Int. J. Nano Dimens., 11 (4): 337-345, Autumn 2020

ORIGINAL ARTICLE

Biogenic synthesis of Copper nanoparticles using aquatic pteridophyte *Marsilea quadrifolia* Linn. rhizome and its antibacterial activity

Madhavan Ramyajuliet[1], Gnana raj Gnana Priyanka Beulah[2], Pious Soris Tresina[3], Asirvatham Doss[3] , Veerabahu Ramasamy Mohan[*3]

[1] Research Scholar (19212232262038), PG and Research Department of Botany, V. O. Chidambaram College, Thoothukudi, Tamil Nadu, India, Affiliated to Manonmaniam Sundaranar University, Tirunelveli
[2] Research Scholar (18112232262009), PG and Research Department of Botany, V. O. Chidambaram College, Thoothukudi, Tamil Nadu, India, Affiliated to Manonmaniam Sundaranar University, Tirunelveli
[3] Ethnopharmacology Unit, PG and Research Department of Botany, V. O. Chidambaram College, Thoothukudi, Tamil Nadu, India

Received 18 April 2020; revised 07 August 2020; accepted 19 August 2020; available online 22 August 2020

Abstract

The spread of contagious diseases and the increase in the drug resistance amongst pathogens has enforced the researchers to synthesize biologically active nanoparticles. Development of eco-friendly practice for the synthesis of nanoparticles is growing bit by bit in the field of nano-biotechnology. The present investigation outlines the development of a method to biosynthesize copper nanoparticles (CuNPs) by mixing copper chloride solution with aqueous rhizome extract of Marsilea quadrifolia. The synthesized nanoparticles were characterized by using the UV-vis spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), X-ray Diffraction (XRD) and Atomic Force Microscope (AFM). The UV-vis spectra showed an absorption band at 324 nm. The FTIR measurement revealed the presence of all functional groups having control over reduction and stabilization of the nanoparticles. The SEM micrograph depicts the morphology of biogenically synthesized CuNPs with leaf like structure. The X- ray diffraction pattern confirmed the formation of crystalline nature of CuNPs with an average size of 25.20 nm. Regular gravel like structure of CuNPs was displayed in the AFM image. Additionally, the biosynthesized CuNPs were found to be extremely toxic against two gram positive bacterial strains Bacillus thuringiensis and Streptococcus faecalis.

Keywords: *Antibacterial; Biogenic Synthesis; Copper Nanoparticles; Marsilea quadrifolia; Scanning Electron Microscopy (SEM).*

How to cite this article

Ramyajuliet M, Gnana Priyanka Beulah GR., Soris TresinaP., Doss A., Ramasamy Mohan V.. Biogenic synthesis of Copper nanoparticles using aquatic pteridophyte Marsilea quadrifolia Linn. rhizome and its antibacterial activity. Int. J. Nano Dimens., 2020; 11 (4): 337-345.

INTRODUCTION

Today, there is an ever-increasing attention of the biosynthesis of nanoparticles as an emerging highlight of the intersection of nanotechnology and biotechnology. It is due to a rising need to expand environment-friendly technologies in material synthesis. Biomolecules as reductants are found to have a significant advantage over their counterparts as protecting agents [1]. The need for biosynthesis of nanoparticles rises for the reason that the physical and chemical processes are expensive, unsafe, take a longer time, and they are tedious process to isolate nanoparticles. The application of plant extracts to the biosynthesis reaction is one of the vital branches of biosynthesis

* Corresponding Author Email: *vrmohanvoc@gmail.com*

of nanoparticles. Based on this method, the plant extract is used as a capping and reducing agent for the synthesis of nanoparticles [2-4].

Among different metal nanoparticles, copper nanoparticles (CuNPs) are used in a wide array of applications such as in electronics, air and liquid filtration, ceramics, wool preservation, textiles, bioactive coatings, skin products, films, lubricants oil and in inks [5-7]. Copper (Cu) is an active transition metal involved in many redox process in animal and plant cells. In plants, copper is a component of regulatory proteins, a cofactor of phenol oxidases, ascorbate oxidase, superoxide dismutase (SOD) and participates in electron transport in the respiratory and photosynthetic chains [8].

Marsilea quadrifolia Linn, a member of Marsileaceae is a creeping herbaceous perennial plant widely distributed in tropical and temperate regions of world and originated in marshy places and along the banks of canals and rivers [9]. As per the traditional claims the plant has been employed for astringent, hypnotic, diuretic, psychopathy, leprosy, hemorrhoids, skin diseases, expectorant, aphrodisiac, anodyne, ophthalmic, constipating, fever, insomnia, febrifuge, reduces mental tension inducing sleep, reduces anxiety and stress in emotional conditions [10]. The phytoconstituents like marsilin (1-triacontanol-cerotate), 3-hydoxytri-acontan-11-one, hentriacontan-6-ol, methylamine, betasitosterol, marsileagenin A, flavonol-O-mono-and-diglycoside, C-glucoylflavones and C-glucosylxanthones have been isolated from the plant [11]. Due to the presence of above active constituent, plant has been reported to possess various pharmacological activities. Hence, the present study was undertaken with an aim to synthesize the CuNPs via novel biological method using aquatic pteridophytic plant extract of *Marsilea quadrifolia* rhizome, in addition, the antibacterial activity of the CuNPs and the *M.quadrifolia* rhizome extract against pathogenic bacteria is assessed.

MATERIALS AND METHODS

Collection of Marsilea quadrifolia Rhizome

Marsilea quadrifolia Linn. rhizome was collected from Puthallam, Kanya Kumari District. The collected samples were cut into minute fragments and shade dried till the fracture is uniform and smooth. The dried material was granulated or powdered by using a blender and sieved using sieve No. 60 to get uniform particles. The powder finally obtained was employed for the extraction of active ingredients of the plant material. The plant samples were identified with the aid of local flora and validated by Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu, India.

Preparation of Extracts for Phytochemical Screening

Hot Maceration Method Using Soxhlet Apparatus

Freshly collected plant materials were dried in shade, and after that coarsely powdered in a blender. The coarse powder (100 g) was extracted successively with water, 250 mL in a Soxhlet apparatus for 24 hrs. The extract was filtered using Whatman No. 41 filter paper. The aqueous extract was subjected to qualitative tests for the identification of various phytochemical constituents as per standard procedures [12].

Green Synthesis of Nanoparticles

Preparation of Rhizome Extract (Reducing Agent)

The rhizome extracts were prepared by taking 20 gm of rhizome. Rhizome was washed thoroughly with double distilled water and cut into fine pieces. Then, the pieces are boiled in 100 ml double distilled water for 20 minutes at 60 °C in a glass beaker. After boiling the extract was filtered using Whatman No. 1.

Preparation of Precursors

Precursors for copper nanoparticles ($CuCl_2$ respectively) were purchased from Hi-media chemicals, India and prepared freshly. Precursor for preparing copper nanoparticles was 2 mM of copper chloride using double distilled water.

Synthesis of Copper Nanoparticles

In a typical reaction mixture, 10 mL of aqueous 2 mM copper chloride dehydrate $CuCl_2.2H_2O$ was treated with 10 mL of aqueous rhizome extract of *M. quadrifolia* and stirred magnetically at room temperature, until the light blue colour changes to light green colour. Then, the mixture is heated at 80 °C for 2 minutes. Afterwards, the mixture was treated with 1 M sodium hydroxide drop by drop. As soon as, the sodium hydroxide comes in contact with copper ions that spontaneously change the green mixture into brown precipitate, indicating the formation of water soluble mono dispersed copper oxide nanoparticles.

Characterization of the Synthesized Copper Nanoparticles

UV – Vis Spectroscopy

Ultraviolet-visible spectroscopy (UV-Vis) submits to absorption spectroscopy in the UV-visible spectral area. The copper nanoparticles were characterized in a Shimadzu V 650 UV- vis spectrophotometer. Being the scanning range for the samples at 300-700 nm, the double distilled water is made use as a blank reference.

Fourier Transform Infra-red Spectroscopy (FTIR)

The nanoparticles were characterized using a Fourier Transform Infra red Spectrophotometer (FTIR Thermoscientific iS5). Two milligrams of the sample were mixed with 100 mg Potassium bromide (KBr). Compressed to prepare a salt disc roughly 3 mm in diameter, the discs were at once kept in the sample holder. The FTIR spectra were recorded in the absorption range between 400 and 4000 cm^{-1}.

Scanning Electron Microscope (SEM) Analysis

The SEM is a kind of electron microscope (Carl Zeiss EVO 18) that images a sample by scanning it with an elevated energy beam of electrons in a raster scan models. This film of the sample was prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid. The extra solution was removed by a blotting paper and then the films on the SEM grid were permitted to dry by putting it below a mercury lamp for 5 min.

X-Ray Diffraction (XRD) Analysis

The particle size and nature of the copper nanoparticles were determined by using the XRD. This was done using Shimadzu XRD–6000/6100 model with 30 kv, 30 mA with Cukα radians at 2θ angle. X-ray powder diffraction is a fast analytical technique. This is chiefly used for phase identification of a crystalline material and can supply information on unit cell dimensions. The analyzed material is thinly ground, and the normal bulk composition is established. The particle or grain size of the copper nanoparticles was determined using Debye Sherrer's equation.

$D= 0.94 \lambda / B \cos \theta$

AFM Analysis

Surface topology of the synthesized copper nanoparticles were studied by 1µm x 1 µm Atomic Force Microscopy (AFM Nanosurf 2) analysis, 0.01 g synthesized nanoparticles were mixed with 20 ml of acetone and sonicated for 5-10 minutes using ultra sonicator. The solution was poured in a clean glass slide and was allowed to dry until all the acetone gets evaporated. Now, this glass slide is studied by using the Atomic Force Microscopy (AFM) in a non contact mode and the captured image was processed using the XEI software.

Antibacterial Activity

Antibacterial activity of synthesized nanoparticles was carried out by disc diffusion method [13]. The test bacteria *Bacillus thuringiensis, Streptococcus faecalis, Salmonella paratyphi* and *Escherichia coli* was obtained from the Research Laboratory, Department of Microbiology, Bharathidasan University, Tiruchirapalli, Tamil Nadu. The overnight incubated bacterial culture was spread over the freshly prepared nutrient agar plates. The 6 mm sterile disc (Hi media) was kept at the centre and different concentrations of synthesized nanoparticles (40 µg/mL, 80 µg/mL and 100 µg/mL) were poured on disc and then placed on the plate. The tetracycline disc (reference or positive control), copper solution without extracts and plant aqueous extract were also kept and then incubated at 37 °C for 24h and after incubation the zone of inhibition was measured.

RESULT AND DISCUSSION

Phytochemical Screening

The resulting preliminary phytochemical screening of aqueous extract of *M.quadrifolia* rhizome reveals the presence of various phytochemicals like, alkaloids, coumarin, flavonoid, phenol, saponin, tannin, terpenoid, sugar, glycoside and xanthoprotein (Table 1).

Synthesis of Copper Nanoparticles (CuNPs)

The first step in nanoparticles characterization is the transformation of solution colour (Fig. 1a, b, c). The reduction of copper ions into CuNPs using aqueous extract of *M.quadrifolia* rhizome is indicated in Fig. 1c. It has occurred so because of visual colour change of solution from light blue to green colour and then brownish (brown precipitate), indicating the formation of water soluble nanodispersed CuNPs. Colour change of reacting solution from green to reddish brown using fruit extract of *Zizyphus spina-christi* and

Table 1. Preliminary Phytochemical Screening of Aqueous Extract of *M. quadrifolia* Rhizome.

Phytochemicals	MR
Alkaloid	+
Anthraquinone	-
Catechin	-
Coumarin	+
Flavonoid	+
Phenol	+
Quinone	-
Saponin	+
Steroids	-
Tannin	+
Terpenoids	+
Sugar	+
Glycoside	+
Xanthoprotein	+
Fixed oil	-

+ Present - Absent MR - *Marsilea* Rhizome.

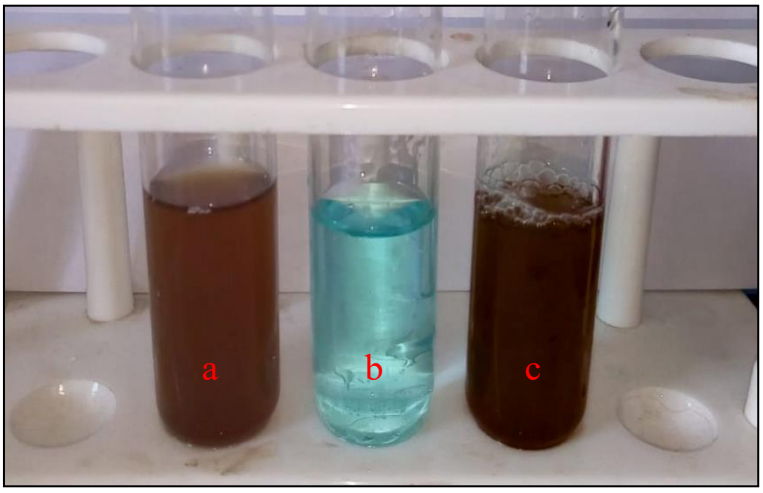

Fig.1. Synthesis of CuNPs from *M. quadrifolia* rhizome a) Plant extract b) Copper Chloride c) Copper nanoparticles.

leaf extract of *Ageratum houstonianum* was also confirmed by Khani *et al*. and Chandrakar *et al*. [14, 7].

Characterization Study of CuNPs

UV-Vis Spectroscopy

The formation of the CuNPs was confirmed mainly based on the colour change of reaction mixture and also by UV-Visible spectroscopy. The change of colour in aqueous solution is due to Surface Plasmon Resonance (SPR) Phenomenon. The position of the peak and shape of the spectra are using sensitive to metal size, dielectric constant of metal, nature of the reducing agent and stabilizer(s). In the present investigation, the UV-Vis spectrum of CuNPs confirmed absorption band at 324 nm (Fig. 2). This absorption band can be attributed to the plasma resonance absorption of the copper particles [15]. Similar observation has been reported in previous studies showing the absorption maxima of CuNPs at 384, 354, 350 and 326 nm using the flower extract of *Millettia pinnata* [16], leaf extracts of *Nerium oleander* and *Ageratum houstonianum* [17], *Cissus arnotiana* [18] respectively.

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR analysis is used to identify the possible bioactive molecule, which are accountable for the reduction of the copper ions, and the capping ability of the bioreduced CuNPs using *M.quadrifolia* rhizome extract. The FTIR absorption spectra have been shown in Fig. 3 and 4 to confirm the occurrence of different functional groups (Table 2)

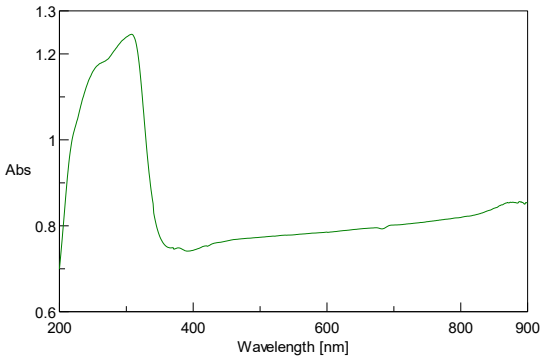

Fig. 2. UV-visible spectrum of CuNPs of *M. quadrifolia* rhizome.

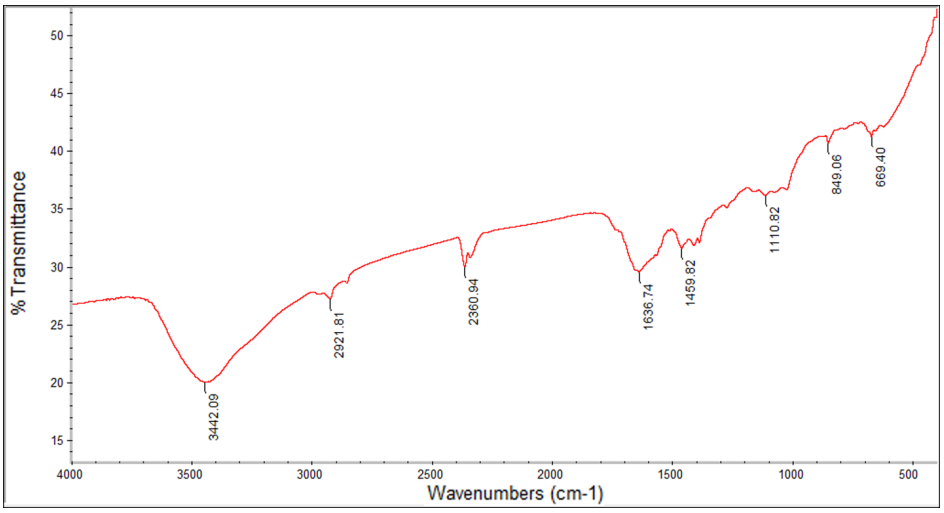

Fig. 3. FT-IR Spectrum of *M. quadrifolia* rhizome powder.

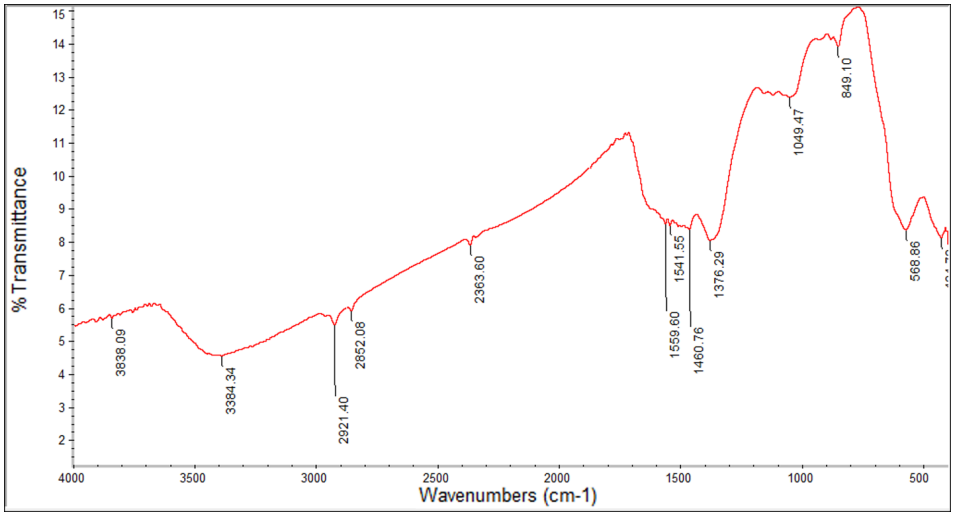

Fig. 4. FT-IR spectrum of CuNPs of *M. quadrifolia* rhizome.

Table 2. FT-IR Analysis of Powder and Synthesized CuNPs of *M. quadrifolia* Rhizome.

S.No.	Frequency (cm^{-1})	Chemical Bond	Phytoconstituents present	Peak Observed (Rhizome Powder)	Peak Observed (CuNPs)
1	3850-3500	O-H Stretch	Hydroxyl group	-	3838
2	3500-3200	O-H Stretch	Alcohols or Phenols	3420	3384
3	3300-2500	O-H Stretch	Carboxylic acid	2924	2921
4	3000-2850	C-H Stretch	Alkanes	2853	2852
5	2700-2250	NH^+ Stretch	Tertiary amine salt	-	-
6	1650-1550	>N-H bend	Secondary amine	1628	-
7	1600-1585	C-C Stretch (in ring)	Aromatics	-	1559,1541
8	1500-1400	C-C Stretch	Aromatics	1441	-
9	1390-1350	C-H rock	Alkanes	1383	1376
10	1360-1290	N-O Symmetric Stretch	Nitro Compound	-	-
11	1320-1000	C-O Stretch	Esters, Ethers	1155	-
12	1250-1020	C-N Stretch	Aliphatic amines	1028	1049
13	910-665	N-H Wag	1*,2* amines	-	-
14	900-675	C-H"OOP"	Aromatics	767	849
15	690-400	C-Br Stretch	Alkyl halides	617	568

in adsorbent. The FTIR spectra of *M.quadrifolia* rhizome powder (Fig. 3), which clearly shows the peak 3420 cm^{-1} corresponds to the O-H stretching hydroxyl group, peak at 2924 cm^{-1} represent O-H stretching carboxyl acid, peak at 2853 cm^{-1} assigned as C-H stretching of alkanes, peak at 1628 cm^{-1} represent > N-H band of secondary amine, peak at 1441 cm^{-1} corresponding to the C-C stretching of aromatics, peak at 1155 cm^{-1} represent C-O stretching of ester and esters, peak at 1028 cm^{-1} represent C-N stretching of aliphatic amines, peak at 767 cm^{-1} represents C-H 'oop' of aromatics and peak at cm^{-1} corresponds to the C-Br stretching of alkyl halides. Fig. 4 shows the FTIR spectrum of the biosynthesized CuNPs, peak at 3838 and 3884 cm^{-1} corresponds to the O-H stretching hydroxyl group/alcoholics or phenolics, peak at 2921 cm^{-1} represents O-H stretching of carboxylic acid, peak at 1599 and 1541 cm^{-1} represents C-C stretching (in ring) of aromatics, peak at 1376 cm^{-1} assigned as C-H rock of alkanes, peak at 1049 cm^{-1} represents C-N stretching of aliphatic amines, peak at 849 cm^{-1} represents C-H 'oop' of aromatics peak at 568 cm^{-1} assigned as C-Br stretching of alkyl halides. The small shift is observed in the absorbance peak in loaded nanoparticles on to *M. quadrifolia* rhizome powder in comparison with *M.quadrifolia* rhizome extracts (Fig. 3 and 4) .The broad band 3420 cm^{-1} is changed to 3838 and 3384 cm^{-1}. In addition, the peak 1628 cm^{-1}, 1441 cm^{-1},1383 cm^{-1}, 1028 cm^{-1}, 767 cm^{-1} are shifted to 1559 cm^{-1}, 1541 cm^{-1}, 1376 cm^{-1}, 1049 cm^{-1} and 849 cm^{-1} respectively. The results obtained from FTIR studies indicate that the phenolic compounds and flavonoids act as capping agents, thus presenting agglomeration and stabilize the formation of nanoparticles [19].

SEM Analysis of Copper Nanoparticles

The morphological analysis of Cu based nanoparticles, driven from *M.quadrifolia* rhizome by SEM, was observed to be leaf like structure (Fig. 5). According to the previous reports nanoparticles synthesized by plant extracts show different shapes which depend on the extract chemical compositions, concentrations and pH of media [20-22].

XRD Analysis of Copper Nanoparticles

Fig. 6 represents the XRD pattern of the synthesized the CuNPs using the aqueous extract of *M. quadrifolia* rhizome. The size, phase identification and crystalline nature of the CuNPs are determined by the XRD analysis. One intense and sharp peak at 2q=35.52°can then indexed to the 111 plane of Bragg's reflection of copper. Therefore, the XRD pattern indicates that the CuNPs are organized by the reduction of Cu^{2+} and Cu^+ ions. This reduction is by the aqueous extract of *M. quadrifolia* rhizome which is crystalline in nature. The size range of the synthesized CuNPs is 25.20 nm. This is consistent with findings published by Rama Devi *et al*. [23].

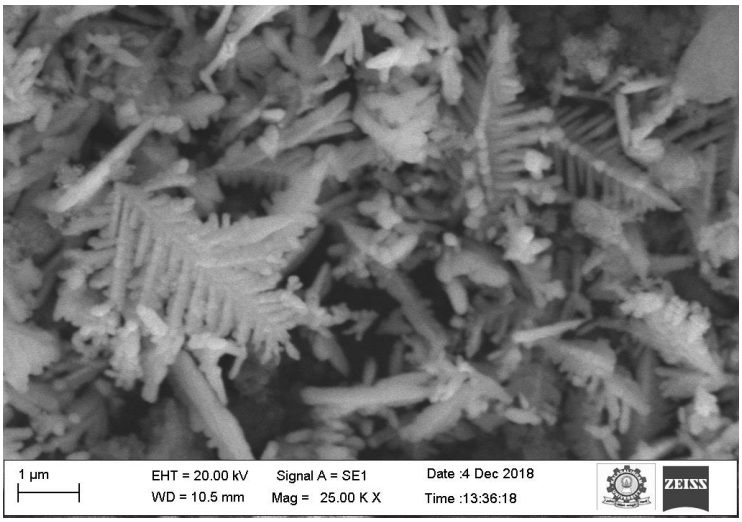

Fig. 5. SEM image of CuNPS of *M. quadrifolia* rhizome.

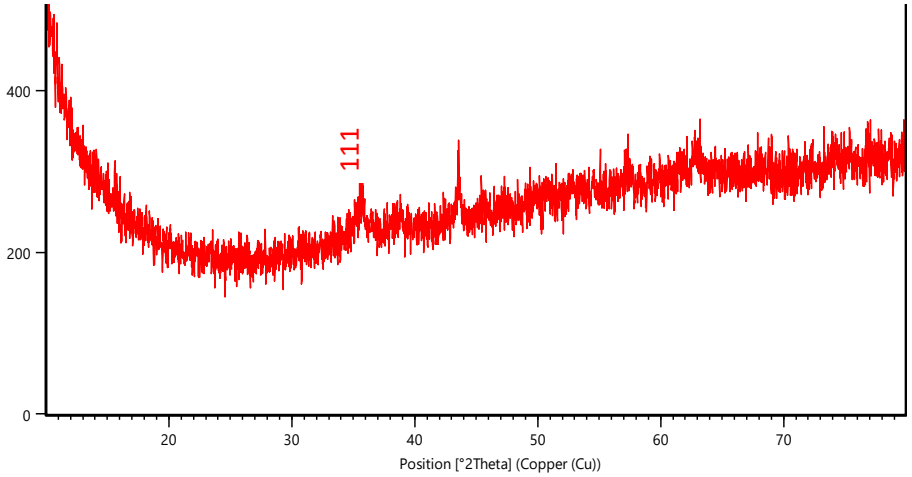

Fig. 6. XRD pattern of synthesized CuNPS of *M. quadrifolia* rhizome.

AFM Analysis of Copper Nanoparticles

An Atomic Force Microscopy (AFM) technique was employed to characterize the copper nanoparticles for its detail size and morphology of copper. The topographical image of the CuNPs synthesized by plant extract *M. quadrifolia* rhizome, are shown in Fig. 7 (a, b). The AFM image of CuNPs exhibited regular gravel like structure.

Antibacterial Activity

The antimicrobial activity of the CuNPs synthesized by *M. quadrifolia* rhizome extract was investigated against various pathogenic organisms such as *Bacillus thuringiensis* (+), *Streptococcus faecalis* (+), *Salamonella paratyphi* (-) and *Escherchia coli* (-). The diameter of inhibition zones (nm) around each disc with the CuNPs solution is represented in Table 3. Copper nanoparticles synthesized from *M. quadrifolia* rhizome showed greater inhibition zone 14mm and 15mm against *Bacillus thuringiensis* and *Streptococcus faecalis* at 100µg/ml concentration respectively. The antimicrobial activity was due to the fact that the copper ions released from the CuNPs permeated the bacterial cell membrane and damaged the structure of the cell membrane by disturbing the negatively charged cell wall [24, 25]. Copper ions are involved in cross linkage of nucleic acid strands by binding them with the DNA molecule of bacteria. These outcomes in a disordered helical

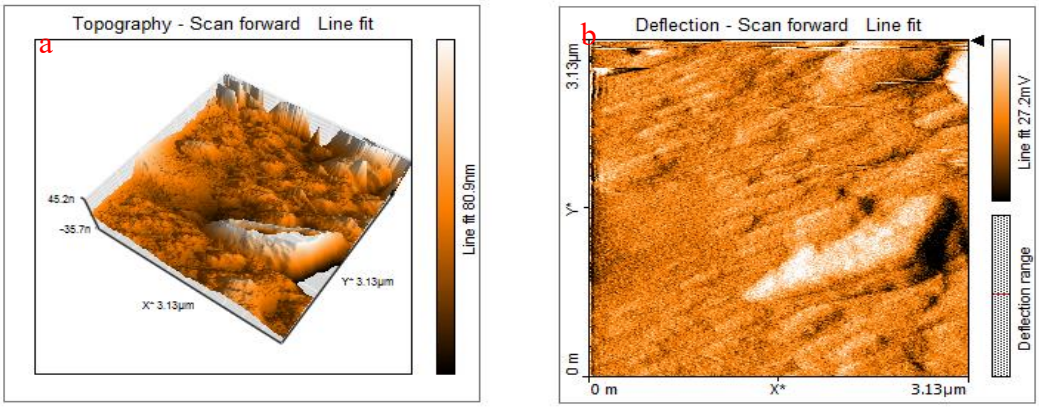

Fig. 7. AFM structure of CuNPs of *M. quadrifolia* rhizome. a)2D Topography, b) 3D Topography.

Table 3. Antibacterial Activity of Synthesized CuNPs of *M. quadrifolia* Rhizome.

Name of the Bacteria	*M. quadrifolia Rhizome* Aqueous Extract (100 µl)	Zone of Inhibition (mm) Tetracycline (30 mcg/disc)	Copper Chloride (100 µl)	CuNPs Different Concentration 40 µl	80 µl	100 µl
Bacillus thuringiensis	3	24	9	11	12	14
Streptococcus faecalis	4	22	8	10	13	15
Salamonella paratyphi	4	25	8	10	11	13
Escherichia coli	5	21	9	9	11	12

structure of DNA molecule, roots denaturation of proteins and some other biochemical procedures in the cell lead to total destruction of the bacterial cell [26].

In general, CuNPs synthesis has attracted particular interest, compared with other NPs, as their useful properties are achievable at cost lower than silver and gold [27]. Research into CuNPs has made significant progress in the areas of nanotechnology and nanomedicine with in the last decade due to their excellent antifungal/ antibacterial applications. In recent years, plant mediated biological synthesis of nanoparticles has gained interest due to its simplicity and eco-friendliness.

CONCLUSION

Biological synthesis of nanoparticles has increased in the field of nanobiotechnology. It creates novel materials that are eco-friendly cost effective and with a great significance to apply in the different areas. In this research, *M. quadrifolia* rhizome extract is used in green chemistry approach towards the synthesis of CuNPs. Phytochemicals studies confirm the presence of flavonoids and phenolics compounds, which are responsible for the formation of CuNPs. The structure of the synthesized nanoparticles has been appropriately characterized using UV-Vis spectroscopy, FT-IR, SEM, XRD, and AFM analysis. UV-vis spectra of CuNPs synthesized by *M. quadrifolia* rhizome extract revealed characteristic absorption peak at 324 nm. FT-IR analysis confirmed that phenolics compounds and protein molecules in the extracts are responsible for capping of the Cu nanoparticles. XRD pattern revealed the crystalline nature of the CuNPs. SEM studies confirmed the structure of the synthesized CuNPs. The AFM image had also confirmed the morphology of the CuNPs. Moreover, the synthesized CuNPs showed considerable antibacterial activity against the treated gram positive and gram negative bacterial strains. This is the first on the synthesis of copper nanoparticles using *M. quadrifolia* rhizome extract and their effect as antibacterial agents.

ACKNOWLEDGEMENT

The authors acknowledge Kalasalingam University, Krishnankoil for rendering their support to carry out the SEM analysis and the Department of Physics, Manonmaniam Sundaranar University, Tirunelveli to carry out the XRD analysis.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors

REFERENCES

[1] Nahar K., Aziz S., Beshar M. S., Haque Md. A., Al-Reza, S. Md., (2020), Synthesis and characterization of silver nanoparticles from *Cinnamomum tamala* leaf extract and its antibacterial activity. *Int. J. Nano Dimens.* 11: 88-98.

[2] Seku K., Ganapuram R. G., Pejjai B., Kotu, G. H., Narasimha G., (2018), Hydrothermal synthesis of copper nanoparticles characterization and their biological application. *Int. J. Nano Dimens.* 9: 7-14.

[3] Mohamed F., Yousefi M., Ghabremanzadeh R., (2019), Green synthesis, characterization and antimicrobial activity of silver nanoparticles (AgNPs) using leaves and stems extract of some plants. *Adv. J. Chem. Sec. A.* 2: 266-275.

[4] Tude S. C., Zubku M., Kusz J., Bhathacharjee A., (2020), Structural, morphological and optional characterization of green synthesized ZnS nanoparticles using *Azadirachta indica* (Neem) leaf extract. *Int. J. Nano Dimens.* 11: 99-111.

[5] Elisma N., Labanni A., Emriadi, Zrilda Y., Asorfi M., Arief S., (2019), Green synthesis of copper nanoparticles using *Uncaria gambir* Roxb. leaf extract and its characterization. *RASAYAN J. Chem*. 12: 1752-1756.

[6] Amatya S. P., Joshi L. P., (2020), Biosynthesis of copper nanoparticle (CuNPs) using garlic extract to investigate antibacterial activity. *BIBECHANA*. 17: 13-19.

[7] Chandraker S. K., Lal M., Chosh M. K., Tiwari V., Ghorai T. K., Shukla R., (2020), Green synthesis of copper nanoparticles using leaf extract of *Ageratum houstonianum* Mill and study of their photocatalytic and antibacterial activities. *Nano Exp.* 1: 010033.

[8] Nekrasova G. F., Ushakova O., Ermakov A. E., Uimim M. A., By Zov I. V., (2011), Effects of copper (II) ions and copper oxide nanoparticles on *Elodea densa* planch. *Russ. J. Ecol*. 42: 458-463.

[9] Pullaiah T., (2002), Medicinal Plants of India, vol. 2 (Regency Publications, Jodhpur, India, 2002) pp. 348-349.

[10] Longman, O., (1997), Indian medicinal plants, vol. 4 (Orient Longman Pvt. Ltd., Chennai, India, 1997) pp. 5-9.

[11] Khare C. P., (2004), Encyclopedia of indian medicinal plants, rational western therapy, ayurvedic and other traditional uses, botany (Springer, London, 2004) p. 303.

[12] Brindha P., Sasikala P., Purushothaman K. K., (1981), Pharmacognostic studies on merugan kizhangu. *Bull. Med. Eth. Bot. Res*. 3: 84-96.

[13] Bauer A. W., Kiruby W. M., Sherris J. C., Turck M., (1996), Antibiotic susceptibility testing by a standardized single disc method. *Am. J. Clin. Pathol.* 45: 493-496.

[14] Kheni R, Rosstaei B, Bagherzade G, Moudi M., (2011), Green synthesis of copper nanoparticles by fruit extract of *Ziziphus spina-christi* (L.) Willd: Application for adsorption on triphenylmethane dye and antibacterial assy. *J. Mol Liq.* 255: 541-549.

[15] Heera P., Shanmugam S., Ramachandran J., (2015), Green synthesis of copper nanoparticle using *Gymnema sylvestre* by different solvent extract. *Int. J. Curr. Res. Accd. Rw.* 3: 268-275.

[16] Thiruvengadam M., Ehung I. M., Gomathi T., Anseri M. A., Khanna V. G., Babu V., Rajakumar G., (2019), Synthesis, characterization and pharmacological potential of green synthesized copper nanoparticles. *Bioprocess Biosyst. Eng*. 42: 1769-1777.

[17] Gopinath M., Subbaiya R., Selvam M. M., Suresh D., (2014), Synthesis of copper nanoparticles from *Nerium oleander* leaf aqueous extract and its antibacterial activity. *Int. J. Curr. Microbio. App. Sci.* 3: 814-888.

[18] Rajeshkumar S., Menon S., Kumar S. V., Tambuwala, M. M., Bakshi H. A., Mehta M., Satijia S., Gupta G., Chellappan D. K., (2019), Antibacterial and antioxidant potential of biosynthesized copper nanoparticles mediated through *Cissus arnotiana* plant extract. *J. Photochem. Photobiol.* 197: 111531.

[19] Elumalai K., Velmurugen S., Ravi S., Kathiravan S., Ashok Kumar S., (2015), Green synthesis of zinc oxide nanoparticles using *Moringa oleifera* leaf extract and evaluation of its antimicrobial activity. *Spectrochim Acta A.* 143: 158-164.

[20] Chandran S. P., Chaudary M., Pasricha R., Ahamad A., Sastry M., (2006), Synthesis of gold nanotriangles and silver nanoparticles using *Aloe vera* plant extract. *Biotechnol. Prog.* 22: 577-583.

[21] Aromal S. A., Vidhu V., Philip D., (2012), Green synthesis of well-dispersed gold nanoparticles using *Macrotyloma uniforum*. *Spetrochim. Acta Mol. Biomol. Spectrosc.* 85: 94-104.

[22] Khalil M. M., Ismail E. H., Magdoub E. I. F., (2012), Biosynthesis of Au nanoparticles using olive leaf extract: 1st nano updates. *Arab. J. Chem*. 5: 431-437.

[23] Ramyadevi J., Jeyasubramanian K., Marikani A., Rajakumar G., Rahuman A. A., (2012), Synthesis and antimicrobial activity of copper nanoparticles. *Mar. Left.* 71: 114-116.

[24] Ren G., Hu D., Chang E. W., Vangas-Reus M. A., Reio P., Allakar R. P., (2009), characterization of copper oxide nanoparticles for antimicrobial applications. *Int. J. Antimicrob. Agents.* 33: 587-590.

[25] Sankar R., Manikandan P., Malarvizhi V., Fathima T., Shivashangari K. S., Rajkumar V., (2014), Green synthesis of colloidal copper oxide nanoparticles using *Carica papaya* and its application in photocatalytic dye degradation. *Spectrochim. Acta Mol. Biomol. Spectrosc*. 121: 746-750.

[26] Yallappa S., Manjanna J., Simdhe M. A., Sathyanarayan N. D., Pramod S. N., Nagara K., (2013), Microwave assisted rapid synthesis and biological evaluation of stable copper nanoparticles using *T. arjuna* bark extract *Spectrochim Acta Mol. Biomol. Spectrosc.* 110: 108-105.

[27] Chung I. M., Rahuman A. A., Marimuthu S., Kirthi A. V., Anbarasan K., Padmini P., Rajakumar G., (2017), Green synthesis of copper nanoparticles using *Eclipta prostrata* leaves extract and their antioxidant and cytotoxic activities. *Exp. Therap. Med.* 14: 18-24.