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# Phytochemical analysis, synthesis, antitumor and antimicrobial activity of Silver Nanoparticles using flower extracts of *Ixora coccinea*

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**Abstract:** This study aims to analyse aqueous, ethanolic and methanolic extracts of flowers of *Ixora coccinea* for the presence of various phytochemicals and synthesised silver nanoparticles using the aqueous extract of *Ixora coccinea* flowers and also checked the antimicrobial activity, TLC studies using both the extracts. The presence of various phytochemicals viz., alkaloids, tannins, carbohydrates, flavonoids, terpenes and glycosides were analysed by standard biochemical screening methods. The synthesised silver nanoparticles (SNPs) were characterised by using UV-Vis Spectroscopy, FTIR, XRD, SEM, TEM The synthesised nanoparticles were found to be spherical in shape with average size in the range of 5-10 nm and also showed inhibitory zones to the bacterial cultures. The results revealed that the aqueous extract of *Ixora coccinea* flowers is a very good bioreductant for the synthesis of silver nanoparticles.
**Keywords:** Phytochemical analysis, silver nanoparticles(SNPs),UV-Vis,FTIR,XRD,SEM,TEM,TLC and antimicrobial studies, *Ixora coccinea* flower extracts.

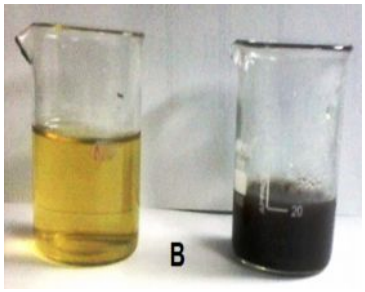
## Introduction

Natural products have been the basis of the treatments of human diseases for a long period of time. Modern medicine or allopathy has gradually developed over the years due to the scientific and observational efforts of scientists- however the basis for its development remains in the roots of traditional medicine and therapies [1]. Herbal medicinal preparations and their proprietary products are also being used more and more widely throughout the world [2]. *Ixora coccinea* is cultivated for ornamental purpose and reported for diverse pharmacological properties including antitumor, hepatoprotective, chemo protective anti-inflammatory[3] cytotoxic, antidiarrheal, antimicrobial, wound healing [4] and antimitotic activities. [5] Leaves and flower extracts *Ixora coccinea* of were reported to possess antimicrobial activitties. [6] Flower extracts of these plants contains ursolic acid and triterpenoids [7] and have shown protective effect against systemic toxicity induced by cyclophosphamide and cisplastin [8-9]. They are useful in dysentery, dysmenorrheal, leucorrhoea, haemoptysis, catarrhal bronchitis and opthalmopathy [10,11,12]. Beyond these phytochemicals present in these flower extracts acts as bioreducing and capping agent in the chemical reduction of metal nanoparticles like silver nanoparticles [13-17]. The present ecofriendly process for the biosynthesis of silver nanoparticles using flower extracts of *Ixora coccinea* reveals the presence of phytochemicals, antitumor activity and antimicrobial activity.

## Material and Methods

All the reagents used in the study were of analytical grade. Silver nitrate ($AgNo_3$) was obtained from Sigma Aldrich. Fresh flowers of *Ixora coccinea* were collected from Osmania university botanical garden. Aqueous, methanolic and ethanolic extracts were prepared from these flowers.


The ethanolic, methanolic and aqueous extracts of *Ixora coccinea* flowers were regulated to various chemical tests for the analysing phytochemical constituents such as alkaloids, tannins, glycosides, flavonoids, saponins, terpenes, carbohydrates. The aqueous extract obtained from flowers is used for the synthesis of silver nanoparticles. The extract was added to the 1mM silver nitrate solution in 1:10 ratio and then heated to $60^0$C for 15mins.The colour change from pale yellow to reddish brown was observed due to the reduction of Ag+ ions (Fig B)




The reduction of pure Ag+ ion was monitored by measuring the UV Visible Spectrum using an Elico SL-159 UV spectrophotometer at 300-700nm and Flower extract prepared is used as blank against silver nanoparticles solution for measuring the UV Visible spectrum. To remove any free biomass residue, the residual solution was centrifuged at 20,000 rpm for 10 mins and the resulting suspension was redispersed in 1 ml sterile distilled water. Thereafter the purified suspension was freeze dried to obtain dried powder. Finally, the dried nanoparticles were analyzed by FTIR and X-ray diffraction (XRD) Scanning Electron Microscope (SEM) and TEM

The aqueous extract was checked for Thin Layer Chromatography. For Aqueous Extract, Ethanolic extract and methanolic extract, solvent system used here is Ethyl acetate and Hexane (2:8) ratio were found to be the best for separation. After proliferating TLC of the methanolic extract, Rf values were calculated for the spots were seen under UV illuminator. The antibacterial activity of *Ixora coccinea* flower extract and green synthesized silver nanoparticles and crude extracts were tested by the agar diffusion method. For the determination of antibacterial activity, the antibiotic resistant bacteria namely *Psuedomonas putida*, *Escherichia coli, Staphylococcus aureus, Klebsheilla pnuemonea,* and *Bacillus subtillis*.

**Cell Culture and Nanoparticles Treatment**

The cell lines U937 (human histiocytic lymphoma), COLO205 (human Colon adenocarcinoma), B16F10 (mouse mealanocarcinoma) HepG2 (hepato cellular carcinoma) and HeLa (human cervix carcinoma) cell lines were obtained from the National Centre for Cellular Sciences (NCCS), Pune, India. Cells were used between passages 10 and 20 and cultured either in RPMI -1640 (U937, COLO205), DMEM (B16F10, HepG2) and MEM (HeLa) media, supplemented with 10% heat-inactivated fetal bovine serum (FBS), 1 mM NaHCO3, 2 mM -glutamine, 100 units/ml penicillin and 100 µg/ml streptomycin. All cell lines were maintained in culture at 37° C in an atmosphere of 5% $CO_2$. At 85% confluence, cells were harvested using 0.25% trypsin and the cells (2 x 104) were seeded in each well containing 100µl of medium in 96 well plates.Cells were allowed to attach the surface for 24 h prior to nanoparticles exposure. IXNPs were suspended in complete cell culture medium (8mg/ml stock) and diluted to appropriate concentrations (25, 50, 75, 100, 150 and 200µg/ml). The dilutions of IXNPs were then sonicated using a sonicator bath at room temperature for 15 min at 40W to avoid nanoparticles agglomeration prior to administration to the cells. Selection of 25–200µg/ml concentration of IXNPs was based on a preliminary dose-response study (data not shown).

### Cytotoxicity

Cytotoxicty was measured using the MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] assay, according to the method of Mossman (1983). Briefly, the cells (2 x 104) were seeded in each well containing 100µl of medium in 96 well plates. After overnight incubation at 37 °C in 5% CO2, exactly 100µl of different test concentrations (25µg to 200µg/ml) of IXNPs were added to the cell suspension, which is equivalent to 5 to 40µg per 200µl of assay volume. The viability of cells was assessed after 24h, by adding 10µl of MTT (5 mg/ml) per well and incubated at 37°C for additional three hours. The medium was discarded and the formazan blue, which formed in the cells, was dissolved in 100 µl of DMSO. The intensity of colour formation was measured at 570 nm in a spectrophotometer (Spectra MAX Plus; Molecular Devices; supported by SOFTmax PRO-5.4). The percent inhibition of cell viability was determined with reference to the control values (without test nanoparticles). The data were subjected to linear regression analysis and the regression lines were plotted for the best straight-line fit. The IC50 (inhibition of cell viability) concentrations were calculated using the respective regression equation. Flower extracts of *Ixora coccinea* were evaluated to investigate their anti-proliferative/cytotoxic activities in five different types of human cancer cells including U937 (human histiocytic lymphoma), COLO205 (human Colon adenocarcinoma), B16F10 (mouse mealanocarcinoma) HepG2 (hepato cellular carcinoma) and HeLa (human cervix carcinoma) cells.

## Results and Discussion

The results of the qualitative screening of the phytochemical components such as alkaloids, glycosides, tannins, terpenes, flavonoids, saponins, carbohydrate in the aqueous, ethanolic and methanolic flower extracts of the plant species are shown in table 1. The presence of bioactive plant product visualises the separation of chemical constituents from the mixture of compounds through suitable chromatographic techniques. In the present study, in aqueous extract carbohydrates, terpenes show positive. In ethanolic extract the tests for the presence of alkaloids, carbohydrates, flavonoids, terpenes and glycosides shows positive. In methanolic extract the tests for the presence of alkaloids,carbohydrates,flavonoids,terpenes,tannins and glycosides shows positive These Phytochemical examinations focus on the distinct separation of the natural chemical constituents from plant extracts as they may be used for the clinical practices.

**Table 1: Results of phytochemical screening of Aqueous, Ethanolic and Methanol extracts of *Ixora coccinea***

| S.No | Primary and Secondary metabolites | Aqueous Extract | Ethanolic Extract | Methanolic Extract | Test Method |
|---|---|---|---|---|---|
| 1. | Alkaloids | - | + | + | Siddiqui and Ali, (1997) |
| 2. | Tannins | - | - | + | Mukherjee PK; 2002 |
| 3. | Glycosides | - | ++ | + | Trease and Evans (1989) |
| 4. | Flavonoids | - | + | + | Siddiqui and Ali (1997) |
| 5. | Saponins | - | - | - | Siddiqui and Ali(1997) |
| 6. | Terpenes | ++ | ++ | ++ | Harborne,1973 |
| 7. | Carbohydrates | ++ | ++ | + | Krishnaveni et al.,(1984) |

### Characterisation of the Silver Nanoparticles

### UV-Vis Study

The addition of *Ixora coccinea* flower extract to 1mM silver nitrate solution led to the appearance of a reddish brown colour solution after 15mins indicating the formation of silver nanoparticles. Further UV-Vis spectral analysis showed surface Plasmon resonance (SPR) band at 459 nm, a typical of silver nanoparticles. Fig. 1A shows the UV-Vis spectra of silver nanoparticles synthesized by using *Ixora coccinea* flower extract.

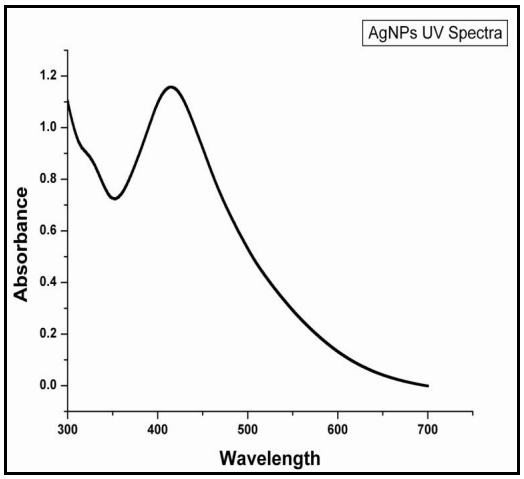



**Fig. 1A: UV-Vis spectra recorded reaction aqueous solution of AgNO3 with *ixora coccinea flower* extract;**

**FTIR Spectroscopy**

The FTIR analysis was carried out to identify the possible biomolecules responsible for the reduction of Ag+ ions and capping of the bioreduced nanoparticles synthesized by the aqueous flower extract of *Ixora coccinea*. FTIR spectra (Fig. 2A) of Ag nanoparticle formed by reduction of Ag+ ions using *Ixora coccinea flower* extract showed peak at 3248 cm-1, 1704 cm-1, 1682 cm-1, 1514 cm-1, 1381 cm-1 and 1139 cm-1.

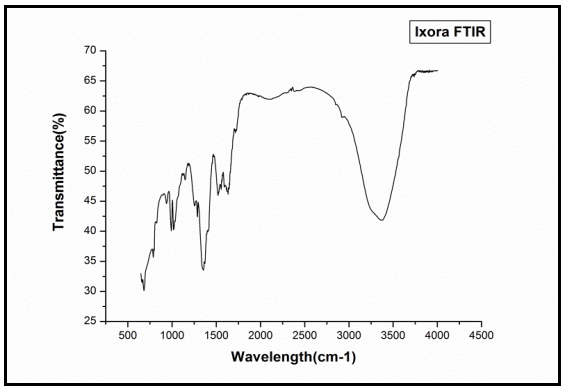



**Fig. 2A.FTIR spectra of Ag nanoparticles synthesized by reduction of Ag+ion by *Ixora coccinea***

**SEM Analysis**

The SEM image of silver nanoparticles synthesized by using *Ixora coccinea flower* extract is shown in Fig. 4A which shows distinct and clear image of synthesized silver nanoparticles in the range between 5-10 nm. Silver nanoparticles are not aggregated i.e monodisperse in nature and EDS analysis confirmed the presence of elemental silver as the major constituent using *Ixora coccinea flower* extract and The EDS results are shown in Fig.4B and Table 1).

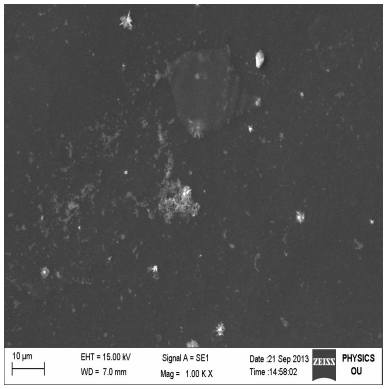



**Fig. 4A: SEM image of the silver nanoparticles synthesized by**

TEM Analysis

The TEM image of silver nanoparticle synthesised by using *Ixora coccinea* flower extract is shown in the Fig., 5 which predominantly shows spherical shape with smooth surface morphology. The Histogram figure of the particles shows number of particles formed and also size of the particles. ranging within 0.5nm. using *Ixora coccinea flower* extract and 5B.showing histogram of particle size distribution of silver nanoparticles.

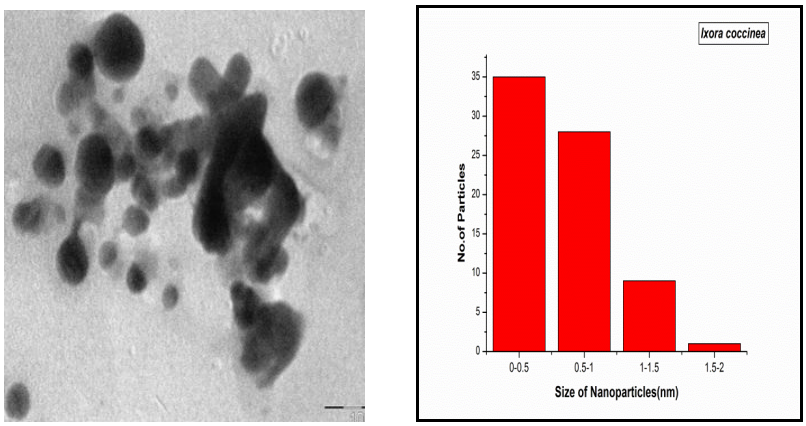



**Fig. 5A: TEM image of the silver nanoparticles synthesized by**

**TLC Analysis**

The aqueous extract was checked for Thin Layer Chromatography. For Aqueous Extract and Ethanolic extracts, solvent system used here is Ethyl acetate and Hexane (2:8) ratio were found to be the best for separation. Rf values were calculated (Table. 2)


**Fig., 6 TLC of Ixora coccinea Methanolic extract**

**Table .2**

| Plant Extract | Solvent System | Rf Values |
|---|---|---|
| Methanolic Extract | Ethyl acetate and Hexane (2:8)ratio | 0.6mm,0.4mm,0.2mm,0.1mm |

**Antibacterial Studies**

The antibacterial studies were tested with strains *Psuedomonas putida,Escherichia coli,Staphylococcus aureus,Klebsheilla pneumonia,* and *Bacillus subtillis* (Fig.,7A,B,C,D,E). The plates are examined for the presence of growth inhibition (Table 3) which is indicated by a clear zone surrounding each disc.

**Table.3: Antibacterial activity of the synthesized nanoparticles**

| S.no | Name of the Organism | Inhibitory zones diameter in mm | | | |
|---|---|---|---|---|---|
| | | Extract 5µl | IxNps 5µl | IxNps 10µl | Ampicillin 5µl |
| 1. | P.putida | - | 18.74 | 24.39 | 24.04 |
| 2. | K.pneumonia | - | 14.85 | 20.16 | 21.21 |

| | | | | | |
|---|---|---|---|---|---|
| 3. | E.coli | - | 12.03 | 24.04 | 24.40 |
| 4. | S.aureus | 10.97 | 13.79 | 18.03 | 20.51 |
| 5. | B.subtillis | - | 10.96 | 16.27 | 18.03 |

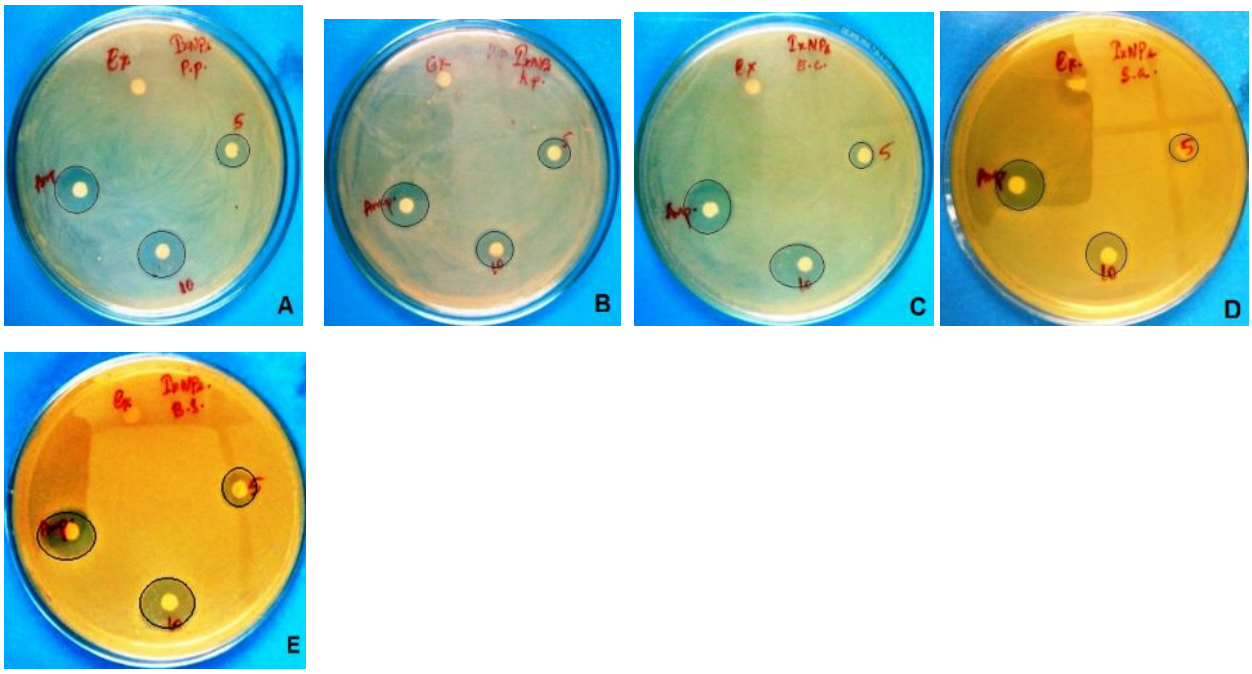



**Fig. 7: A-P.putida, B-K.pneumonia, C- E.coli, D- S.aureus, E- B.subtillis…,showing the antimicrobial activity and zones of Inhibition.**

Flower extracts of *Ixora coccinea* are active against all cell lines including U937, Colo205, B16F10, HepG2 and HeLa at below 200µg/ml concentration (Table-4). COLO205 cells are more sensitive for IXNPs (IC50: 65.40±2.41) followed by U937 (IC50: 84.17±2.13), HeLa (IC50: 93.27±2.53), HepG2 (IC50: 95.52±4.08) and B16F10 (IC50: 196.5±4.19). The order of sensitivity of human cancer cell lines towards the IXNPs is Colo205> U937 >HeLa >HepG2 >B16F10. The biologically synthesized IXNPs were exhibited cytotoxic properties against all the cell lines (<200 µg/ml) in a concentration-dependent manner. It is evident from the overall results, that the biologically synthesized IXNPs showed the potent anti-proliferative activity against Colo205 cell line, which is less cytotoxicity than the positive control, Etoposide. Exponentially growing cells were treated with different concentrations of IXNPS for 24h and cell growth inhibition was analyzed through MTT assay. $^{\S}IC_{50}$ is defined as the concentration, which results in a 50% decrease in cell number as compared with that of the control cultures in the absence of an inhibitor and were calculated using the respective regression analysis. The values represent the mean ± SE of three individual observations. Etoposide is a standard drug molecule employed as positive control.

**Table-4 *In vitro* Cytotoxicity of biologically synthesized IXNPS against U937, Colo-205, B16F10, Hep G2 & Hela cells by MTT assay**.

| S.NO | Cell line | $^{\S}IC_{50}$ values of IXNPs (µg/ml) | $^{\S}IC_{50}$ values of $^{a}$Etoposide (µg/ml) |
|---|---|---|---|
| 1 | U937 | 39.49±1.23 | 5.02±0.62 |
| 2 | Colo205 | 36.50±2.03 | 1.07±0.12 |
| 3 | B16F10 | 105.5±2.10 | 4.12±0.42 |
| 4 | HepG2 | 63.12±2.22 | 3.12±0.26 |
| 5 | HeLa | 72.142±1.4 | 2.12±0.32 |

## Conclusions

Differential anticancer properties of the biologically synthesized IXNPS against cell lines (U937, COLO205, B16F10, HepG2 and HeLa) may be due to different mechanism of action. These preliminary results

indicated that slight modification of methodology for biologically synthesis of IXNPS may yield as prospective anticancer drugs. Based on the present results, it is warranted that these IXNPS to be further evaluated on other cancer cell lines.

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