Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(1): 2436-2440
Received: 01-11-2017
Accepted: 02-12-2017

U Lakshminarayana
Department of Entomology, S. V. Agricultural College, Tirupati, Acharya N.G. Ranga Agricultural University, Andhra Pradesh, India

NC Venkateswarlu
Department of Entomology, S. V. Agricultural College, Tirupati-517 502 Acharya N.G. Ranga Agricultural University, Andhra Pradesh, India

KV Hariprasad
Department of Entomology, S. V. Agricultural College, Tirupati-517 502 Acharya N.G. Ranga Agricultural University, Andhra Pradesh, India

R Sarada Jayalakshmi Devi
University librarian, Guntur, Acharya N.G. Ranga Agricultural University, Andhra Pradesh, India

TNVKV Prasad
Institute of Frontier Technology, RARS, Tirupati, Andhra Pradesh, India

Correspondence
U Lakshminarayana
Department of Entomology, S. V. Agricultural College, Tirupati, Acharya N.G. Ranga Agricultural University, Andhra Pradesh, India

Preparation of different concentrations of nano scale NSKE formulations

U Lakshminarayana, NC Venkateswarlu, KV Hariprasad, R Sarada Jayalakshmi Devi and TNVKV Prasad

Abstract
Nanotechnology is an emerging field in the area of interdisciplinary research especially in biology. The advancement of nanotechnology mainly requires the development of reliable and eco-friendly protocols for the synthesis of nano material over a range of biological composition, sizes, shapes and high mono disparity. Nanoparticles possess exceptional physical and chemical properties which lead to rapid commercialization. Nanoparticles are considered as fundamental molecular building blocks for nanotechnology. They are pre-requisites for preparing many nanostructure materials and devices and synthesized nanoparticles containing were characterized using UV-Vis spectrophotometer, Particle size and Zeta potential analyzer and Fourier Transform Infrared Spectrophotometer (FTIR). Different concentrations of nano scale NSKE formulations are prepared among all the nano treated NSKE (neem seed kernel extract) formulations the zinc oxide and silver nitrate solution treated with different concentrations of NSKE showed the spectrum absorbance peak of zinc oxide nanoparticles at 265 nm for 5 g of neem seed kernel extract, 272 nm for 10 g, 278 nm for 20 g, 280 nm for 30 g and 284 nm for 50 g of neem seed kernel extract and silver recorded maximum absorbance peak at 400 nm.

Keywords: ancymidol, *A. officinalis. Echinacea*, embryo maturation

Introduction
The Neem tree, *Azadirachta indica* A. Juss (Meliaceae), has long been studied as a source of medicines and insecticides. Neem greatest potential usefulness is in the subsistence agricultural systems of the tropics. In many tropical regions, the neem tree grows readily and is an abundant multipurpose resource. Biosynthesis of nanoparticles is an attractive possibility of advancement of green nanotechnology which has potential to find numerous applications in biology, agriculture in particular. Bansal *et al.*, (2011) [1] reported that the biosynthesis of nanoparticles is a kind of bottom-up approach which involves reduction or oxidation process. Recently the utilization of biological systems provide a novel idea for the production of nano materials.

Material and Methods
1. Preparation of neem seed kernel extract
The neem fruits were collected from nearby areas of S.V. Agricultural College, Tirupati and fruits were allowed to shade dry. Later fruits were soaked in water overnight to get rid of pulp from fruit, and then clean the seed with fresh water and allowed them to dry under shade condition. The neem seed crushed in mixer grinder to get powder and powder was soaked in fresh water for 24 hours, later the Neem Seed Kernel solution was filtered with muslin cloth and stored at room temperature. The NSKE solution was mixed with Nano particles before spraying of the NSKE against leaf miner on groundnut crop.

2. Preparation of nano zinc oxide particles
Zinc oxide nanoparticles, of mean size of 25 nm were prepared using oxalate decomposition method. Zinc oxalate was prepared by mixing equimolar (02 M) of Zinc acetate and oxalic acid. The resultant precipitate was collected and reused extensively with de ionized double distilled water and dried in air. The oxalate then ground and decomposed in air by placing it in pre-heated furnace for 45 minutes at 500°C. 0.1 g of nano oxide particles were mixed in 100 ml of water and heated to 60°C with vigorous stirring for 1 hour. Then 100 ml of Nano Zinc oxide hydrosol was added to 400 ml of NSKE to make up final solution to 500 ml and stirred for 30 minutes at 60°C then the solution is ready for spraying.

3. Preparation of nano silver particles

Ninety ml of 1 nm silver nitrate solution was added to 10 ml of 5% NSKE and heated to 45^0C. Then change of color of the solution was observed to confirm the formulation of the silver nanoparticles. The prepared nanoparticles characterized using the techniques like UV-VIS Spectroscopy, Dynamic Light Scattering (DLS) for hydrodynamic diameter and Zeta potential measurement.

4. Characterization of synthesized nanoparticles

1. UV-Visible Spectroscopy

The reduction of silver ions was monitored by UV-Vis spectrum of the reaction mixture at 24 hrs. The spectra of the surface plasmon resonance of AgNPs and ZnONPs in the reaction mixture were recorded using UV–Vis spectrophotometer (Shimadzu, UV-2450) at wavelengths between 200 to 800 nm.

2. Fourier transform infrared spectroscopy

For Fourier transform infrared (FTIR) spectroscopy measurements, the bio-transformed products present in cell-free filtrate after 72 h of incubation were freeze-dried and diluted with potassium bromide in the ratio of 1: 100. FTIR spectrum of samples was recorded on FTIR instrument mode Nicolet 6700 spectrometer of resolution 4 cm^{-1}) attachment. All measurements were carried out in the range of 400-4000 cm^{-1} at a resolution of 4 cm^{-1}. IR spectroscopic study has confirmed that amino acid and peptides have formed a coat covering the silver nanoparticles to prevent agglomeration.

3. Particle size and zeta potential analyzer

The aqueous suspension of the synthesized silver nanoparticles was filtered through a 0.22μm syringe driven filter unit and the size of the distributed silver nanoparticles were measured by using the principle of Dynamic Light Scattering (DLS) technique made in a Nano partica SZ-100 series compact scattering spectrometer.

UV- Vis spectroscopy analysis

The absorption spectrum was recorded for the sample in the range of 200-800 nm. The spectrum showed the absorbance peak of zinc oxide nanoparticles at 265 nm for 5 g of neem seed kernel extract, 272 nm for 10 g of neem seed kernel extract, 278 nm for 20 g of neem seed kernel extract, 280 nm for 30 g of neem seed kernel extract and 284 nm for 50 g of neem seed kernel extract. The absorbance is due to the localized surface plasmon resonance (LSPR) of the pure and neem seed kernel extract encapsulated nano scale zinc oxide particles. The bio matrix present in the neem seed kernel extract may leads to the change in the absorbance UV-Vis micrograph (Gunalan *et al*., 2012) [2]. It is clear that as the quantum of encapsulated biomass increases, the absorbance is shifted towards higher wave lengths.

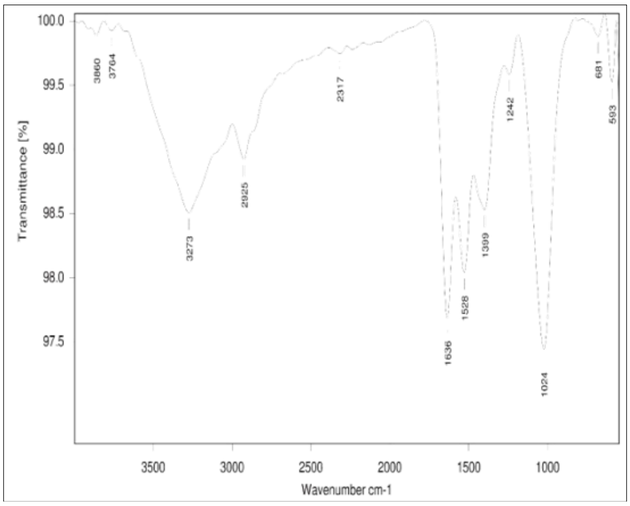
Table 1

S. No	Material	Hydrodynamic diameter (nm)	Zeta potential (mv)
1	5g NSKE Zno NPs	20.2	-2.8
2	10g NSKE Zno NPs	182.6	-15.2
3	20g NSKE Zno NPs	205.7	-29.6
4	30g NSKE Zno NPs	231.8	-11.80
5	50g NSKE Zno NPs	296.0	-11.9
6	50g NSKE Ag NPs	77.50	9.2

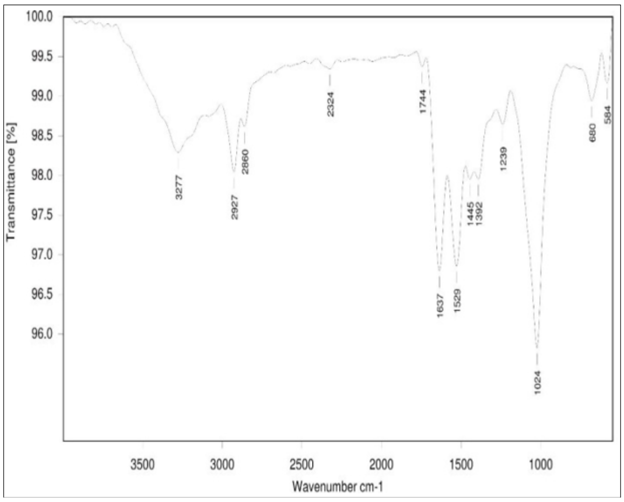
The reduction of silver ions into silver nanoparticles during exposure to neem seed kernel extract was also observed which is evident from change in color due to the localized surface plasmon resonance phenomenon (LSPR) of the formed silver nanoparticles. The metal nanoparticles have free electrons, which give the SPR absorption band; due to the combined vibration of electrons of metal nanoparticles in resonance with light wave UV–Vis spectroscopy of neem seed kernel extract (50 g) showed a maximum absorbance peak at 400 nm. The absorbance may be influenced by several factors such as concentration and combination of extract, pH, incubation temperature, reaction time and electrochemical properties of metal ion.

Fourier transform-infra red spectroscopic analysis

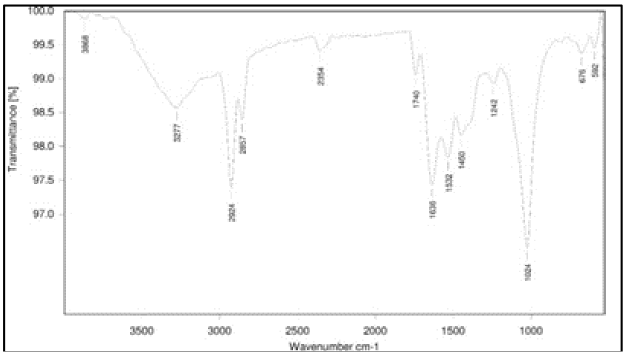
The functional groups – alcohols, alkynes, alkanes, nitriles, aromatics, nitro compounds, Primary amines, alkyl halides and aliphatic amines were identified from FT-IR spectrum recorded from the neem seed kernel extract The spectrum showed the bands for the functional groups located at 5 g of ZnO nanoparticles neem seed kernel extract, the peak at 3860 and 3764 cm^{-1} reveals the presence of O-H group, indicating the presence of alcohols. The strong band of C-H stretch alkynes was recorded at 3273 cm^{-1}. The medium bands of C-H- stretch were recorded at 2925 cm^{-1}. Peak at 2317 cm^{-1} show stretching



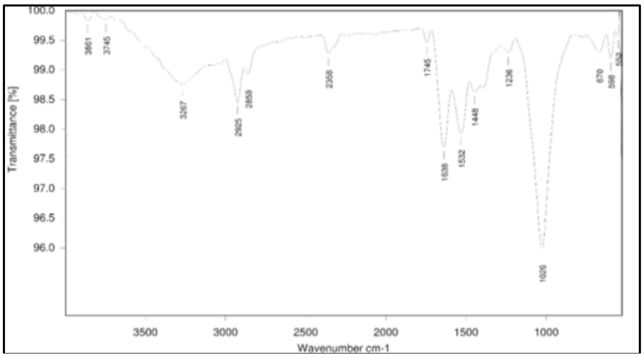
FTIR spectrometer for 5g NSKE Zn



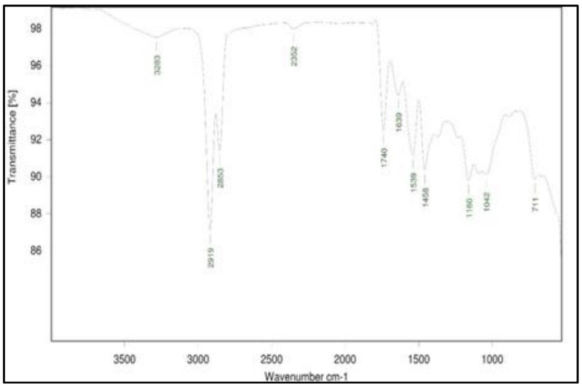
FTIR spectrometer for 10g NSKE Zn



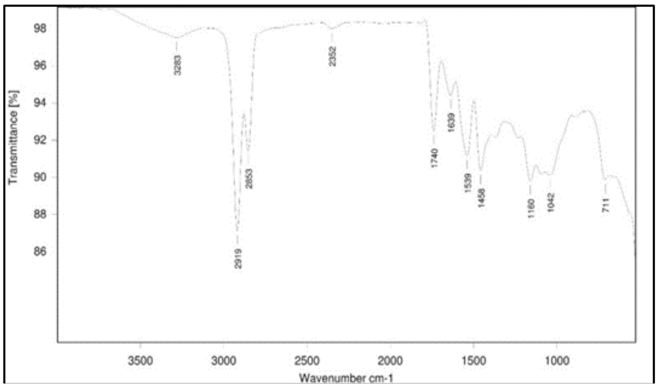
FTIR spectrometer for 20g NSKE Zn



FTIR spectrometer for 30g NSKE Zn



FTIR spectrometer for 50g NSKE Zn



FTIR spectrometer for 50g NSKE ag

vibration of C ≡ N indicating presence of nitriles, 1636 cm^{-1} show stretching vibration of N-H bend indicating presence of primary amines, 1528 cm^{-1} show stretching vibration of N-O indicating presence of nitro compounds, 1399 cm^{-1} show stretching vibration of C-H indicating presence of alkanes, 1242 cm^{-1} and 1024 cm^{-1} show stretching vibration of C-N indicating presence of aliphatic amines, 681 cm^{-1} and 593 cm^{-1} show stretching vibration of C-Br indicating presence of alkyl halides.

The functional groups – alkynes, alkanes, alkynes, ester saturated aliphatic, nitro compounds, Primary amines, alkyl halides and aliphatic amines were identified from FT-IR spectrum recorded from the neem seed kernel extract. The spectrum showed the bands for the functional groups located at 10 g of ZnO nanoparticles neem seed kernel extract, the peak at 3277 cm^{-1} show stretching vibration of C-H indicating presence of alkynes, 2927 cm^{-1} and 2860 cm^{-1} show stretching vibration of C-H indicating presence of alkanes, 2324 cm^{-1} show stretching vibration of C-H indicating presence of alkynes, 1744 cm^{-1} show stretching vibration of C=O indicating presence of ester saturated aliphatic, 1637 cm^{-1} show stretching vibration of N-H indicating presence of primary amines, 1529 cm^{-1} show stretching vibration of N-O indicating presence of nitro compounds, 1445 cm^{-1} show stretching vibration of C-H indicating presence of alkanes, 1392 cm^{-1} show stretching vibration of C-H indicating presence of rock alkanes, 1239 cm^{-1} show stretching vibration of C-H wag indicating presence of alkyl halides, 1024 cm^{-1} show stretching vibration of C-N indicating presence of aliphatic amines, 680 cm^{-1} and 584 cm^{-1} show stretching vibration of C-Br indicating presence of alkyl halides (Fig. 4.7.2). The functional groups – alcohol, alkynes, alkanes, nitriles, aldehydes, saturated aliphatic, nitro compounds, Primary amines, alkyl halides and aliphatic amines were identified from FT-IR spectrum recorded from the neem seed kernel extract. The spectrum showed the bands for the functional groups located at 20 g of ZnO nanoparticles neem seed kernel extract, the peak at 3868 cm^{-1} show stretching vibration of free O-H indicating presence of alcohol, 3277 cm^{-1} show stretching vibration of free C-H indicating presence of alkynes, 2924 cm^{-1} and 2857 cm^{-1} show stretching vibration of free C-H indicating presence of alkanes, 2354 cm^{-1} show stretching vibration of C≡N indicating presence of nitriles, 1740 cm^{-1} show stretching vibration of C=O indicating presence of aldehydes, 1636 cm^{-1} show stretching vibration of N-H indicating presence of primary amines, 1532 cm^{-1} show stretching vibration of N-O indicating presence of nitro compounds, 1450 cm^{-1} show stretching vibration of C-H indicating presence of alkanes, 1242 cm^{-1} and 1024 cm^{-1} show stretching vibration of C-N indicating presence of aliphatic amines, 676 cm^{-1} and 592 cm^{-1} show stretching vibration of C-Br indicating presence of alkyl halide.

N-O indicating presence of nitro compounds, 1448 cm^{-1} show stretching vibration of C-H indicating presence of alkanes, 1236 cm^{-1} show stretching vibration of C-H indicating presence of alkyl halides, 1026 cm^{-1} show stretching vibration of C-N indicating The functional groups – alcohol, alkynes, alkanes, nitriles, ester saturated aliphatic, nitro compounds, Primary amines, alkyl halides and aliphatic amines were identified from FT-IR spectrum recorded from the neem seed kernel extract. The spectrum showed the bands for the functional groups located at 30 g of ZnO nanoparticles neem seed kernel extract, the peak at 3861 cm^{-1} and 3745 cm^{-1} show stretching vibration of C-H indicating presence of alcohols, 3267 cm^{-1} and 3745 cm^{-1} show stretching vibration of C-H indicating presence of alkynes, 2925 cm^{-1} and 2859 cm^{-1} and 3745 cm^{-1} show stretching vibration of C-H indicating presence of alkanes, 2358 cm^{-1} show stretching vibration of C≡N indicating presence of nitriles, 1745 cm^{-1} show stretching vibration of C=O indicating presence of ester saturated aliphatic, 1638 cm^{-1} show stretching vibration of N-H indicating presence of primary amines, 1532 cm^{-1} show stretching vibration presence of aliphatic amines, 670 cm^{-1}, 598 cm^{-1} and 552 cm^{-1} show stretching vibration of C-Br indicating presence of alkyl halide.

The functional groups – alkanes, nitriles, ester saturated aliphatic, nitro compounds, Primary and secondary amines, alkyl halides and aliphatic amines were identified from FT-IR spectrum recorded from the neem seed kernel extract. The spectrum showed the bands for the functional groups located at 50 g of ZnO nanoparticles neem seed kernel extract, the peak at 3283 cm^{-1} show stretching vibration of N-H indicating presence of primary and secondary amines, 2919 cm^{-1} and 2853 cm^{-1} show stretching vibration of C-H indicating presence of alkanes, 2352 cm^{-1} show stretching vibration of C≡N indicating presence of nitriles, 1740 cm^{-1} show stretching vibration of C=O indicating presence of ester saturated aliphatic, 1639 cm^{-1} show stretching vibration of C=C indicating presence of alkenes, 1539 cm^{-1} show stretching vibration of N-O indicating presence of nitro compounds, 1458 cm^{-1} show stretching vibration of C-H indicating presence of alkanes, 1160 cm^{-1} show stretching vibration of C-H indicating presence of alkyl halides, 1042 cm^{-1} show stretching vibration of C-N indicating presence of aliphatic amines, 711 cm^{-1} show stretching vibration of C-H rock indicating presence of alkanes.

The functional groups – alkanes, nitriles, aldehydes, alkenes, nitro compounds, primary and secondary amines, alkyl halides and aliphatic amines were identified from FT-IR spectrum recorded from the neem seed kernel extract. The spectrum showed the bands for the functional groups located at 50 g of silver nanoparticles neem seed kernel extract, the peak at 3283 cm^{-1} show stretching vibration of N-H indicating presence of primary and secondary amines, 2919 cm^{-1} and 2853 cm^{-1} show stretching vibration of C-H indicating presence of alkanes, 2352 cm^{-1} show stretching vibration of C≡N indicating presence of nitriles, 1740 cm^{-1} show stretching vibration of C=O indicating presence of aldehydes, 1639 cm^{-1} show stretching vibration of C=C indicating presence of alkenes, 1539 cm^{-1} show stretching vibration of N-O indicating presence of nitro compounds, 1458 cm^{-1} show stretching vibration of C-H indicating presence of alkanes, 1160 cm^{-1} and 1042 cm^{-1} show stretching vibration of C-N indicating presence of aliphatic amines, 711 cm^{-1} show stretching vibration of C-Br indicating presence of alkyl halides.

Fourier transform infrared (FT-IR) analysis was also included for characterization of the synthesized ZnO NPs and AgNPs confirmed that the bio-reduction of silver ions to AgNPs. This is due to the reduction of capping material of neem seed kernel extract. In the present study the FT-IR analysis showed the involvement of amines, alkanes, alkenes, aromatics, aldehydes, alkyl halides, and nitriles in the synthesis of ZnO NPs and AgNPs. Further, the FT-IR studies confirmed that the Primary and secondary amines residues and proteins has the strong ability to bind the metals indicating that proteins could play a vital role in capping of silver and zinc oxide nanoparticles and preventing agglomeration and thereby stabilizes the nanoparticles. This strongly suggests that the biological molecules could possibly perform the dual functions of formation and stabilization of zinc oxide and silver nanoparticles. These issues can be addressed once the various fractions of neem seed kernel extracts are separated, identified and assayed individually for the reduction of metal ions.

Silver nanoparticles were prepared by chemical reduction method and silver nitrate was taken as the metal precursor. The formation of silver nanoparticles were was monitored using UV-visible spectroscopy it is reveal formation of silver nanoparticles by exhibiting typical surface plasmon absorption at 418-420 nm. The silver nanoparticle was characterization carried by Energy-dispersive Spectroscopy (EDX), X-ray Diffraction (XRD), Transmission Electron Microscopy (TEM) and UV-vis. Spectroscopy showed that theoretical and practical comparison of diameter of silver nanoparticles in the colloidal solution is about 60 nm (Maribel *et al*., 2009) [4].

Hussain *et al.* (2011) [3] reported effects of aniline concentration on the growth and size of silver nanocrystals using aniline silver nitrate as reductant and oxidant, respectively. Nanocrystals were characterized by using the methods UV-vis. Spectroscopy, Transmission Electron Microscopy (TEM) and Selected Area Electron Diffraction (SAED). TEM images showed the size and shape. Average size of the silver nanocrystals size was 25 nm broad band resonance appears at 400 nm.

Nagajyothi *et al.* (2011) [6] reported the biosynthesis method of employing plant extracts have drawn attention as a simple and viable alternative to chemical procedures a physical method. Bio reduction of silver ions to yield metal nano particles using living plants geranium leaf, neem leaf.

Marinho *et al*. (2012) [1] reported that the preparation of nanometer-sized structures of zinc oxide (ZnO) from zinc acetate and urea as raw materials was performed using conventional water bath heating and a Microwave Hydrothermal (MH) method in an aqueous solution. The oxide formation is controlled by decomposition of the added urea in the sealed autoclave. The influence of urea and the synthesis method on the final product formation are discussed. Broadband photoluminescence behavior in visible-range spectra was observed with a maximum peak centered in the green region which was attributed to different defects and the structural changes involved with ZnO crystals which were produced during the nucleation process.

References

1. Bansal V, Ramanatham R, Bhargava SK. Fungus mediated biological approaches towards green synthesis of oxide nanomaterials. Australian Journal of Chemistry. 2011; 64(1):279-293.
2. Gunalan Sivaraj R, Rajendran V. Green synthesized ZnO nanoparticles against bacterial and fungal pathogens. Progress in Natural Science: Materials International-Journal. 2012; 22(6):693-700.
3. Hussain JI, Sunil Kumar, Hashmi AA, Zaheer Khan. Silver nanoparticles preparation, characterization and kinetics. Advanced Materials Letters. 2011; 2(3):188-194.
4. Maribel G, Guzman Dille J, Godet S. Synthesis of silver nanoparticles by chemical reduction method and their antimicrobial activity. International Journal of Chemical and Biomolecular Engineering. 2009; 2(3):104-111.
5. Marinho JZ, Romeiro FC, Lemos SCG, Motta FV, Riccardi CS, Li MS *et al*. Urea based synthesis of Zinc oxide nano structure at low temperature. Hinawi Publishing Corporation Journal of Nanomaterials. 2012, 1-7.
6. Nagajyothi PC, Prasad TNVKV, Sreekanth TVM, Kap Duk Lee. Biofabrication of nanoparticles using leaf extracts of Saururus chinensis. Digest Journal of Nanomaterials and Biostructures. 2011; 6(1):121-133.