Recent Advances in Nanoscience and Nanotechnology

Volume 1

NANOMEDICINE AND DRUG DELIVERY

Edited By

Mathew Sebastian, MD, Neethu Ninan, and A. K. Haghi, PhD

Apple Academic Press

TORONTO NEW JERSEY

Apple Academic Press Inc.
3333 Mistwell Crescent
Oakville, ON L6L 0A2
Canada

Apple Academic Press Inc.
1613 Beaver Dam Road, Suite # 104
Point Pleasant, NJ 08742
USA

Exclusive worldwide distribution by CRC Press, a Taylor & Francis Group

International Standard Book Number: 978-1-926895-17-8 (Hardback)

Printed in the United States of America on acid-free paper

0 9 8 7 6 5 4 3 2 1

Library of Congress Control Number: 2012935667

Library and Archives Canada Cataloguing in Publication

Nanomedicine and drug delivery/edited by Mathew Sebastian, Neethu Ninan and A.K. Haghi.
(Recent advances in nanoscience and nanotechnology; v.1)

Includes bibliographical references and index.
ISBN 978-1-926895-17-8
1. Nanomedicine. 2. Drug delivery systems. 3. Nanotechnology.
I. Sebastian, Mathew II. Ninan, Neethu III. Haghi, A. K IV. Series:
Recent advances in nanoscience and nanotechnology; v.1

R857.N34N363 2012 610.28 C2011-908741-3

Recent Advances in Nanoscience and Nanotechnology

Series Editors-in-Chief

Sabu Thomas, PhD

Dr. Sabu Thomas is the Director of the School of Chemical Sciences, Mahatma Gandhi University, Kottayam, India. He is also a full professor of polymer science and engineering and Director of the Centre for nanoscience and nanotechnology of the same university. He is a fellow of many professional bodies. Professor Thomas has authored or co-authored many papers in international peer-reviewed journals in the area of polymer processing. He has organized several international conferences and has more than 420 publications, 11 books and two patents to his credit. He has been involved in a number of books both as author and editor. He is a reviewer to many international journals and has received many awards for his excellent work in polymer processing. His h Index is 42. Professor Thomas is listed as the 5th position in the list of Most Productive Researchers in India, in 2008.

Mathew Sebastian, MD

Dr. Mathew Sebastian has a degree in surgery (1976) with specialization in Ayurveda. He holds several diplomas in acupuncture, neural therapy (pain therapy), manual therapy and vascular diseases. He was a missionary doctor in Mugana Hospital, Bukoba in Tansania, Africa (1976-1978) and underwent surgical training in different hospitals in Austria, Germany, and India for more than 10 years. Since 2000 he is the doctor in charge of the Ayurveda and Vein Clinic in Klagenfurt, Austria. At present he is a Consultant Surgeon at Privatclinic Maria Hilf, Klagenfurt. He is a member of the scientific advisory committee of the European Academy for Ayurveda, Birstein, Germany, and the TAM advisory committee (Traditional Asian Medicine, Sector Ayurveda) of the Austrian Ministry for Health, Vienna. He conducted an International Ayurveda Congress in Klagenfurt, Austria, in 2010. He has several publications to his name.

Anne George, MD

Anne George, MD, is the Director of the Institute for Holistic Medical Sciences, Kottayam, Kerala, India. She did her MBBS (Bachelor of Medicine, Bachelor of Surgery) at Trivandrum Medical College, University of Kerala, India. She acquired a DGO (Diploma in Obstetrics and Gynaecology) from the University of Vienna, Austria; Diploma Acupuncture from the University of Vienna; and an MD from Kottayam Medical College, Mahatma Gandhi University, Kerala, India. She has organized several international conferences, is a fellow of the American Medical Society, and is a member of many international organizations. She has five publications to her name and has presented 25 papers.

Dr. Yang Weimin

Dr. Yang Weimin is the Taishan Scholar Professor of Quingdao University of Science and Technology in China. He is a full professor at the Beijing University of Chemical Technology and a fellow of many professional organizations. Professor Weimin has authored many papers in international peer-reviewed journals in the area of polymer processing. He has been contributed to a number of books as author and editor and acts as a reviewer to many international journals. In addition, he is a consultant to many polymer equipment manufacturers. He has also received numerous award for his work in polymer processing.

Contents

Book chapter

Chapter 7

Biosynthesis of Silver Nanoparticles, Characterization and Their Antimicrobial Activity

K. Mallikarjuna, G. Narasimha, B. V. Subba Reddy, B. Sreedhar,
G. R. Dillip, and B. Deva Prasad Raju

INTRODUCTION

There has been an increasing interest in the development of a clean synthetic procedure often known as "green chemistry" for nanoproducts which are targeted as potential applications in the fields of catalysis in chemical reactions, drug delivery in medical field, biolabelling, microelectronic, information storage, and optoelectronic devices (Crooks et al., 2001; Bhumakar et al., 2007; Dai and Bruening, 2002; Gittins et al., 2000; Hayat, 1989; Murray et al., 2001). The broad spectrum of silver nanoparticles was produced by different physical and chemical methods. For environmental concerns, there is a need to develop benign nanoparticles using non-toxic chemicals in the synthesis protocols in order to avoid adverse effects in medical applications. At present, several groups of researchers concentrate on biomimetic approaches such as plant or plant leaf extracts, microorganisms and yeast to synthesize the metal nanoparticles called as "green chemical or phytochemical" approach (Sinha et al., 2009). One of the synthesis procedure such as leaf extracts of *geranium, lemon grass, neem and several others* has been reported (Dubey et al., 2010; Rajesh et al., 2009; Shankar et al., 2003, 2004a, 2004b; Song et al., 2009;). The *Piper betle* is a traditional medicinal plant of India. The betel leaf is used in a number of traditional remedies for the treatment of stomach ailments, infections, and as a general tonic. It is often chewed in combination with the betel nut *(Areca catechu)*, as a stimulatory. Some evidence suggests that betel leaves have immune boosting properties as well as anti-cancer properties. It is also well known for its phenolic content, and also for its antibacterial and antioxidant activities as well. So far, there have been no reports on the synthesis of nanoparticles by using *piper betle* leaf extract. In this paper, we report on the synthesis of silver nanoparticles using *piper betle* leaf, their characterization and their antibacterial activity.

EXPERIMENTAL DETAILS

Preparation of Leaf Extract

The fresh leaves of *piper betle* were collected from a retail shop in Tirupati, Andhra Pradesh, India. Silver nitrate ($AgNO_3$, 99.99%) was purchased from Sigma-Aldrich chemicals.10g of fresh leaves were washed thoroughly under the running tap water, while finely cut leaves were added with 50 ml of distilled water in a 250 ml Erlenmeyer

flask, and then boiled for 10min, before decanting it. The extract was filtered and stored at 4°C for further experiments.

Synthesis of Silver Nanoparticles

The leaf broth with various concentration levels, ranging from 50 to 150 µl was added to 3 ml of 1mM aqueous $AgNO_3$ solution kept at room temperature. The bioreduced silver nitrate solution was monitored by periodic sampling of aliquots (0.3 ml). It wasdiluted to the ratio of 1:10 with distilled water, to avoid errors due to high optical density of the solution for measuring UV-Vis spectra.

Antifungal Activity of Silver Nanoparticles

The antifungal activity of the silver nanoparticles was checked against *Aspergillussps*. The pure cultures of the fungi were obtained by sub culturing. The culture slants were subjected to a 3 ml sterile distilled water containing 0.01 ml of TritionX-100. 100 µl of fungal spore suspension was loaded into the Czapek-Dox agar medium plates. Later cavities of 0.5 cm diameter were made and filled with 100 µl of silver nanoparticle solution.

Antibacterial Activity of Silver Nanoparticles

The antibacterial property of silver nanoparticles was checked on both gram positive and gram negative bacteria by following the agar diffusion method. Gram positive bacteria like *Staphylococcus sps, Bacillus sps* and gram negative bacteria like *Escherichia coli* (*E.Coli*) *Psedomononassps*were used for the present study. The 24 hr active cultures of the above bacterial strains were seeded in the Agar plates by pour plate technique. Cavities of 0.5 cm diameter were made using a borer and the bottoms of the cavities were sealed. Each cavity was filled with 100 µl of silver nanoparticles solution and then incubated at 37°C in an incubator.

RESULTS AND DISCUSSION

UV-Visible Absorbance Spectroscopy

The concentration variation with bioreduced Ag^+ ions, in aqueous component were measured with an UV-V is spectrometer, (Perkin-Elmer lambda 25)which operated at a resolution of 1nm in the range of 370800 nm. The progress of the reaction between the *betle* leaf broth and the metal ions were observed by UV-V is spectra of silver nanoparticles which are shown in Figure 1. A Bathochromatic shift in the surface plasmon resonance band of silver nanocolloid, with an increasing concentration of leaf extract and consequent color change was observed. From the spectrum, we observed that the peak blue shift was at 477440 nm while the amount of leaf extract was constantly increased. The reduction of silver ions and the synthesis of stable nanoparticles occurred with a concentration variation reaction, making it one of the smart phytofabricationmethods; in order to produce Ag nanoparticles reported nowadays (Dwivedi and Gopal, 2010; Gils et al., 2010; Konwarh et al., 2011; Philip and Unni, 2011).

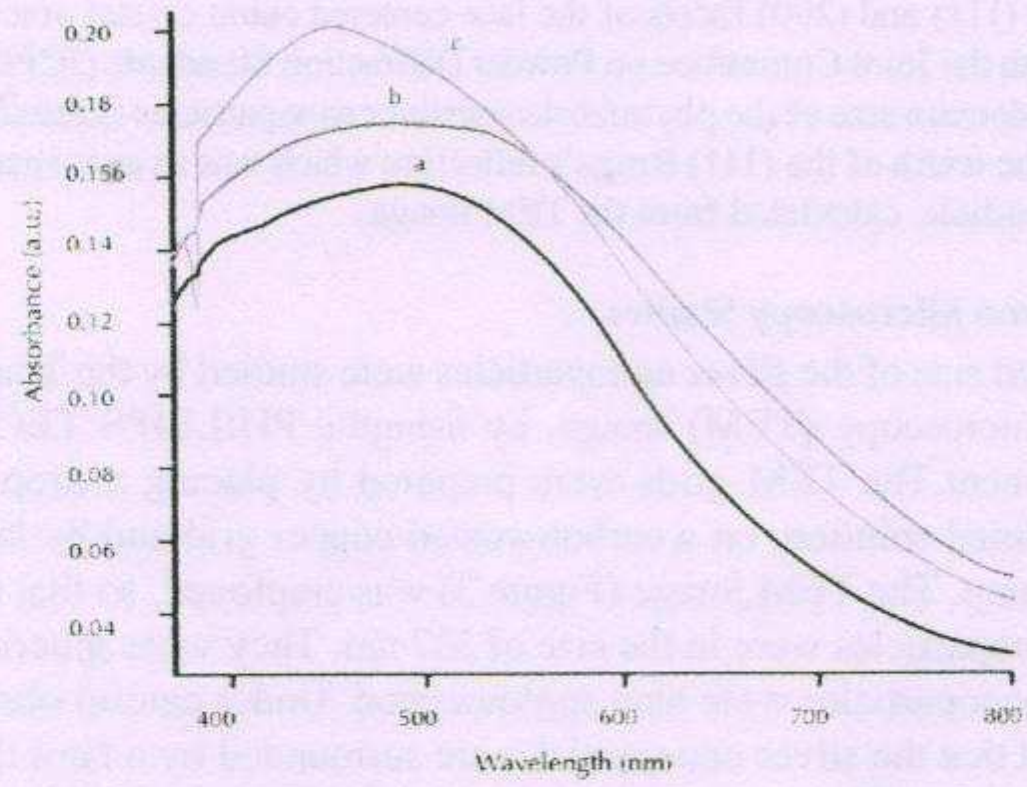

Figure 1. UV-Vis spectra of silver nitrate with *piper betle* leaf extract at different concentrations. a) 50 L, b) 100 µL and c) 150 µL.

X-Ray Diffraction Spectral Analysis

An X-ray diffraction (XRD) measurement of a thin film of the bioreduced silver ions' aqueous solution was drop coated onto a glass slide and carried out on an INEL X-ray diffractometer. The diffraction pattern was recorded by Co-kα_1 radiation with λ of 1.78A° in the region of 2θ from 20 to 90° at 0.02/min. and the time constant was 2 sec. The size of the nanoparticles was calculated through the Scherer's equation (Mulvaney, 1996). The Crystalline nature of Ag nanoparticles was studied with the aid of an X-ray diffraction (Figure 2).The diffracted peaks were observed at 37.6° and 44.4°

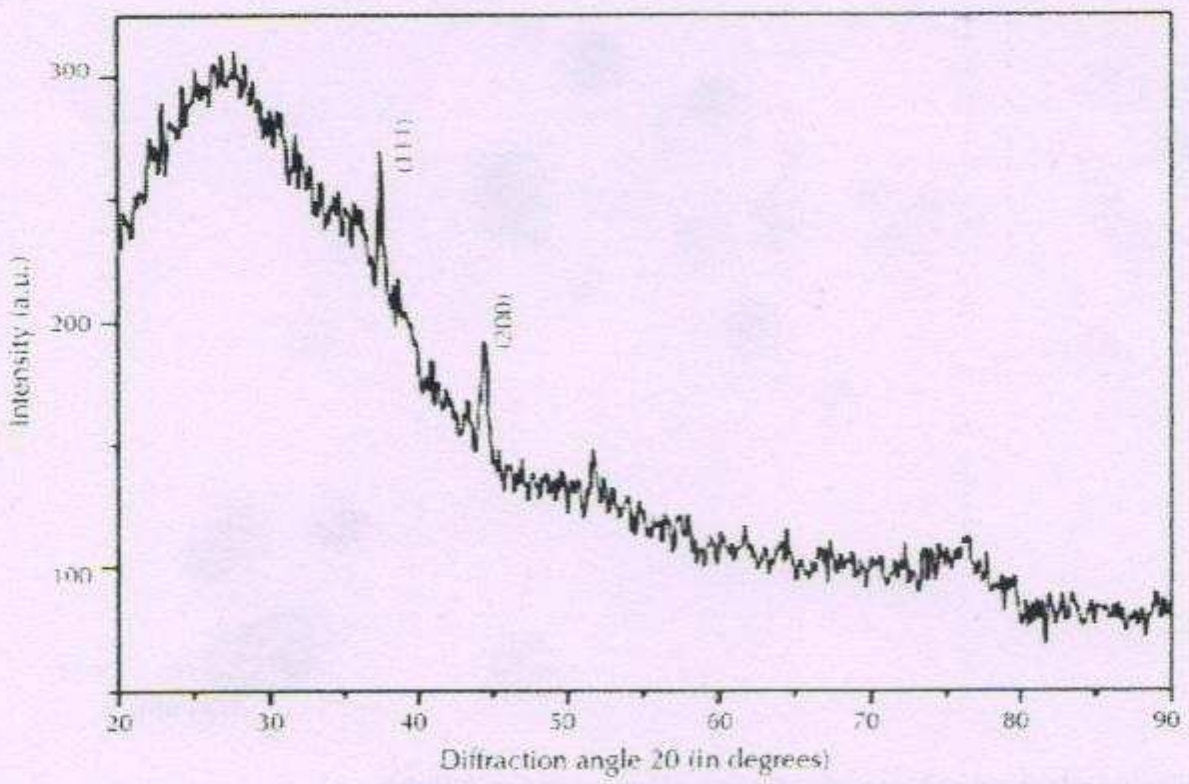

Figure 2. X-Ray diffraction spectrum of synthesized silver nanoparticles.

corresponding to the (111) and (200) facets of the face centered cubic crystal and the data was matched with the Joint Committee on Powder Diffraction Standards (JCPDS) file No.030921. The domain size of the phytofabricatedsilver nanoparticles is found to be 5.4 nm,by using the width of the (111) Bragg's reflection which was in consonance with the size of the particle, calculated from the TEM image.

Transmission Electron Microscopy Studies

The morphology and size of the silver nanoparticles were studied by the Transmission electron microscopy (TEM) image, by usingthe PHILLIPS TECHNAI FE 12 instrument.The TEM grids were prepared by placing a drop of the bio reduced diluted solution, on a carbon-coated copper grid and by later drying it under a lamp. The TEM image (Figure 3) was employed, so that the bio synthesized nanoparticles were in the size of 337 nm. They were spherical in shape and few nanoparticles were also agglomerated. Under careful observation, it is evident that the silver nanoparticles are surrounded by a faint thin layer of other materials. The histogram of fabricated silver nanoparticles is shown in Figure 4. It is evident that there is a variation in particle sizes and the estimated average size is 12 nm.The small sized nanoparticles were able to easily penetrate across the membrane; and similar results have been reported in literature (Jaidev and Narasimha, 2010; Pal et al., 2007; Morones et al., 2005).

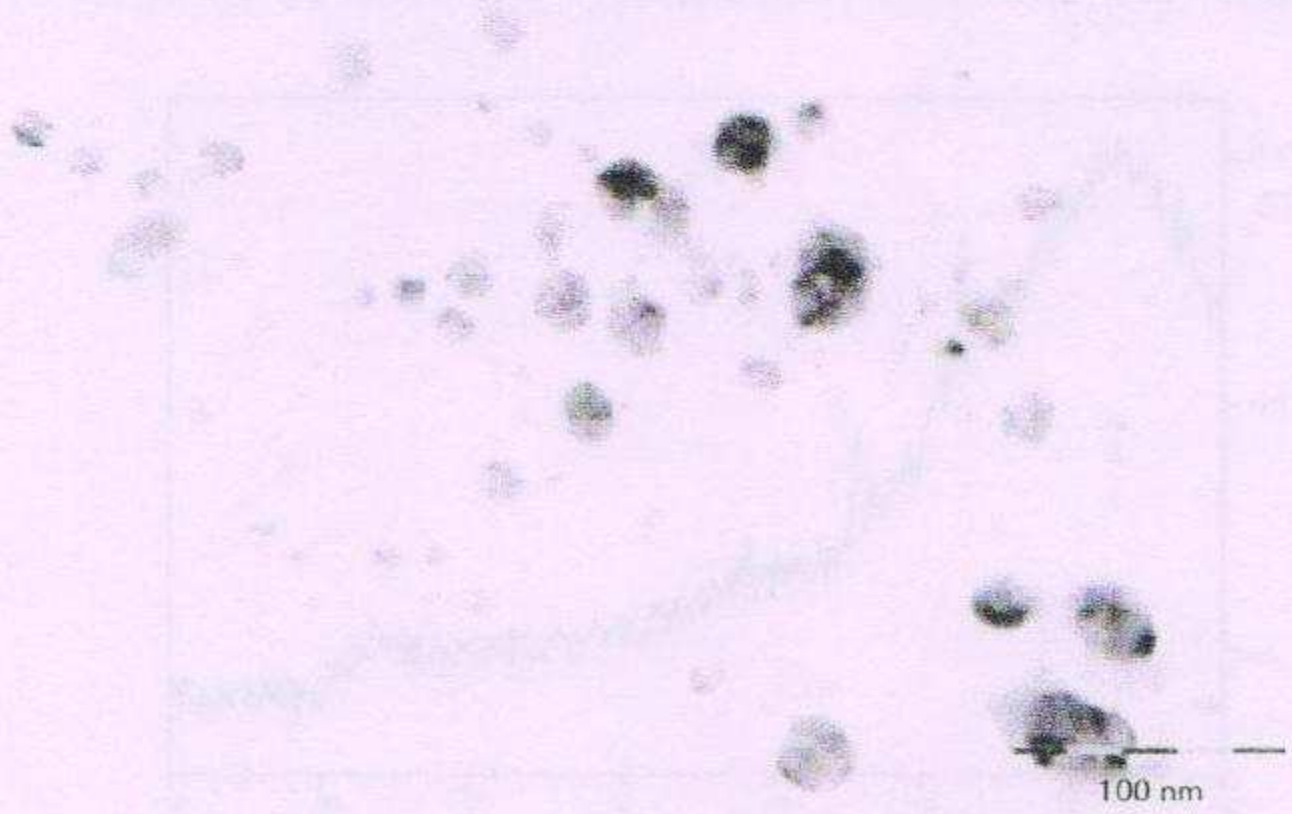

Figure 3. Transmission electron microscopy image of silver nanoparticles.

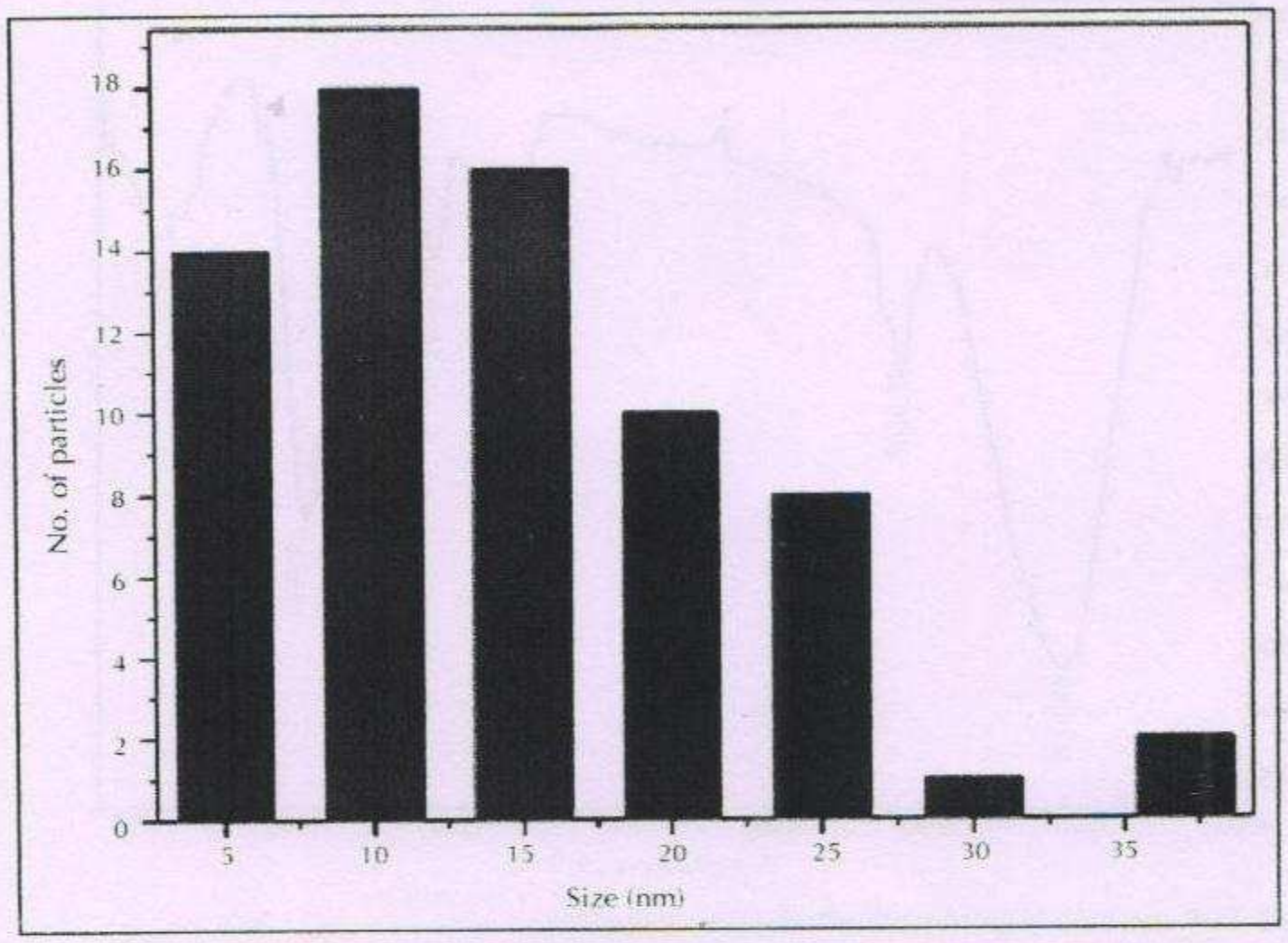

Figure 4. Histogram of synthesized silver nanoparticles.

Fourier Transformed Infra-Red Spectroscopy

For Fourier transformed infrared (FTIR) measurements, the bioreduced Ag^+ ion aqueous component was centrifuged at 10,000 rpm for 15 min. The dried sample was grinded with KBr pellets and analyzed on Thermo Nicolet Nexus 670 IR spectrometer which was operatedat a resolution 4 cm^1 in the region of 4,000400 cm^{-1}. The FTIR spectrum of synthesized silver nanoparticles by using *Piper betle* leaf extract is shown in Figure 5. It confirmedthe fact that to identify the bimolecular for reducing and efficient stabilization of the metal nanoparticles, the band at 3,419 cm^1 corresponds to O-H, as also the H-bonded alcohols and phenols. The peak at 2,920 cm^1 indicates carboxylic acid. The band at 1,640 cm^1 states primary amines. The band at 1,431 cm^1 corresponds to C-C stretching aromatics, while the peak at 1,378 cm^1 states C-H rock alkenes and 1,163, 1,113 and 1,058 cm^1 indicatethe C-O stretching alcohols, carboxylic acids, esters and ethers. Therefore, the synthesized nanoparticles were encapsulated by some proteins and metabolites such as terpenoids having functional groups of alcohols, ketones, aldehydes and carboxylic acids.

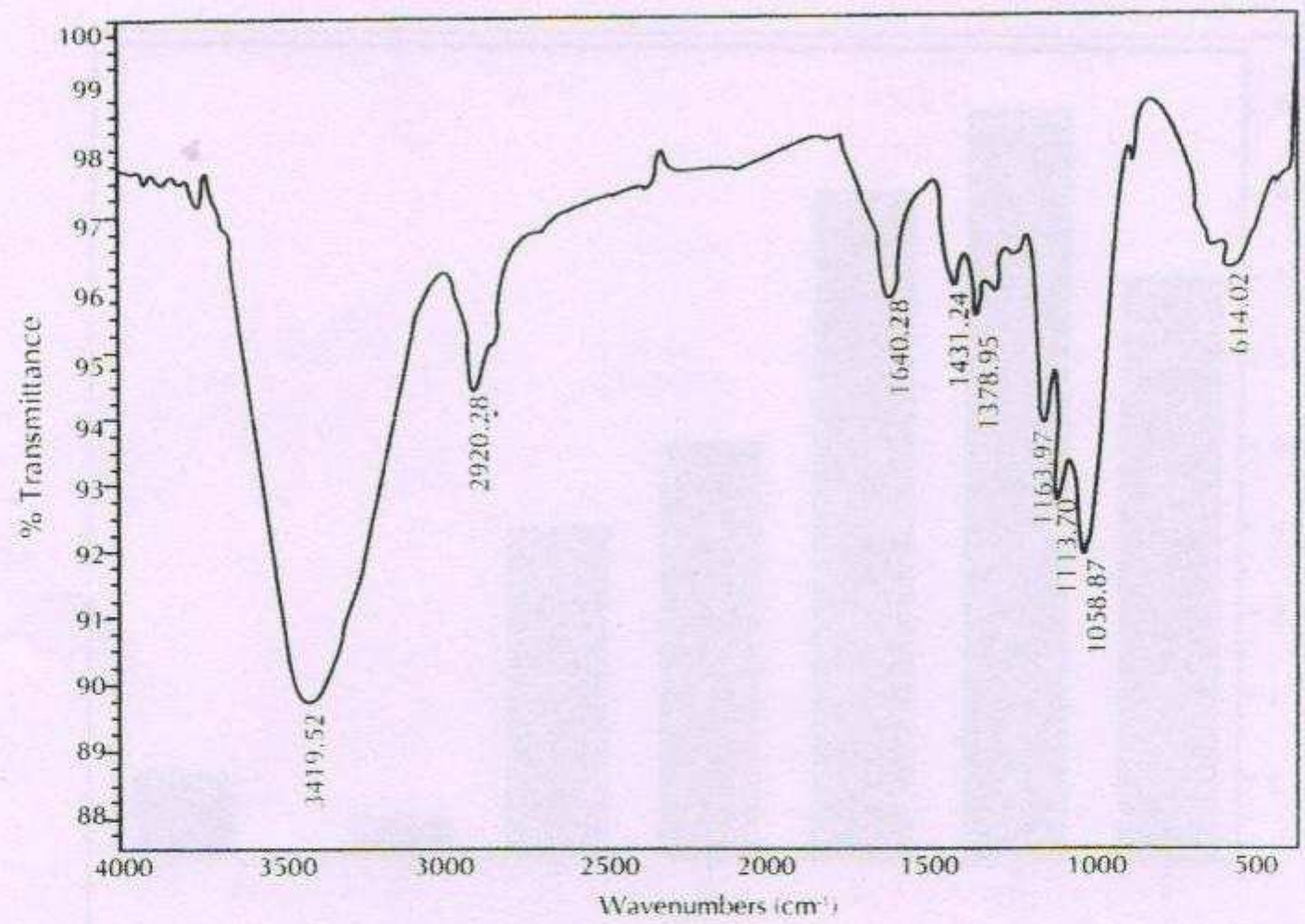

Figure 5. FTIR spectrum of green synthesized silver nanoparticles.

Table 1. Inhibitiory activity of silver nanoparticles (100µl) on bacterial strains.

S. No	Organism	Zone of inhibiton (cm)
1.	*Aspergillussps*	1.2
2.	*E.coli*	1.8
3.	*Pseudomonas sps*	1.12
4.	*Staphylococcus sps*	1.01
5.	*Bacillus sps*	0.89

*All values represented the in the table are average of conducted experiment.

The antifungal property of silver nanoparticles checked against the fungi *Aspergillussps.* was shown in Table 1. The Agar plate with fungal culture having the cavities filled with nanoparticles suspension showed a clear zone of diameter 1.2 cm. This indicates that silver nanoparticles have the antifungal activity and are very effective against the pathological fungi like *Aspergillus* which cause the disease Aspergillosis. Similar reports were made by Devendra, et al. 2009. According to his studies, papaya fruit extract mediated silver nanoparticles exhibited antibacterial activity against *E.coli* and *Pseudomonasaeruginosa.* The antifungal activity of silver nanoparticles could be disruption of transmembrane energy metabolism and membrane electron transport chain by formation of insoluble compounds in the cell wall; the formation of insoluble compound may be due to the inactivation of cell wall sulfhydryl group; silver ions can create mutation in fungal DNA by displacing the hydrogen bonds; silver

ions can dissociate the enzyme complexes which are essential for respiratory chain and membrane permeability, disruption of membrane bound enzymes and lipids could cause the cell lysis(Velmurugan et al., 2009).

These bacterial strains in petriplate with the clearing zones around the cavities reveal that the silver nanoparticles inhibit the growth of the bacterial strains. The formation of inhibition zones around the wells containing silver nanoparticles against the test strains *Staphylococcus* sp. was measured as 1.01 cm, *Bacillus* sp. was 0.89 cm and *E.coli* was 1.8 cm (Table 1). This shows that the silver nanoparticles were more effective on *E.coli* (gram negative bacteria). Several studies have investigated and interpreted the interaction of the silver nanoparticles with bacteria. Similarly Sondi, et al. (2004) and Morones, et al. (2005), revealed that majority of the silver nanoparticles were localized on the membranes of treated *E. coli* cells. According to Lok et al. (2006), the treatment of *E.coli* cells with nanomolar concentrations of silver nanoparticles results in an immediate dissipation of the proton motive force, killing the cells. The gram positive and gram negative bacteria in antibiotic enhanced the action of silver nanoparticles and showed that the silver nanoparticles got bound to the DNA of the bacteria which caused their inactivation.

With reference to the above studies, the possible mechanism of the action of silver nanoparticles on both bacteria and fungi may be generalized as their accumulation in the cell membrane caused lysis of the cell by some unknown mechanism.

CONCLUSION

The bio synthesis of silver nanoparticles using leaf broth of *piper betle* provides an environment friendly, simple, cost effective and efficient route for the synthesis of benign nanoparticles. The size of the silver nanoparticles was estimated to be 337 nm. The reduced silver nanoparticles effectively inhibited the growth of microorganisms including bacterial and fungal strains which cause diseases in human beings. Further works needs to be conducted to investigate the cellular mechanism and toxicity of nanoparticles in microorganism.

KEYWORDS

- **Fourier transformed infrared**
- **Green chemistry**
- **Nanoparticles**
- **Transmission electron microscopy**
- **X-ray diffraction**