J Nanostruct 7(3): 165-174, Summer 2017

RESEARCH PAPER

Phytosynthesis and Biological Activities of Fluorescent CuO Nanoparticles Using Acanthospermum hispidum L. Extract

Shreyas Pansambal [1], Keshav Deshmukh [1], Ajay Savale [2], Suresh Ghotekar [2]*, Onkar Pardeshi [3], Gotan Jain [4], Yogesh Aher [4] and Dattaprasad Pore [5]

[1] Department of Chemistry, S.N. Arts, D.J.M. Comm. and B.N.S. Science College, Sangamner, Savitribai Phule Pune University, Maharashtra 422 605, India

[2] Department of Chemistry, KKHA Arts, SMGL Comm. and SPHJ Science College, Chandwad, University of Pune, Maharashtra 423 101, India

[3] Department of Electronics, KKHA Arts, SMGL Comm. and SPHJ Science College, Chandwad, University of Pune, Maharashtra 423 101, India

[4] Department of Physics, KKHA Arts, SMGL Comm. and SPHJ Science College, Chandwad, University of Pune, Maharashtra 423 101, India

[5] Department of Chemistry, Shivaji University, Kolhapur 416, India

ARTICLE INFO

Article History:
Received 28 April 2017
Accepted 10 June 2017
Published 01 July 2017

Keywords:
Acanthospermum hispidum L.
Biological activity
CuO-NPs
Nanotechnology
Phytochemical screening

ABSTRACT

Copper oxide nanoparticles (CuO-NPs) synthesized by an implicitly environmentally benign process using *Acanthospermum hispidum* L. aqueous plant extract as an effective bio-oxidizing/bio-reducing agent. Phytochemical screening of the fresh aqueous leaves extract showed the presence of coumarins, tannins, saponins, phenols, flavonoids, sterols and volatile oils. Fourier transform infrared spectroscopy confirmed the possible biomolecules responsible for the formation of copper oxide nanoparticles. X-ray diffraction patterns revealed the monoclinic phase of the synthesized copper oxide nanoparticles. The average size, shape and the crystalline nature of the nanoparticles were determined by field emission scanning electron microscopy and transmission electron microscopy analysis. Energy-dispersive X-ray spectroscopy analysis confirmed the presence of elements in the synthesized nanoparticles. Photoluminescence and fluorescence life-time spectroscopy showed luminescence properties of copper oxide nanoparticles. Furthermore, Copper oxide nanoparticles evinced highly robust antimicrobial, antimalarial and antimycobacterial activity against Pseudomonas aeruginosa, Streptococcus pyogenes, Staphylococcus aureus, Escherichia coli, Plasmodium falciparum and Micobacterium tuberculosis $H_{37}RV$. The current study demonstrates convenient utilization of *Acanthospermum hispidum* L. extract as a fuel for the efficient synthesis of copper oxide nanoparticles through a green synthesis method to obtain significantly biologically active material.

How to cite this article
Pansambal S, Deshmukh K, Savale A, Ghotekar S, Pardeshi O, Jain G, Aher Y, Pore D. Phytosynthesis and Biological Activities of Fluorescent CuO Nanoparticles Using Acanthospermum hispidum L. Extract. J Nanostruct, 2017; 7(3):165-174.
DOI: 10.22052/jns.2017.03.001

INTRODUCTION

Copper is a 3d transition metal (coinage metal) and has some significant physical and chemical properties. Copper based nanomaterial can promote and undergoes a variety of reaction due to its wide range of accessible oxidation states (Cu^0, Cu^I, Cu^{II} and Cu^{III}), which enable reactivity both one and two-electron pathways. Because

* *Corresponding Author Email: ghotekarsuresh7@gmail.com*

of their significant characteristics and properties, copper based nanomaterials have found many applications in electronics, gas sensors, catalysis and solar energy transformation. Moreover, CuO-NPs have attracted remarkable curiosity due to its miraculous features causing eclectic applications such as organic catalysis [1], gas sensors [2], CO oxidation of automobile exhaust gases [3], catalysts for the water-gas shift reaction [4] and cancer therapy [5].There are several reports on the formation of CuO-NPs using microwave irradiation, sonochemical, electrochemical, sol-gel technique and pyrolysis [6-10]. However, these methods have disadvantages like the use of toxic chemical, need of special instruments, use of drastic synthetic conditions like high temperature and pressure, long reaction time, and requirement of external additives during the reaction. Hence there is sufficient scope for the development of facile, rapid, environmentally benign and additive free synthesis of CuO-NPs. Currently plant extract mediated nonmaterial synthesis have attacted attention to the several advantages offered by chemical and physical methods [11,12]. The scrutiny of the literature revealed some notable plant extract used for facile synthesis of CuO-NPs. For example, Gloriosa superba [13], Tinospora cordifolia [14], Calotropis gigantean [15], Aloe barbadensis [16], Ficus religiosa [17], Citrus limon [18] and Ziziphus mouritiana [19] have been reported. Therein, CuO-NPs have been studied as potential antimicrobial agents against infectious organisms such as E. coli, Bacillus subtilis, Vibria cholera, Pseudomonas aeruginosa, Syphillis typhus, and Staphylococcus aureus [20].

Acanthospermum Hispidum L. belongs to family Asteraceae is an annual plant which is native to tropical America. This plant is also used as a medicinal plant. Amongst them, leaves (Fig. 1) and flowering tops of the plant have antimicrobial activity, crushed herb is use in the form of the paste to treat the skin aliments and leaf juice is used to relieve the fever. A scrutiny of the literature revealed some notable pharmacological activities of the drug like antimicrobial, antiviral, antiplasmodial, antitumour and antibacterial activity [21-25]. Herein, we report the cost effective and ecofriendly green synthesis of CuO-NPs using plant extracts of *Acanthospermum hispidum* L. and their antibacterial, antimalarial and antimycobacterial activity against bacterial pathogens has been evaluated. It was found that synthesized CuO-NPs exhibited biomedical applications.

MATERIALS AND METHODS

Materials

Copper acetate monohydrate [$Cu(CH_3COO)_2.H_2O$, 98%, LR grade, Sigma-Aldrich), sodium bicarbonate ($NaHCO_3$, Analytical grade, 99.7%, Sigma-Aldrich) and dimethyl sulfoxide (DMSO, ACS reagent, 99.9%, Sigma-Aldrich) were used. All chemicals were used as such without any further purification. All the solutions were prepared using

Fig. 1. Leaves of *Acanthospermum hispidum* L.

deionized water during the synthesis. The fresh leaves of *Acanthospermum hispidum* L. were sourced from Chandwad college campus, Nashik, Maharashtra, India. The collected leaves were washed with deionized water, snick into small pieces. All glassware's are washed with distilled water and acetone and dried in oven before use.

Biogenic synthesis of CuO-NPs

5g small wizened pieces of *Acanthospermum hispidum* L. leaves were transferred into 250 mL beaker containing 100 mL deionized water. The mixture was refluxed at 100 °C for 20 minutes and cooled at room temperature followed by filtration through ordinary filter paper. Then, resultant filtrate was again filtered through Whatmann No. 1. The filtered extract was stored in refrigerator at 4 °C and used for synthesis of CuO-NPs. The aqueous solution of 0.01 M copper acetate monohydrate was prepared in deionized water. *Acanthospermum hispidum* L. leaf extract was mixed to 2 mM aqueous copper acetate solution in 1:8 ratios in a 250 ml beaker with constant stirring on magnetic stirrer at 500 rpm for 25 minutes. After time of period the color of solution turns to dark yellow. The mixture was kept in a muffle furnace at 400 ºC and subjected for combustion. The reaction was completed within 5 min. A fine black colored material was obtained and this was carefully collected and packed for characterization purposes.

Characterization techniques

The morphology and composition of the synthesized CuO-NPs were examined by field emission scanning electron microscopy (FESEM, FEI, Nova Nano SEM 450), FESEM coupled energy-dispersive X-ray spectroscopy (EDX, Bruker, XFlash 6I30). Find the exact morphological structures and size of the CuO-NPs using transmission electron microscopic (TEM) analysis is done by using a PHILIPS, CM200. The crystallinity and crystal phases were characterized by X-ray diffraction (XRD, Bruker, D8-Advanced Diffractometer) pattern measured with Cu- Kα Radiation (λ= 1.5406 Å) in the range of 20–80°. The Fourier transform Infrared (FTIR) spectrum was recorded by JASCO 4100 in the range of 4000–400 cm^{-1}. Photoluminescence studies were evaluated by using fluorescence spectrophotometer (JOBIN YVON FLUROLOG-3-11, Spectroflurimeter). Fluorescence lifetime were acquired using a JOBIN-VYON M/S operating at excitation wavelength 390 nm as the light source to trigger the fluorescence of CuO-NPs.

Phytochemical Screening

The fresh aqueous extract of *Acanthospermum hispidum* L. leaves was investigated for the presence of phytochemicals viz. coumarins, saponins, tannin, flavonoids, volatile oils, sterols and phenols by standard biochemical method [26].

Antimicrobial Activity of Synthesized CuO-NPs

The antimicrobial activity of synthesized CuO-NPs was examined by using Disc diffusion method. This method was employed against human pathogens i.e. Pseudomonas aeruginosa, Streptococcus pyogenes, Staphylococcus aureus and Escherichia coli obtained from Institute of Microbial Technology, Chandigarh, India. The nutrient agar medium (g/l) plates were prepared, well sterilized and solidified. After solidification, bacterial cultures spread over the plate, and CuO-NPs solution was poured into each plate. These plates were incubated in incubator at 37 ^{0}C for 24 hrs and zone of inhibition against bacterial strains was measured.

In Vitro Antimalarial Screening of Synthesized CuO-NPs

The in vitro antimalarial assay was carried out in 96 well microtitre plates according to the microassay protocol [27]. The cultures of falciparum strain were maintained in medium of RPMI-1640 supplemented with 25 mM HEPES, 0.23% $NaHCO_3$, 1% d-glucose and 10% heat inactivated human serum. The asynchronous parasites of Plasmodium falciparum were synchronized after 5% d-sorbitol treatment to obtain only the ring stage parasitized cells. For carrying out the assay, an initial ring stage parasitaemia of 3% haematocrit in a total volume of 200 µl of medium RPMI-1640 was resolved by Jaswant Singh Bhattacharya (JSB) staining [28] to assess the percent parasitaemia and uniformly maintained with 50% RBCs (O^{+ve}). The culture plates were incubated at 37 °C in a candle jar. After 36 hrs incubation, thin blood smears from each well were prepared and stained with JSB stain. The slides were microscopically observed to record maturation of the ring stage parasites into schizonts and trophozoites in the presence

of various concentrations of the synthesized CuO-NPs. The synthesized CuO-NPs concentration which inhibited the complete maturation into schizonts was recorded as the minimum inhibitory concentration (MIC). Chloroquine and Quinine were used as the reference drugs.

In Vitro Antimycobacterial Screening of Synthesized CuO-NPs

The antimycobacterial screening for synthesized CuO-NPs was obtained for Mycobacterium tuberculosis $H_{37}RV$, by using L. J. (Lowenstein and Jensen) MIC method [29]. Stock solutions of primary 1000, 500, 250 and secondary 200, 100, 62.5, 50, 25, 12.5, 6.25, 3.25 µg/ml of CuO-NPs in DMSO were added in the liquid L. J. Medium and then media were sterilized. A culture of Mycobacterium tuberculosis $H_{37}RV$ growing on L. J. medium was harvested in 0.85% saline in bijou bottles. These tubes were then incubated at 37°C for 24 hrs followed by streaking of Mycobacterium tuberculosis $H_{37}RV$. Th. Growth of bacilli was seen after 12 days, 22 days and finally 28 days of incubation respectively. Tubes having CuO-NPs were compared with control tubes where medium alone was incubated with M. tuberculosis $H_{37}RV$. The concentration at which no development of colonies occurred or < 20 colonies was taken as MIC concentration of test compound. The standard strain M. tuberculosis $H_{37}RV$ was tested with known drug isoniazid.

RESULTS AND DISCUSSION

Structural & crystallographic analysis

The CuO-NPs biosynthesized from *Acanthospermum hispidum* L. leaf extract were confirmed by the characteristic peaks observed in the XRD patterns, as shown in Fig. 2. Such a powder XRD was carried out using monochromatic CuKα1 radiation (wavelength 1.5406Å), operating at a voltage of 40 KV and a current of 40 mA, in the angular range 2θ of 20-80 deg. XRD analysis showed a series of diffraction peaks at 32.5º, 35.4°, 38.7°, 48.7°, 53.5°, 61.5° and 66.2°, corresponding to (110), (002), (111), (202), (020), (113) and (311) of face-centered-cubic structure of copper oxide nanoparticles with a monoclinic phase (JCPDS No. 45-0937). The XRD pattern exposed that synthesized copper oxide nanoparticles are crystalline in nature [30].

Morphological studies & elemental analysis

From the FESEM image as shown in Fig. 3(a and b) the synthesized CuO-NPs present uniform and define quasi-spherical morphology. Each CuO-NPs possesses the average particles size of 9-21 nm. It is noticed that green synthesis of CuO-NPs produces the small and uniform size of spherical particles. Therein, the composition of synthesized CuO-NPs has been analyzed by investigating the energy-dispersive X-ray spectroscopy (EDS), as shown in Fig. 4. EDS spectrum displays the Cu (24.64%) and O (28.00%) peaks. Other peaks corresponding to C (44.16%) in the EDS is an artifact of the C-grid on which the sample was coated while peaks for Phosphorous (0.93%), Nitrogen (1.63%), Silicon (0.41%), Sulphur (0.18%) and Chlorine (0.05%) correspond to the phenols, flavonoids, coumarins

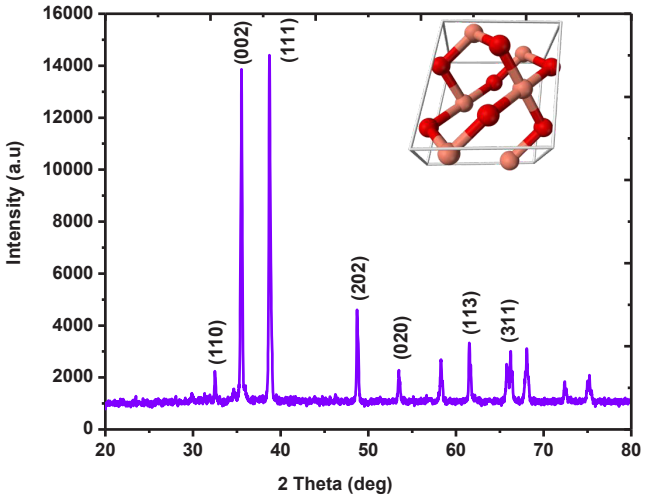

Fig. 2. X-Ray diffraction profile of synthesized CuO-NPs at room temperature

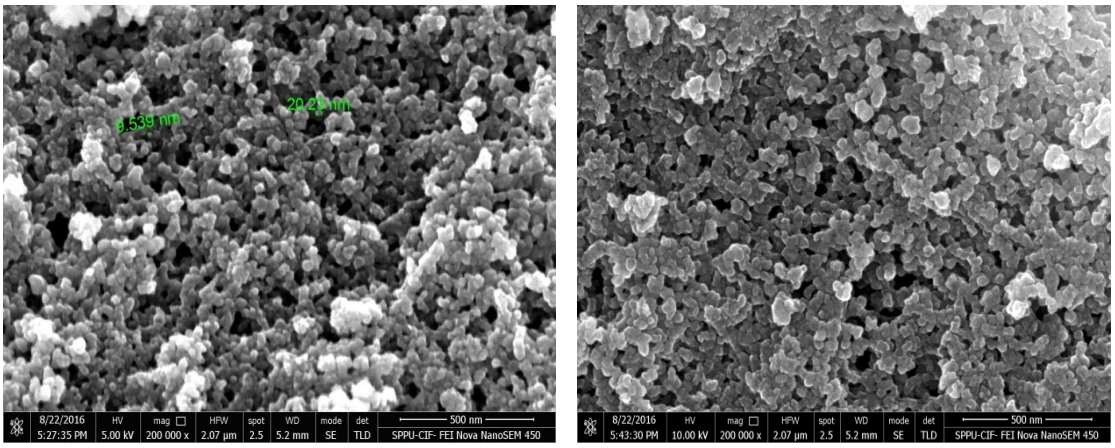

Fig. 3. FE-SEM microphotographs of CuO-NPs deposited on a carbon strip.

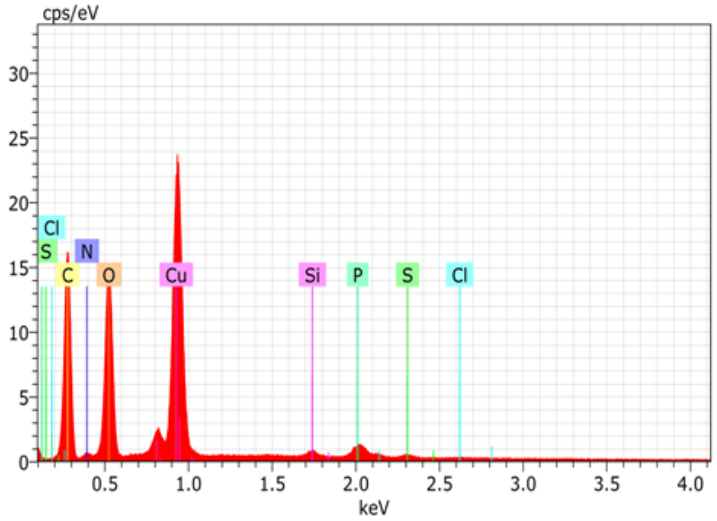

Fig. 4. EDS spectrum of synthesized CuO-NPs.

and enzymes capping over the synthesized CuO-NPs. Furthermore, TEM provided further insight into the morphology and size details of the synthesized CuO-NPs. The fig. 5(a&b) shows the TEM images of synthesized CuONPs. From TEM image, the average particle size is estimated to be 5-25 nm spherical particles, which is consistent with the FESEM results. From the TEM image of CuO-NPs as shown in fig. 5(a), the particles are aggregated and interconnected to each other, resulting in the less visible lattice fringes. The low magnification TEM image [fig. 5(b)] reveals almost similar spherical morphology of Cu-ONPs as seen in FESEM image. Therefore, the morphological characterizations confirm the spherical morphology of CuO-NPs biosynthesized from the leaves of *Acanthospermum hispidum* L. plant.

Vibrational properties

Fig. 6 represents the FTIR spectrum of CuO-NPs synthesized from leaves of *Acanthospermum hispidum* L. The broad band seen at 3416 cm^{-1} reveals the presence of an OH group, resulting from either alcoholic or phenolic stretching, while the peaks around 2919 cm^{-1} are attributed to an asymmetric stretching vibration of the C-H bond in alkanes. The peaks around 1611 cm^{-1} may be attributed to C=C in aromatic compounds, and those at 1385 cm^{-1} correspond to the O-H bend of polyphenol, confirm the presence of an aromatic group [31]. The peak observed at 1079 cm^{-1} corresponds to C-O stretching frequency of ester

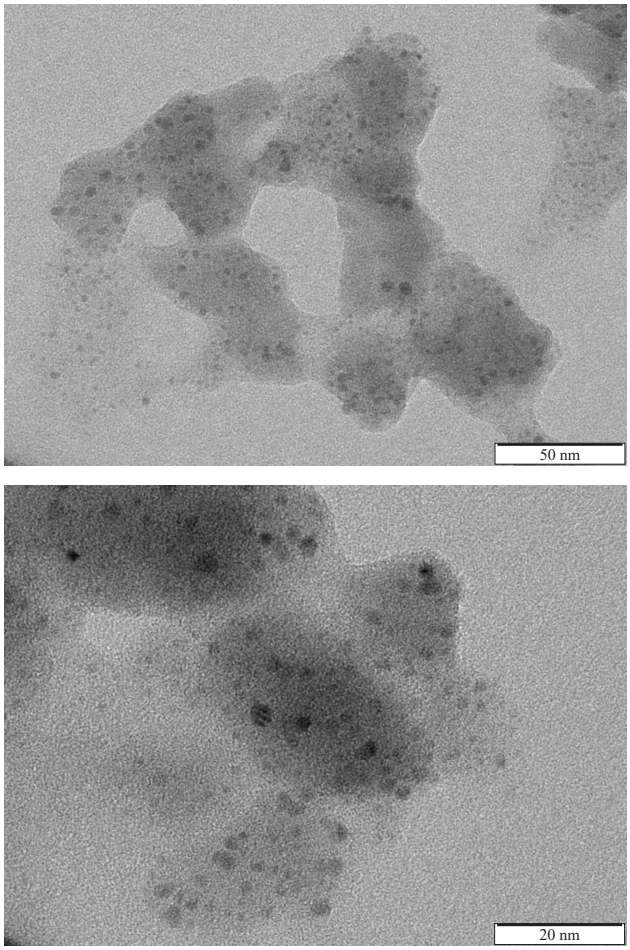

Fig. 5. TEM images indicating the presence of spherical CuO-NPs recorded at various magnifications (a-50 nm and b-20 nm).

in the aqueous leaves extract of Acanthospermum hispidum L. Additionally, the stretching bands at 601 and 667 cm^{-1} are related to Cu-O absorption, further indicating CuO-NPs formation. The FTIR results confirm the presence of phytochemicals in the plant extract such as, which further act as reducing/ capping agents for the synthesis of CuO-NPs and is in good agreement with the phytochemical screening of aqueous leaves extract of *Acanthospermum hispidum* L.

Phytochemical screening

The results of qualitative pharmocognostic assess of aqueous leaf extract of Acanthospermum hispidum L. are shown in table 1. Phytochemical profile of Acanthospermum hispidum L. leaves revealed and highlighted the presence of saponins, coumarins, phenols, flavonoids, volatile oils, tannins and sterols which may be responsible for the efficient capping and chelating agent of nanoparticles and this was further confirmed by FTIR spectrum. The physical and chemical processes driving the reaction of the natural extract of the *Acanthospermum hispidum* L. and the copper acetate precursor and the reaction dynamic will be explored via adequate characterization techniques in view of identifying the exact mechanisms of formation of the CuO-NPs. More precisely, the bioactive compounds from the natural extract as highlighted in Fig. 7.

Photoluminescence study

CuO-NPs are reported to exhibit visible photoluminescence and their fluorescence spectra are shown in Fig. 8. The CuO-NPs were found to be luminescent with four emissions at

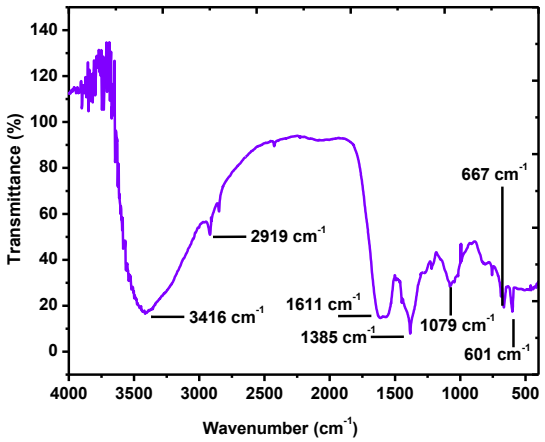

Fig. 6. FT-IR spectrum of synthesized CuO-NPs

Table 1. Phytochemical profile of aqueous leaves extract of *Acanthospermum hispidum* L.

Phytoconstituents	Test	Phytoconstituents	Test
Tannin	+	Saponins	+
Coumarins	+	Emodins	-
Proteins	-	Flavonoid	+
Glycosides	-	Anthraquinone	-
Anthocyanin	-	Sterols	+
Phenols	+	Volatile oils	+

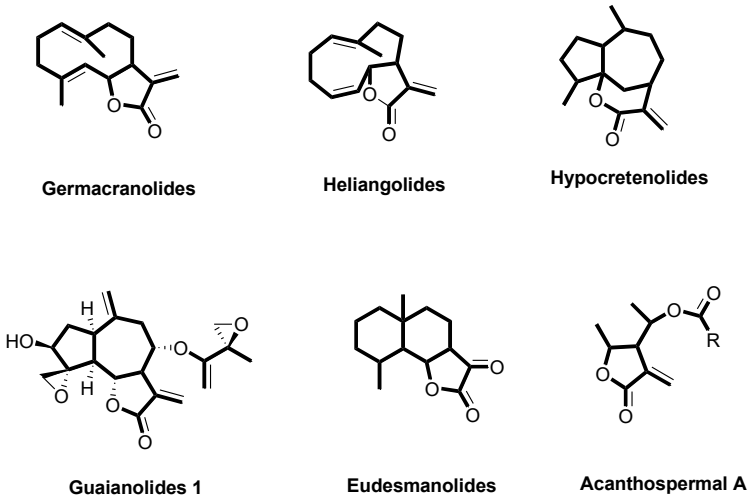

Fig. 7. Major bioactive compounds in the natural extract of *Acanthospermum hispidum* L.

412 and 438 nm for an excitation at 390 nm. The luminescence at 390 nm may be due to presence of phytoconstituents or antioxidants present in the plant extract.

Fluorescence life-time studies

The chemical information can often be obtained from the same experiment by exploiting the time-dependent nature of fluorescence. Time-resolved fluorescence provides more information about the molecular environment of the fluorescent compound than steady state fluorescence measurements [32]. It is important to remember that the fluorescence lifetime is an average time for a molecule to remain in the excited state before emitting a photon. Each individual molecule emits randomly after excitation. Many excited molecules will

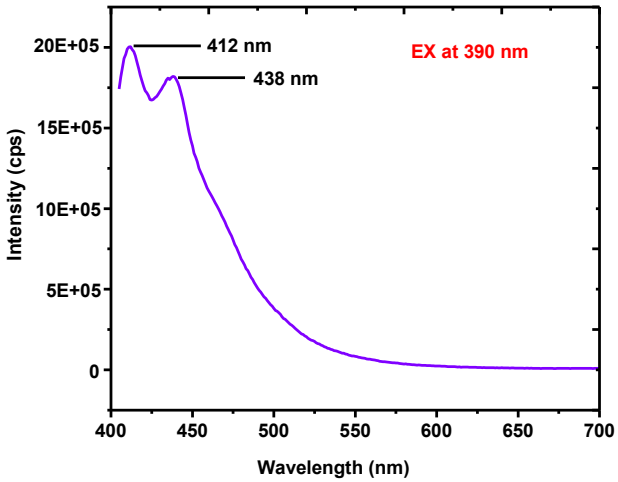

Fig. 8. Fluorescence spectra of synthesized CuO-NPs formed with excitation at 390 nm.

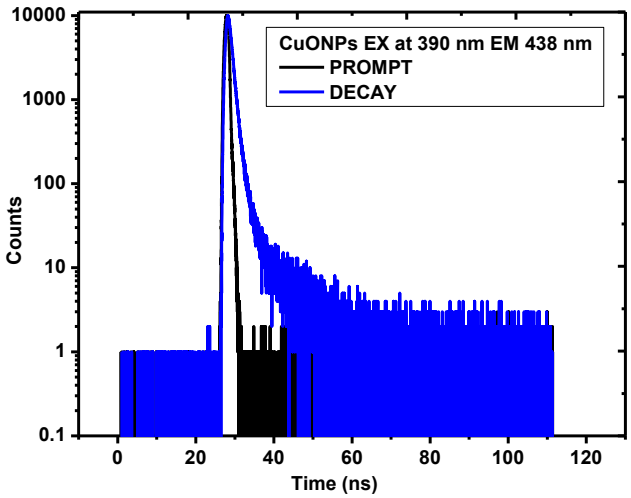

Fig. 9. Fluorescence decay profile of synthesized CuO-NPs.

fluorescent before the average lifetime, but some will also fluorescent long after the average lifetime. Fluorescence lifetimes are generally on the order of 1^{-10} ns, although they can range from hundreds of nanoseconds to the sub-nanosecond time scale. Fig. 9 shows fluorescence decay profile for CuO-NPs. The fluorescence lifetime of scanned samples were fitted in multi-exponential decay curves having more than one value. The average fluorescence lifetimes of CuO-NPs are 1.5851 ns (emission at 412 nm). It is known that aggregation and molecular interactions lead to a prolonged lifetime [32,33].

Antimicrobial activity of CuO-NPs

Literature reports reveal that CuO-NPs are highly toxic to most of the human pathogens [34]. In this context, we decided to investigate antimicrobial activity of biosynthesized CuO-NPs against various pathogens *viz* Pseudomonas aeruginosa, Streptococcus pyogenus, Staphylococcus aureus and Escherichia coli. These bacterial and fungal strains namely P. aeruginosa MTCC 1688, S. pyogenus MTCC 442, S. aureus MTCC 96 and E. coli MTCC 443 were added on nutrient agar plate and spread over the plate with the help of glass spreader and the "well" was made with the help of disc difussion method. The various concentrations of synthesized CuO-NPs (25, 50, 100, 250, 500 µg/ml.) were tested for antimicrobial activity against these pathogen with amplicilline has positive control. The plates were then kept at 4-5 °C for 1

Table 2. Zone of inhibition (mm) of biosynthesized CuO-NPs against bacterial pathogens

Test pathogens	Inhibition zone (mm) of CuONPs (µg/ ml)					Control
	25	50	100	250	500	
E. coli	13	15	16	19	23	19
P. aeruginosa	11	12	14	16	19	18
S. pyogenus	14	15	17	18	22	14
S. aureus	15	16	18	19	21	16

Table 3. Minimum inhibition concentration (MIC) of biosynthesized CuO-NPs against Plasmodium falciparum

Sr. No	Compound Name	Mean IC_{50} values
1)	CuONPs	1.08 µg/ ml
2)	Chloroquine (Standard)	0.020 µg/ ml
3)	Quinine (Standard)	0.268 µg/ ml

Table 4. Minimum inhibition concentration (MIC) of biosynthesized CuO-NPs against Micobacterium tuberculosis

Sr. No	Compound Name	MIC (µg/ ml)
1)	CuO-NPs	100 µg/ ml
2)	Isoniazide (Standard)	0.20 µg/ ml

hr, followed by incubation at 37 °C for 24 hrs. After 24 hrs, exact zone of inhibition was measured with respect to positive controls (Table 2). Gratifyingly, it was observed that biosynthesized CuO-NPs exhibited moderate antibacterial activity against the selected strains.

Antimalarial activity of CuO-NPs

The synthesized CuO-NPs were screened for their significant in vitro antimalarial activity against Plasmodium falciparum by measuring the minimum inhibitory concentration (µg/mL) against standard Quinine and Chloroquine, as shown in Table 3.

Antimycobacterial activity of CuO-NPs

The antimycobacterial screening was performed using Lowenstein-Jensen MIC method (Table 4) and it is worthwhile to note that biosynthesized CuO-NPs was the only displaying inhibition of Micobacterium tuberculosis $H_{37}RV$ completely (99%) at the MIC of 100 µg/ml.

CONCLUSION

Phytoassisted synthesis of CuO-NPs with an aqueous extract of *Acanthospermum hispidum* L. is an environmentally safe, facile and cost-effective method. The results of this study clearly show that the pathogenic strains tested are susceptible to CuO-NPs, which confirms their potential upshot against other bacterial strains. This result can be utilized to expand the use of these nanoparticles in biomedical applications and will play vital role in medical devices in future.

ACKNOWLEDGEMENT

We are thankful to CIF Savitribai Phule Pune University, SAIF IIT Powai, SAIF IIT Madras and Microcare Laboratory Gujrat for providing the technical, instrumental and biological activities supports. We are also thankful to S R Nagare for his help during experimental work.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

REFERENCES

1. Jingfa D, Qi S, Yulong Z, Songying C, Dong W. A novel process for preparation of a $Cu/ZnO/Al_2O_3$ ultrafine catalyst for methanol synthesis from CO2 + H2: comparison of various preparation methods. Appl. Catal. A. 1996; 139(1): 75-85.
2. Frietsch M, Zudock F, Goschnick J, Bruns M. CuO catalytic membrane as selectivity trimmer for metal oxide gas sensors. Sens. Actuators B. 2000; 65(1): 379-381.
3. Cao JL, Shao GS, Wang Y, Liu Y, Yuan ZY. CuO catalysts supported on attapulgite clay for low-temperature CO oxidation. Catal. Commun. 2008; 9(15): 2555-2559.
4. She Y, Zheng Q, Li L, Zhan Y, Chen C, Zheng Y, Lin X. Rare earth oxide modified CuO/CeO_2 catalysts for the water-gas shift reaction. Int. J. Hydrogen Energy. 2009; 34(21): 8929-8936.
5. Rehana D, Mahediran D, Senthil Kumar R, Kalilur Rahiman A. Evaluation of antioxidant and anticancer activity of copper oxide nanoparticles synthesized using medicinally important plant extracts. Biomed. & Pharmaco. 2017; 89: 1067–1077.
6. Wang H, Xu J Z , Zhu J J, Chen H Y. Preparation of CuO nanoparticles by microwave irradiation. J. Cryst. Growth. 2002; 244: 88–94.
7. Kumar R V, Diamant Y, Gedanken A. Sonochemical synthesisand characterization of nanometer-size transition metal oxids from metal acetates. Chem. Mater. 2002; 12: 2301-2305.
8. Borgohain k, Singh J B, Rama Rao M V, Shripathi T, Mahamuni S. Quantum size effects in CuO nanoparticles. Phys. Rev. 2000; 61: 11093-11096.
9. Eliseev A A, Lukashin A V, Vertegel A A, Heifets L I, Zhirov A I, Tretyakov Y D. Complexes of Cu (II) with polyvinyl

alcohol asprecursor for the preparation of CuO/SiO_2 nanocomposites. Mater. Res. Innov. 2000; 3: 308-312.
10. Fan H, Yang L, Hua W, Wu X, Wu Z, Xie S, Zou B. Controlled synthesis of monodispersed CuO nanocrystals. Nanotechnology. 2004; 15: 37–42.
11. Hebbalalu D, Lalley J, Nadagouda Mallikarjuna N, Varma Rajender S. Greener techniques for the synthesis of silver nanoparticles using plant extracts, enzymes, bacteria, biodegradable polymers, and microwaves. ACS Sustainable Chem. Eng. 2013; 1: 703-712.
12. Pande S N, Bharati K T, Wakchure S K, Ghotekar S K, Gujrathi D B, Phatangare N D. Green synthesis of silver nanoparticles by carallua fimbriata L. and its characterization. Ind. Jou. App. Res. 2015; 5: 749-750.
13. Ghosh B, Mukherjee S, Jha T B, Jha S. Enhanced colchicine production in root cultures of Gloriosa superba by direct and indirect precursors of the biosynthetic pathway. Biotechnol. Lett. 2002; 24: 231-234.
14. Udayabhanu, Nethravathi P C, Pavan Kumar M A, Suresh D, Lingaraju K, Rajanaika H, Nagabhushana H, Sharma S C. Tinospora cordifolia mediated facile green synthesis of cupric oxide nanoparticles and their photocatalytic, antioxidant and antibacterial properties. Materials Science in Semiconductor Processing. 2015; 33: 81-88.
15. Sharma J, Akhtar J K, Ameen M S, Srivastava S, Singh P G. Green synthesis of CuO nanoparticles with leaf extract of Calotropis gigantea and its dye-sensitized solar cells applications. J. Alloys Compd. 2015; 632: 321.
16. Gunalan S, Sivaraj R, Venckatesh R. Aloe barbadensis Miller mediated green synthesis of mono-disperse copper oxide nanoparticles: optical properties. Spectrochim. Acta A. 2012; 97: 1140.
17. Sankar R, Manikandan P, Malarvizhi V, Fathima T, Shivashangari K S, Ravikumar V. Anticancer activity of Ficus religiosa engineered copper oxide nanoparticles. Spectrochim. Acta A. 2014; 121: 746.
18. Prathna T C, Chandrasekaran N, Raichur A M, Mukherjee A. Biomimetic synthesis of silver nanoparticles by Citrus limon (lemon) aqueous extract and theoretical prediction of particle size. Colloids Surf. B Biointerfaces. 2011; 82: 152-159.
19. Ghotekar Suresh K, Savle Ajay R, Pardeshi Onkar M, Pagar Bapu R. Robust biogenic synthesis of copper oxide nanoparticles using leaves extract of Ziziphus mauritiana L. and their biological activity. NINM. 2016; 71-75.
20. Hassan M S, Amna T, Yang O B, El-Newehy M H, Al-Deyab S S, Khil M S. Smart copper oxide nanocrystals: synthesis, characterization, electrochemical and potent antibacterial activity. Colloids Surf B: Biointerfaces. 2012; 97: 201-205.
21. Fleischera T C., Ameadea E P K., Sawerb I K. Antimicrobial activity of the leaves and flowering tops of Acanthospermum hispidum. Fitoterapia. 2003; 74: 130–132.
22. Artur Summerfield, Gunther M K., Thomas C., Mettenleiter B., Hanns-Joachim R., Armin S. Antiviral activity of an extract from leaves of the tropical plant Acanthospermum hispidum. Antiviral Research. 1997; 36: 55–62.
23. Sanon S, Azas N, Gasquet M, Ollivier E, Mahiou V, Barro N, Cuzin-Ouattara N, Traore AS, Esposito F, Balansard G, Timon-David P. Antiplasmodial activity of alkaloid extracts from Pavetta crassipes (K. Schum) and Acanthospermum hispidum (DC), two plants used in traditional medicine in Burkina. Faso. Parasitol Res. 2003; 90: 314-317.
24. Deepa N and Rajendran N N. Anti-tumor Activity of Acanthospermum hispidum DC on dalton ascites lymphoma in mice. Natural Product Sciences. 2007; 13(3): 234-240.
25. Mario E. Arena, Elena Cartagena, Nadia G., Mario B., Juan C V, Alicia B. In vivo and in vitro antibacterial activity of acanthospermal B, a sesquiterpene lactone isolated from Acanthospermum hispidum. Phytotherapy Research. 2011; 25(4): 597–602.
26. Fransworth N R. (1996). Biological and phytochemical screening of plants. Jou. of Pharma. Sci. 1996; 55: 225-227.
27. Riekmann K H, Campbell G H, Sax L J, Mrema J E. Drug sensitivity of Plasmodium falciparum: an in-vitro microtechnique. Lancet. 1978; 1: 22-23.
28. Singh J S B. J. S. B stain- A Review. Indian Journal of Malariology. 1956; 10(2): 117-129.
29. Anargyros P, Astill D S, Lim I S. Comparison of improved BACTEC and Lowenstein-Jensen media for culture of mycobacteria from clinical specimens. 1990 Jou. of Clin Microbio. 1990; 28(6): 1288-1291.
30. Ethiraj A S and Kang D J. Synthesis and characterization of CuO nanowires by a simple wet chemical method. Nanoscale Res. Lett. 2012; 7: 70-75.
31. Rao K J and Paria S. Green synthesis of silver nanoparticles from aqueous Aegle marmelos leaf extract. Mater. Res. Bull. 2013; 48: 628-634.
32. Mahajan P G, Bhopate D P, Kolekar G B, Patil S R. N-methyl isatin nanoparticles as a novel probe for selective detection of Cd2+ ion in aqueous medium based on chelation enhanced fluorescence and application to environmental sample. Sensor. Actuat. B: chem. 2015; 220: 864-872.
33. Mahajan P G, Bhopate D P, Kamble A A, Dalavi D K, Kolekar G B, Patil S R. Selective sensing of Fe2+ ions in aqueous solution based on fluorescence quenching of SDS capped rubrene nanoparticles: application in pharmaceutical formula. Anal. Methods. 2015; 7: 7889-7898.
34. Rajgovind, Sharma G, Deepak Gupta K, Jasuja N D, Suresh Joshi C. Pterocarpus marsupium derived phyto-synthesis of copper oxide nanoparticles and their antimicrobial activities 2015 Jou. Microb. Biochem. Technol. 2015; 7: 140-144.