Staphylococcus aureus* (MTCC3160) and Gram negative bacteria Viz. *Escherichia coli* (MTCC 723, 1554) microorganisms. Initially, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37ºC for 18 hrs. The agar plates are poured by muller hinton media and wells were made in the plate. Each plate was inoculated with 18 h old cultures (100 µl, 10^{-4}cfu) and spread evenly on the plate. After 20 min, the wells were filled with of compound at different volumes. All the plates were incubated at 37ºC for 24 h and the diameter of inhibition zone were noted

RESULTS AND DISCUSSION

The silver nanoparticles synthesized by rhizome extract of *M. arundinacea* colour change was obtained after 2 hrs. of reaction period. Similar results were perceived in the rhizome extract of *Acorus calamus* L. [27] The appearance of brown colour was arising due to the silver nanoparticles which absorb radiation in the visible region of electromagnetic spectrum due to excitation of surface plasmon vibrations and it provides a convenient spectroscopic signature to indicate the formation of silver nanoparticles. [28,29,30] The absorption spectra showed an intense peak at 406 nm due to the surface plasmon resonance

band of silver nanoparticles and broadening of peak indicated that the particles are polydispersed. Different parameters were optimized including pH, reaction temperature, rhizome extract concentration and silver nitrate concentration were identified as factors affecting the synthesis of silver nanoparticles.

Factors influencing the synthesis of nanoparticles

Effect of pH

The acidic pH favored over the nucleation and aggregation of nanoparticles and decreases the synthesis of silver nanoparticles, while basic pH increases the synthesis of silver nanoparticles. It is also conceivable that the addition of hydroxide (OH^-) ions deprotonated the active biomolecules with hydroxyl and carboxyl functional groups supplying electrons for the reduction of the Ag^+ ions. Similar result was found in FTIR confirms the hydroxyl and carboxyl groups are involved in the bioreduction. The raising in pH value at basic condition enhances the repulsive barrier and keeping the nanoparticles physically apart from each other. The excess of H^+ ions at low pH, on the contrary, depresses ionization of the active reducing agent and gates electron transfer due to protonation (Stenesh, 1998).[30] The optimum temperature for the synthesis of silver nanoparticles is 9 pH (Fig. 2).

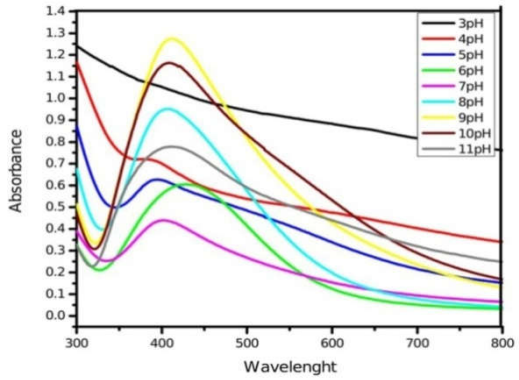

Fig. 2 UV-Vis absorption spectra of silver nanoparticles synthesized by rhizome extract of *M. arundinacea* at different pH

***Effect of temperature*:** The synthesis of silver nanoparticles depends on the temperature, increased in temperature, increases the rate of formation of silver nanoparticles. The size is reduced initially due to the reduction in aggregation of the growing nanoparticles. The peak sharpness increases with an increase in the reaction temperature. Reaction temperatures play an important role to control the nucleation process of nanoparticles configuration. The optimum temperature for the synthesis of silver nanoparticles is 90^0C (Fig. 3).

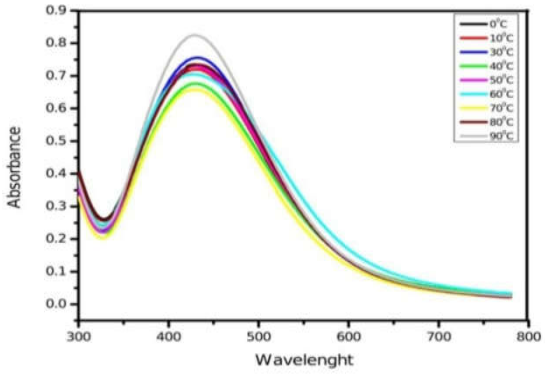

Fig 3 UV-Vis absorption spectra of silver nanoparticles synthesized by rhizome extract of *M. arundinacea* at different temperature

***Effect of rhizome extract concentration*:** The *M. arundinacea* showed that increases in the quantity of rhizome extract SPR/ absorption peak was shifted from red shift to blue shift which indicates that mean diameter of the silver nanoparticles are decreased. The sharp intense peak was produced in blue shift region which indicates that particles are spherical and smaller sized further confirmed by HR-TEM. The quantity of leaf and fruit extract increases more number of biomolecules are available to reduce silver ion to silver nanoparticles and forms maximum number of small sized particles. [32,33,34] The optimum condition for the synthesis of silver nanoparticles is 4 ml (Fig. 4)

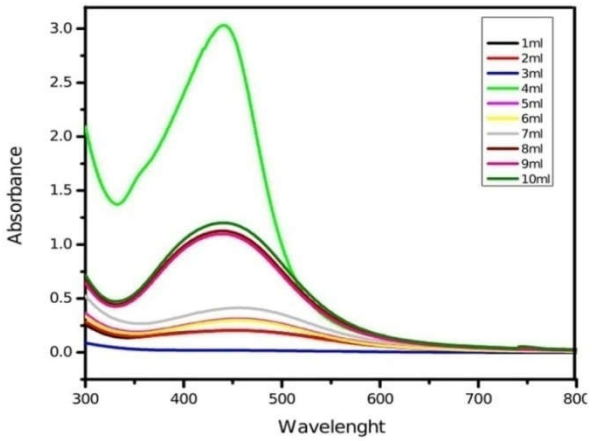

Fig. 4 UV-Vis absorption spectra of silver nanoparticles synthesized by different rhizome extract of *M. arundinacea*

***Effect of salt concentration*:** Variation in silver nitrate concentration (1-5 mM) varies with the size of nanoparticles. According to Henglein, (1993) [35] the SPR band shifted to the blue shift when electron are donated to the particles and the Surface Plasmon Resonance (SPR) band shifted to the red shift when holes are injected to the clusters. The spectrum can exhibit a shift towards the red end or the blue end depending upon the particle size, shape, state of aggregation and the surrounding dielectric medium[36](Fig. 5). Physico-chemical conditions of reaction mixture such as pH, reaction temperature, leaf extract concentration and silver nitrate concentration have been standardized to obtain sharp intense absorption peaks at 410 nm in *M. arundinacea* respectively which indicate the formation of small sized monodispersed silver nanoparticles.

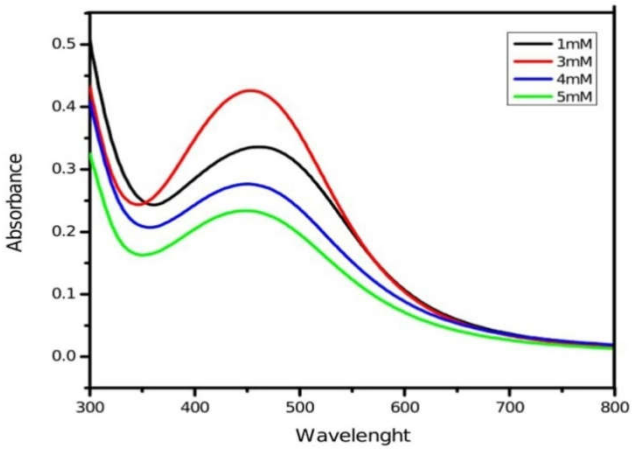

Fig 3 UV-Vis absorption spectra of silver nanoparticles synthesized by rhizome extract of *M. arundinacea* at Silver nitrate concentration

***FTIR Study*:** FTIR analysis was carried out to identify the possible reducing biomolecules in rhizome extract responsible for the formation of silver nanoparticles and to identify the chemical change of the functional groups involved in bioreduction. The FTIR spectra of *M. arundinacea* rhizome extract and silver nanoparticles has shown broad absorption band at ~3400 $cm^{-1}$was due to the O-H stretching (Fig. 6). The spectra show two small peaks at ~2900 and ~2800 cm^{-1} attributed due to methylene antisymmetric and symmetric vibrational mode. The shifting of peaks from 1414.10-1384.53 cm^{-1} and 1079.20-1018.15 cm^{-1} attributed to C=C and C-N stretching vibrations of aromatic amines. The spectrum of rhizome extract shows bands at 1595.88, 1457.25, 1319.00, 1269.25, 1121.34, 927.50, 541.84 cm^{-1} which were arised due to N-H bending of primary amines, methylene scissoring vibrations, -C-O- stretching vibration mode, $-NO_3$ stretching which comes from silver nitrate, Amide band III, -C-O-C- linkages, alkane group, C-Cl or C-Br stretching vibration of alkyl halides. The bands at 1745, 1625, 1542, 1384 and 661 cm^{-1} assigned due to carbonyl (C=O) stretching, -NH stretch vibrations of amide linkages, C=C stretching of aromatic amines, Alpha-glucopyranose rings deformation of carbohydrate these functional groups appears only after the bioreduction of silver nanoparticles. The above results reveal that functional groups viz. amide group, carbonyl group, -NH phenolic compounds etc. were involved in the synthesis of silver nanoparticles which impart characteristic special signatures in the infrared region of the electromagnetic spectrum.

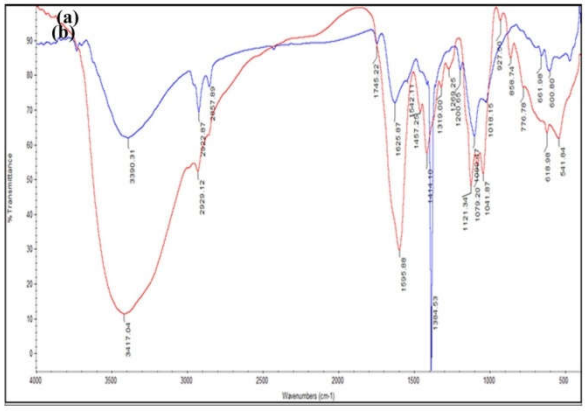

Fig 6 FTIR spectra of silver nanoparticles synthesized by rhizome extract of *M. arundinacea* (a) rhizome extract (b) silver nanoparticles solution

XRD: The XRD pattern of *M. arundinacea* silver nanoparticles synthesized by rhizome extract showed four diffraction peaks 37.86°, 44.06°, 64.22°, 77.19° corresponding to (111), (200), (220) and (311) planes respectively (Fig. 7). All the peaks in XRD pattern can be readily indexed to a face centered cubic structure of silver as per data base of Joint committee on powder diffraction standards (JCPDS) (file number 893722/ 04-0783). It confirms the FCC crystalline nature of elemental silver and shape of nanoparticles are spherical and hexagonal. The bragg angle which give the mean size of silver nanoparticles are 39.67 nm Many unassigned peaks were observed which might have occurred due to the crystallization of the biogenic phases that remained attached on the surface of the synthesized nanoparticles.

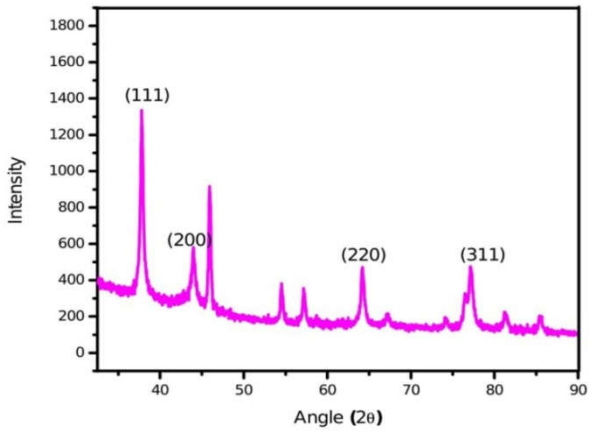

Fig 7 X-ray diffraction spectrum of silver nanoparticles synthesized by rhizome extract of *M. arundinacea*

EDX: EDX spectrum (Fig. 8) revealed that the clear identification of the elemental composition profile of silver nanoparticles synthesized by *M. arundinacea* rhizome extract which suggests the presence of elemental silver. It gives qualitative as well as quantitative status of elements that may be involved in the formation of nanoparticles. The strong signal of elemental peak at approximately 3 kev exist the absorption of metallic silver nanoparticles due to surface plasmon resonance peak.[36]

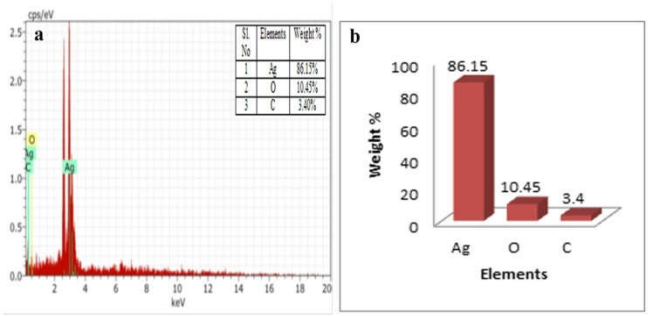

Fig 8 a) EDX spectrum b) quantitative analysis of biosynthesized silver nanoparticles by using rhizome extract of *M. arundinacea*

***AFM*:** AFM image reveals that surface morphology, shape and size of silver nanoparticles. The silver nanoparticles sizes ranges from 35 to 80 nm and are uniformly sized and spherical in shape (Fig.9). The morphology, size and shape of silver nanoparticles were further asserted by HR-TEM (Fig. 10). It was confirmed that silver nanoparticles possess uniform size 10 to 70 nm, almost spherical and hexagonal shape and uniformly sized.

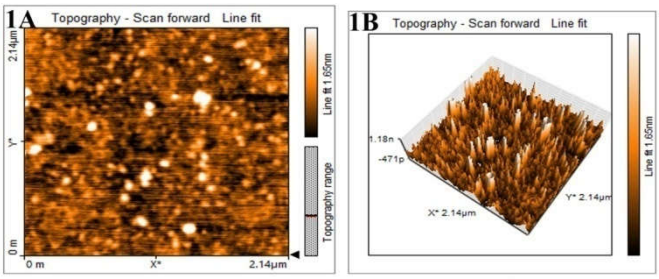

Fig 9 AFM images of silver nanoparticles synthesized by rhizome extract of *M. arundinacea* (1A) Two-dimensional image (1B)-Three-dimensional images

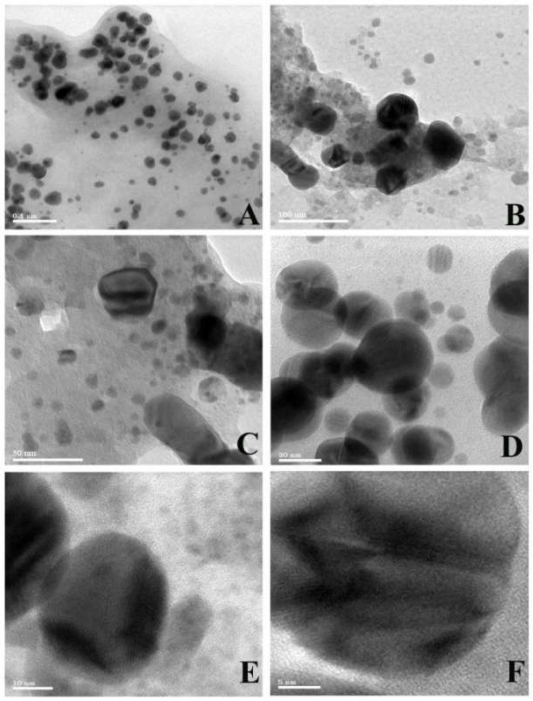

Fig 10 HR-TEM Images of silver nanoparticles synthesized by rhizome extract of *M. arundinacea* (A- 0.1 µm, B-100 nm, C- 50 nm, D-20 nm, E-10 nm, F-5 nm)

***Antimicrobial activity*:** Silver nanoparticles synthesized by *M. arundinacea* rhizome extract was used to test the efficacy of antimicrobial activity. Silver nanoparticles have shown toxicity against *E. coli,* and *S. aureus*. Silver nanoparticles at different concentration (25 µl, 50 µl, 100 µl and 250 µl) showed different zone of inhibition (ZOI) with respect to different microorganism. The zone of inhibition 6.1 mm and 2.5 mm was observed in *E. coli* and *S. aureus* at 250 µL respectively (Table 2). The control plant extract has not showed any zone of inhibition up to 250 µL.

The mechanism behind the activity of silver nanoparticles on bacteria not yet fully explored, the three most common mechanisms proposed to date are (1) the disruption of ATP production and DNA replication by uptake of free silver ions, (2) Reactive Oxygen Species (ROS) generation by silver and silver nanoparticles and (3) direct damage of silver nanoparticles to the cell membranes. [38] Feng *et al*., (2008) and Matsumura *et al*., (2003) proposed that silver nanoparticles release silver ions which interact with thiol groups of many enzymes thus inactivating most of the respiratory chain enzymes leading to the formation of reactive oxygen species enzymes which causes the self-destruction of the bacterial cells and interruption of DNA replication due to damage of the DNA. [39,40,41,42] Gram-negative bacteria are more sensitive to silver nanoparticles due to difference in the cell wall structure of bacteria. The cell wall of the gram positive bacteria is composed of a thick layer of peptidoglycan, consisting of linear polysaccharide chains cross-linked by short peptides thus forming more rigid structure leading to difficult penetration of the silver nanoparticles compared to the gram negative bacteria where the cell wall possesses thinner layer of peptidoglycan.[43]

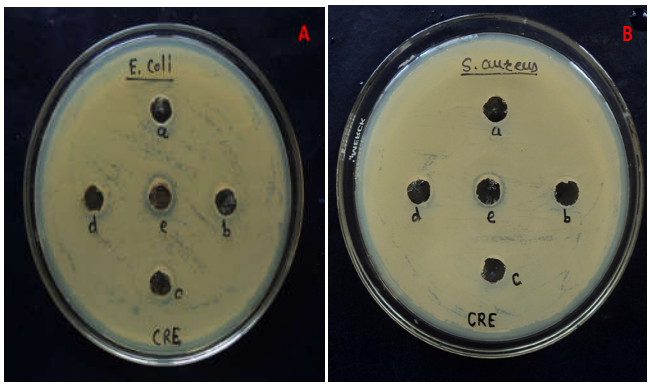

Fig 11 Antimicrobial activity of concentrations of silver nanoparticles (a) Control, (b) 25, (c) 50, (d) 100 and (e) 250 µl

CONCLUSION

In the present investigation rhizome extract of *M. arundinacea* was used to synthesize biogenic silver nanoparticles. The influences of physico-chemical factors increase reduction of silver precursor due to increased activity of leaf extract constituent. As a result, the number of nucleus and thus size of the silver nanoparticles decreased with basic pH higher temperature of the reaction. The synthesized silver nanoparticles show good antibacterial activity against *E. coli* and *S. aureus.* Silver nanoparticles were more sensitive to gram negative than the gram positive.

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