Nanostructural characterization of antimicrobial and antioxidant copper nanoparticles synthesized using novel Persea americana seeds

S. Rajeshkumar , G. Rinitha

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Highlights

- Preparation of copper nanoparticles using Avocado seed extract
- Structural characterization of CuNPs using SEM, TEM, AFM and XRD
- Antioxidant, antibacterial and antifungal efficacy of CuNPs

Nanostructural characterization of antimicrobial and antioxidant copper nanoparticles synthesized using novel *Persea americana* seeds

Rajeshkumar S * and Rinitha G

Nano-Therapy Lab, School of Bio-Sciences and Technology
VIT, Vellore- 632014, TN, India

***Corresponds to: ssrajeshkumar@hotmail.com,**

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ABSTRACT

In this present investigation, we have synthesized the copper nanoparticles using the eco-friendly green method. This work explains that biosynthesis of copper nanoparticles by using *Persea americana* of seed extract with easier and green technique. The nanoparticles can be characterized by UV-Vis spectroscopy, XRD, FT-IR, SEM, and TEM. Copper nanoparticles are the spherical shape and crystalline in nature, the size range of these particles around 42 to 90 nm the elemental and chemical composition was characterized by FT-IR. The copper nanoparticles are having good antifungal and antibacterial activity against the diseases causing pathogens. The antioxidant activity of *Persea americana* mediated copper nanoparticles was evaluated by using DPPH assay.

Keywords: Copper nanoparticles; green synthesis; structure; antifungal; antibacterial; Antioxidant.

INTRODUCTION

Recently, the research works are mostly increases in the field of Nanotechnology because it plays an important role in many fields such as pharmaceutical, electrical, electronic industries, environmental remediation, consumer products and medical field etc. Nanoparticles are defined as the particles size less than 0.1µm the properties of the nanoparticles is based on the size of the nanoparticles [1]. Many researchers have attracted by metallic nanoparticles because of their excellent physiochemical [2], electronic chemical, [3] catalytic, optical, antifungal applications [4,5] mechanical, electrical, and thermal conduction properties [6] for past few decades much focus on CuNPs. Many routes are available to synthesis of copper nanoparticles those are physical, chemical, biological, thermal reduction, chemical reduction, polyol method, vacuum vapor deposition, microwave irradiation method, solvothermal method, microemulsion techniques, sonochemical reduction, microwave heating [7–9] compare to other methods chemical method is easy and cost-effective. Many researchers reported that the use of toxic materials leads to affect human and biological synthesis of copper nanoparticles is the best way to fabricate nanostructured materials, and also it reduces the toxic substance which affects the human health and environment [10,11,12]. Biosynthesis of metal nanoparticles has been done by using various source are micro-organisms like bacteria, fungi, yeast, and enzyme. The problem of this biosynthesis of metal copper nanoparticles was to purification and yield. Many plants are used to synthesize copper nanoparticles such as *Aloe barbadensis* [13], *Murraya Koenigii L* [14], *Acalypha indica* [15], *Calotropis procera* [10], *Ficus religiosa* [16], *Cyprinus carpio* [11], *Citrus medica Linn* [17], Among the above sources plant extract is the best route to synthesize the copper nanoparticles and also free from toxic chemicals as well as natural capping agent. Comparison to microorganism synthesis plant synthesis is cost effective method [18,19]. The copper nanoparticles can be easier and cost effective material and also is considered an alternative for gold and silver nanoparticles [8,20,21] mostly copper nanoparticles are insoluble form this may release copper ion into surrounding effects on aquatic organisms [10].In this study deals with the synthesis of copper nanoparticles by using *Persea Americana* (Avocado). Avocado belongs to the family Lauraceae and commonly known as alligator pear and butter fruit. Avocado contains carotenoid such as alpha-carotene, beta-carotene, zeaxanthin, neoxanthin, violaxanthin [22,23] lipophilic carotenoids has anti-carcinogenic effects [24] leaf of avocado has an activity to apoptosis in human breast cancer cells. The prostate cell line was inhibited by avocado tocopherols and carotenoids are present in that [25].The seed and root of the avocado plant contain antibiotic to prevent bacterial spoilage of food. It is used in pharmaceutical and cosmetics industry the avocado has benefit to human health as a balanced diet. It helps to reduce cholesterol and preventing cardiovascular diseases. This avocado fruits and leaves have activity against human lymphocytes [22,25].The beta-sitosterol in avocado has an immunity against diseases such as cancer, HIV, and infection. Long ago the plant avocado was used as medicine for the treatment of stomach ache, diarrhea, hypertension, bronchitis. Avocado contains potassium (345mg) and sodium (140mg) which help to maintain normal blood pressure and also rich in antioxidant and vitamins (vitamin C, E) reduce blood pressure in the hypertensive patient. It is a natural antioxidant due to the presence of glutathione and vitamin E [24]. Avocado has nutrition value as potassium, magnesium, zinc, copper, potassium, manganese, iron and vitamin such as A and B complex contains monosaturated fatty acid as well as lipid stigmasterol, campesterol, phytosterols and β-sitosterol. This β-sitosterol reduces LDL level and increase HDL level. The seed of avocado increases the immunity. This avocado has more phenolic compound help for dysentery and other GI tract problem and also prevents gastric ulcer, bacterial and viral infection. The flavonol in avocado protects from tumor growth. Avocado seed oil has the ability to slow aging process because it increases collagen in the human skin and free from wrinkle and the hair looks shiny. It has monounsaturated fats and low levels of sugar and sodium and improves glucose tolerance for the diabetic patient [22,24,26].

MATERIALS AND METHOD

Preparation of seed extract

Avocado fruits were purchased from Vellore market and the seed was collected and washed thoroughly using distilled water the seed was ground well by mortar and pestle and 1g of seed powder boiled with 100ml of deionized water for 5mins and allowed to cool at room temperature. The mixture of the extract was filtered through filter paper (what man No 1) and stored in the refrigerator for further use.

Synthesis of the CuNPs

The synthesis of copper nanoparticles, 20ml of seed extract was added with 80 ml of CuSO4 kept under constant stirring using magnetic stirrer at 45 to 50 °C for 6-7 hrs. At the end of the step brownish black color was obtained after centrifugation process the product was washed twice with de-ionized water and dried in hot air oven at 100°C for 3hrs. Finally, the dried powder was stored in properly labeled and used for further analysis.

Characterization of copper nanoparticles

The aqueous copper nanoparticles and the optical properties were characterized by UV-spectrophotometer (UV-2450, Shimadzu). The synthesized copper nanoparticles were identified by X-ray diffraction the Fourier transform infrared spectroscopy were analyzed the functional and chemical group (range of 4000-400cm) the size, shape and size distribution of the nanoparticles can be characterized by scanning electron microscopy (Model JSM 6390cm, JOEL, USA) and transmission electron microscopy (JEOL JEM-3100F).

Antimicrobial and antioxidant activity of CuNPs

The centrifuged aqueous copper nanoparticles were tested its antimicrobial efficiency by agar well diffusion method. The antibacterial activity of copper nanoparticles tested against four different bacterial isolates like *E. coli, Streptococcus sp, Klebsiella sp* and *Rhizobacterium* and antifungal activity was tested against some plant pathogens like *Aspergillus flavus, Aspergillus fumigates* and *Fusarium oxysporium*. Finally, the antioxidant activity of the copper nanoparticles was analyzed by DPPH assay. The experiments were conducted based on our previous studies [27,28].

Results and discussion

The study reports that the synthesis of nanoparticles when exposed to *P. americana* by observing changes the color from green to brownish black color shown in figure 1. At the first synthesis green color appeared and at the end of the synthesis brownish black color appeared. Due to the excitation of surface Plasmon vibration (SPR) color change arises with the metal nanoparticles.

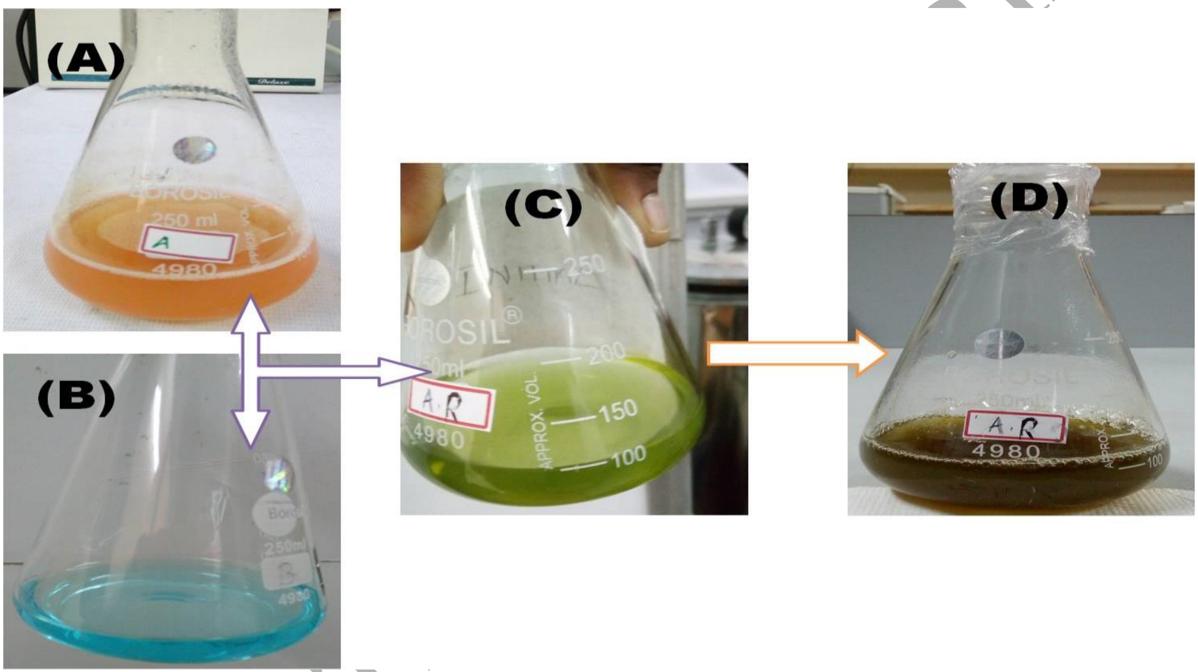

Figure 1: Visual observation of Copper nanoparticles synthesis (A) Seed extract (B) Copper sulfate solution (C) Initial color change (D) final color

UV-Vis studies

Fig .2 shows UV-VIS is the absorption spectra of biosynthesized Cu nanoparticles synthesized from *P. americana*. The photograph that shows a blue-green solution gradually turns to brownish black color the changing of color from the bluish green color of the CuSO4 solution to brown color is because of the surface Plasmon resonance. The surface plasma resonance absorbance was very sensitive to size and shape of the particles. So, it is observed that the SPR bands are located at the range 357nm which is characteristic absorption peak for copper nanoparticles. It is recognized that UV-Vis spectra are used to identify the size and shape of nanoparticles.

Ashtaputrey et al (2017) observed that the absorption peaks at 340 nm for copper nanoparticles by using *Murraya Koenigii* L leaves extract synthesis method [14].

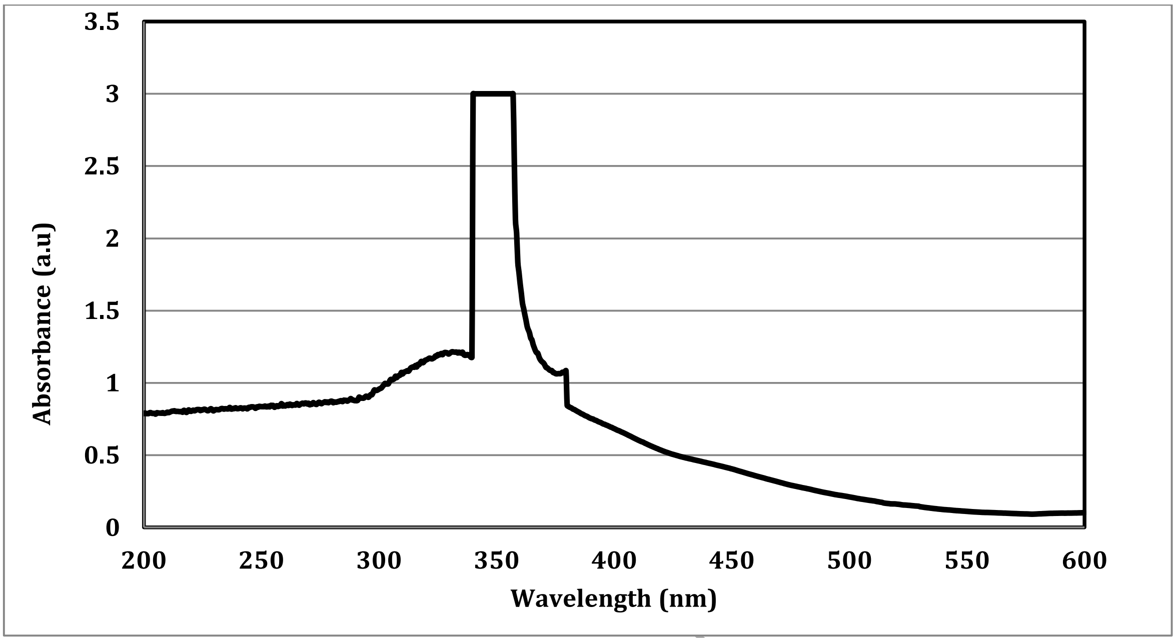

Figure 2. UV- Vis spectra of Cu nanoparticles

FT-IR studies

FTIR analysis was carried out to identify the biomolecules for capping and efficient stabilization of the metal nanoparticles synthesized by *P.americana* seed extract the FTIR spectrum of copper nanoparticles is shown in Figure 3 spectra of green synthesized copper nanoparticles. The peaks at 3250.20 cm-1, 2920.88 cm-1, 1618.69 cm-1, 1310.93 cm-1correspond to O-H stretch of carboxylic acid, H-C-H symmetric stretch of alkanes, C-C-C symmetric stretch of Alkenes respectively. Similar spectra are obtained for the nanomaterials produced via synthesis (B) the band observed at 3356.14 cm⁻¹, 1604.77 cm⁻¹, 1442.75 cm⁻¹, 1282.66 cm⁻¹, and have been referred to as O-H stretch of carbolic acid, C-C-C stretch of Alkenes, H-C-H bend of Alkanes and C-O stretch of Esters respectively. Reddy et al., 2016 reported that the occurrence of bands relevant to aromatic O-H stretching(3444 cm-1), carbonyl C=O bending(1780 cm-1) and C=O stretching (1087 cm-1) attributed to the asymmetric and symmetric C-H stretching vibration of flavonoids or phenolic compounds [29].

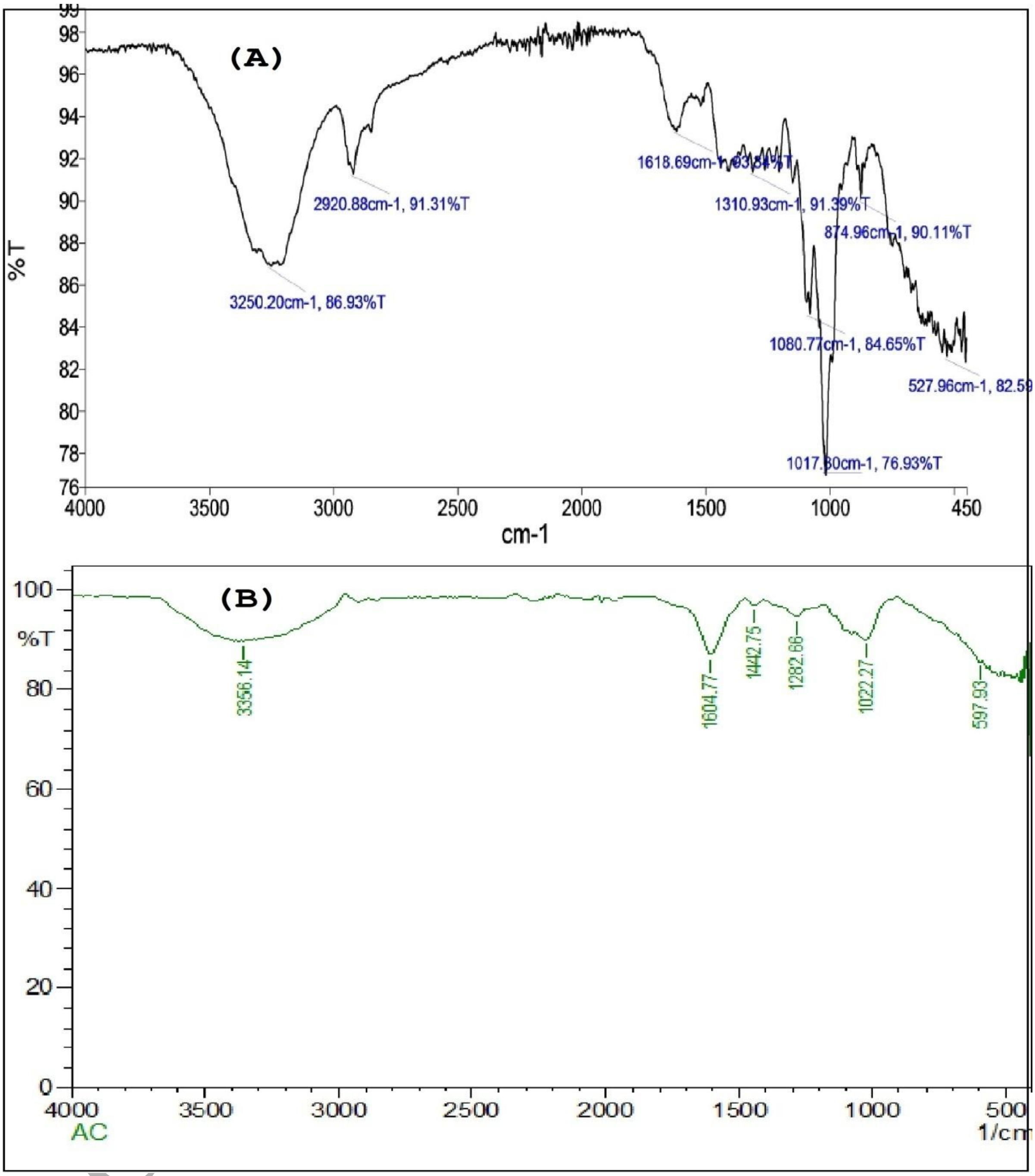

Figure 3. FT-IR spectrum of (A) Avocado (B) copper nanoparticles synthesized by avocado seed extract

EDS studies

The energy dispersive spectrum of biosynthesized copper nanoparticles recorded using Oxford software on SEM distinctly analyzes the elemental composition of the nanoparticles. Fig. 4 shows the EDS spectra of biosynthesized nanoparticles deposited on the carbon coated aluminum sheet. The signal from the EDS spectrum confirms the presence of copper. It indicates that the reduction of copper sulfate by P.americana seed extract. The weight composition of copper (Cu) is 26.06% and the atomic composition is then calculated as 21.36% respectively. The other impurity is found such as carbon, oxygen, potassium was identified, because of the interaction with the extract during bioprocessing. Patel, et al 2016 reported that the weight composition of copper and oxygen were 4.45% and 75.42% respectively. Atomic compositions were 1.29% and 86.65% and sodium, potassium, and carbon are considered as impurities [30].

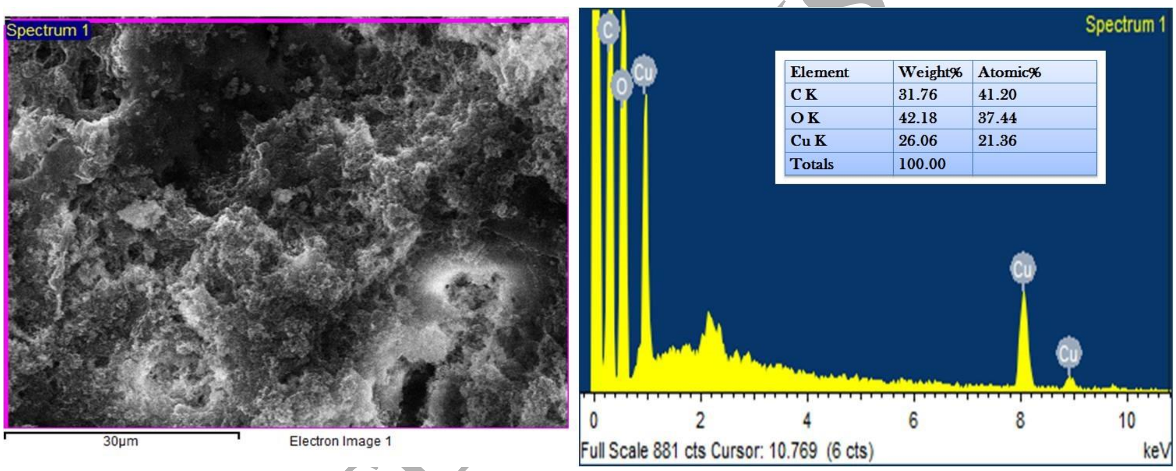

Figure 4: EDX spectrum of Cu NPs

XRD spectrum of Cu NP

XRD was performed for copper nanoparticles, using powder X-ray diffractometer Instrument shown in figure 5. There are Three diffraction peaks for Cu were observed 2θ = 24°, 30°, 42° correspond to (111), (111), (200) this proves that the formation of crystalline CuNPs. The pattern of XRD shows for the synthesis of copper nanoparticles at optimum conditions at pH 11. The average diameter of the copper nanoparticles is calculated and found to be in range 42 to 90nm by Scherrer formula are used to calculate FWHM value get from the diffraction peaks.

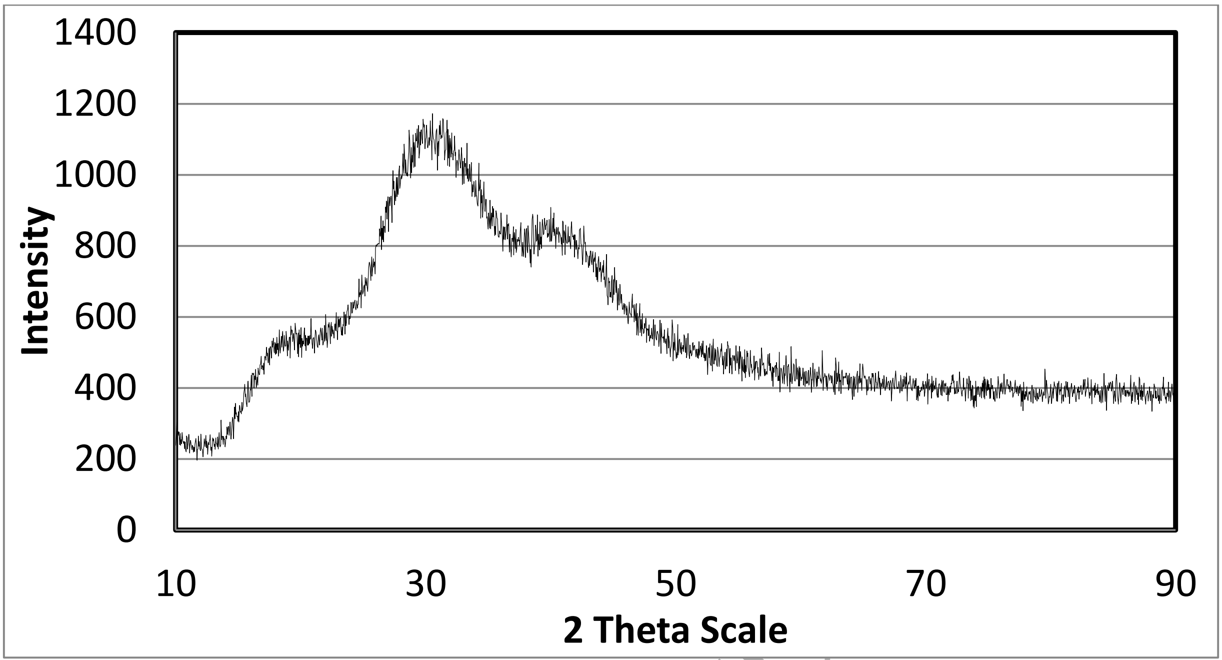

Figure 5 : XRD spectrum of Cu NP

SEM studies

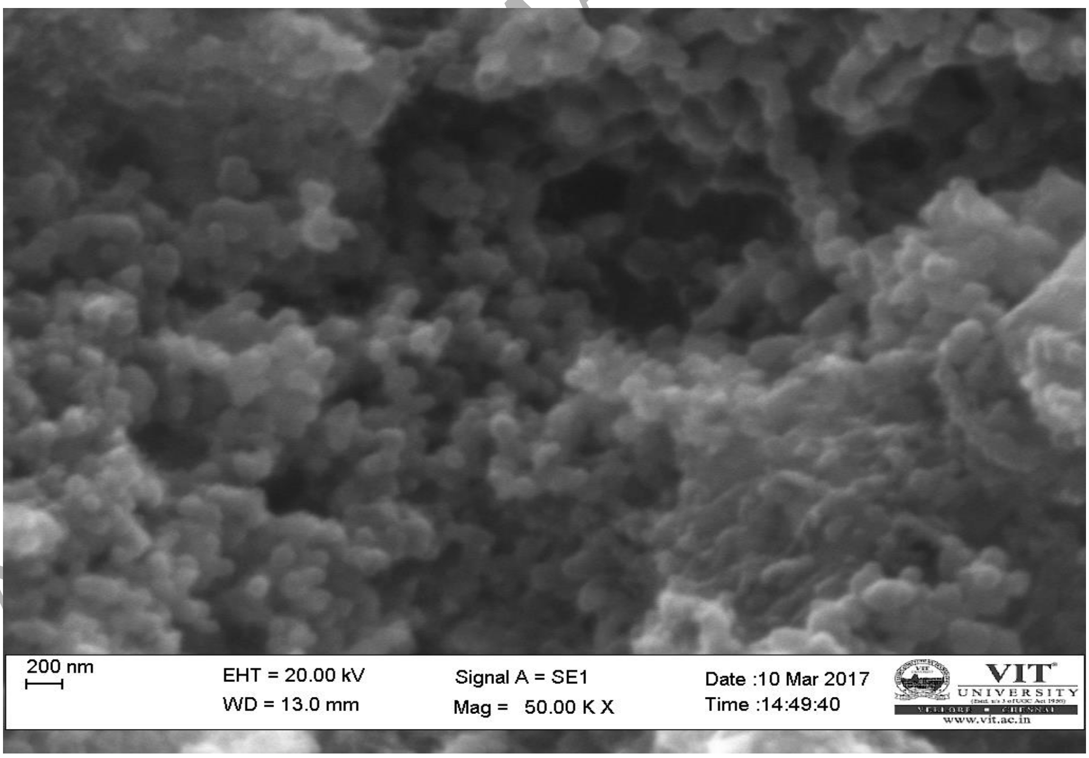

Figure 6: SEM images of copper nanoparticles synthesized using *Persea americana* seed extract

The SEM micrographs in Fig.6 explain well dispersed, versatile and spherical shape of Cu nanoparticles prepared with P. americana extract with the size range of particles from 42 to 90 nm. When the P. americana extract added with copper sulfate does not change nanoparticles shape but it increases the size of the nanoparticles mostly in higher concentration (50%) and aggregation for (0%) Persea americana extract. The nanoparticles assembled into very open, quasi-linear superstructure than a dense closely packed assembly Palaniselvan et al,.2017 state that the copper nanoparticles are spheroid in shape and polydisperse in nature and the particle size range between 45 and 100 nm [31].

TEM studies

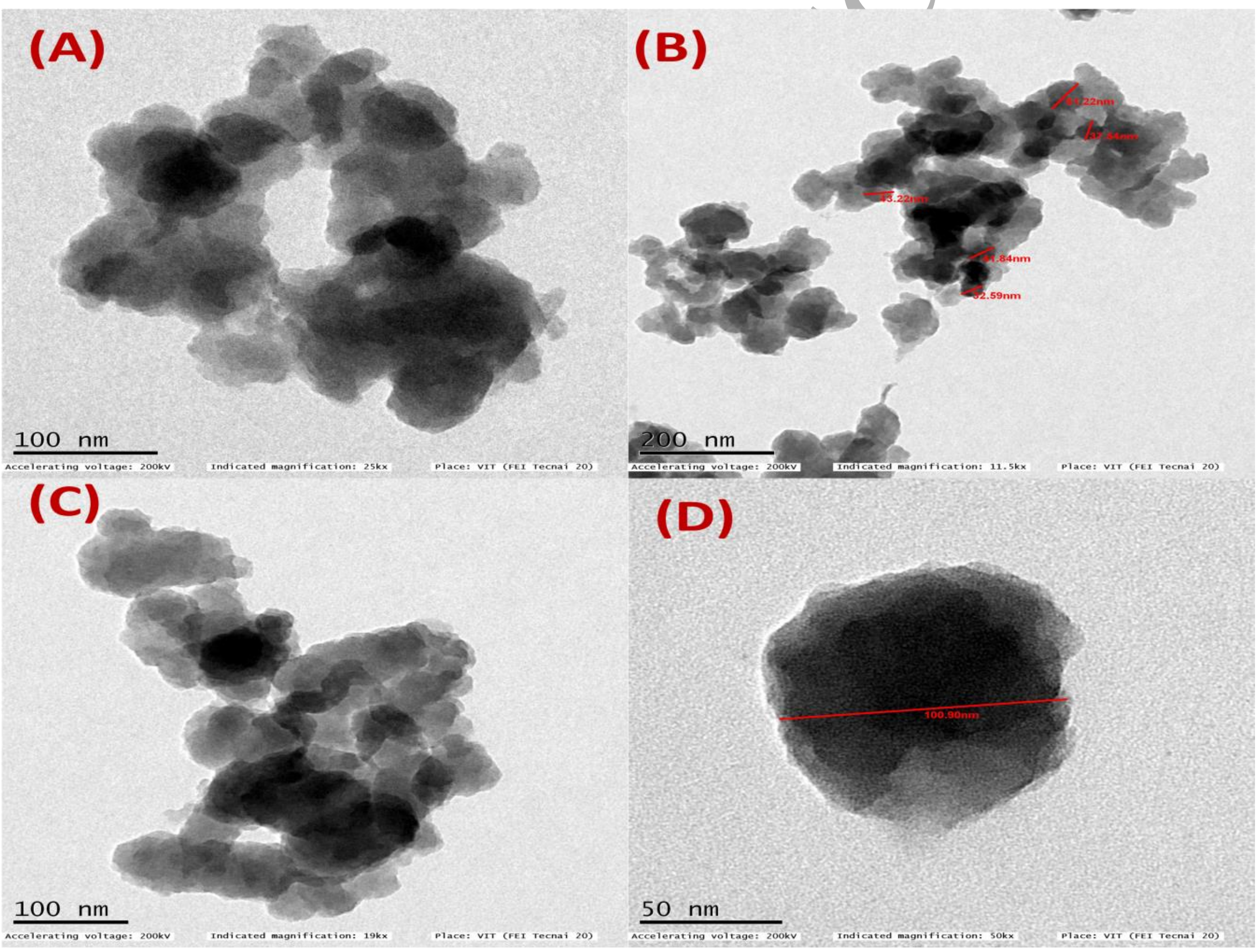

Figure 7: TEM Images of copper nanoparticles

TEM images show that particles are well dispersed, crystalline in nature clearly shown in figure 7. Copper nanoparticles are spherical in nature. The particles size was ranging from 40 to 90 nm, The TEM image shows that nanoparticles are not combined but are separated by equal interspace between the particles, which was confirmed by microscopy visualizing under the higher resolution. TEM image confirms that shape of copper nanoparticles is spherical in shape. This image explains that the copper nanoparticles are bounded with the phytochemicals of the plant extract [16].

AFM images of CuNPs

The AFM results are shown in figure 8 closely match with the SEM and TEM images. Most of the nanoparticles are spherical and some particles are the undefined shape. The SEM and image like backgrounds are found may be the phytochemicals of avocado seed extract.

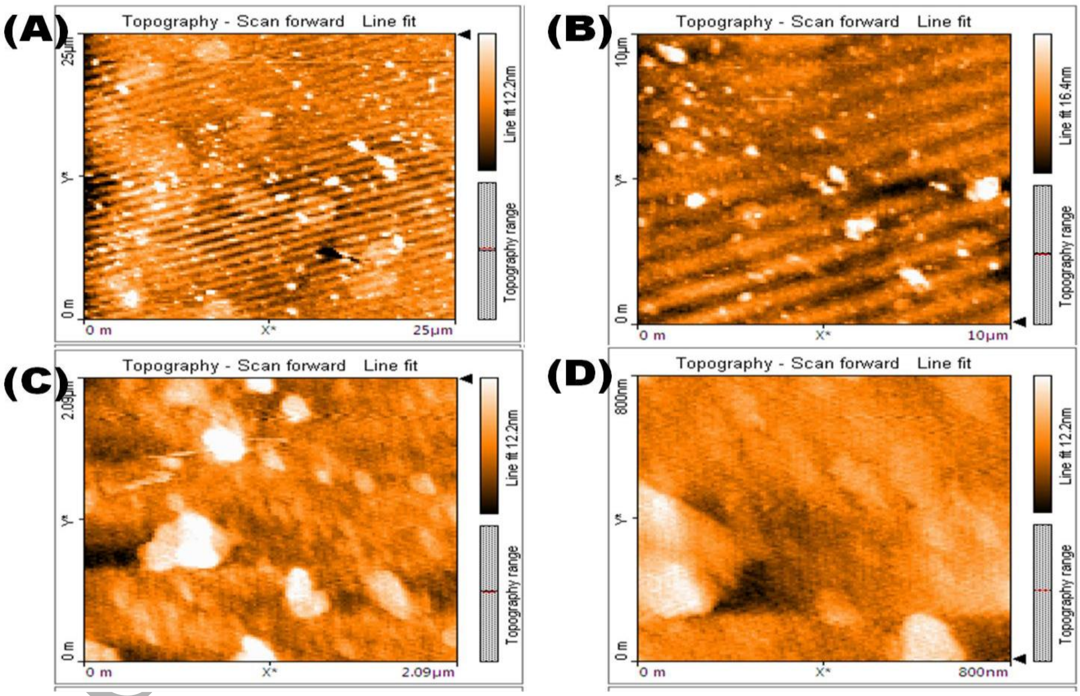

Figure 8: AFM images of CuNPs (A) 25 µm (B) 10 µm (C) 2.09 µm (D) 800nm

Antibacterial activity

The copper oxide nanoparticles having good antibacterial activity against both gram-positive and negative bacterial strains [19,30]. Figure 9 shows the results of antimicrobial activity of avocado mediated copper nanoparticles against pathogenic organisms. Maximum zone of inhibition was obtained in gram-positive bacteria *Streptococcus* with a zone diameter of 22.23±0.15 mm at a concentration of 75µl, and lowest zone of inhibition was observed in *Rhizobacterium* with a zone diameter of 9.27±0.15 mm at 25µl. Because of the maximum zone of inhibition in *Streptococcus* shows that the Avocado copper nanoparticles have the ability to control the *Streptococcus* causing infections such as wound and skin infection, sepsis, and endocarditis. The results of seed-mediated synthesis of copper nanoparticles show effective antimicrobial activity against disease-causing pathogenic bacteria.

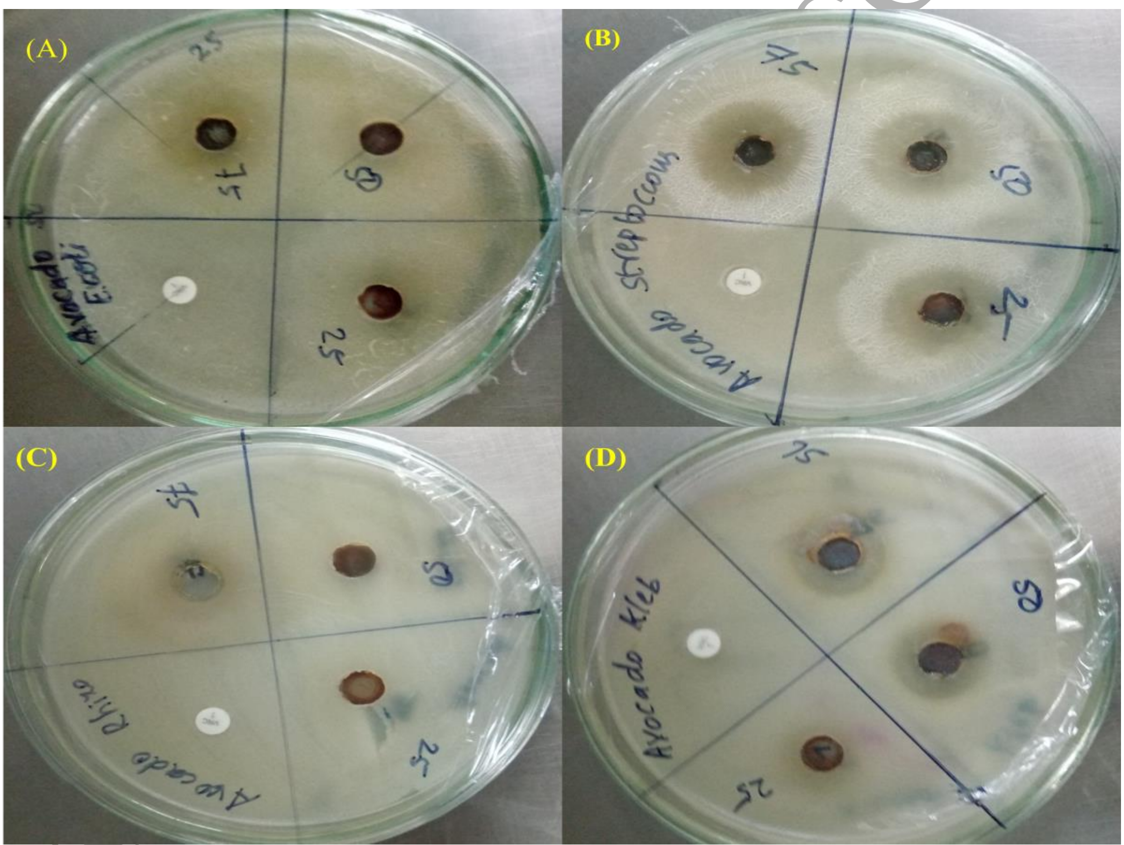

Figure 9: Antibacterial activity of Avocado of copper nanoparticles *(A) E.coli (B) Streptococcus (C) Rhizobacterium (D) Klebsiella sp*

Table 3: Zone of inhibition of copper nanoparticles against microorganisms

Concentration	**Zone of Inhibition (mm in diameter)**			
	E.coli	***Klebsiella sp***	***Rizhobacterium***	***Streptococcus sp***
25µL	09.30±0.33	12.05±0.55	9.27±0.15	14.08±0.63
50µL	11.15±0.65	18.63±0.33	10.23±0.54	18.56±0.33
75µL	15.06±0.13	20.16±0.13	12.09±0.16	22.23±0.15
Seed extract	07.00±00*	06.00±00*	07.33±0.67*	06.00±00*
Sterile water	06.00±00*	06.00±00*	06.00±00*	06.00±00*

* - No activity was noted

Antifungal activity

The antifungal activity was determined using the agar well diffusion assay method for different concentration of CuNPs (Fig. 10) against *A. niger*, *A. fumigatus*, and *F. oxysporum*. CuNPs have antifungal activity against fungal strains tested, similarly, these results indicate that CuNPs have excellent potential antifungal activity in treating fungal infectious diseases [14].

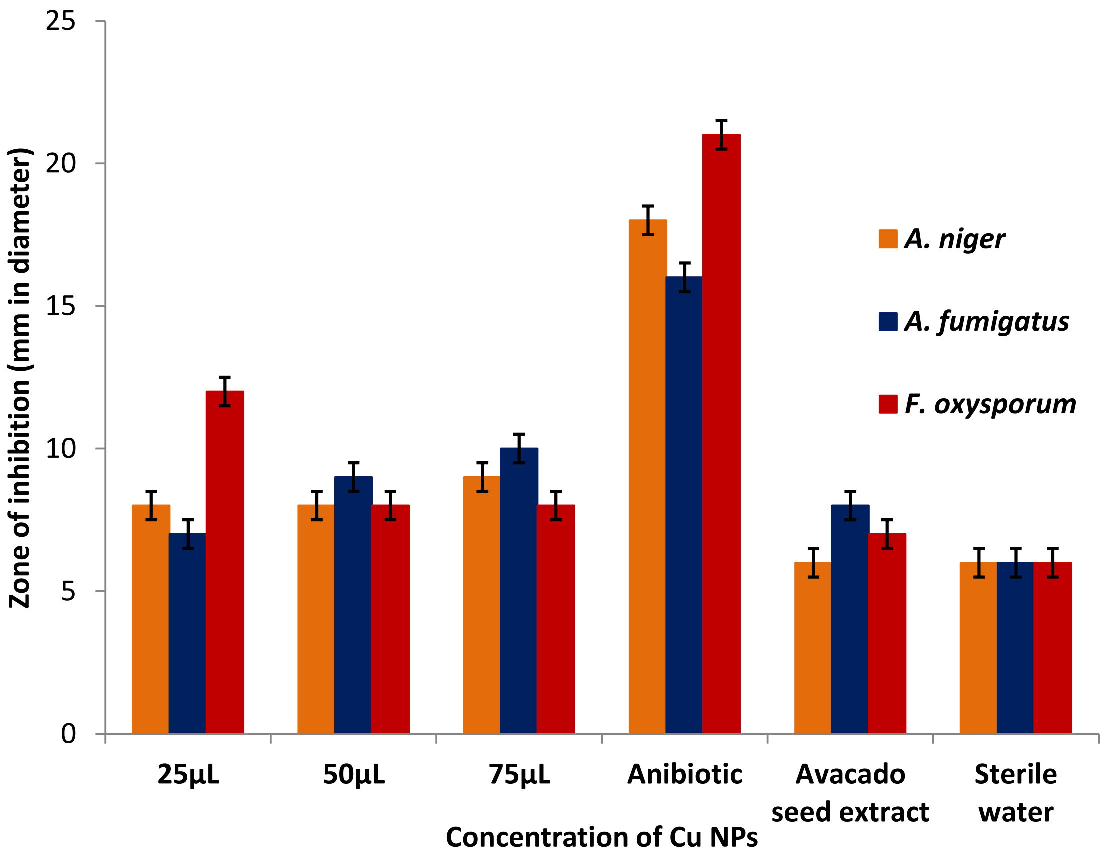

Figure 10: Antifungal activity of CuNPs

Antioxidant activity

The copper nanoparticles are continuously used for the advanced biomedical applications [32]. DPPH has been used extensively as a stable free to evaluate reducing substances and its useful reagent for investigation free radical scavenging activity of the component. The DPPH free radical scavenging activity of copper nanoparticles was shown in figure 11. The free radical scavenging activity of copper nanoparticles are very close to the standard ascorbic acid.

Table 4: Antioxidant activity of nanoparticles

	% of scavenging activity	
Sample concentration (µl)	**Ascorbic acid**	**Nanoparticles**
20	23	17
40	22	22
60	23	21
80	22	23
100	23	22

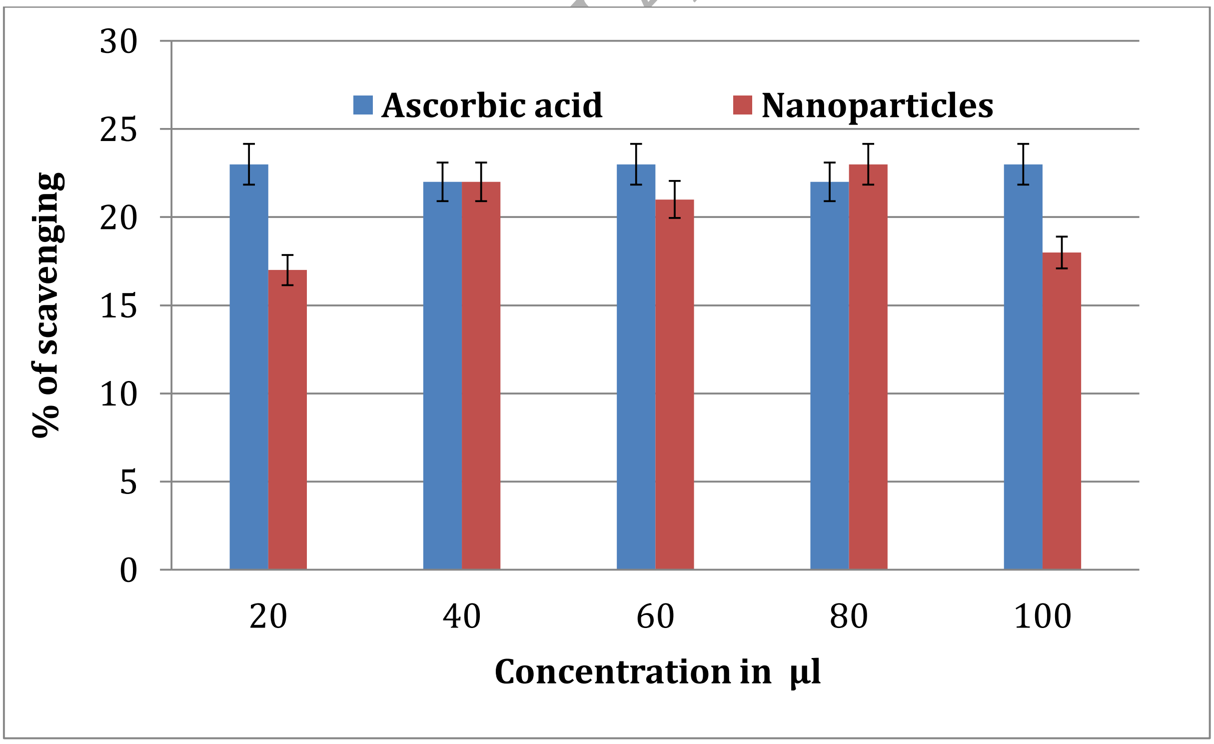

Figure 11: Antioxidant activity CuNPs by DPPH assay

Conclusion

In this work, we synthesized copper nanoparticles using the green route with avocado seed extract. It is the simple, cost-effective and eco-friendly method. The products of the nanoparticles are stable and reproducible. The synthesized nanoparticles can be characterized by UV-Vis, FTIR, XRD, EDS, AFM, SEM and TEM analysis. The biosynthesized copper nanoparticles are spherical in shape with the size range from 42 to 90 nm. These nanoparticles have an excellent antimicrobial activity against the plant disease pathogens are *Aspergillus niger, Aspergillus fumigatus, Fusarium oxysporum* and performed the antioxidant activity by using DPPH assay. Based on my study the copper nanoparticles are bio-medically important was proved. It may use as a very good medicine in future.

Conflict of interest

The author declared there is no conflict of interest

Acknowledgment

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