IJPSR (2017), Vol. 8, Issue 4 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 11 September, 2016; received in revised form, 13 January, 2017; accepted, 20 March, 2017; published 01 April, 2017

ANTIMICROBIAL, CYTOTOXICTY AND ANTI CANCER ACTIVITY OF SILVER NANOPARTICLES FROM *GLYCYRRHIZA GLABRA*

N. Pandian and Soundhari Chidambaram *

Department of Microbiology, Valliammal College for Women, Chennai - 600102, Tamilnadu, India.

Keywords:

Silver Nanoparticles, *Glycyrrhiza Glabra*, Anticancer activity, Cytotoxicity

Correspondence to Author:
Soundhari Chidambaram

Head
Department of Microbiology,
Valliammal College for Women,
Chennai - 600102, Tamilnadu, India.

E-mail: drcsoundhari@gmail.com

ABSTRACT: In this study, the plant *Glycyrrhiza glabra was* explored for the synthesis of silver nanoparticles (SNPs). The SNPs were characterised by ultra-violet-visible spectroscopy, FTIR analysis and scanning electron microscopy. SEM analysis revealed the formation of SNPs of average size of 46 nm with aqueous extract of *G. glabra*. The SNPs synthesised by aqueous extract of *G. glabra* showed strong antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* and significant inhibition to other bacteria and few fungi tested. The SNPs also exhibited potential anti cancer activity. The SNPs was observed to be nontoxic from a concentration of 500 µg/ml in vero cell line. The results document SNPs can be synthesised using aqueous plant extracts for applications in the biomedical field.

INTRODUCTION: Synthesis of nanoparticles and their characterization is evolving into an important branch of nanotechnology. Particles with a size less than 100 nm are referred as nanoparticles (Nps). Nanotechnology is applied widely to offer targeted drug delivery, therapy, diagnostics, tissue regeneration, cell culture, biosensors and other tools in the field of molecular biology. The most widely used and known application of silver nanoparticles are in the medical industry. Nanotechnology is applied greatly to minimize the drug degradation and loss, to prevent harmful side effects and to increase the drug bioavailability [1]. Green synthesis of nanoparticles is an emerging branch of nanotechnology which has a potential future for enhancing the activity and overcoming the problems associated with plant medicine.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.8(4).1633-41

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.8 (4).1633-41

Nanoparticles synthesis is usually carried out by physical, chemical, electrochemical, irradiative and biological techniques. Drawbacks associated with physico- chemical methods of silver nanoparticles synthesis include the use of toxic chemicals high temperature, pressure, production of hazardous by-products etc. Therefore, it becomes necessary to search for safer alternative methods of silver nanoparticles synthesis.

Among the various methods known, plant mediated nanoparticles is preferred due to its cost effectiveness and safety for human therapeutic use and due to a single step method for biosynthesis [2]. The most effectively studied nanoparticles today are those made from noble metals, in particular silver, platinum, gold and Pd. Among the above form, silver nanoparticles play a significant role in the field of biology and medicine.

It has been reported that since ancient times silver is known to have antimicrobial activities, such as antiviral and antiangiogenesis and silver nanoparticles are now extensively in the field of medicine and drug delivery [3].

The main advantage of silver nanoparticles is that they have an inhibitory effect on microbes. Among all the well known activity of silver ions and silver based compound is that they kill microbes effectively. Silver nanoparticles interact with the bacterial membrane proteins and DNA as they possess sulphur and phosphorus compounds and silver have higher affinity to react with these compounds [4].

The World Health Organization (WHO) in 2008 estimated that approximately 80% of the world's population relies on traditional medicines mainly plant based drugs in their health care. India is rich in indigenous herbal resources and hence the world is now looking towards India for new drugs to manage various challenging disease because of its biodiversity of medicinal plants and vast traditional knowledge such as siddha, ayurveda.

Plant mediated green synthesis of silver nanoparticles is a widely acceptable technology for rapid production of silver nanoparticles for successfully meeting the excessive need. On the other hand, plant extract could be utilized as a source of nanoparticles as they are non toxic safe to handle and having a broad variability of metabolites which are required for reduction and a single step technique or production [5]. When the use of whole plant extracts and plant tissues are evaluated, it is found that the use of plant extracts are simpler, readily scalable and may be less expensive for preparation of nanoparticles.

Licorice (*Glycyrrhiza glabra* Linn; family: papilionaceae) is a traditional medicinal herb grows in the various part of the world. Licorice is a hardy herb or under shrub, erect grows to about 2 cm height. The roots are long, cylindrical, thick and multibranched. The rhizome of *Glycyrrhiza glabra* Linn., is a reputed drug of ayurveda. Licorice is used to relieve inflammation, eye diseases, throat infections, peptic ulcers, arthritic conditions and liver diseases in Indian ayurveda system. Licorice has been used in medicine for more than 4000 years. Licorice is reported to have antiviral, anticancer, antiulcer, anti diabetic, antithrombic, anticonvulsant, antiallergenic, and expectorant activities [6]. The adverse effect of the present synthetic antibiotics and poor bioavailability of the administered drugs highlight the need of the use of green synthesized nanomedicine. The present investigation has focused on the biosynthesis of highly stable silver nanoparticles using the aqueous extract of rhizome extract from *Glycyrrhiza glabra* Linn.

MATERIALS AND METHODS:

Materials: Rhizome of *G. glabra* were collected locally and the accuracy of the plant part was ascertained at PG & Research Department of Plant Biology and Biotechnology, Presidency College, Chennai. Silver nitrate and other solutions were obtained from Sigma-Aldrich. Preparation of the rhizome extract was carried out by the methodology adapted by Soundhari and Rajarajan 2013 [7].

Preparation of plant extracts: The rhizome (**Fig. 1)** was rinsed thoroughly first with tap water followed by distilled water to remove all dust and unwanted visible particles. It was cut into small pieces and dried at room temperature. The dried parts were ground and collected by sieving as fine powder.

FIG. 1: *GLYCYRRHIZA GLABRA* RHIZOME

20 grams of the specified powdered plant parts weighed and soaked in 100 ml water and stored overnight at 4 ºC filtered and centrifuged to get clarified extract. The method was repeated three times more, all extracts pooled together filtered through a 0.22 µm pore sized millipore filter. The sterile extract was transferred into a sterile lyophilisation flask & frozen at -80 ºC in a deep freezer. The frozen extract was loaded to Lyophilizer. The lyophilised extract was stored in – 20 ºC till bioevaluation.

Qualitative phytochemical screening of *Glycyrrhiza glabra:* The different qualitative chemical test was performed for establishing profile of the extract for its chemical composition.

The following standard tests were performed on extracts to detect various phyto constituents present in the extract.

Test for alkaloids: One mg of the extract was dissolved in 1ml of sterile distilled water. To that 1 ml of dragendorff's reagent was added and observed for prominent yellow color precipitate.

Test for anthraquinones: One mg of the extract was dissolved in 1ml of sterile distilled water. To that aqueous ammonia was added and observed for change in color of aqueous layer.

Test for flavonoids: One mg of the extract was dissolved in 1ml of sterile distilled water. To that few drops of concentrated HCl and Magnesium chloride was added and observed for pink to tomato red colour.

Test for tannins: One mg of the extract was dissolved in 1ml of sterile distilled water and few drops of 0.1% ferric chloride was added and observed for blue colourization/brownish green.

Test for terpenoids: One ml of sample was taken (100µl of compound in 900 µl of methanol). 1ml of concentrated H_2SO_4. Was and added observed for appearance of red ring.

Test for Saponins: The extract was diluted with distilled water and made up to 20 ml. The suspension is shaken in a graduated cylinder for 15 mins. Observed for foam indicates the presence of saponins.

Test of phytosterols: The extract was dissolved in 2ml acetic anhydride. To this, one or two drops of concentrated sulphuric acid was added slowly along the sides of the test tubes colour change shows the presence of phytosterols.

Chemical characterization

UV-visible spectra analysis: UV-vis spectroscopy is an important technique to ascertain the formation and stability of metal nanoparticles in aqueous solution. UV-visible analysis was done to monitor the completion of bioreduction of silver ions in aqueous solution. The formation and completion of silver nanoparticles was characterized by using Shimadzu UV visible spectrophotometer. The bioreduction of the silver ions in the solution was monitored by periodical sampling of aliquots and the UV visible spectra of these aliquots were monitored in 200-600 nm range operated at a resolution of 1 nm. Silver nitrate solution was used as a blank.

FTIR analysis: FTIR measurement was carried out for functional group characterization.

Scanning Electron Microscope: Scanning electron microscope was done to observe clear images of the particles in colloid well dispersed with uniform size.

Antibacterial activity assay:

Agar disc diffusion method: (Bauer *et al*., 1966) Antibacterial activity of the extracts was determined by disc diffusion method on Muller Hinton agar (MHA) medium. Muller Hinton Agar (MHA) medium is poured in to the petriplate. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the bacterial suspension. The sterile discs were soaked with plant rhizome extract and solution containing silver nanoparticles. Ampicillin is taken as positive control. 20 µl of diluted samples and positive control were added in sterile discs and placed in MHA plates. The plates were incubated at 37 ºC for 24 hrs. Then antibacterial activity was determined by measuring the diameter of zone of inhibition.

Antifungal activity assay:

Agar disc diffusion method: Antifungal activity of the extracts was determined by disc diffusion method on Sabouraud Dextrose agar (SDA) medium. Sabouraud Dextrose agar (SDA) medium is poured in to the petriplate. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the fungal suspension. Samples were diluted for 1000 µg/ml.

The sterile discs were soaked with plant extracts and the solution containing silver nanoparticles. Amphotericin-B is taken as positive control. 20 µl of diluted samples and positive control were added in sterile discs and placed in SDA plates. The plates were incubated at room temperature for 24 hrs. Then antifungal activity was determined by measuring the diameter of zone of inhibition.

Cell viability:

Viabilty test by MTT assay: The viability test was performed as per the method adopted by Mosmann [8]. HeLA Cells (1×10^5/well) were plated in 24-well plates and incubated in 37 ^{0}C with 5% CO_2 condition. After the cell reached confluence, various concentrations of the samples and L-ascorbic acid were added and incubated for 24hrs. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4) or Minimal Essentail Medium (MEM) without serum. 100µl/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)- 2, 5 - diphenyl—tetra zolium bromide (MTT) was added and incubated for 4 hours. After incubation, 1ml of DMSO was added in all the wells. The absorbance at 570 nm was measured with UV- Spectrophotometer using DMSO as the blank. Measurements were performed and the concentration required for a 50% inhibition (IC_{50}) was determined graphically.

The % cell viability was calculated using the following formula:

% cell viability = A570 of treated cells / A570 of control cells × 100

Graphs are plotted using the % of Cell Viability at Y-axis and concentration of the sample in X-axis. Cell control and sample control is included in each assay to compare the full cell viability assessments.

Toxicity assay: Preparation of stock solution of lyophilized nanoparticle 1 mg of the lyophilized nanoparticle was added to 1 ml of plain MEM and dissolved completely by cyclomixer. The solution was filtered through 0.22 µm sartorius syringe filter to ensure sterility.

Serial two fold dilution of nanoparticle: Assaying the toxicity of the SNPs on cell lines was carried out in tissue culture 96 well microtitre plates following the methods adopted and described by Soundhari and Rajarajan 2013 [7]. Cytotoxicity was done on vero cells for evaluating the safety of the extract and for determining the toxic free concentration on the cell lines.

200µl of nanoparticle mixed medium from the stock was transferred to first well and was serially diluted in two fold manner in 5% FBS MEM, from an initial compound concentration of 500µg / ml to a final concentration of 3.9 µg/ml. 0.1ml of the serially diluted nanoparticle was added into the wells containing a confluent monolayer of vero cells. For standard, adopting the same procedure, similar preparation of ampicilin (standard drug) was added to another row of cells. The plate was incubated at 37 ^{0}C in 5% CO_2 atmosphere for 72 hours observed under inverted phase contrast microscope for determination of toxic free concentration. Important observations were recorded by microphotography.

RESULTS:

Synthesis of silver nanoparticles:

UV-visible spectrum analysis: Reduction of silver nitrate to silver nanoparticles during exposure to plant extracts is followed by a gradual increase in color development from clear to reddish brown after 30 minutes, of the reaction, which indicated the formation of silver nanoparticles (**Fig. 2**). The formation and stability of the reduced SNPs in the colloidal solution were monitored by UV-Vis spectrophotometer analysis. The UV-Vis spectra showed maximum absorbance at 424 nm (**Fig. 3**).

FIG. 2: SYNTHESIS OF SILVER NANOPARTICLES (a) PLANT EXTRACT (b) SYNTHESIZED SILVER NANOPARTICLES IN BROWN COLOR SOLUTION

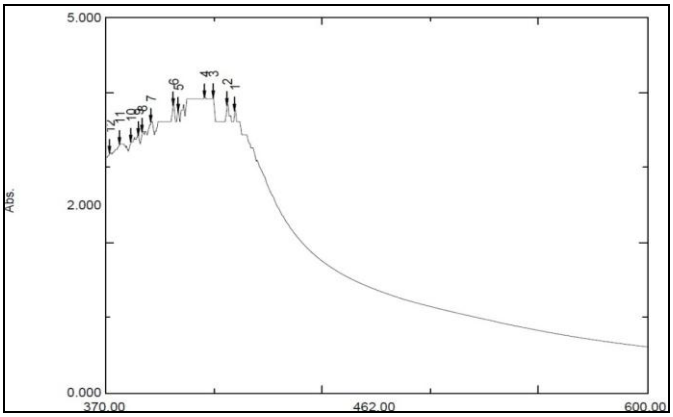

FIG. 3: UV–VIS SPECTRUM ANALYSIS: PLASMON RESONANCE OF SILVER NANOPARTICLES REDUCED BY *G. GLABRA* AT 424 NM

Characterization of silver nanoparticles:

Fourier transform infra-red spectrum: FTIR measurement was carried out for functional group characterization (**Table 1**) (**Fig. 4**).

TABLE 1: FTIR SPECTRA VALUES WITH PEAK HIT ASSIGNMENTS

S. no	Peak Hit (Cm^{-1})	Inference/Assignment
1.	538.16	Carbon halide group
2.	571.92	Carbon halide group
3.	648.11	Carbon chloride group
4.	670.29	Carbon chloride group
5.	817.85	Carbon fluoride group
6.	1239.32	C-N stretch medium weak band
7.	1399.32	C=C aromatic group
8.	1419.67	C=C aromatic group
9.	1435.1	C=C aromatic group
10.	1543.12	N-O stretch strong single bond
11.	1632.81	N-H bending medium bond
12.	1938.54	C-N stretch weak
13.	2200.87	C=C- alkyne stretch
14.	2500- 3300	O-H stretch, acid, strong bond
15.	3391.97	O-H stretch, alcohol functional group
16.	3611.86	O-H stretch free strong/sharp intensity.
17.	3834.65	-O-H alcohol group

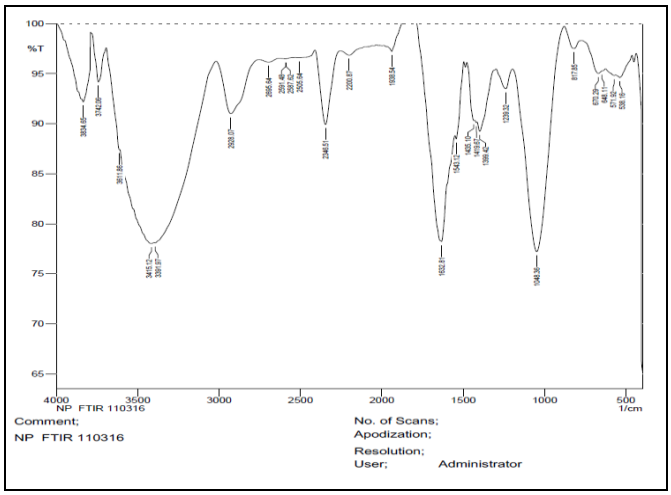

FIG. 4: FTIR ANALYSIS OF *G. GLABRA* SILVER NANOPARTICLES

Scanning electron microscope: The presence of synthesized SNPs were confirmed by Scanning electron microscope (SEM) images at the magnification of 35,000X and it is found the nanoparticles size in the range of 46 nm (**Fig. 5**).

Phytochemical screening of *G.glabra* silver nanoparticles: Phytochemical screening of *G.glabra* SNPs revealed the presence of saponins, Flavanoids and alkaloids. (**Table 2**).

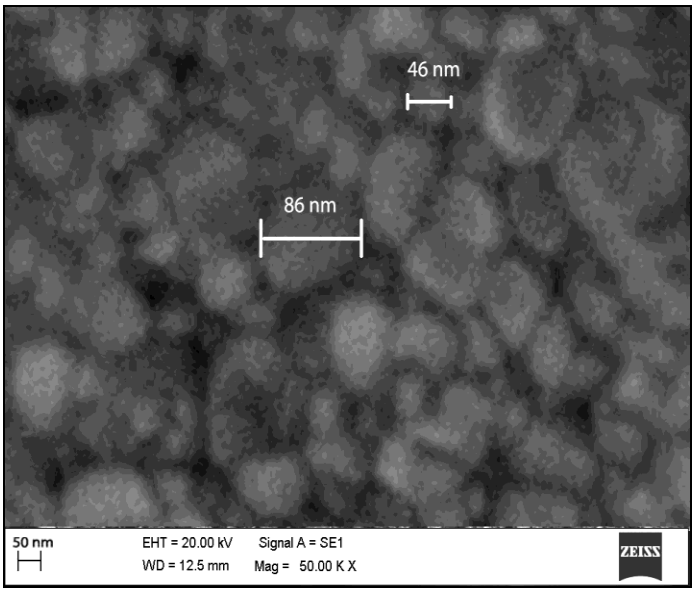

FIG. 5: SEM ANALYSIS OF SYNTHESISED SILVER NANOPARTICLES

TABLE 2: PHYTOCHEMICAL COMPOUNDS OF *GLYCYRRHIZA GLABRA*

S.no	Phytochemical compound	*G.glabra* silver nanoparticles
1	Tannins	_
2	Saponins	+
3	Flavonoids	+
4	Alkaloids	+
5	Proteins	+
6	Steroids	_
7	Anthraquiones	_

Antimicrobial activity: The antimicrobial activity of the synthesized SNPs were carried out against gram positive (*staphylococcus aureus*, *Bacillus subtilis*) and gram negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi*) and some fungal species (*Trichoderma*, *Rhizopus*, *Aspergillus niger*, *Candida)* using disc diffusion agar method.

The diameter of inhibition zone (mm) around each well with silver nanoparticles and rhizome extract is represented in the table. The zone of inhibition of synthesized silver nanoparticles were found to be more than that of the plant rhizome extract (**Table 3**).

TABLE 3: ANTIMICROBIAL ACTIVITY

S.no	Microorganisms	Zone of inhibition in mm		
		20µl of silver	20µl of plant	Standard
1.	*Pseudomonas aeruginosa*	11	7	12
2.	*Staphylococcus aureus*	12	8	14
3.	*Bacillus subtilis*	9	8	19
4.	*Salmonella typhi*	9	7	18
5.	*Escherichia coli*	8	7	17
6.	*Trichoderma*	18	12	30
7.	*Rhizopus*	17	11	23
8.	*Aspergillus niger*	17	14	28
9.	*Candida spp.,*	14	11	29

Viability test by MTT assay: The synthesized SNPs showed a potent cytotoxic activity against HeLa cells. The concentration of synthesized silver nano particles at 7.8µg/ml, 15.6µg/ml, 31.2µg/ml, 62.5µg/ml, 125µg/ml showed cytotoxic activity of 60.8%, 57.2%, 54.13%, 51.13% (**Table 4**) (**Fig. 6**) respectively that was comparable to that 0f positive control, whereas the concentration of aqueous extract at 7.8µg/ml, 15.6µg/ml, 31.2µg/ml, 62.5µg/ml, 125µg/ml, 250µg/ml showed cytotoxic activity of 69.13%, 66.7%, 62.2%, 57%, 54%, 47%. (**Table 5**) (**Fig. 7**)

The IC_{50} value SNPs after 24 hours was found to be 51.3% at 62.5µg/ml concentration and for the aqueous extract it is found to be 47.2% at 125µg/ml. It is shown that SNPs showed a significant anticancer effect.

TABLE 4: ANTICANCER EFFECT OF NANO PARTICLE ON HeLa CELL LINE

S.no	Concentration (µg/ml)	Duplicate (%)			Cell Viability (%)
		I	II	Mean	
1	1000	0.217	0.225	0.221	38.10
2	500	0.242	0.253	0.247	42.58
3	250	0.261	0.270	0.2965	45.68
4	125	0.273	0.281	0.277	47.75
5	62.5	0.298	0.305	0.301	51.13
6	31.2	0.311	0.318	0.314	54.13
7	15.6	0.326	0.339	0.332	57.24
8	7.8	0.348	0.359	0.353	60.86
9	Cell control	-	-	0.580	100

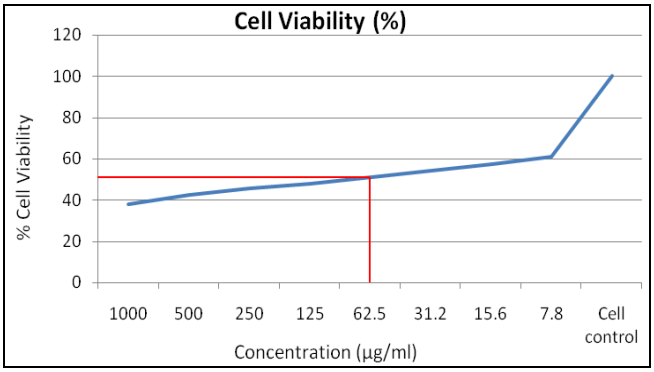

FIG. 6: ANTICANCER EFFECT OF SILVER NANOPARTICLES ON HeLa CELL LINE

TABLE 5: ANTICANCER EFFECT OF *G. GLABRA* AQUEOUS EXTRACT ON HeLa CELL LINE

S.no	Concentration (µg/ml)	Duplicate (%)			Cell Viability (%)
		I	II	Mean	
1	1000	0.245	0.217	0.231	39.82
2	500	0.264	0.241	0.252	43.44
3	250	0.281	0.267	0.274	47.24
4	125	0.323	0.308	0.315	54.31
5	62.5	0.342	0.321	0.331	57.06

6	31.2	0.374	0.349	0.361	62.24
7	15.6	0.398	0.376	0.387	66.72
8	7.8	0.409	0.393	0.401	69.13
9	Cell control	-	-	0.580	100

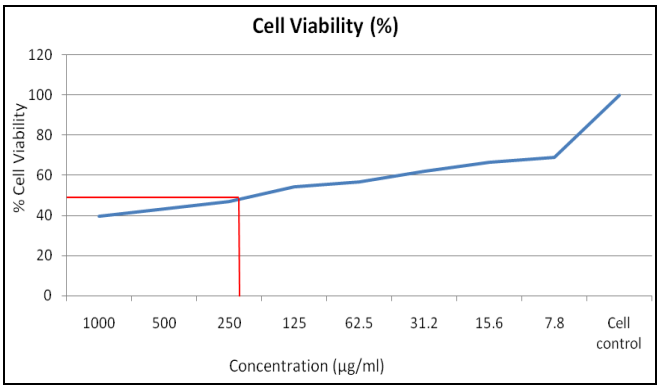

FIG. 7: ANTICANCER EFFECT OF AQUEOUS EXTRACT ON HeLa CELL LINE

Estimation of *in vitro* maximal cytotoxic free concentration on Vero cell line: The synthesized SNPs was evaluated for cytotoxicity effect. The effect of the nanoparticles on vero cell line at different concentration. The synthesized silver nanoparticles were found to be non toxic at 500 mg/ml concentration and was recorded as micrographs.

DISCUSSION: In recent years, plant extracts from different parts of various plants have been investigated in the synthesis of nanoparticles. *Ocimum sanctum* [9], *Dioscorea oppositifolia L.* rhizome extract [10], *Ananas comosus* extract [11], *Catharanthus roseus Don* leaves [2], *Alpinia galanga* rhizome extract [12], *Rosamarinus officinales* leaves extract [13] and *Glycyrrhiza glabra* root extract [14].

In this present study, *G. glabra* rhizome extract was used to synthesis silver nanoparticles and was tested for its antimicrobial, cytotoxic and anticancer activities probably the first attempt to the best of our knowledge.

The rhizome of this plant is reported to have antimicrobial antiulcer, antioxidant, antimalarial, antiplasmodic, anti-inflammatory and anti hyperglycemic properties [15].

Because of its wide usage in traditional system of medicine and availability in the rural area, this study was adopted to investigate the antimicrobial and anticancer efficacy of the nanoparticles synthesized from the rhizome of the plant.

Reduction of silver ion into silver nanoparticles during exposure to the plant extracts were followed by color change. Reduced silver nanoparticles exhibited dark brown color in aqueous solution. These characteristic color changes is due to the excitation of the surface Plasmon resonance in the metal nanoparticles [13].

When the rhizome extract was mixed with silver nitrate solution and incubated at room temperature within 30 minutes color change from yellow to reddish brown color was observed indicating the formation of silver nanoparticles. The results are in conformity with Dinesh *et al.*, 2012 [14], who reported color change after 30 minutes of incubation. A rhizome extract of *Acorous calamus* were reported to reduce silver ions into silver nanoparticles after 48 hours [3]. The dissimilarities in the rate of bioreduction observed may be due to the differences in the activities of the enzyme present in the plant extracts [16].

The UV-visible spectrophotometer showed the maximum absorbance at 424 nm using the *G. glabra* rhizome extract. The result reflects that the silver nanoparticles prepared by *G.glabra* rhizome extract are stable without aggregation. Dinesh *et al.*, 2012, reported the UV-visible spectra maximum absorbance at 440 nm using the root extract of *G.glabra* [14].

From the SEM images, it was observed that most of the silver nanoparticles are spherical in shape and few agglomerated. The figure also showed there is

variation in particles size and distribution. It is shown that average particle estimated was 46nm.

From the study carried out by Dinesh *et al.*, 2012 using *G.glabra* root extract, the size was found to be 20-30 nm [14]. Using *Catharanthus roseus*, the particle size was found to be 35-55 nm [2] using *Paederia foetida* leaf extract, the size was about 22-40 nm [17]. Renuga devi K and Venus Aswini R 2014 [18], using *Azardicta indica* leaf extract reported the SEM image of 41-130nm.

FTIR measurements were carried out to find the chemical groups and possible biomolecules present in the silver nanoparticles. Dinesh *et al.*, 2012 reported the infrared bands of *G. glabra* at 2914, 2847, 1708, 1601, 1464, 1376, 1008, 880, 716 and 469 cm^{-1} [14]. Most of the IR bands are flavonoids and terpenoids present in the root. The vibrational bands corresponding to bonds such as –C=C, -C=0, -C-0, -C-0-C and –C-N are derived from the water soluble compounds such as flavonoids, terpenoids and thiamine present in the *G. glabra* root.

In the present study, the stretching at the wave number 2500 to 3300 cm^{-1} shows the presence of O-H functional group, acid, strong and broad peak. The sharp peak was observed at the 3834.65 cm^{-1} shows the O-H stretch free. According to Dinesh *et al.*, 2012 the FTIR suggest that the flavonoids, terpenoids present in the plant extract acting as a capping agent [14].

The FTIR spectra of *Acorus calamus* AgNps rhizome extract indicate the presence of amino, carboxylic, hydroxyl and carbonyl groups. Strong broad O-H stretch carboxylic bands in the region 1564cm^{-1} and carboxylic stretching bands in the regions 1645cm^{-1} were observed [3]. These bands denote stretching vibrational bands responsible for efficient capping and stabilization of silver nanoparticles.

The antimicrobial activity of *G.glabra* silver nanoparticles were analysed for the first time against *Psedomonas aeruginosa, Staphylococcus aureus, Escherichia coli*, *Bacillus spp., Salmonella spp., Trichoderma spp., candida albicans, Rhizopus spp., Aspergillus niger* by disc diffusion method. The culture plates treated with *G.glabra* silver nanoparticles exhibited significant antimicrobial activity of 1.2cm to 1.1cm zone of inhibition to *S. aureus* and *P.aeruginosa* respectively which was nearly comparable to standard antibiotic and also inhibited other bacteria. A zone of 1.8 cm to 1.4 cm to fungal species was also observed which was superior over the plant extract. This shows the nanoparticle are better drug leads than the extracts.

The earlier study on antimicrobial activity carried out in *Terminalia arjuna* silver nanoparticles by Ahmed S and Ikram S 2015 [5] showed 1.4 cm and 1.3 cm zone of inhibition against *Staphylococcus aureus* and *Escherichia coli* at different concentration of the extracts. The bactericidal activity of the biosynthesized *Acorus calamus* rhizome silver nanoparticles showed maximum zone of inhibition against *Staphylococcus aureus* (1.5 cm) and minimum zone of inhibition against *Salmonella enterica* 0.8 cm [3]. AgNPs exhibit antibacterial activity by attaching to the sulphur containing proteins of the cell membrane, thereby causing membrne damage and depleting the levels of intracellular ATP of the microorganism. Silver can also interact with the DNA of microorganisms, preventing cell reproduction [19].

The *in vitro* cytotoxic effects of *G.glabra* silver nanoparticles were screened against Hela cell line and viability of tumour cells was confirmed using MTT assay. The cytotoxic potential of *G. glabra* silver nanoparticles analyzed by AO/EB staining for apoptotic cell death in HeLa and Vero cells. The cells were treated with appropriate IC_{50} concentration of *G. glabra* silver nanoparticles for 24 and 48 hour and evaluated for apoptotic changes under flurorescent microscope. The synthesized silver nanoparticles showed a potent cytotoxic activity against HeLa cells. The IC_{50} value of silver nanoparticles after 24 hours was found to be 51.13% at 62.5 µg/ml concentration and for the aqueous extract it was found to be 47.24% at 250µg/ml concentration.

Parallel study in detecting maximal cytotoxic free concentration on Vero cell lines was carried out. The morphology of the cells were inspected microscopically after 48 hours for detectable alteration (*i.e.*) loss of monolayer, granulation. The results showed safer profile at a concentration of 500 µg/ml which shows it's significant to be used for clinical purposes.

CONCLUSION: To summarize, we succeeded in synthesising Silver Nanoparticles from aqueous extract of *G. glabra*. The biological reduction of silver nanoparticles was carried out in appropriate condition and characterization of synthesized nanoparticles was carried out by UV–Vis spectroscopy, FT IR and SEM. The phytochemicals present in the extract of *G. glabra* reduced the silver ions into metallic nanoparticles. The synthesized silver nanoparticle exhibited a strong antibacterial activity against *P.aeruginosa* and *S. aureus*. The synthesised SNPs exhibited potential anticancer activity and were also non toxic on mammalian Vero cell line. Hence the SNPs from plant extracts may be used to develop nanomedicine against pathogens.

CONFLICT OF INTEREST: We declare that we have no conflict of interest.

ACKNOWLEDGEMENT: The authors gratefully acknowledges the support rendered by Dr. I. Seetha Lakshmi for SEM and FTIR analysis.

REFERENCES:

1. Ajithadas Aruna, Ramraj Nandhini, Venkatachalam Karthikeyan, Pandi Bose. Synthesis and characterization of Silver Nanoparticles of Insulin plant (*Costus pictus d. don*) leaves. Asian Journal of Biomedical and Pharmaceutical Sciences 2014; 4: 1-6.
2. Ponaruselvam S, Panneerselvam C, Murugan K, Aarthi N, Kalimuthu K and Thangamani S. Synthesis of silver nanoparticles using leaves of *Catharanthus roseus Linn*. G. Don and their antiplasmodial activities. Asian Pacific Journal of Tropical Biomedicine 2012; 2: 574-580.
3. Chinnappan Sudhakar, kandasamy Selvam, Muthusamy Govarthanan, Balakrishnan Senthil Kumar, Arumugam Sengottaiyan *et al*. *Acorus calamus* rhizome extract mediated biosynthesis of silver nanoparticles and their bactericidal activity against human pathogens. Journal of Genetic Engineering and Biotechnology 2015; 10: 03.
4. Probin Phanjom *et al*. Plant mediated synthesis of silver nanoparticles using *Eleagnus latifolia* leaf extract. Digest Journal of Nanomaterials and Biosciences 2012; 7: 1117-1123.
5. Shakeel Ahmed and Saiqa Ikram. Silver nanoparticles: One pot green synthesis using *Terminalia arjuna* extract for biological application. Journal of Nanomedicine and Nanotechnology 2015; 6: 309-314.
6. Rajandeep Kaur, Harpreet Kaur and Ajaib Singh Dhindsa. *Glycyrrhiza glabra*: A Phytopharmacological review. International Journal of Pharmaceutical Science and Research 2013; 4: 2470-2477.
7. Soundhari Chidambaram and Rajarajan Swaminathan. Detrmination of antitubular activity of four Indian medicinal plants against *Mycobacterium tuberculosis* by broth dilution micro dilution method. International Journal of Pharmaceutical Science and Research 2013; 4: 3832-3937.
8. Mosmann Tim. Rapid calorimetric assay for cellular growth and survival application to proliferation and cytotoxicity assays. Journal of Immunological Methods. 1983; 65: 55-63.
9. Yogeswari Rout, Sikha Behera, Akshya Kumar Ojha and Nayak P L. Green synthesis of silver nanoparticles using *Ocimum santum* (tulashi) and study of their antibacterial and antifungal activities. Journal of Microbiology and Antimicrobials 2012; 4: 103-109.
10. Uma maheswari R, Lakshmi Prabha A, Nandagopalan V and Anburaja V. Green synthesis of silver nanoparticles by using rhizome extract of *Dioscorea oppositifolia L.* and their antimicrobial activity against human pathogens. Journal of Pharmacy and Bioallied Sciences 2012; 1: 38-42.
11. Naheed Ahmad and Seema Sharma. Green synthesis of silver nanoparticles using extracts of *Ananas comosus*. Green and sustainable chemistry. 2012; 2: 141-147.
12. Siby Joseph and Beena Mathew. Microwave assisted biosynthesis of silver nanoparticles using the rhizome extract of *Alpinia galangal* and evaluation of their catalytic and antimicrobial activities. Journal of Nanoparticles 2014; 1-9.
13. Ghassan M Sulaiman, Arieg AW, Mohammad, Hamssa E, Abdul Wahed, Mukhlis Ismai M. Biosynthesis, antimicrobial and cytotoxic effects of silver nanoparticles using *Rosmarinus officinalis* extract. Digest Journal of Nanomaterials and Biosciences 2013; 8(1): 273-280.
14. Dinesh S, arthieyan S, Arumugam P. Biosynthesis of silver nanoparticles from *Glycyrrhiza glabra* root extract. Archives of Applied Science Research 2012; 4(1): 178-187.
15. Vivek k Gupta, Atiya fathima, Uzma Faridi, Arvind S Negi, Karuna Shanker, kumar JK, *et al*., Antimicrobial potential of *Glycyrhhiza glabra* roots. Journal of Ethnopharmacology 2008; 116: 377-380.
16. Kumara Swamy M, Sudipta KM, Jayanta K, Balasubramanya S. The green synthesis, characterization, and evaluation of the biological activities of silver nanoparticles synthesized from *Leptadenia reticulata* leaf extract. Applied Nanoscience 2015; 5:73-81.
17. Madhavaraj Lavanya, Sethumadhavan Vishnu Veenavardhini, Geun Ho Gim, Mathur Nadarajan Kathiravan and Siwouk Kim. Synthesis, characterization and evaluation of antimicrobial efficacy of silver nanoparticles using *Paederia foetida* L. leaf extract. International Research Journal of Biological Sciences 2013; 2: 28-34.
18. Renuga Devi K and Venus Aswini. Microwave irradiation assisted synthesis of silver nanoparticles using *Azadirachta indica* leaf extract as a reducing agent and in vitro evaluation of its antibacterial and anticancer activity. International Journal of Nanomedicine and Biostructures 2012; 2: 5-10.
19. Saranya Devi J and Valentine Bhimba. Antimicrobial potential of silver nanoparticles synthesized using *Ulva reticulate*. Asian Journal of Pharmaceutical and Clinical Research 2014; 7: 82-85.

How to cite this article:
Pandian N and Chidambaram S: Antimicrobial, cytotoxicty and anti cancer activity of silver nanoparticles from *Glycyrrhiza glabra*. Int J Pharm Sci Res 2017; 8(4): 1633-41.doi: 10.13040/IJPSR.0975-8232.8(4).1633-41.

All © 2013 are reserved by International Journal of Pharmaceutical Sciences and Research. This Journal licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License.

This article can be downloaded to **ANDROID OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Playstore)