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GREEN SYNTHESIS OF SILVER NANOPARTICLES USING *LUFFA ACUTANGULA* ROXB. VAR. *AMARA*. LIN. AND IT'S ANTIBACTERIAL ACTIVITY.

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ABSTRACT

Aqueous leaf extract of *Luffa acutangula* Roxb. Var. *amara*. Lin. was used for the synthesis of silver nanoparticles using antibacterial activity. The silver nanoparticles formation was confirmed by the color change of plant extracts (SNPs) and further confirmed with the help of UV – Vis spectroscopy and Fourier Transform Infra Red (FTIR). The absorption spectra of silver nanoparticles studied using the UV-VIS spectroscopy had absorbance peak at 420 nm. The antibacterial activities of silver nanoparticles were studied using human pathogenic bacterial strains was used in *Escherichia coli, Klebsiella pneumonia, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella paratyphi,* and *Staphylococcus aureus*. The zone of inhibition was higher in *Klebsiella pneumonia* and zone of inhibition lower in *Staphylococcus aureus*. The move towards an extracellular synthesis of Ag^+ nanoparticles using dried biomass appears to be cost effective, eco-friendly method of nanoparticles synthesis.

KEY WORDS: ***Luffa acutangula*, Silver nitrate, UV, FTIR, FE-SEM, Microorganisms.**

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INTRODUCTION

Silver has been known for antibacterial activity since the times of ancient Greece. Among the noble metals, silver (Ag) is the metal of choice in the field of biological system, living organisms and medicine. Green synthesis of nanoparticles is an emerging branch of nanotechnology. Currently, the investigation of this phenomenon is regained importance due to the increase of bacterial resistance to antibiotics, caused by their over use.[1] Recently silver nanoparticles[2-4] as well as various silver-based compounds containing ionic silver (Ag^+)[5,6] or metallic silver (Ag^0)[7-11] exhibiting antimicrobial activity have been synthesized. Antibacterial activity of the silver ion containing materials can be used, for example, in medicine to reduce infections in burn treatment[12, 13] and arthroplasty, [14] as well as to prostheses, [15] catheters, [16] vascular grafts, [17] dental materials, [18] stainless steel[19] materials and human skins.[20-22] Silver-containing materials can be employed to eliminate micro organisms on textile fabrics[23, 24] or they can be used for water treatment.[25] Silver nanoparticles also exhibit a potent cytoprotective activity toward HIV infected cells.[26] Because of such wide range of application, numerous synthetic methods have been developed.[28] Contrary to bactericide effects of ionic silver, the antimicrobial activity of colloid silver particles are influenced by the dimensions of the particles[3, 4] the smaller particles, the greater antimicrobial effect. Therefore, in developing routes of synthesis, an emphasis was made to control the size of silver nanoparticles. The research in nanotechnology highlights the possibility of green chemistry route to produce prevalence resistance to anti-microbial agents has emerged as a major problem[29]. Bio synthesis of metallic nanoparticles is an eco friendly process and important step in the field of applied nanotechnology[30]. The most common synthesis of silver nanoparticles is the chemical reduction of a silver salt solution by a reducing agent such as NaBH, citrate, and ascorbate. [31-33] the use of a strong reductant such as borohydride, resulted in small particles that were somewhat monodisperse, but controlled synthesis of silver colloid particles was attempted using a two-step reduction process in order to control the particle size. In this process, a strong reducing agent was first applied to produce small silver particles, which were then enlarged in a secondary step using a weaker reducing agent.[34, 35] Though the enlargement of particles in the secondary step from about 20-45nm to 120-170nm has been reported, the initial solution was not reproducible, and the resulting particles were considerably polydisperse. Moreover, such reducing agents may be associated with environmental toxicity or biological hazards. It has been, therefore, of increasing interest to develop green synthesis of silver colloid nanoparticles. In recent studies, a synthesis using a Tollens process was used to demonstrate the formation of silver particles with controlled size in a one-step process.[36, 37] The basic reaction in this process involves the reduction of a silver ion solution using glucose. In this modified Tollens procedure, films with colloidal silver particles ranging from 50 to 200 nm, and silver hydrosols with particles in the order of 20-50 nm, were obtained. In a more recent study, the size of the particles in our laboratory was further controlled by using a difference in the redox potentials of the silver ions undergoing reduction. This difference was easily controllable by changing the concentration of complexing agent, ammonia, of the silver ion, and using a range of reducing saccharides (xylose, glucose, fructose, and maltose). This process gave silver colloid particles with controllable sizes ranging from 45 to 380 nm. This synthetic route is environmentally friendly because of the use of non - toxic chemicals. The wild variety of *Luffa acutangula* Lin. Var. *amara*. Roxb. is an important wild medicinal plant. Plants have been used in traditional medicine for several years. The whole plant is used to cure many diseases, especially in anti-cancer, anti-diabetic, anti-jaundice and many ailments. The seed oil is also known for curing serious skin diseases and prevention of other skin ailments. All parts of the plants are a crystalline bitter principal compound with cucurbitacin B and amarinin. In addition of the luffin and colocynthin is also present. The plant possesses laxative and purgative property. The fruit shows presence of cucurbitacin B and E and Oleanalic acid. It is tonic to intestine cures vata, kapha and anemia. In addition, the cucurbitacins have received great deal of attention because of their cytotoxic and anti-cancer activities. Scientifically; it is proved as CNS Depressant activity. In India, liquid from the leaves and fruit the wild variety of *Luffa acutangula* Var. *amara*. (Roxb) a wild variety, were used to treat jaundice and it is also possess in anti-oxidant and larvicidal activity. The plant contains β-carotenes, flavonoid acutosides A-G, Oleanane type of triterpene, saponins, acutosides H and I, oleanalic acid saponins.

MATERIALS AND METHODS

The wild variety of *Luffa acutangula* Lin. Var. *amara*. Roxb. leaves were collected from the experimental garden in the Department of Plant Science, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India. The Collected leaves were shade dried and ground into a fine powder. For the synthesis of silver nanoparticles, 100 µl of $AgNO_3$ aqueous solution was added to the leaf extract (200 ml) and gently heated until the colour of the solution changed from pale yellow to light brown indicating the formation of Ag nanoparticles. The bio-reduced Ag nanoparticles solution was collected and monitored by periodic sampling of aliquots (5 ml) of aqueous component and measuring UV-Visible spectra of the solution. The remaining leaf extract was centrifuged at 10,000 rpm for 5 minutes. The collected pellets were stored at 4^0C.

(i) UV-VIS Spectra Analysis

The reduction of pure Ag+ ions was monitored by measuring the UV-Vis spectrum of the reaction at 420nm. UV-Visible Spectrum analysis was carried out on UV-VIS Spectrophotometer: 119 Absorption Spectroscopy.

(ii) FT – IR spectroscopy analysis of Silver nanoparticles

In Fourier Transform Infrared (FTIR) analysis, the FTIR spectrum of the dried sample was recorded on a shows bands at 400 – 4000 cm^{-1} at 3441.62, 1637.18, 706.46, 623.35cm^{-1}. The samples were dried and analysed using Perklin Elmer model-Spectrum Rx1 FT-IR Spectrophotometer instrument.

(iii) FE - SEM analysis of Silver nanoparticles

Field Emission Scanning Electron Microscopy (FE-SEM) analysis was done by using Sigma model Carl Zeiss. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min. The SEM grid was allowed to dry and the images of nanoparticles were taken. Spherical and relatively uniform shape of nanoparticles formation was observed (Fig. 5).

(iv) Antibacterial activity study

1. Micro-organisms used

The following microbial strains were obtained from the culture collection centre, Department of Microbiology KAP Medical College, and Department of Microbiology, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India. *Escherichia coli, Klebsiella pneumonia, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella paratyphi,* and *Staphylococcus aureus* were used for the study. The bacteria were maintained on Nutrient Broth (NB) and also Nutrient Agar medium at 37^0C.

2. Preparation of Inoculums

The Gram Positive and Gram Negative bacteria were pre-cultured in Nutrient Broth (NB) on overnight in incubator at 37^0C. After incubation centrifuged at 10,000 rpm for 5 min, pellet was suspended in distilled water (DW). The Petri dishes were flooded, 50 ml of sterile distilled water, using sterile cotton buds, micro tips and forceps.

3. Disc diffusion method

A suspension of the organisms was poured in Nutrient Agar Medium (NAM) in sterile Petri dishes. The mixture was transferred to sterile petri dishes and allowed to solidify. Sterile disc (6mm) (Hi-Media Sterile Disc) was dipped in silver nanoparticles solution of different concentration (20µ and 40µ) of compounds standard and a blank were placed on the surface of agar plate. After the plates were incubated at 37^0C for 24 hrs. Zone of inhibition for control, SNPs and Silver nitrate were measured.

RESULTS AND DISCUSSION

The green synthesis of silver nanoparticles using *Luffa acutangula* Lin. Var. *amara* Roxb. plant extracts were carried out. It is known that silver nanoparticles exhibit brown colour was changed in aqueous solution due to excitation of surface Plasmon vibrations in silver nanoparticles. The appearance of brown colour in the reaction flasks suggests the formation of silver nanoparticles (SNPs) (Fig - 2). Silver nitrate is used as reducing agent as silver has distinctive properties such as good conductivity, catalytic and chemical stability. The aqueous silver ions were exposed to herbal extracts were reduced in solution, thereby leading to the formation of silver hydrosol.

Figure 1
Luffa acutangula Lin. Var. amara. Roxb. Plant

Figure 2
Reduction Silver Nanoparticles indicating by colour change

(i) UV-Vis spectroscopy
The reaction mixture, *Luffa acutangula* Lin. Var. *amara*. Roxb. leaf extracts with aqueous solution of the silver nitrate, started to change its colour and the formation of silver nanoparticles with the reduction of Ag^+ ions. The characteristic surface Plasmon absorption bands were observed at 420 nm with increasing reaction time from 30 min to 60 min. Extinctions spectra of silver synthesized form $AgNO_3$ were shown in figure – 3.

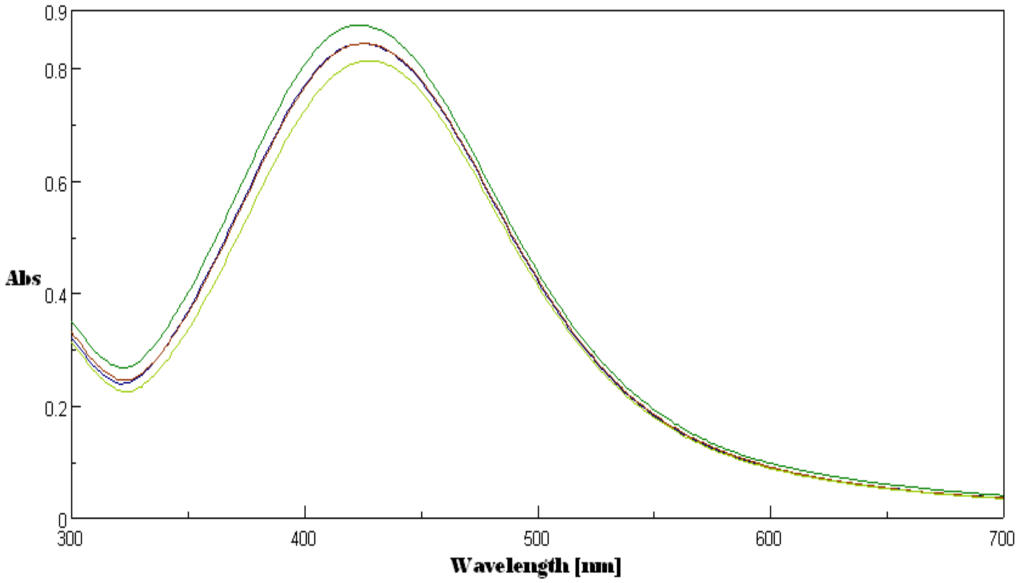

Figure 3
nanoparticles UV-Vis absorption spectroscopy of silver

FT-IR Spectroscopy

FTIR analysis was carried out in order to identify the possible reducing and stabilizing biomolecules, described in (Figure – 4) in the wild variety of *Luffa acutangula* Lin. Var. *amara*. Roxb. extract. FTIR absorption spectra of water extract before and after reduction of silver ions are shows bands at 400 – 4000 cm^{-1} at 3441.62, 1637.18, 706.46, 623.35cm^{-1}. These absorbance bands were known to be associated with stretching vibrations for –C C=C O, respectively in particular, new peaks were presents in the analysis in 623.35 cm^{-1} bands are most probably from the C=C reduction of silver ions. The total disappearance of this band after the bioreduction might be due to the fact that the polyols were mainly responsible for the reduction of silver ions, whereby they themselves got oxidized to unsaturated carbonyl groups leading to a broad peak at 1637 cm^{-1} for reduction group of aromatic hydroxyflavones and catachins.

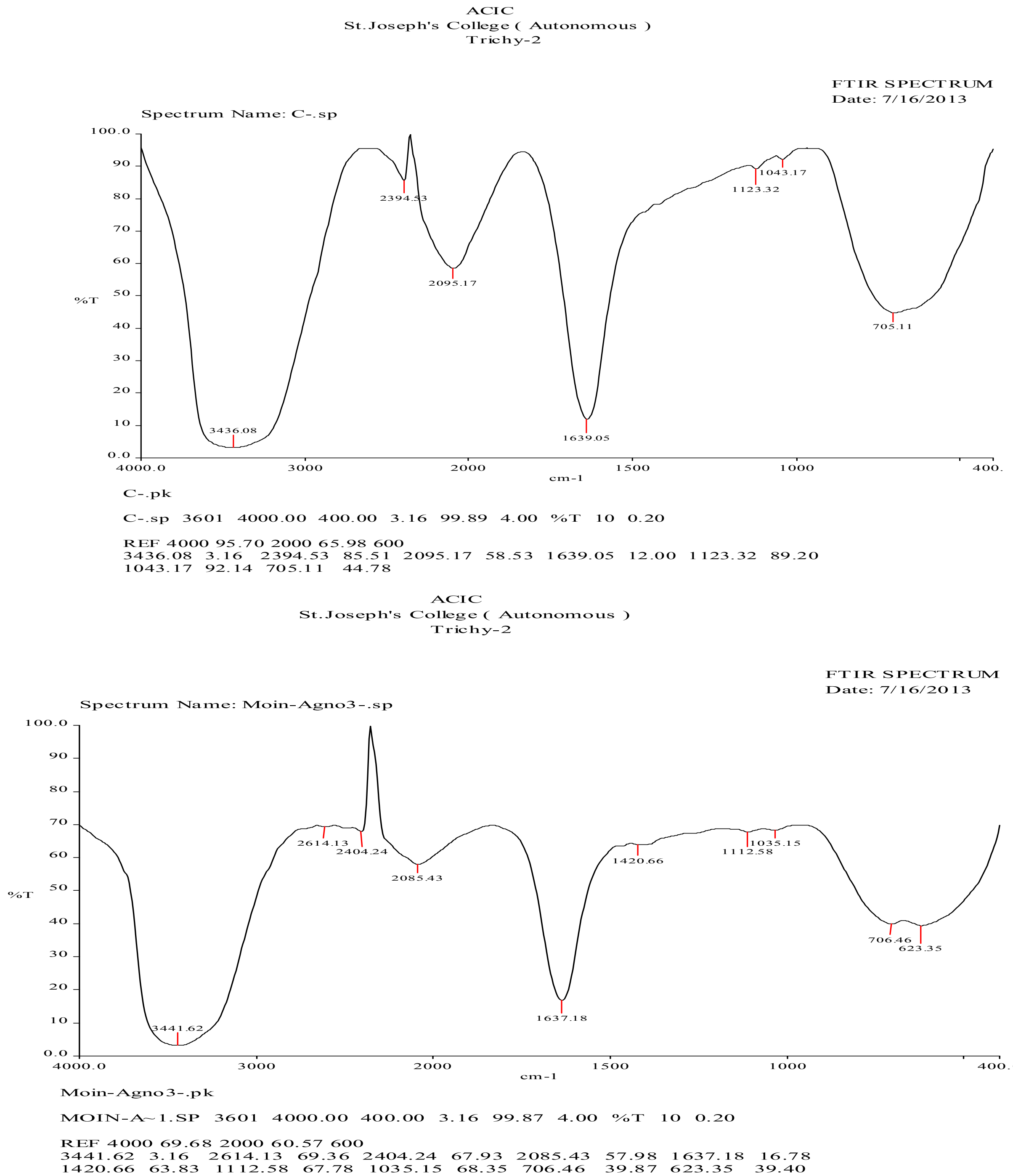

Figure 4
FTIR spectrum of silver nanoparticles synthesized using Luffa acutangula Lin. Var. amara. Roxb. a). Control b). Synthesized silver nanoparticles using plant

(ii) FE-SEM analysis of Silver nanoparticles

Field Emission Scanning Electron Microscopy (FE-SEM) has been employed to characterize the size, shape and morphology of formed silver nanoparticles. From the images, it is evident that the morphology of silver nanoparticles is nearly spherical it is in agreement with the shape of surface plasma resonance band in the UV – Visible spectra. The average particle size analysed from the SEM images is observed to be 10 – 100 nm in the wild variety of *Luffa acutangula* Lin. Var. *amara*. Roxb. (Figure - 5).

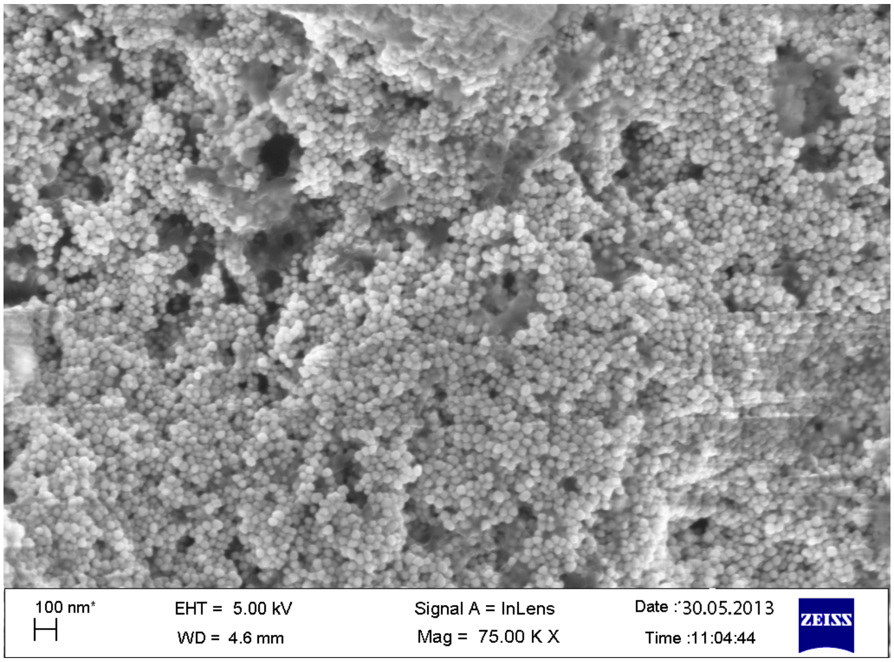

Figure 5
SEM image of $AgNO_3$ by Luffa acutangula Lin. Var. amara. Roxb.

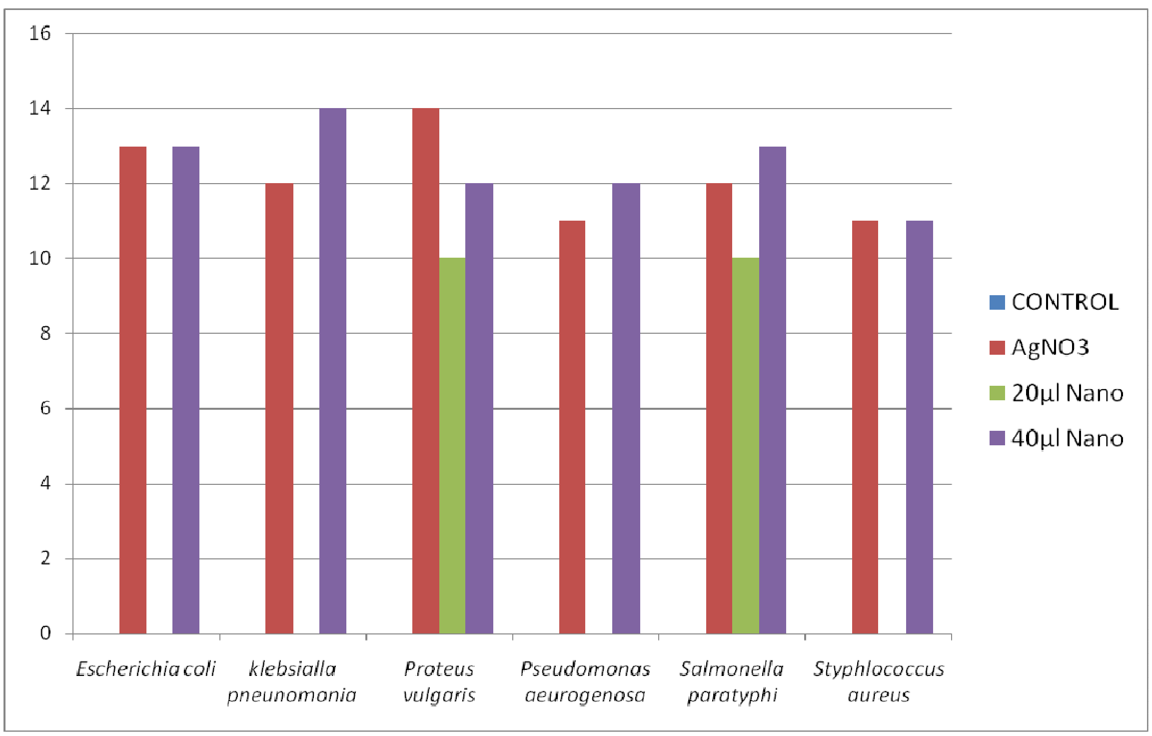

Figure 6
Antibacterial activity and its zone of inhibition of silver nanoparticles synthesized using Luffa acutangula Lin. Var. amara. Roxb.

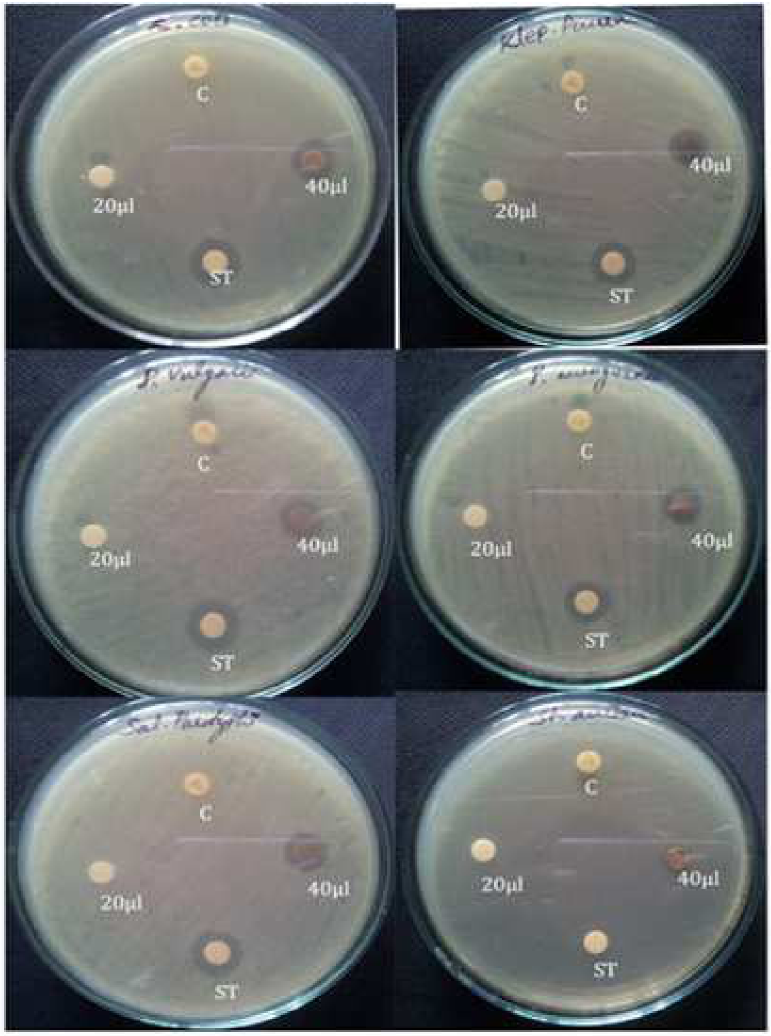

Figure 7
Antibacterial activity of silver nanoparticles synthesized by leaf extract of Luffa acutangula Lin. Var. amara. Roxb. against various pathogenic bacterial strains

Microbial Susceptibility Testing (Disc diffusion assay)

Silver is well known as one of the most universal antimicrobial substances. The results of the antimicrobial activity of wild variety of *Luffa acutangula* Lin. Var. *amara*. Roxb. water extracts and the prepared silver nanoparticles using different v/v ratios of the water extract and aqueous $AgNO_3$ solution (20µl, 40µl) were mixed thoroughly and subjected to UV – irradiation tested against pathogenic microbial strains by disc diffusion assay are summarized in table- 1. The extract of wild variety of *Luffa acutangula* Lin. Var. *amara*. Roxb. plants was found to possess antimicrobial activity. The antibacterial activity of silver nanoparticles were analysed by disc diffusion assay. The maximum 14 mm clear inhibitory zone appeared around 40 µl silver nanoparticles against *Klebsiella pneumonia* and minimum 11 mm in *Staphylococcus aureus* (Table 1). After incubation for 24 h followed by suggesting that synthesized nanoparticles showed phenomenal bactericidal effect. This study clearly demonstrates that the bactericidal effect depends on the concentration of the silver nanoparticles. The result demonstrates that the zone of clearance increased according to concentration of silver nanoparticles in all microbes.[41] Silver nanoparticles may attach to the surface of the cell membrane and disturb its power function such as permeability and respiration. It is reasonable to state that, the binding of the particles to the bacteria depends on the surface area available for interaction. Some nanoparticles penetrate into the cell and bind with DNA interrupting some gene expression necessary for important metabolism. Smaller particles having the larger surface area available for interaction will give more bactericidal effect than the larger particles.

Table 1
Antibacterial activity of silver nanoparticles synthesized using Luffa acutangula Lin. Var. amara. Roxb. against some human pathogenic organisms.

S. No	Tested Pathogenic Microbial Strains	Antibiotic Disc (Rifampicin)	Silver nanoparticles synthesized using Leaf extract of *Luffa amara L.* Zone of Inhibition (mm)		
			$AgNO_3$ Stock	20µl	40µl
1.	*Escherichia coli*	Resistant	13	Resistant	13
2.	*Klebsiella pneumonia*	Resistant	12	Resistant	14
3.	*Proteus vulgaris*	Resistant	14	10	12
4.	*Pseudomonas aeruginosa*	Resistant	11	Resistant	12
5.	*Salmonella paratyphi*	Resistant	12	10	13
6.	*Staphylococcus aureus*	Resistant	11	Resistant	11

Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) values of the leaf extract against tested bacteria were shown in table – 1. The values were present in 40µg/ml and respectively, against the tested Gram-positive bacteria, ranged from 20 to 40µg/ml and against Gram-negative bacteria. Potency of antibacterial activity of plant extract against these bacteria exposed in MIC indicated the plant extract is more effective against Gram-positive at lower concentration than that against Gram-negative bacteria.

CONCLUSION

The Ag nanoparticles were synthesized using leaf extract of *Luffa acutangula* Lin. Var. *amara*. Roxb. Further, the above Ag nanoparticles revealed to possess an effective antibacterial property against human pathogenic organisms. The present study emphasizes the use of plant medicine for the synthesis of Ag nanoparticles with potent antibacterial effects.

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