

GREEN SYNTHESIS, CHARACTERIZATION AND APPLICATIONS OF SILVER NANOPARTICLES FROM *XANTHIUM STRUMARIUM* LINN

Sharmila. S and Dr. S. Yamini Sudha Lakshmi*

Department of Medical Biochemistry, University of Madras, Taramani Campus,
Chennai-600 113.

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***Correspondence for
Author**

**Dr. S. Yamini Sudha
Lakshmi**

Department of Medical
Biochemistry, University
of madras, Taramani
campus, Chennai -600 113

ABSTRACT

To develop a novel approach for the green synthesis of silver nanoparticles using aqueous leaves extracts of *Xanthium strumarium* Linn. which has been proven active against MCF-7 cell line. In this study, the silver nanoparticles were synthesized biologically using *Xanthium strumarium* Linn. leaf extract as reducing agent. The extract was used for the study of characterization, antibacterial and free radical activities. The antibacterial study was made against *Protease vulgaris*, *Enterococcus faecalis*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*. The formation of silver nanoparticles was indicated by the colour change from colourless to reddish brown and the particles were characterized by UV-vis, FTIR, ERD, SEM, and TEM analysis. These nanoparticles were found to have significant antimicrobial

activity against *Pseudomonas aeruginosa*. The nanoparticles also showed a potent cytotoxic activity against MCF7 cell line *invitro*.

KEYWORDS: UV-vis, FTIR, ERD, SEM, TEM analysis, *Xanthium strumarium*.

INTRODUCTION

The term “cancer” was used for the first time by Hippocrates, Father of western medicine, who applied Greek words “carcinoma” and “Karakinos” to describe tumor (Nobili *et al.*, 2009). Cancer is uncontrolled growth of abnormal cells in the body (Jena *et al.*, 2012). Normally, meiosis and cell death procedure occurs to protect stable condition of tissues in balanced state (Dhorajiya *et al.*, 2012). Carcinogenesis is a multistage or multi mechanism procedure. Deformed cell mass could remain inside of tissue from that tissue it has been

produced and it is called *insitu* cancer, or it can be spread to neighboring tissues, which is called malignant cancer. There are more than 100 types of cancer have been identified. Surgery, chemotherapy and radiotherapy are considered as the most common methods of cancer treatment (Qi *et al.*, 2010). Although chemotherapy and radiotherapy are highly effective methods of cancer treatment, these methods exert severe side effects in use. One of the main problems in cancer treatment is gradual resistance of cancer cells against treatment (Wang *et al.*, 2012). Hence, achieving a new approach is one of the aims of nanotechnology studies to improve cancer treatment results (Azadmehret *et al.*, 2011). Nanotechnology is an emerging techniques in the area of research with important applications. Nanoparticles act as the bridge between bulk materials and atomic or molecular structures. Nanoparticles are made from noble metals such as silver, gold, platinum, and palladium (Mercy Ranjitham *et al.*, 2013). Silver nanoparticles are in the range of 1 and 100 nm in size. They have unique properties which help in molecular diagnostics, in therapies, as well as in devices that are used in several medical procedures. (Sukumaran Prabhu *et al.*, 2012). Since the nineteenth century, silver-based compounds have been used in many antimicrobial applications. Nanoparticles have been known to be used for numerous physical, biological, and pharmaceutical applications (Sukumarprabhu *et al.*, 2012).

Botanical description

X. strumarium L. grows as weed throughout on waste lands. Locally it is known as Gokharu and Kuttazad (Bhogaonkar and Ahmad., 2012). Whole plant is used as blood purifier and in the treatment of scabies. In Ayurveda, it is called 'Shankeshwara' and 'Arishta', and is considered anthelmintic, antipyretic, diuretic, cooling, laxative, alexiteric, tonic, digestive, appetizer, improves voice, complexion, used in epilepsy, leucoderma and as antidote for insect bite. (Bhogaonkar and Ahmad., 2012). The Whole plant was found to contain anthraquinone, cardenolide, leucoanthocyanin, simple phenolics (Catechol) and triterpenoids. (Bhogaonkar and Ahmad., 2012). The leaves were used in herpes, malaria, ringworm, scrofula; fruits and seeds used as cooling; in eye diseases, headache, piles, cancerous wounds (Bhogaonkar and Ahmad., 2012).

Hence, In the present study, synthesis of silver nanoparticles from the leaf extract of *X. strumarium* Linn. was carried out and analysed for characterization and other applications.

MATERIALS AND METHODS

COLLECTION OF PLANT MATERIALS

Based on the documented ethanopharmacological knowledge on the use of medicinal plants in the treatment of pathological diseases, fresh leaves of *X.struamrium* were collected from Thiruvallur district, and the taxonomic identification was done by Department of CAS Botany, University of madras, Guindy campus, Chennai-600 025.

PREPARATION OF AQUEOUS EXTRACTION

The leaves were washed 2-3 times in distilled water, and shade dried until the water molecules evaporated (Umer Farooq *et al.*, 2014). 20g of leaf were taken and crushed gently and add 100ml of distilled water. Then the extract was filtered and stored in a airtight container (Kantha Arunachalam *et al.*, 2014).

PREPARATION OF SILVER NANOPARTICLES

Aqueous solution of 3Mm silver nitrate was prepared to synthesis silver nanoparticles. 10 ml of extract is added drop by drop into the 90ml of 3 Mm silver nitrate solution. The formations of silver nanoparticles were confirmed by the colour change from colourless to reddish brown. (Mercy Ranjitham *et al.*, 2013), then the incubated solution were centrifuge for about 10 to 20 minutes at 10000 rpm. To the pellet, add 1ml of acetone for recovery of silver nanoparticles, then the solution were transformed into the petridish for observation of nanoparticles. The dried nanoparticle powder were stored in a airtight container.

CHARACTERIZATION OF SILVER NANOPARTICLES

Nanoparticles are characterized by their size, morphology, and surface charge, using techniques such as UV- visible spectroscopy, scanning electron microscopy(SEM), transmission electron microscopy (TEM), XRD, FTIR.(Konwar Ranjit *et al.*, 2013).

UV-VIS SPECTROPHOTOMETER

The test sample was subjected to optical measurements carried out by UV-Vis spectrophotometer between 400-460 nm (Shreshtaverma *et al.*, 2013). The UV-visible spectroscopic measurements were performed on a Shimadzu dual-beam spectrophotometer (model UV-1601 PC).

SEM ANALYSIS OF AgNPs: (Panchanathan Manivasagan *et al* 2013).

FE-SEM determinations of the above-mentioned sample showed the formation of nanoparticles, which were confirmed to be of silver by EDX. EDX analysis also showed a peak in the silver region, confirming the formation of silver nanoparticles.

TEM ANALYSIS OF AgNPs: (Panchanathan Manivasagan *et al* 2013).

The TEM image analysis the shape of the silver nanoparticles. The micrograph showed NPs with variable shape; most of them present in spherical in nature. The TEM micrograph also confirmed the size of NPs.

X-RAY DIFFRACTION (XRD) ANALYSIS: (S Ponarulselvam *et al.*, 2011).

The particle size and nature of the silver nanoparticles were determined using XRD. X-ray powder diffraction is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions

FOURIER TRANSMISSION INFRA RED SPECTROSCOPY (FTIR): (Panchanathan Manivasagan *et al* 2013).

A Fourier Transform Infra Red Spectrometer is used to obtain the infra red spectra of absorption and emission of the formed silver nanoparticles.

PROCEDURE FOR ANTI BACTERIAL STUDY

The antibacterial activity was based on disc diffusion method (Thitilertdech et al., 2008) using different microorganisms such as *P.aeruginosa*, *enterococcus faecalis*, *klebsiellapneumoniae*, *protease vulgaris*.

FREE RADICAL SCAVENGING ACTIVITY BY DPPH ASSAY

The ability of the AgNPs to annihilate the DPPH radical (1,1-diphenyl-2-picrylhydrazyl) was investigated.

CYTOTOXIC STUDY ON CANCER CELL LINE

MTT assay

MTT assay is called as (3-(4, 5-diphenyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide. MTT assay was first proposed by Mossmann in 1982.

MTT is known as (3-(4, 5-diphenyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide. MTT assay was first proposed by Mossmann in 1982. MTT is cleaved by mitochondrial

dehydrogenize in viable cells, yielding a measurable purple product formazan. The formazan production is proportionate to the viable cell number and inversely proportional to the degree of cytotoxicity. The MTT assay was carried out in a multiwell plate and each well was washed with MEM (w/o) FBS. And 200 micro liter of MTT conc. of (5mg/ml) was added. It was incubated for 6-7hrs in 5% CO₂ incubator. After incubation 1ml of DMSO was added in each well and mixed by pipette and leave for 45sec. And it showed the purple color formation. The suspension is transferred into the cuvette of spectrophotometer and O.D values were read at 595nm. % of cell viability was calculated using the formula. (OD of sample/OD of cell control)(y)*100=%cell viability. Graph was plotted using the % of cell viability at Y-axis and concentration of the sample in X-axis. Cell control and sample was included in each assay to compare the full cell viability in cytotoxic and antitumor activity assessments.

RESULTS AND DISCUSSIONS

PREPARATION OF SILVER NANOPARTICLES

The leaf extract of *X.strumarium* L.was mixed in the aqueous solution of the 3Mm silver ion complex, it started to change the colour from colourless to yellowish brown due to reduction of silver ion. which shows the formation of silver nanoparticles. The reddish brown colour formed due to the reduction of silver ions is shown in Fig.1 &2.



Figure: 1: Before Centrifuge.



Figure: 2: After centrifuge.



Figure: 3: AgNP sin petridish.



Figure: 4: Dried AgNPs.

CHARACTERIZATION STUDY

UV-Visible Spectroscopy

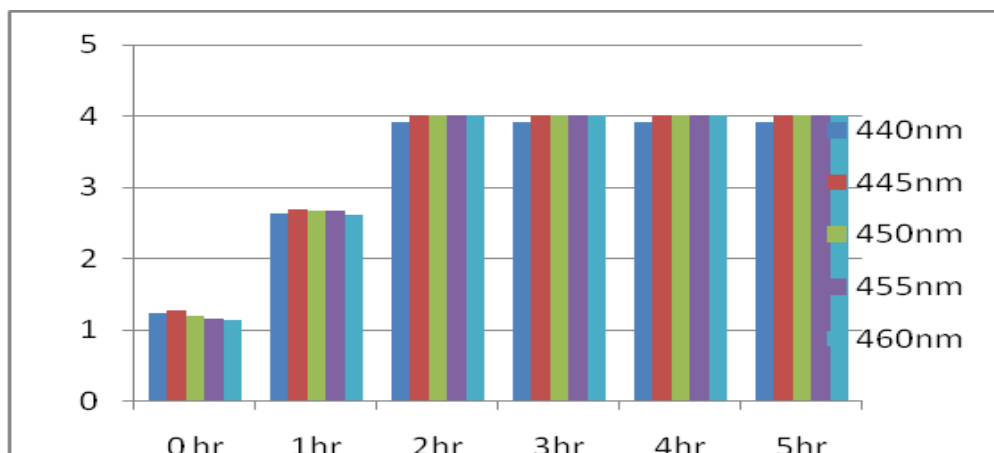


Figure 5: UV-Vis Spectral Analysis

From Figure.5, the result obtained for UV- Visible spectrum for the silver nanoparticle prepared from *X.strumarium L.* shows that the maximum absorption was found to be at 445nm. This was in related to the work done by ponauselvam (2012), where the reduction of silver ions and the formation of stable nanoparticles occurred rapidly within 2 h of reaction making it one of the fastest bioreducing methods to produce silver nanoparticles.

SEM Analysis

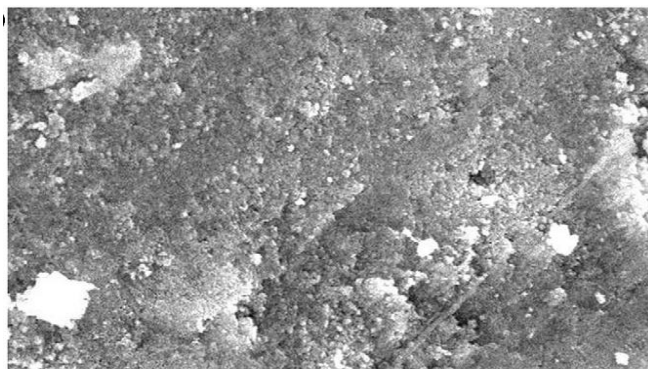


Figure: 6: SEM Analysis

From the Fig.6.the result obtained for SEM for the silver nanoparticle prepared from *X.strumarium L.* shows that the size of the AgNPs was found to be in the range of 100 nm. The shape appeared to be spherical. SEM analysis shows uniformly distributed silver nanoparticles on the surface of the cells, because those dispersing in the solution may also deposit onto the surface of the cells. This was in relation to the work done by Aupam Sing (2012).

X- Ray diffraction (XRD)

XRD analysis showed three distinct diffraction peaks at 38.1°, 44.1° and 64.1°. The average grain size of the silver nanoparticles formed in the bio reduction process was determined using Scherrer's formula, $d = (0.9 \times 180^\circ) / \sqrt{\cos}$. Hence XRD pattern thus clearly illustrated that the silver nanoparticles formed in this present synthesis are crystalline in nature. Which was compared with the study carried out by (Ponarulselvam *et al.*, 2012).

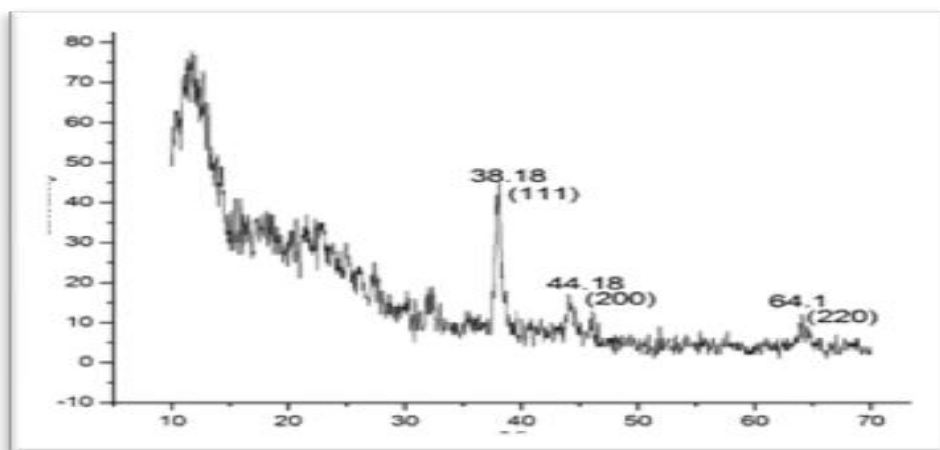


Figure: 7. XRD Analysis

TEM analysis of Silver Nanoparticle

Fig.8. shows that the TEM images of the nanoparticles synthesized with leaf extract of *X.strumrium L.* is dominated by spherical nanoparticles in varying sizes mostly 100nm in size and also a small number of quasi spherical and uneven shaped nanoparticles were observed.

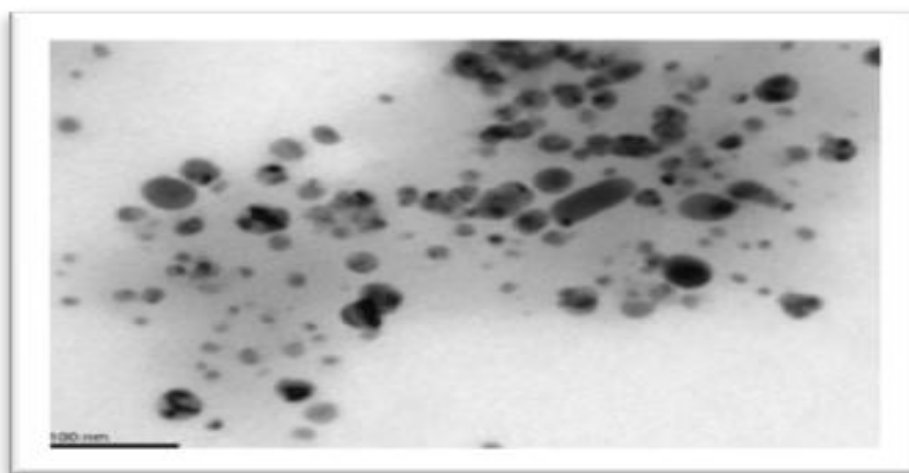


Figure: 8: TEM Analysis

These nanoparticles are polydisperse and the average particle sizes were about 100 nm. The aggregation of few cells was due to the accumulation of nanoparticles at one particular area. This was compared to the work done by Aruna Jyothi Kora (2012).

FTIR Analysis of AgNPs

FTIR spectrum analysis of AgNPs showed intense absorption bands at 1800, 1600, 1400, 1200, 1000, 800, 600 and 400 cm^{-1} . It was observed from the FTIR spectrum of AgNPs that the bands at 1650 correspond to a primary amine NH band; similarly, 1540 and 1060 correspond to a secondary amine NH band and primary amine CN stretch vibrations of the proteins, respectively. The intense medium absorbance at 1655 cm^{-1} ($-\text{C}=\text{C}-$ stretch) is the characteristic of the alkenes group. The intense medium absorbance at 1460 cm^{-1} (C–H bend) is the characteristic of the alkanes group. The intense broad absorbance at 685 cm^{-1} ($-\text{C}=\text{C}-\text{H}$: C–H bend) is the characteristic of the alkynes group. The presence of the bond stretching for the various atoms confirms the functional groups at various compounds being present in the nanoparticle of *X. strumarium* Linn. which was compared to the work done by Panchanathan Manivasagan (2013). Who shows that the alcohol, phenolic, alkynes, alkanes groups have a strong ability to interact with nanoparticles.

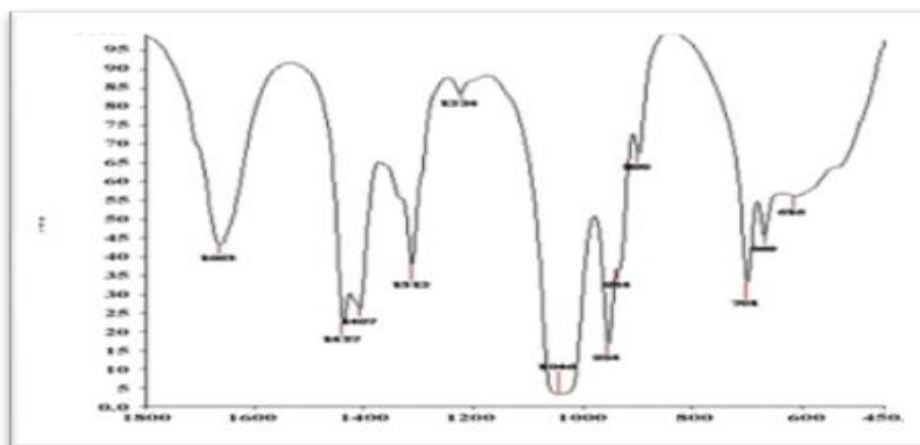


Figure: 9. FTIR Analysis

ANTIMICROBIAL ACTIVITY OF SYNTHESIZED NANOPARTICLE FROM *XANTHIUM STRUMARIUM* LINN

The result obtained in this study indicates that biologically synthesized AgNPs possess tremendous antimicrobial properties. The antimicrobial activity was observed against *Pseudomonas aeruginosa*, *K. pneumonia*, *Protease vulgaris* and *Enterococcus faecalis*, among these bacteria the maximum zone of inhibition was noticed against *P. aeruginosa* for both 50 μl and 100 μl concentration. Silver has been used for its well known antimicrobial properties, the advances in generating Ag-NPs have made possible a revival of the use of silver as a powerful bactericide.

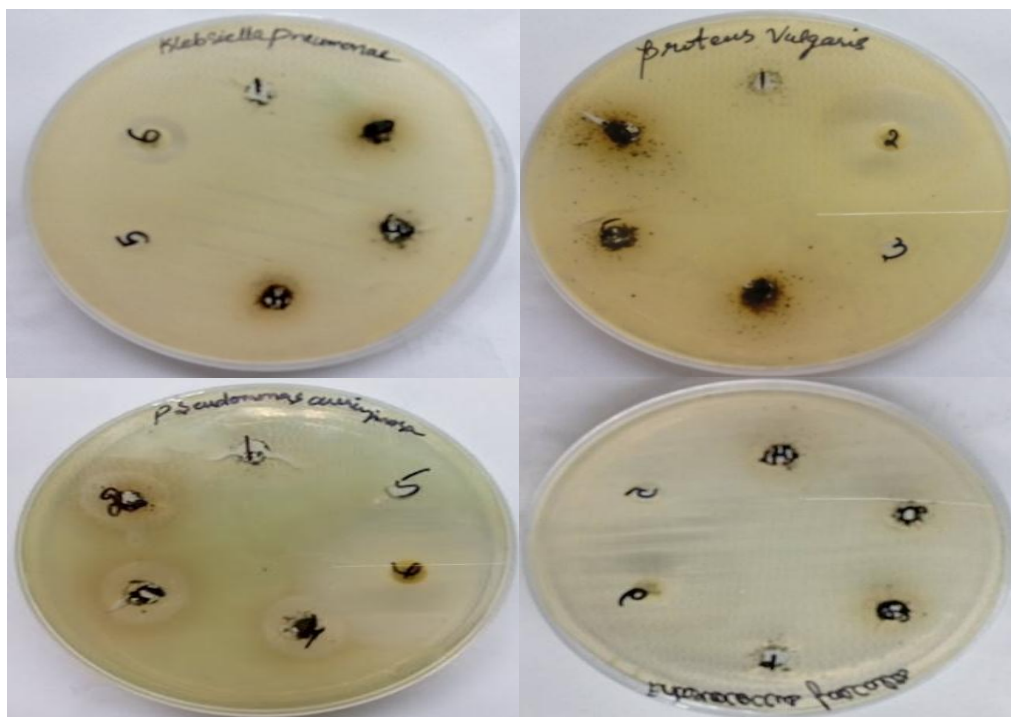


Figure: 10 Zone of inhibition on four different species

Table1: shows the Antibacterial activity of biosynthesized silver nanoparticle from the leaf extract of *X. strumarium* L. was carried out on *P. aeruginosa*, *Streptococcus faecalis*, *Klebsiella pneumoniae*, *Protease vulgaris*.

Micro organisms	50 μ l	100 μ l
<i>Pseudomonas aeruginosa</i>	13mm	14mm
<i>K.pneumoniae</i>	12mm	13 mm
<i>Protease vulgaris</i>	13mm	12 mm
<i>Enterococcus faecalis</i>	12mm	13 mm

FREE RADICAL SCAVENGING ACTIVITY MEASURED BY DPPH

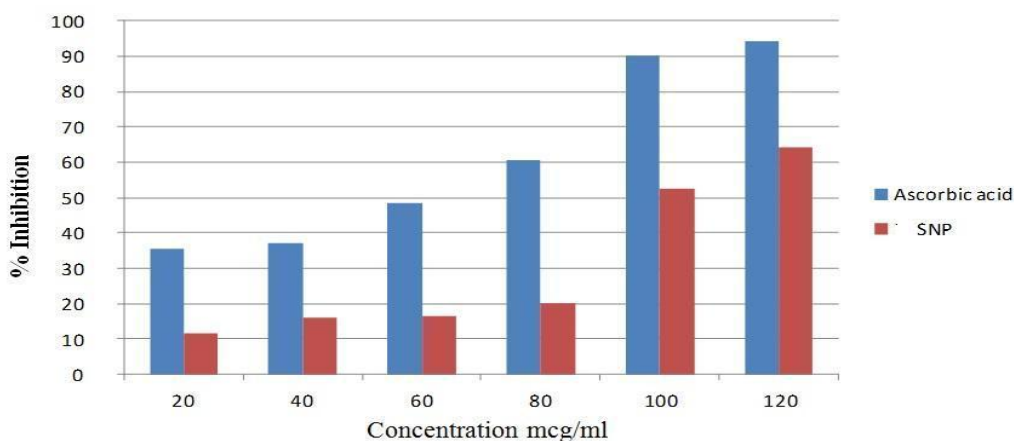


Figure: 11 DPPH scavenging activity of silver Nanoparticle

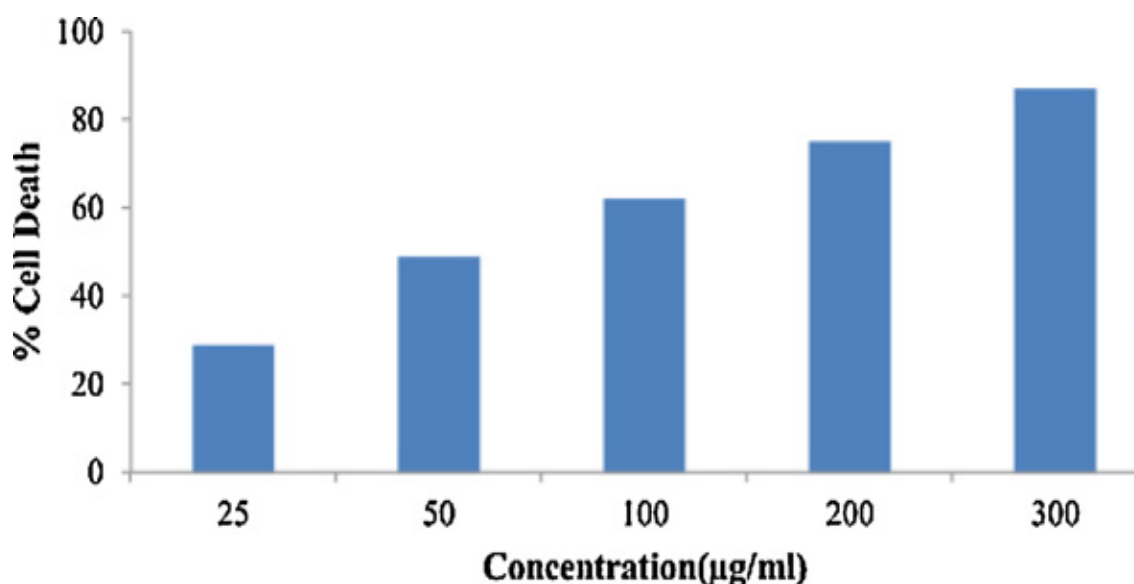
The free radical scavenging property as measured by DPPH method showed that the percentage of inhibition increase in concentration of synthesized silver nanoparticles as indicated in fig 11. this result is similar to the work done by Bar *et al.*, 2009. This confirms the antioxidant activity of biosynthesized silver nanoparticles.

CYTOTOXICITY OF SILVER NANOPARTICLES ON MCF-7 CELL LINE

The *invitro* cytotoxicity of the AgNPs was evaluated against MCF-7 Cell line at different concentrations. Cytotoxicity analysis of the sample shows a direct dose relationship; cytotoxicity increased at higher concentrations. The sample demonstrated a considerable cytotoxicity against MCF-7 cell lines. Table:2. shows that, as the concentration increases, the toxicity against cancer cells was found to be increased (92.74% at 300µg) at certain limit. This preliminary study using Silver Nanoparticles synthesized from *X.strumarium L.* predicted that the toxic effects against cancerous cells by the Silver Nanoparticles is found to be appreciated to a certain limit. which was compared to the work done by (Mercy Ranjitham *et al.*,2013).

Table: 2: Cell toxicity of Silver Nanoparticle

S. No.	Concentration (µg)	Cell toxicity (%)
1	25	30.12
2	50	51.04
3	100	60.01
4	200	75.42
5	300	92.74



Figur: 12 (cell proliferation using MTT method for synthesized nanoparticles).

CONCLUSION

In the present study, the silver nanoparticle synthesized from the leaf extract of *X. strumarium L.* showing a promising result after characterizing by UV-Visible Spectroscopy, XRD, TEM, SEM, FTIR to be used for a medicinal purpose. Assessing the Nanoparticle for various applications like antimicrobial, Free radical Scavenging activity, preliminary cytotoxic study proved that the leaf extract of *X. strumarium L.* could be used as one of the medicinal plant.

Future studies

- Further study can be carried out by isolating the compound with respect to the specific activity of the plant. Various extracts like, aqueous extract, acetone extract, ethanolic extract, etc., could be prepared and compound identification could be carried out using column chromatography. Isolation and Purification of the identified compound could be carried out. Nanoparticles can be prepared from that isolated compound based on the activity to be studied and structure of the purified compound could be predicted by using NMR, ESR studies. The Nanoparticle synthesized from the purified compound could be tested against *in vitro*, *in vivo*, and human cell line studies. Bio informative tools could be used for the drug docking study.

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