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Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2021 18(2): 1752-1757. **OPEN ACCESS**

Green synthesis of Fe and Cu nanoparticles from leaf extract of *Euphorbia heliscopia* and their antibacterial activity

Taj Ur Rahman*, Zohra Khatoon, Muhammad Aurang Zeb, and Wajiha Liaqat

Department of Chemistry, Mohi-Ud-Din Islamic University, AJ&K, **Pakistan**

***Correspondence:** taj_urrehman81@yahoo.co.uk **Received: 21-02-2021, Revised: 25-06-2021, Accepted: 27-06-2021 e-Published: 29-06-2021**

Nanotechnology is very effective in every field of life. The current research aims to prepare NP's of Fe and Cu using plant extract of *E. heliscopia*. The synthesized NP's were characterized by established modern characterization techniques such as XRD, EDX, and SEM. XRD technique was applied to examine phase detection and unit cell dimensions of prepared NP's. EDX was used for elementary reasoning or chemical description of NP's. SEM images showed that the synthesized Fe and Cu NP's are sphere-shaped with a particle size range of 1µm in clustered conditions. The prepared NP's were tested against different bacterial strains. Both Fe and Cu NP's exhibit good antibacterial activity. The antibacterial results obtained revealed that the Fe NP's possess maximum inhibition of 18.91mm against *P. aereginosa* while Cu NP's showed maximum inhibition of 17.6 mm against *B. cereus.*

Keywords: Nanoparticles; Plant extract, Metals, *E. heliscopia*, Antibacterial

INTRODUCTION

The family *Euphorbiaceae*, also known as the spurge family, is one of the largest families of flowering plants conspicuously throughout the tropics and composed of over 300 genera and 8000 species (Scholz, 1964; Webster, 1994). The family is very diverse in range, distribution, and morphology and is composed of diverse species that grow on mainland as small/large shrubs, small/large weeds, and large/woody trees, or as climbing lianas (Webster, 1994). *Euphorbia* is among the largest genera of flowering plants reported in this family (*Euphorbiaceae*) and contains several other subgenera and sections with over 2000 species (Andrea and Judit, 2014). The species in this genus, and to a larger extent of the spurge family, are characterized by the production of a milky irritant latex (Scholz, 1964).

E. helioscopia is an important Chinese medicinal herb belong to the family *Euphorbiaceae*. Plants of Euphorbia have been used in the traditional medicine for treatment of tumours, cancers, and warts for hundreds of years. It is well known that they contain irritant and tumor-promoting ingredients (Waheed et al. 2021). *E. helioscopia* is used in the treatment of various ailments like migraine, intestinal parasites, warts cure, gonorrhea, skin diseasesm (Webster, 1994; (Kind and Evans, 1975). Traditional uses of stems and leaves of *E. helioscopia* are febrifuge and vermifuge actions, seeds oil is used in constipation, roosted pepper mixed with seeds are used in cholera, and roots are being used as anthelmintic (Wu et al. 1991; Panda, 2004).

The ethanolic extract of this specie has been reported to possess strong antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeroginosa* (Mohammad et al. 2018). The secondary metabolites of *E. helioscopia* are reported which include tannins, amino acids, flavonoids, terpenoids, and steroids (Zhang and Gou, 2006; Aslam et al. 2014).

The present work aimed to synthesize Fe and Cu NP's from leaf extract of *E. heliscopia* and to evaluate their antibacterial activity.

MATERIALS AND METHODS

Plant material

The medicinal plant botanically classified as *E. heliscopia* was collected from hilly areas of Azad Jammu and Kashmir in the month of June and July 2018. A voucher specimen No ZK-1234 was deposited in the Herbarium of Botany Department, Abdul Wali Khan University Mardan, Khyber Pakhtunkhwa, Pakistan. The plant sample was shade dried for two months. The dried roots were chopped, crushed, and powdered. The powdered material was soaked in ethanol, with occasional stirring at room temperature for two weeks.The ethanol extract was filtered, separated, and concentrated by vacuum rotary evaporator. The dark brown extract obtained was further processed for various experiments.

Preparation of Fe and Cu nanoparticles

Preparation of Fe nanoparticles

50 ml of sample of *E. heliscopia* extract was taken in a beaker and 30 ml of iron chloride solution (0.1mM) was added and mixed well until precipitate was formed. The colour of precipitate was changed to brownish black. As the precipitate appeared, the solution was stirred using electric stirrer until the NP's formed came to the surface of solution. The NP's were filtered through filter paper and the NP's formed were washed with distilled water. The particles were allowed to get dried for one or two days and were stored for further analysis.

Preparation of Cu nanoparticles

50 ml of sample from *E. heliscopia* extract was taken in a beaker and 30 ml of $CuCl_2$ solution (0.1mM) was added and mixed with the help of a stirrer. Stirring was continued until precipitates formed in the solution. 5 ml of 0.01 solution of NaOH was added to keep the pH constant. Precipitates showed a salient alteration in the colour of the solution. Precipitates formed were filtered with the help of filter paper followed by washing with distilled water (Umar et al. 2012). The prepared NP's were seen in wet form. The NP's were dried for about 2 days in shade and were kept for further analysis.

Characterization of nanoparticles

Nanoparticles were characterized by using different modern characterization techniques like Powder X-ray Diffractometer (XRD), EDX and Scanning Electron Microscopy (SEM).

Antibacterial Activity

The antibacterial activity of synthesized nanoparticles was evaluated by Ager well diffusion method (Kora et al. 2012).

RESULTS AND DISCUSSION

XRD analysis of Fe nanoparticles

X-ray diffraction (XRD) technique was applied to examine changes of phase construction and crystalline amount of prepared NP's. The diffracto gram of XRD (Figure 1) of Fe NPs showed hexagonal wurtzite structure with the data 11.02, 18.48, 14.01, 19.19 and 18.34. Through Debye - Scherrer formula the average size of Fe Nanoparticle calculated was 16.208 nm. In these analyses XRD peaks were wide which showed the crystal-lite size in minute variety. The diameter D of nanoparticles using XRD was designed by using Debye -Scherrer formula $D = K\lambda / (\beta\cos\theta)$, in this equation D represented average crystal-lite size, K was Scherrer constant. The value of K was 0.94. The λ tells X-ray wavelength and its value was 0.15418, β showed peak thickness of half paramount and θ represent Bragg diffracting angle. The d spacing was found in various values ranging from 1.24Å-2.6Å (Kim and Hayashi, 2005).

Pos.[°2Th.]	Height[cts]	FWHM[°2Th.]	d-spacing [Å]	Rel.Int.[%]
33.8455	26.40	0.7872	2.64852	69.30
36.0392	33.55	0.4723	2.49218	88.08
39.5703	38.09	0.6298	2.27755	100.00
47.3295	31.60	0.4723	1.92069	82.96
76.4054	19.24	0.5760	1.24554	50.52

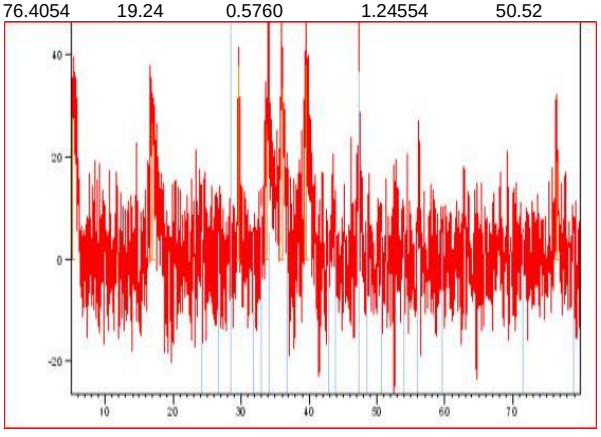

Figure 11: XRD analysis of Fe nanoparticles

XRD Analysis of Cu nanoparticles

The XRD technique was also applied to conclude the magnitude of Cu NP's. The X-ray diffraction peaks in the X-ray diffractogram (Figure 2) of synthesized Cu NP's using the *E. heliscopia* extract were 21.12, 13.32, 36.35, 11.02 and 18.48. The average size of copper NP's were obtained by using Debye–Scherer's rule D = Kλ/ (βcos θ), where D illustrated precious crystal magnitude, K is the Scherr's constant and its value is 0.9, λ represent the X-ray wavelength and its value is 0.15418. All the values were obtained from data given below. The XRD spectrum justified that the synthesized Cu NP's using the *E. heliscopia* extract are crystalline in nature. The crystal magnitude was measured using Scherrer' formula and was found to be almost 20.58 nm. The d spacing was found in various values ranging from 2.4Å to 16.9Å (Surmawar et al. 2011).

Pos.[°2Th.]	Height [cts]	FWHM[°2Th.]	d-spacing [Å]	Rel.Int.[%]
5.2023	27.74	0.3936	16.98745	72.82
16.8675	26.62	0.6298	5.25641	69.89
29.6387	37.88	0.2362	3.01415	99.44
33.8455	26.40	0.7872	2.64852	69.30
36.0392	33.55	0.4723	2.49218	88.08

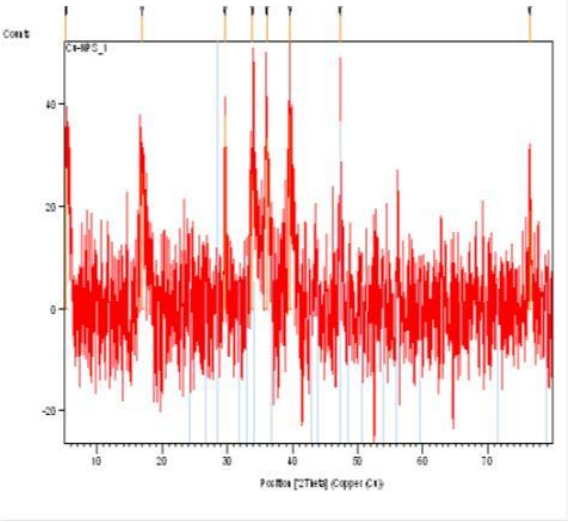

Figure 2: XRD analysis of Cu nanoparticles

EDX results of Fe nanoparticles

The EDX spectrum of Fe NP's obtained confirms the presence of synthesized Fe NP's (Figure3).

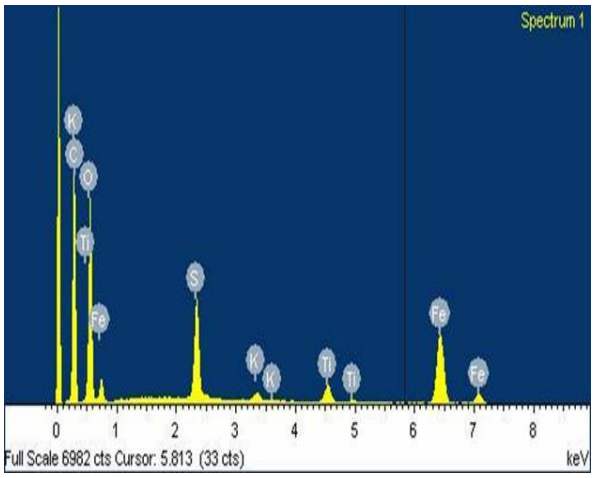

Figure 3: EDX of Fe nanoparticles

EDX results of Cu nanoparticles

The EDX spectrum of Cu NP's obtained confirms the presence of Cu NPs (Figure 4).

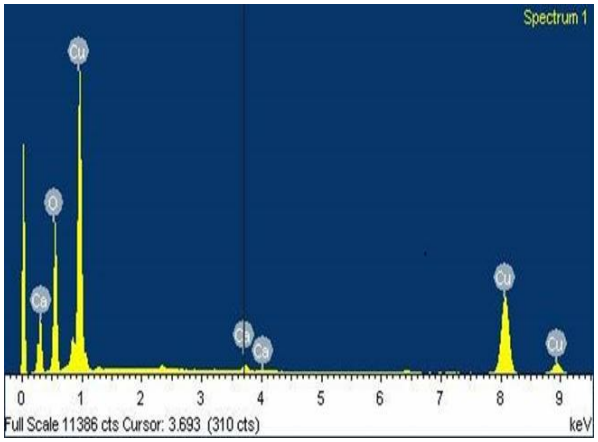

Figure 4: EDX of Cu nanoparticles

SEM analysis of Fe nanoparticles

Scanning electron microscopy (SEM) was used to study the synthesized Fe NP's from *E. heliscopia* extract. SEM images of Fe NP's are given in (Figure 5). The SEM image showed that synthesized Fe NP's are sphere shaped with particle size range 1µm in clustered condition. The data obtained was compared with already reported literature data of synthesized iron nanoparticle and was found to be in close agreement with the literature value (Feng and Lim, 2007).

SEM images of Cu nanoparticles

The SEM results indicated the existence of sphere-shaped Cu NP's. The crystalline particles range in size of 1µm in clustered form is shown in the (Figure 6).

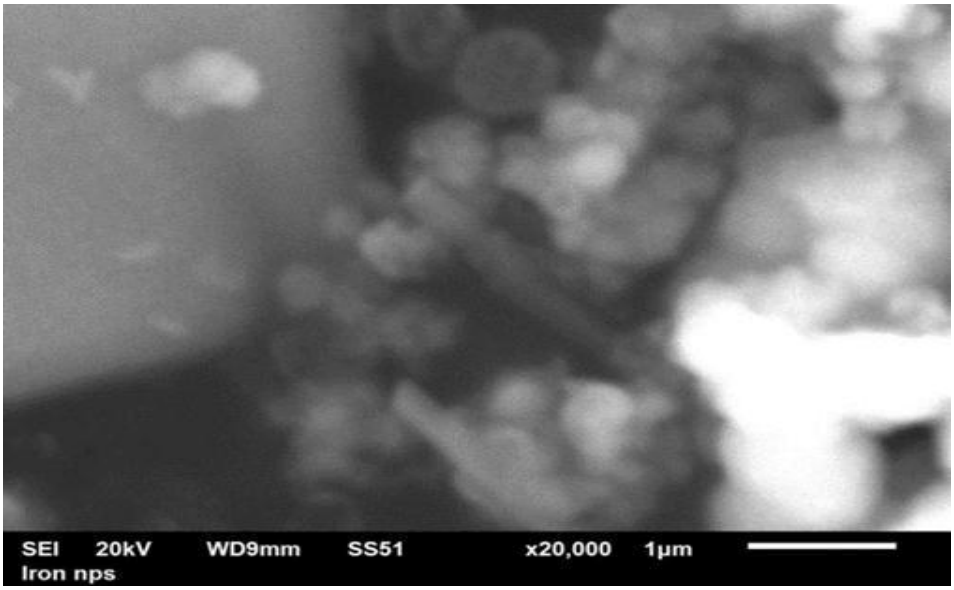

Figure 5: SEM image of Fe nanoparticles

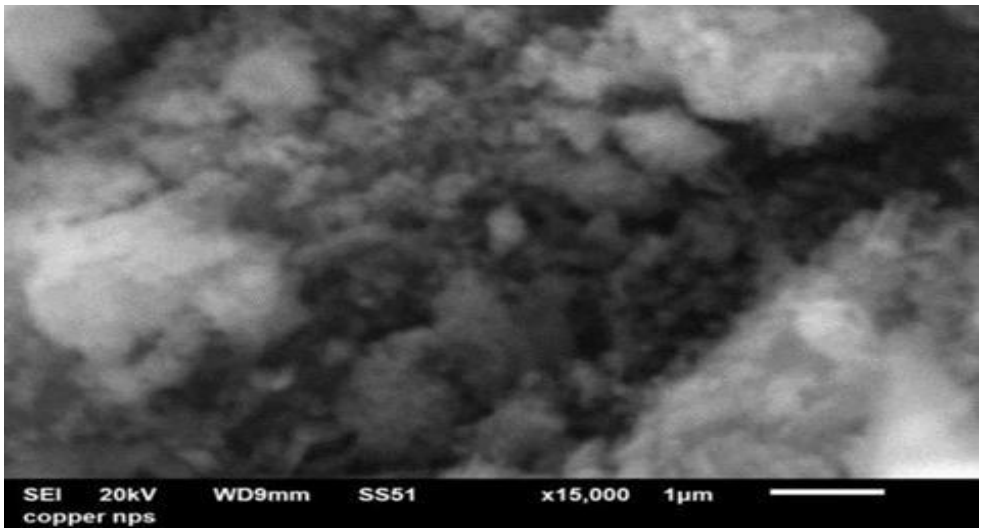

Figure 6: SEM images of Cu nanoparticles

Antibacterial activity of synthesized nanoparticles

The green synthesized nanoparticles of Fe and Cu were tested against different strains of bacteria such as *S. aureus, B. cereus, P. aereginosa, K. pneumoniae*, *E. coli* and *H. influenza.* The results obtained are given in (Table 1) which showed that Fe nanoparticles exhibit maximum inhibition of 18.91 mm against *P. aereginosa* while Cu nanoparticles displayed maximum inhibition of 17.6 mm against *B. cereus*. In this bioassay Ampicillin was used as standard drug a positive and DMSO was used as negative control.

Table 1: Antibacterial activity of synthesized NP's

S.No.	*S. aureus*	*B. cereus*	*P. aereginosa*	*K. pneumoniae*	*E. coli*	*H. influenza*
FeNPs	16.33±1.25	16.516.00±1.39	18.91±0.52	17.91±0.14	17.16±0.520	18.33±0.52
CuNPs	17.5±1.32	17.66±0.76	16.5±1.80	16.41±2.03	16.66±0.76	16.75±1.7
DMSO	0	0	0	0	0	0
Ampicillin	18.41±0.41	18.16±0.52	-	-	-	-

CONCLUSION

In the current study, Fe and Cu NP's were prepared using plant extract of the medicinally important herb named *E. heliscopia*. The synthesized NP's were characterized by various characterization techniques and were tested against different bacterial strains. The results of antibacterial activity revealed that the Fe NP's exhibit maximum inhibition of 18.91mm against *P. aereginosa* while Cu NP's displayed maximum inhibition of 17.6mm against *B. cereus*. The results obtained exposed the medicinal importance of this plant. However, further in vivo experiments must be carried out to explore its hidden medicinal importance.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

The authors are grateful to the Department of Chemistry, Mohi-Ud-Din Islamic University, AJ&K, Pakistan for providing lab facilities.

AUTHOR CONTRIBUTIONS

All the authors contributed equally.

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