Green Synthesis of Copper Nanoparticles from *Ixora coccinea* leaves and *Momordica charantia* fruit extracts

Rima Lad [1], Shruti Singh [2]
[1,2] Dolat-Usha Institute of Applied Sciences and Dhiru-Sarla Institute of Management & Commerce, Valsad, Gujarat, India

ABSTRACT

The development of nanotechnology interests the research for synthesis of nanoparticles with various bio-applications. The green synthesis of copper nanoparticles using *Momordica charantia* and *Ixora coccinea* act as both reducing and capping agents. Copper nanoparticles have attracted huge attention due to catalytic, optical, photonic, antibacterial activity depending on the basis of size and shape. It is eco-friendly, non toxic and cost effective approach. Formation of copper nanoparticles has been confirmed by anti bacterial, anti fungal and phytochemical analysis. The antimicrobial activity of CuNps was established using disc diffusion and agar well method. The CuNps new eco-friendly approach of synthesis is a novel, cheap and convenient technique for large scale commercial production.

Keywords: Copper nanoparticles, *Momordica charantia, Ixora coccinea*, antimicrobial activity

INTRODUCTION

Bio nanotechnology utilizes biological principles and physical and chemical approaches to yield nanosized particles with specific functions.The development of nanotechnology interest the research for synthesis of nanoparticles with various bio-applications. The green synthesis of copper nanoparticles using *Momordica charantia* and *Ixora coccinea* act as both reducing and capping agents. Physical and chemical methods are more popular for nanoparticle synthesis but the use of toxic compounds limits their application [1] and also not economically feasible one. An array of physical, chemical and biological methods has been used for synthesis of noble metal nanoparticles of particular shape and size for various applications, but they remain expensive and involve the use of hazardous chemicals [2].

Research into CuNPs has made significant progress in the areas of nanotechnology and nanomedicine within the last decade due to their excellent catalytic, optical, electrical and antifungal/antibacterial applications [3]. In recent years, plant-mediated biological synthesis of nanoparticles has gained interest due to its simplicity and eco-friendliness. Formation of copper nanoparticles has been confirmed by anti bacterial, anti fungal and phytochemical analysis.

Among the metallic nanoparticles, copper nanoparticles (CuNPs) have continued to gain public interest due to their optical, electrical and thermal properties [4]. Synthesis of CuNPs is cost effective when compared to silver (Ag), gold (Au) and platinum (Pt) [5]. It is evident from the literature that, synthesis of stable CuNPs is the challenging task and various methods have been developed to synthesize CuNPs including physical, chemical and biological processes [6]. Copper and CuNPs have been synthesized by a variety of plant extracts [7].

The antimicrobial activity of CuNps was established using disc diffusion and agar well method. Although the biosynthesis of CuNPs by plants such as *Momordica charantia* and *Ixora coccinea* has previously been reported, the potential of plants as biological materials for the synthesis of nanoparticles is yet to be fully explored. Copper Nanoparticles are used as an antimicrobial, anti-biotic and anti- fungal agent when incorporated in coatings, plastics, textiles etc. copper and copper based compounds have efficient biocidal properties, which are generally used in pesticidal formulation.

METHODOLOGY

Sample collection

Fresh leaves of *Ixora coccinea* and fruits *Momordica charantia* were collected into clean zipper bags.

Preparation of extracts

The selected leaves and fruits were washed in running tap water and then again washed with distilled water. Then the leaves were dried with absorbent paper. These leaves were cut into small pieces and 10gm of *Ixora coccinea* leaves and *Momordica charantia* fruit extracts were grinding using mortar and pastel and extracts were filtered by Whatmann No.1 filter paper. It filtered was made upto 100ml and 50ml in standard measuring flask respectively. It were then stored in refrigerator for further use.

Synthesis of copper nanoparticles using *Ixora coccinea* leaf extracts

For synthesis of copper nanoparticles from *Ixora coccinea* leaves, 4mM copper choloride was treated with 2 ml aqueous leaf extract of *Ixora coccinea* and stirred at room temperature until the light blue colour changed to light green colour. Then the mixture is heated at 80 °C for 2hrs. Afterwards, the mixture was treated with 1 M sodium hydroxide drop by drop. As soon as, the sodium hydroxide comes in contact copper ions spontaneous change the green mixture to brown precipitate, indicating the formation of water soluble copper nanoparticles.

Synthesis of copper nanoparticles using *Momordica charantia* fruit extracts

For synthesis of copper nanoparticles from *Momordica charantia* fruits extracts, 90 ml aqueous solution of 1.0×10^{-3} M copper nitrate was treated with 10 ml of filtered fruit extracts and incubated at room temperature for 24hrs. The colour change of copper nitrate indicates the formation of CuNps due to reduction of copper ions. The samples were then centrifuged at 15,000 rpm for 15 minutes to get clear supernatants at room temperature. In *Ixora coccinea* after centrifugation, also washed with ethanol to remove the impurities for the final products and also once again centrifuge the sample.

Characterization of Nanoparticles

Determination of Phytochemicals:

Chemical tests were carried out on the aqueous extracts to identify the constituents using standard procedures as described by Njoku and Obi, 2009 [8].

- **Test for tannins:**
  About 2 ml of the aqueous extract was stirred with 2 ml of distilled water and few drops of $FeCl_3$ solution were added. The formation of a green precipitate was an indication for the presence of tannins.
- **Test for saponins:**
  5ml of aqueous extract was shaken vigorously with 5 ml of distilled water in a test tube and warmed. The formation of stable foam was taken as an indication for the presence of saponins.
- **Test for flavonoids:**
  To 1 ml of aqueous extract was added 1 ml of 10% lead acetate solution. The formation of a yellow precipitate was taken as a positive test for flavonoids.
- **Test for terpenoids:**
  2 ml of the organic extract was dissolved in 2 ml of chloroform. 2 ml of concentrated sulphuric acid was then added and heated for about 2 min. A greyish colour indicates the presence of terpenoids.
- **Test for steroids:**
  2ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml H_2SO_4. The colour changed from violet to blue or green in some samples indicating the presence of steroids.
- **Test of alkaloids:**
  3 ml aqueous extract was stirred with 3 ml of 1% HCL on a steam bath. Mayer's and Wagner's reagents were then added to the mixture. Turbidity of the precipitate was taken as evidence for the presence of alkaloids.

Determination of antimicrobial activity

Test microorganisms:

The microbial strain studied were Gram positive *(Bacillus subtilis* and *Bacillus meghaterium* species) and Gram negative (*Salmonella paratyphi A* and *Escherichia coli)* bacterial and fungal strain (*Penicillium* and *Aspergillus* species). These strains were obtained from Laboratory of Microbiology Department of Dolat

Usha Institute of Applied Sciences and Dhiru Sarla Institute of Management & Commerce, Valsad. Microorganisms were maintained on nutrient agar slants at 4°C and sub-cultured every month.

Preparation of inoculums:

Each organism was recovered for testing by sub-culturing on fresh Nutrient agar and Sabouraud dextrose agar medium. A loopful of inoculum of each bacteria and fungi were suspended in nutrient broth and incubated overnight at 37°C and were used for further studies.

Antibacterial activity:

Antimicrobial activity of aqueous extract was determined by using agar well diffusion method. In this technique, about 15ml of sterile melted and cool nutrient agar was used and to it 1ml of inoculums were added, mixed well and poured into sterile Petri dishes. Agar plates were allowed to solidify at room temperature. Further plates were divided into three parts and with the help of sterile cup-borer 6mm three well were made at the centre of different parts in plate. The various samples were carefully placed in the wells and further were subjected to diffusion for 1 hour at 4°C. The plates were then incubated at 37°C for 24 hours for antimicrobial activity study. Next day the plates were observed for microbial zone of inhibition around the environmental samples present in wells.

Antifungal activity:

Antifungal activities of the synthesized silver nanoparticles were determined, using the agar well diffusion assay method (NCCLS, 2000). Approximately 20 ml of molten and cooled media (SDA) was poured in sterilized Petri dishes. The plates were left overnight at room temperature to check for any contamination to appear. The fungal test organisms were grown in dextrose broth for 24 h. A 100 ml Sabouraud dextrose broth culture of each fungal organism was used to prepare fungal lawns. Agar wells of 5 mm diameter were prepared with the help of a sterilized stainless-steel cup borer. Three wells were prepared in the agar plates. The wells were loaded with 50µl of silver nanoparticles, 50µl of leaf extract and 50µl of silver nitrate. The plates containing the fungal and silver nanoparticles were incubated at 37°C. The plates were examined for evidence of zones of inhibition, which appear as a clear area around the wells. The diameter of such zones of inhibition was measured using a meter ruler and the mean value for each organism was recorded and expressed in Millimeter.

RESULT AND DISCUSSION:

Biosynthesis of copper nanoparticles from *Ixora coccinea* leaf and *Momordica charantia* fruits was done under static condition. The extract was mixed in the aqueous solution of the copper ion complex, it stated to change the colour from green to brown due to reduction of copper ion which indicated formation of copper Nanoparticles (Figure 1).

Figure 1: Synthesis of copper nanoparticles from *Ixora coccinea* and *Momordica charantia.*

- **Phytochemicals analysis of *Ixora coccinea* and *Momordica charantia:***

Table 1: Phytochemicals analysis of *Ixora coccinea* and *Momordica charantia*

Phytochemicals	**Observation**	***Ixora coccinea***	***Momordica charantia***
Tannins	Green colour precipitate	+	+
Saponins	Present of foam	+	+
Terpenoids	Greyish colour ring	-	+
Steroids	Green or blue colour precipitate	+	+
Alkaloids	Turbidity of precipitate	–	–
Flavonoids	Yellow colour precipitate	+	+

The results revealed that the medicinally active constituents such as flavonoids, tannins, steroids, saponins were present in *Ixora coccinea leaf* extract while, alkaloid was not found to be present in *Momordica charantia.*

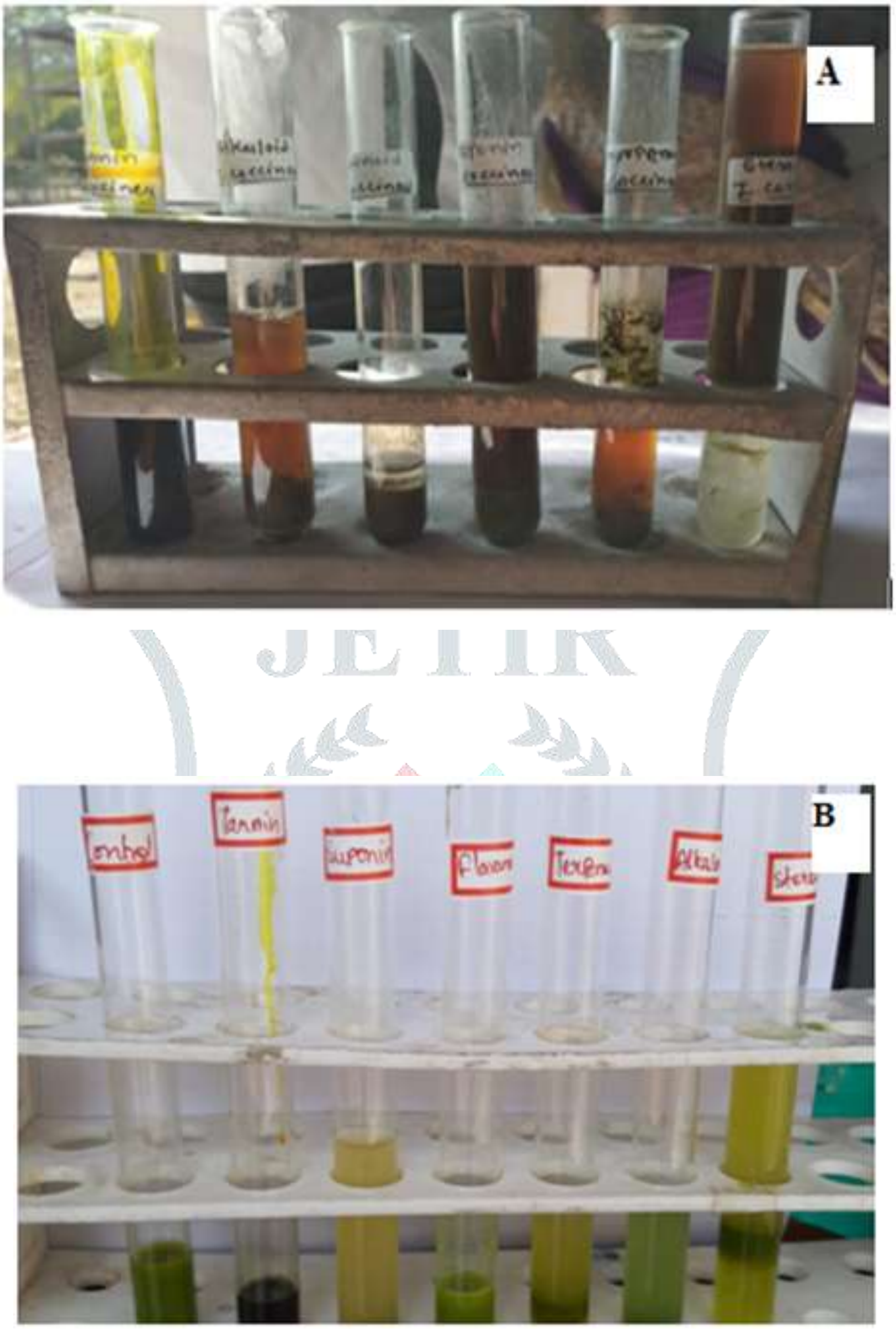

Figure 2: Phytochemicals analysis of (A) *Ixora coccinea* and (B) *Momordica charantia.*

- **Antibacterial activity of *Ixora coccinea* and *Momordica charantia*:**

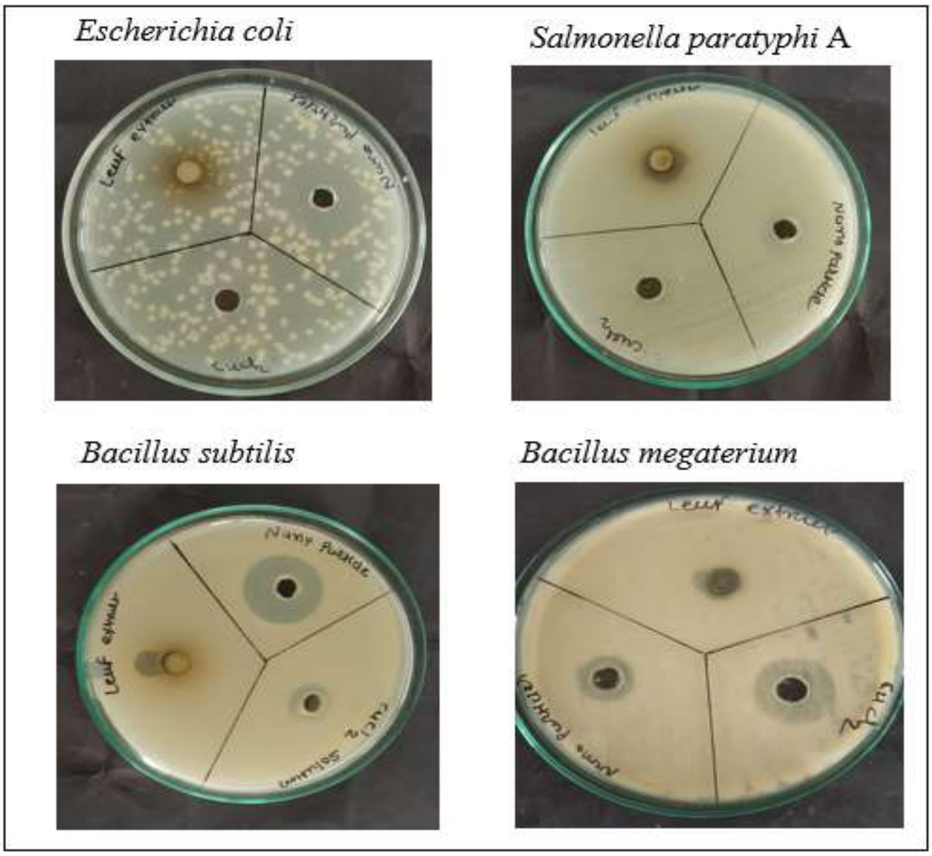

Figure 3: Antibacterial activity of *Ixora coccinea*

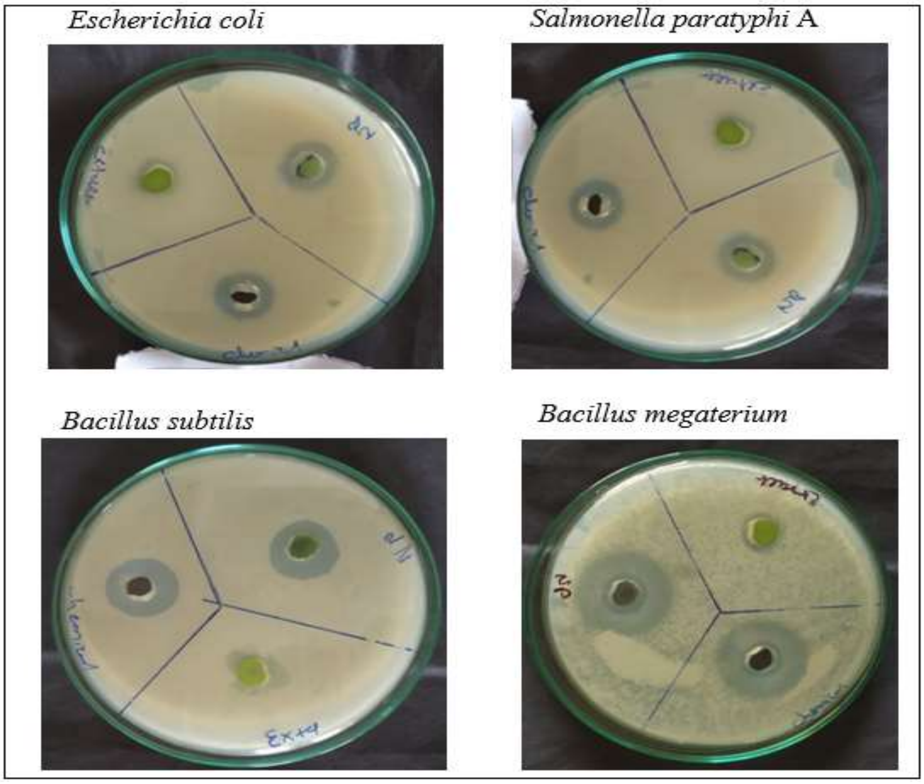

Figure 4: Antibacterial activity of *Momordica charantia*

Table 2: Anti bacterial activity of *Ixora coccinea* leaf extract

Bacteria	**Copper nanoparticles (mm)**	**Copper chloride (mm)**	**Leaf extract (mm)**
Bacillus subtilis	21	13	14
Bacillus megaterium	12	10	12
Escherichia coli	27	00	00
Salmonella paratyphyi A	12	00	00

Table 3: Antibacterial activity of *Momordica charantia* fruits extract

Bacteria	**Copper nanoparticles (mm)**	**Copper nitrate (mm)**	**Leaf extract (mm)**
Bacillus subtilis	19	17	12
Bacillus megaterium	26	25	11
Escherichia coli	16	15	10
Salmonella paratyphyi A	15	14	08

Zone of inhibition were observed with the *Ixora coccine* leaf and *Momordica charantia* fruit extract, copper chloride and copper nitrate against two Gram positive bacteria *Bacillus subtilis, Bacillus meghaterium* and two Gram negative bacteria *Escherichia coli, Salmonella typhy A*. The anti bacterial activity of copper nanoparticles showed more inhibition than the plant extracts. The highest zone of inhibition has reported by the synthesized copper nanoparticles in *Ixora coccinea* against *Escherichia coli*. The results of anti microbial activity shown in table 1.

- **Antifungal activity of *Ixora coccinea* and *Momordica charantia* :**

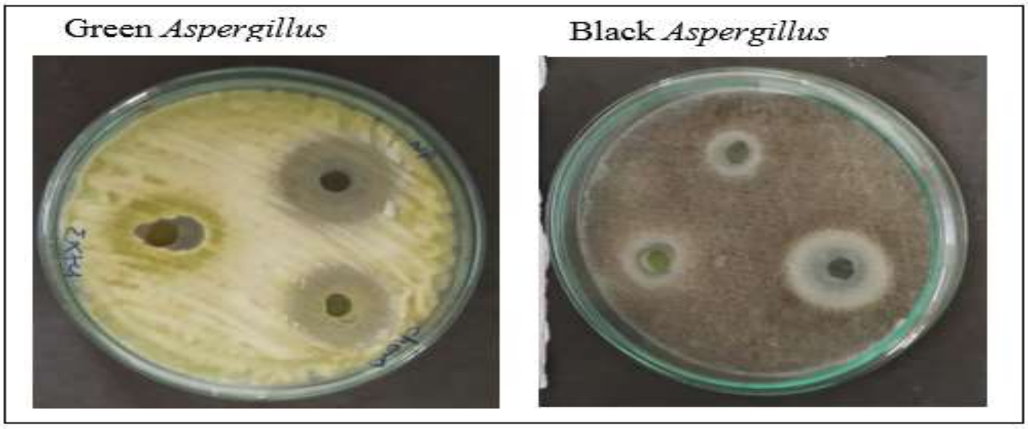

Figure 5: Antifungal activity of *Ixora coccinea*

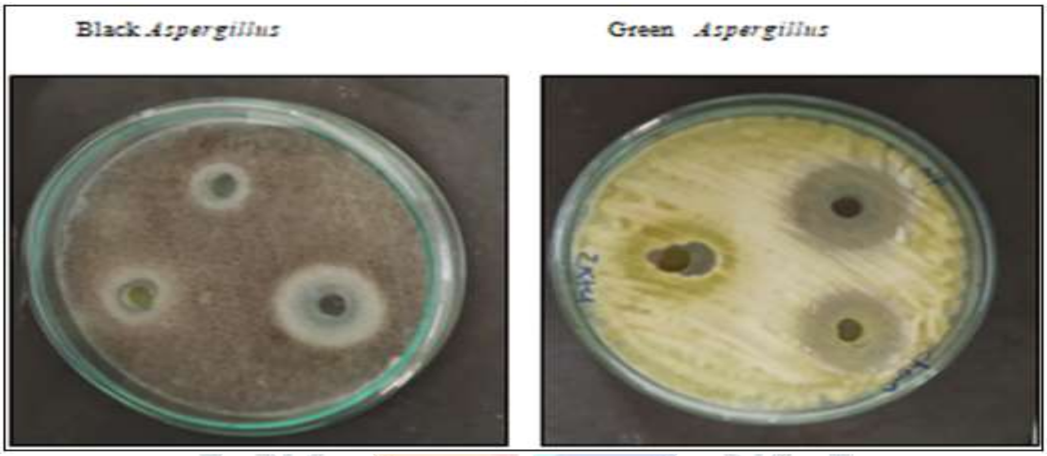

Figure 6: Antifungal activity of *Momordica charantia*.

Table 4: Antifungal activity of *Ixora coccinea* leaf extract

Fungal species	**Copper nanoparticles (mm)**	**Copper chloride (mm)**	**Leaf extract (mm)**
Aspergillus spp. (green)	19	10	08
Aspergillus spp. (black)	18	08	08

Table 5: antifungal activity of *Momordica charantia*

Fungal strain	**Copper nanoparticles (mm)**	**Copper nitrate (mm)**	**Leaf extract (mm)**
Aspergillus spp. (green)	32	27	00
Aspergillus spp. (black)	25	15	13

CONCLUSION:

A simple, fast and biological procedure was introduced to synthesize copper nanoparticles by using *Ixora coccinea* leaf and *Momordica charantia* fruit extract. The extract of *Ixora coccinea* and *Momordica charantia* has reducing agent that can identified by phytochemical test. The antimicrobial and anti fungal activity of the nanoparticles have been evaluated our current study revealed that the CuNps of *Ixora coccinea* and *Momordica charantia* showed significant anti bacterial activity against *Escherichia coli* and *Salmonella paratyphi A*. The CuNps of *Ixora coccinea* has more effective against fungi than *Momordica charantia*. The antifungal activity of CuNps showed significant activity against *Aspergillus niger* where less activity was observed against *Penicillium* species. It is eco-friendly, non toxic and cost effective approach for synthesis of nanoparticles in a field of Nanomedicine.

REFERENCES

1. Hasna Abdul Salam., P. Rajiv, M. Kamaraj, P. Jagadeeswaran, Sangeetha Gunalan and Rajeshwari Sivaraj. 2012. Plants: Green route for nanoparticle synthesis (review) .Inter. Res.J. Biol. Sci. 1(5): 85-90.

2. Balagurunathan, R., M. Radhakrishnan, R. Babu Rajendran and Velmurugan,D. 2011. Biosynthesis of gold nanoparticles by actinomycete Streptomyces viridogens strain HM10 Indian. J. Biochem.Biophy. 48:331335.

3. Ponce, A. A., & Klabunde, K. J. (2005). Chemical and catalytic activity of copper nanoparticles prepared via metal vapor synthesis. *Journal of Molecular Catalysis A: Chemical*, *225*(1), 1-6.

4. Le TTT, Fribourg-Blanc E, Dang MC. Synthesis and optical properties of copper nanoparticles prepared by a chemical reduction method. Adv Nat Sci Nanosci Nanotechnol 2011;2:015009.

5. Singh P, Katyal A, Kalra R, Chandra R. Copper nanoparticles in an ionic liquid: an efficient catalyst for the synthesis of bis-(4-hydroxy- 2-oxothiazolyl) methanes. Tetrahedron Lett 2008;49:727.

6. SoomroRA, SheraziSTH, Sirajuddin, MemonN, ShahMR, KalwarNH, *et al*. Synthesis of stable copper nanoparticles and their use in catalysis. Adv Mat Let 2014;5:191-8.

7. Lee HJ, Lee G, Jang NR, Yun JH, Song JY, Kim BS. Biological synthesis of copper nanoparticles using plant extracts. Nanotechnol 2011;1:371-4.

8. Njoku, V. O., & Obi, C. (2009). Phytochemical constituents of some selected medicinal plants. *African journal of pure and applied chemistry*, *3*(11), 228-233.