

## SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLE FROM *COUROUPITA GUIANENSIS* LEAF EXTRACT AND ITS EFFECT ON CLINICAL PATHOGENS

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### ABSTRACT

**Objective:** In this study, a rapid and simple approach was applied for the synthesis of silver nanoparticle (AgNP) from aqueous leaf extract of *Couroupita guianensis*. The plant extract acts as an antimicrobial agent and is also used to synthesis AgNP.

**Methods:** Ultraviolet (UV)-visible spectrophotometer was used to characterize synthesized AgNP and to identify the compounds responsible for the reduction of silver ions, the functional groups present in plant extract were investigated by Fourier transform infrared. Agar well diffusion method was used for the antibacterial activity of synthesized AgNPs.

**Results:** UV-visible spectrophotometer showed an absorbance peak in the range of 405 nm. The AgNPs showed antibacterial activities against *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Bacillus sp*, *Klebsiella sp*, and *Pseudomonas sp*.

**Conclusion:** Nowadays, silver-based topical dressing has been widely used as a treatment for infections such as wounds, burns, and chronic ulcer.

**Keywords:** Silver nanoparticle, *Couroupita guianensis*, Antimicrobial activity, Ultraviolet-visible spectrophotometer, Fourier transform infrared.

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### INTRODUCTION

Silver nanoparticles (AgNPs) are one of the most vital and fascinating nanomaterials which are involved in biomedical applications. They can be used for both *in vitro* and *in vivo* applications [1]. AgNPs play a major role in nanoscience and nanotechnology, particularly in nanomedicine. AgNPs are highly used in various fields such as medicinal field, food, health care, and industrial purposes [2-4]. Due to their surface to volume ratio, they can change physical, chemical, and biological properties. Therefore, these nanoparticles have been exploited for various purposes [5]. Nowadays, AgNPs are used to coated frequently in biomedical devices, wound dressings, and textiles [6].

Among the several methods to the synthesis AgNP, biological methods show high yield, solubility, high stability and also simple, rapid, non-toxic, and green approaches that can produce well-defined size and morphology under optimized conditions. Moreover, the physicochemical properties of a particle can have a significant impact [7]. They have to use many analytical techniques to evaluate the synthesized nanomaterials such as ultraviolet (UV)-visible spectroscopy, X-ray diffractometry, Fourier-transform infrared (FTIR) spectroscopy, scanning electron microscopy, and transmission electron microscopy [8,9].

*Couroupita guianensis* is an evergreen tropical tree belongs to the family - Lecythidaceae. The various parts of the tree used as an antibiotic, antifungal, antiseptic, antioxidant, anti-inflammatory, analgesic property, treat hypertension, anticancer, reduce tumor pain, antiulcer, antidepressed, antifertility, antibiofilm, wound healing, antidiabetic, stomach ache, dysentery, etc. The juice of *C. guianensis* leaves can be used to cure skin disease. Young leaves are used to cure toothache [10].

### METHODS

#### Collection and preparation of plant

Fresh leaves of *Couroupita guianensis* were collected from Kasi Viswanathar Temple in Kolumam, Udumalpet, during the month of December 2018 and authenticated at Botanical Survey of India (BSI),

Southern Regional Center, Coimbatore. The plant leaves were washed in tap water and dried in the shade for 4-5 days and made to a coarse powder using a mechanical grinder; then, the powder was stored at room temperature in an airtight container. These powdered materials were used for antibacterial activity and synthesis of AgNP.

#### Preparation of 1mM silver nitrate solution

0.017 g of silver nitrate was added to 100 ml of sterile distilled water and stirred well. Then, the 1 mM solution of silver nitrate was stored at 4°C for further studies [11].

#### Green synthesis of AgNP

Ten grams of powdered *Couroupita guianensis* leaf material were weighed and soaked in 100 ml of sterile distilled water. The flask was completely covered with aluminium foil and shaken up to 30 min. Then, the solution was subsequently shaken and filtered using Whatman No 1 filter paper. The aqueous filtrate was centrifuged at 1200 rpm for 5 min to remove the heavy biomaterials. Then, the volume of 10 ml of leaf extract was mixed with 90 ml of 1 mM AgNO<sub>3</sub> solution and incubated at room temperature for 3 days. The color change of the solution from brown to reddish-brown was indicated the reduction of Ag<sup>+</sup> to Ag<sup>0</sup> nanoparticles. The color changes of the solution confirm the presence of AgNP; then, the solution was centrifuged at 5000 rpm for 5 min to separate the AgNP. The supernatant was discarded and the pellet was washed with water and again centrifuged at 8000 rpm for 5 min. The washing step was repeated 3 times to eliminate the presence of any possible contaminants and the pellet was stored for further studies [12].

#### Characterization of AgNP

The synthesized green AgNP was further characterized by UV-visible spectroscopy and FTIR can be used to identify the functional group of AgNP.

#### UV-visible spectrophotometric analysis

The synthesized silver nanoparticle can be characterized using UV-visible spectrophotometer. UV-visible absorption spectra of the silver

colloids were acquired using wavelength scan between 200 nm and 800 nm. On average, a plasmon peak at 400 nm–450 nm implies the formation of approximately 12 nm AgNPs. Larger wavelength points to the formation of larger sized nanoparticles [13].

#### FTIR analysis

FTIR spectroscopy is a salient roll to identify the functional group. FTIR spectrum reveals clear peaks throughout the whole range of observation and expressed visible bands of synthesized AgNPs [14].

#### Antibacterial activity of synthesized AgNPs

The antibacterial activity of AgNPs was evaluated using the agar well diffusion method in triplicate. The solid agar plates were punched with 7 mm diameter wells and the inoculums were spread over the agar plates using sterile cotton swabs. The AgNPs solution was prepared using dimethyl sulfoxide (1 g of AgNP was dissolved in 1ml dimethyl sulfoxide). Then, the wells were filled with 100  $\mu$ l of AgNP. Chloramphenicol was used as a positive control, and silver nitrate was used as a negative control. The plates were incubated at 37°C for 24 h. After incubation, the zone of inhibition was measured.

#### Antibacterial activity of leaf extract

One gram of crude extract was dissolved in 1 ml of dimethyl sulfoxide and the wells were filled with 100  $\mu$ l of leaf extract. Dimethyl sulfoxide was used as negative control and chloramphenicol was used as a positive control. The plates were incubated at 37°C for 24 h, and the zone of inhibition was measured. The experiment was done in triplicate [15].

#### Statistical analysis

Experiments were carried out in triplicate and the results are expressed as mean values with standard deviation.

## RESULTS

The collected plant material was identified as *C. guianensis* (Plate 1a and 1b) and authenticated (BSI/SRC/12/25/2018/ - Tech - 3568) at BSI, Southern Regional Center, TNAU Campus, Coimbatore - 641003. The specimen was deposited in the Department of Microbiology, Dr. N.G.P. Arts and Science College, Coimbatore.

#### Synthesis of silver nanoparticle

Using 1mM  $\text{AgNO}_3$  for reduction of silver ion into silver nanoparticles from the aqueous leaf extract of *C. guianensis*. The synthesis of silver nanoparticles was confirmed by visualizing the color of the reaction mixture. The color of the solution at the starting of the reaction was the pale yellow color which gradually transformed into dark reddish-brown in color after 24 h indicating the formation of the AgNPs (Fig. 1). The appearance of reddish-brown color indicates the formation of AgNPs from aqueous extract of *C. guianensis*.



Plate 1a: *C.guianensis* Tree

plate 1b: Young leaves of *C.guianensis*

#### Characterization of silver nanoparticle

##### UV-visible spectrophotometer

The synthesized of AgNPs was further confirmed by UV-visible spectrophotometer. The characteristics of reddish-brown color appears due to the excitation of surface plasmon vibrations in the AgNPs. This bioreduction of silver nitrate ions was followed by UV-visible spectroscopy (Fig. 2a and b). The leaf extract synthesized AgNP

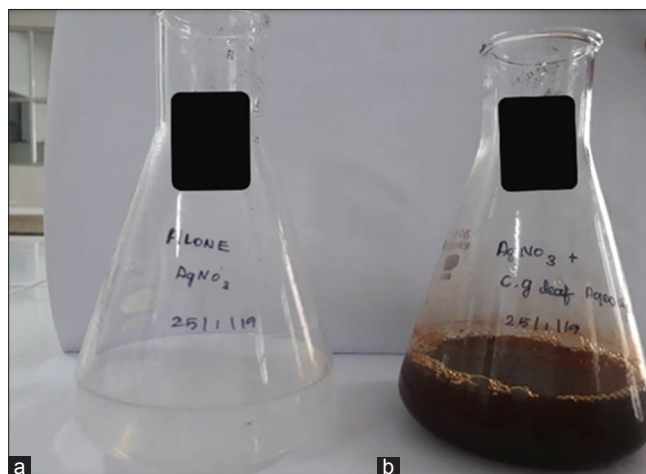


Fig. 1: Synthesis of silver nanoparticle (AgNP) (a) 1mM Silver nitrate solution and (b) AgNP produced in aqueous leaf extract using 1 mM silver nitrate solution.

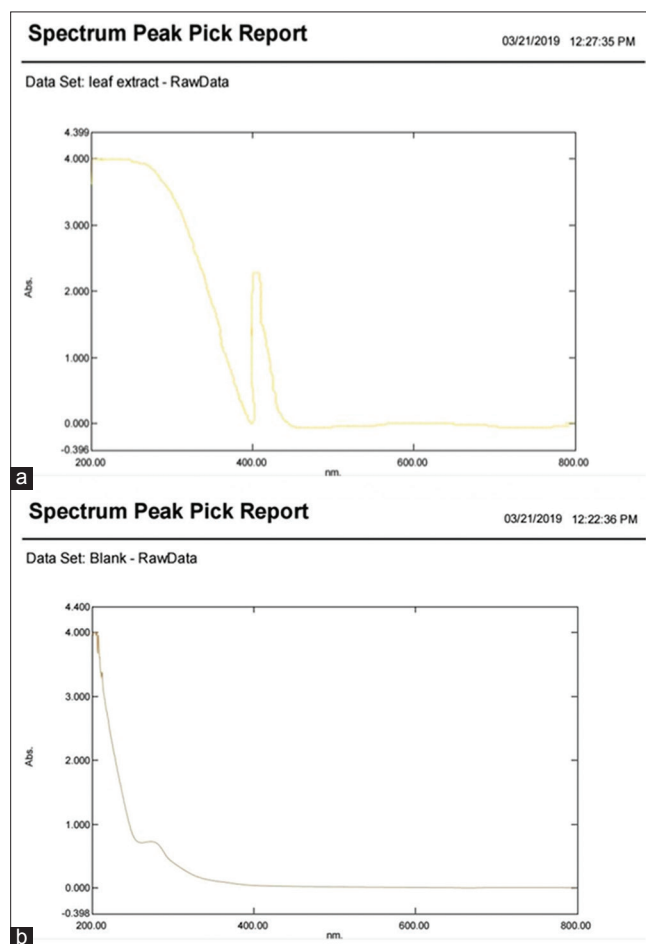


Fig. 2: (a) Ultraviolet (UV) -visible spectrum of silver nanoparticles. (b) UV-visible spectrum of leaf extract

showed absorbance peak at 405 nm, respectively. It is indicating the presence of AgNPs that remained the same through the reaction period.

#### FTIR Analysis

FTIR spectral analysis was carried out to identify the nature of compounds which are present in the AgNPs. In the present study, FTIR spectral measurements were carried out to identify the potential biomolecules in the aqueous extract of *C. guianensis* leaves that may be responsible for reducing and capping the bioreduced AgNPs. The observed intense bands were compared with standard values to identify the functional groups. The FTIR spectra of *C. guianensis* extract synthesized nanoparticles sample were compared, and it showed considerable variation in the peaks of spectra (Fig. 3).

In the purified AgNP, only 17 peaks were found, and loss of certain groups was confirmed by the reduction of certain peaks. The band at 3309.85 in the synthesized nanoparticles corresponds to O-H stretching vibration indicating the presence of alcohol and phenol. A peak at 1720.50 is indicating the presence of the ester group. A peak at 1211.30 is responsible for N-H stretch vibration indicating the presence of amide linkage. These functional groups have a role in the stability or capping of AgNPs as reported in many studies [16]. Therefore, the results concluded these changes in the functional groups responsible for the formation of AgNP.

#### Antibacterial assay

##### Antibacterial activity of AgNP

The antibacterial activity of synthesized AgNP was evaluated by agar well diffusion method. The test organisms are *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Bacillus* sp, *Klebsiella* sp, and *Pseudomonas* sp. Chloramphenicol used as positive control and  $\text{AgNO}_3$  used as a negative control. The results are given in Table 1 and Fig. 4.

The maximum zone of inhibition range between 9 mm and 15 mm was obtained in the synthesized AgNPs. The maximum zone of inhibition ( $15 \pm 0.14$ ) was measured from 2 mg and 1 mg of AgNP in *Pseudomonas* sp.

##### Antibacterial activity of the *C. guianensis* leaf extract

The antibacterial activity of *C. guianensis* leaf extracts was assessed against different bacteria by a well diffusion method in comparison with standard antibiotic chloramphenicol. The test organisms are *S. aureus*, *S. pyogenes*, *Bacillus* sp, *E. coli*, *Pseudomonas* sp, and *klebsiella* sp. The obtained result is given in Table 2.

#### DISCUSSION

The present research was carried out to explore the potential of medicinal plant *C. guianensis*. The study was started from the collection and authentication of plant material. The AgNPs were synthesized from *C. guianensis* and characterization of AgNP, followed by analysis the bioassay. The formation of AgNPs using leaf extract of *C. guianensis* was viewed by the color change from colorless to yellowish-brown. Similarly, the AgNPs exhibited striking colors, from light yellow to brown [17]. Further, silver nanoparticles exhibit dark yellowish brown colour in aqueous solution due to the surface Plasmon resonance phenomenon, and the maximum absorbance of peak for *C. guianensis* seen at 405 nm in UV-visible spectrum [18,19].

FTIR analysis confirmed that the bioreduction of silver ions to AgNPs is due to the reduction by capping material of plant extract. Similarly, the protein present in the extract can bind to AgNPs through either free amino or carboxyl groups in the proteins [20,21]. The reduction function of polyphenols in the synthesis of AgNPs was also reported as earlier [22]. AgNPs obtain from the *C. guianensis* have very strong inhibitory action against *S. aureus*, *S. pyogenes*, *Pseudomonas* sp, *E. coli*, *Bacillus* sp, and *Klebsiella* sp. The phytochemicals are reported to have the capability of increasing the susceptibility of bacteria for various drugs [23]. Hence, the bactericidal property of the nanoparticles is size-

Table 1: Antibacterial activity of *C. guianensis* leaf synthesized silver nanoparticle

S. No.	Test organism	Zone of inhibition in mm				
		Concentration of silver nanoparticle				
		2 mg	1 mg	0.5 mg	Standard drug	DMSO
1.	<i>S. aureus</i>	-	-	-	18±0.86	Nil
2.	<i>Bacillus</i> sp.	15±0.81	13±0.69	12±0.65	19±0.54	
3.	<i>S. pyogenes</i>	-	-	-	18±0.24	
4.	<i>Klebsiella</i> sp.	13±0.72	11±0.81	9±0.76	20±0.45	
5.	<i>E. coli</i>	15±0.82	13±0.72	12±0.54	22±0.12	
6.	<i>Pseudomonas</i> sp.	15±0.14	15±0.21	13±0.92	20±0.43	

*C. guianensis*: Couroupita guianensis, *E. coli*: *Escherichia coli*, *S. aureus*: *Staphylococcus aureus*, *S. pyogenes*: *Streptococcus pyogenes*

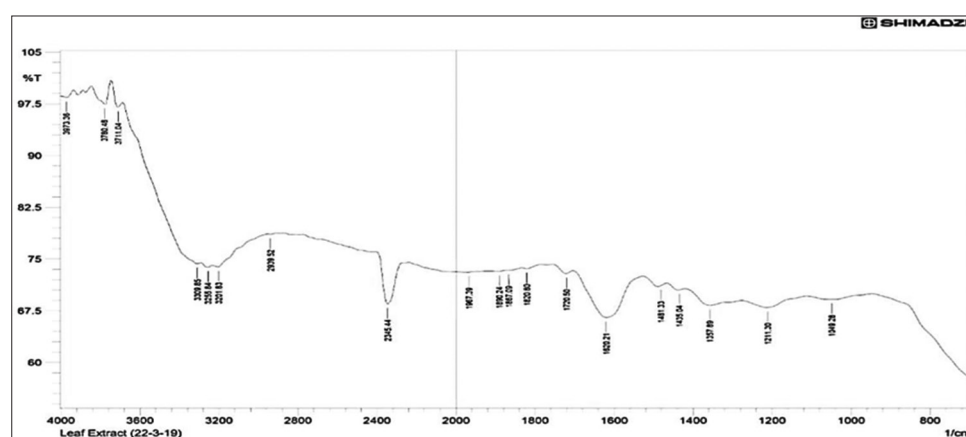


Fig. 3: Fourier transform infrared spectra of silver nanoparticle

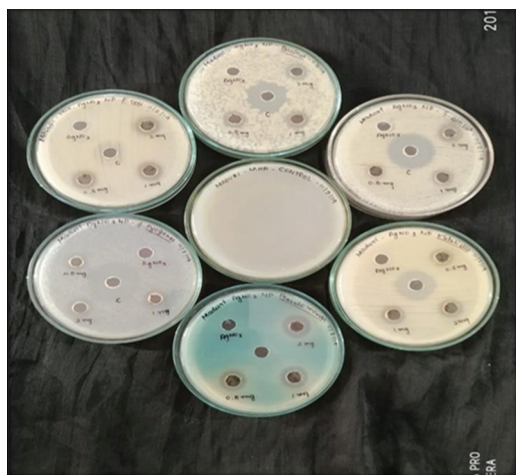


Fig. 4: Antimicrobial activity of silver nanoparticle synthesized from *C. guianensis*

Table 2. Antibacterial activity of *C. guianensis* leaf extract

S. No.	Test organism	Zone of inhibition		
		Aqueous	Standard drug	DMSO
1.	<i>S. aureus</i>	12±0.65	18±0.86	Nil
2.	<i>Bacillus</i> sp	12±0.72	19±0.54	
3.	<i>Streptococcus pyogenes</i>	10±0.34	18±0.24	
4.	<i>Klebsiella</i> sp	13±0.56	20±0.45	
5.	<i>E. coli</i>	11±0.76	22±0.12	
6.	<i>Pseudomonas aeruginosa</i>	13±0.87	20±0.43	

*C. guianensis*: *Couroupita guianensis*, *E. coli*: *Escherichia coli*, *S. aureus*: *Staphylococcus aureus*, *S. pyogenes*: *Streptococcus pyogenes*

dependent, the larger the surface area, the greater the antibacterial activity [24], and biosynthesis method [25].

## CONCLUSION

The development of reliable and eco-friendly process for synthesis of metallic nanoparticles used for the critical need in the field of nanotechnology. AgNPs play a profound role in the field of biology and medicine due to their phytochemical properties. The biosynthesized AgNP using *C. guianensis* leaves extract proved to be good against clinical pathogens. The antimicrobial activity is well demonstrated by the well diffusion method. The use of *C. guianensis* has added advantage and this plant can be used by nanotechnology processing industries. Nanoparticles can be used as bactericidal, water purification, and also used in the field of medicine. Hence, this method can be potentially exciting for the large-scale synthesis of nanoparticles.

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## AUTHORS' CONTRIBUTIONS

Maduri Nagarajan carried out all the laboratory work. Sinduja Baskaran compiled and analyzed the data. Dr. Devakumar Joseph and Dr. Sudha Sivasubramanian wrote the manuscript, compile all the work, and finalize the manuscript. All the authors read and approved the final manuscript

## CONFLICTS OF INTEREST

The authors have no conflicts of interest to publish this research article in this journal.

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## REFERENCES

- Krithiga N, Rajalakshmi A, Jayachitra A. Green synthesis of silver nanoparticles using leaf extract of *Clitoria ternatea* and *Solanum nigrum* and study of its antibacterial effect against common nosocomial pathogens. *J Nanosci* 2015;2015:928204.
- Gurunathan S, Park JH, Han JW, Kim JH. Comparative assessment of the apoptotic potential to nanoparticles synthesized by *Bacillus tequilensis* and *Calocybe indica* in MDA-MB-231 human breast cells: Targeting p53 for anticancer therapy. *Int J Nanomed* 2015;10:4203-22.
- Li WR, Xie XB, Shi QS, Zeng HY, Ou-Yang YS, Chen YB. Antibacterial activity and mechanism of silver nanoparticles on *Escherichia coli*. *Appl Microb Biotechnol* 2010;8:1115-22.
- Mukherjee P, Ahmad A, Mandal D, Senapati S, Sainkar SR, Khan MI, et al. Fungus-mediated synthesis of silver nanoparticle synthesis. *Nano Lett* 2001;1:515-9.
- Sharma VK, Yngard RA, Lin Y. Silver nanoparticles: Green synthesis and their antimicrobial activities. *Coll Interface* 2009;145:83-96.
- Staquinini FI, Ozawa MG, Moya CA, Driessen WH, Barbu EM, Nishimori H, et al. Systemic combinatorial peptide selection yields a non-canonical iron-mimicry mechanism for targeting tumors in a mouse model of human glioblastoma. *J Clin Investig* 2011;121:61-73.
- Lin PC, Lin S, Wang PC, Sridhar R. Techniques for physicochemical characterization of nanomaterials. *Biotechnol Adv* 2014;32:711-26.
- Gurunathan S, Han JW, Kim ES, Prk JH, Kim JH. Reduction of graphene oxide by resveratrol: A novel and simple biological method for the synthesis of an effective anticancer nanotherapeutic molecule. *Int J Nanomed* 2015;10:2951-69.
- Sapsford KE, Tyner KM, Dair BJ, Deschamps JR, Medintz IL. Analyzing nanomaterial bioconjugates: A review of current and emerging purification and characterization techniques. *Anal Chem* 2011;83:4453-88.
- Al-Dhabi NA, Balachandran C, Raj MK, Durairandiyar V, Muthukumar C, Ignacimuthu S, et al. Antimicrobial, antimycobacterial and antibiofilm properties of *Couroupita guianensis*. *BMC Complement Altern Med* 2012;12:242.
- Shekhawat MS, Anusuya P, Kannan N, Manokari M, Revathi J, Illavarasi V. Green synthesis of silver nanoparticles using *Couroupita guianensis* Aubl. and their characterization. *Int J Green Herbal Chem* 2013;2:1041-9.
- Devaraj P, Kumari P, Aarti C, Renganathan A. Synthesis and characterization of silver nanoparticles using cannonball leaves and their cytotoxic activity against MCF-7 cell line. *J Nanotechnol* 2013;2012:598328.
- Kumar TV, Murthy JS, Rao MN, Bhargava Y. Evaluation of silver nanoparticles synthetic potential of *Couroupita guianensis* Aubl. Flower buds extract and their synergistic antibacterial activity. *3 Biotech* 2016;6:92.
- Sivakumar T, Rathimeena T, Shankar T, Ponmanickam P. Production of silver nanoparticles synthesis of *Couroupita guianensis* plant extract against human pathogen and evaluations of antioxidant properties. *Int J Life Sci* 2015;3:333-40.
- Sivakumar T, Shankar T, Vijayabaskar P, Geetha G. Efficacy of *Couroupita guianensis* against selected human pathogens. *Adv Biol Res* 2012;6:59-63.
- Fu M, Li Q, Sun D, He Lu, Deng X, Wang H, et al. Rapid preparation process of silver nanoparticles by bioreduction and their characterizations. *Chin J Chem Eng* 2006;14:114-7.
- Sastry M, Ahmad A, Khan MI, Kumar R. Biosynthesis of metal nanoparticles using fungi and actinomycetes. *Curr Sci* 2003;85:202-6.
- Shankar SS, Ahmad A, Pasricha R, Sastry M. Bioreduction of chloroaurate ions by geranium leaves and its endophytic fungus yields gold nanoparticles of different shapes. *J Mater Chem* 2003;13:1822-6.
- Mulvaney P. Surface plasmon spectroscopy of nanosized metal particles. *Langmuir* 1996;12:788-800.
- Gole A, Dash C, Ramakrishna V, Sainkar SR. Pepsin-gold colloid conjugates: Preparation, characterization and enzymatic activity.

- Langmuir 2001;17:1674-9.
21. Sivaraman SK, Elango I, Kumar S, Santhanam V. A green protocol for room temperature synthesis of AgNPs in seconds. *Curr Sci* 2009;97:1055-9.
  22. Dibrov P, Gosinl JK, Hase CC. Chemiosmotic mechanism of antimicrobial activity of Ag<sup>+</sup> in *Vibrio cholera*. *J Antimicro Agents Chemother* 2002;46:2668-70.
  23. Jayaraman P, Sakharkar MK, Lim CS, Tang TH, Sakharkar KR. Activity and interaction of antibiotic and phytochemical combinations against *Pseudomonas aeruginosa in vitro*. *Int J Biol Sci* 2010;6:556-68.
  24. Jeong SH, Hwang YH, Yi SC. Antimicrobial properties of padded PP/PE nonwovents incorporating nano-sized silver colloids. *J Mater Sci* 2005;40:5413-8.
  25. Logeswari P, Silambarasan S, Abraham J. Synthesis of AgNPs using plants extract and analysis of their antimicrobial property. *J Saudi Chem Soc* 2012;48:007.