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Green Synthesis of Copper Nanoparticles: Evaluation of Catalytic and Antibacterial Activity

M. REDDEPPA[1], R. CHENNA KRISHNA REDDY[2,3], Y. PAUL RAJ[1] and T. SHOBHA RANI[1,*]

[1]Department of Chemistry, Dravidian University, Kuppam-517426, India
[2]Department of Chemistry, Sri Venkateswara University, Tirupati-517502, India
[3]Department of Science & Humanities, Sri Venkateswara College of Engineering, Karakambadi Road, Tirupati-517507, India

*Corresponding author: sobha.kaushu@gmail.com

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The objective of present study is to synthesize copper nanoparticles from *Vitex negundo* Linn., root bark extract as reducing and stabilizing agents. The synthesized nanoparticles were characterized by using UV-visible spectrophotometer, X-ray diffraction, FTIR, SEM and EDX. The results were shown that copper nanoparticles exhibited UV-visible absorption peak at around 550 nm. The XRD study confirmed that nanoparticles are crystalline in nature. In addition to these, FTIR was also used to analyze the various functional groups stabilized and protected the copper nanoparticles. The SEM results revealed that the copper nanoparticles are spherical, cubic and hexagonal in shape and having the particle size ranging from 40 to 60 nm. Catalytic activity of copper nanoparticles were studied in the Huisgen [3+2] cycloaddition of azides and alkynes to obtain 1,4-disubstituted 1,2,3-triazole at room temperature. Antibacterial profile revealed that the biopotent copper nanoparticles exhibited moderate to good activity against bacterial strains.

Keywords: Green Synthesis, *Vitex negundo*, Copper Nanoparticles, Catalytic activity, Antimicrobial activity.

INTRODUCTION

In last few years, nanoparticles owned much importance in scientific field due to its typical size, shape, surface area and colossal technological applications like electrical, optical, magnetic, catalytic, biomedical and antibacterial activities which cannot be achieved for their bulk counter parts [1-9]. There are several traditional synthetic methods developed by various research groups to synthesize nanoparticles by physicochemical methods [10,11]. The use of physicochemical methods for the synthesis of nanomaterials leads to potentially hazardous to environment. Conventional methods for the synthesis of copper nanoparticles (CuNPs) leads to the usage of toxic and expensive chemicals with hazardous organic solvents [12,13]. The presence of these toxic materials on the surface of CuNPs makes them toxic and disposal of hazardous solvents trigger environmental problems. Recently, researchers focus on biosynthesis of metal nanoparticles which is an eco-friendly, green, economic and easy method. Biosynthesis of biocompatible nanoparticles is a better alternative involving biomolecules as reducing and capping agents compared with traditional techniques.

However, synthesis of nanoparticles by various plant extracts is an ecologically friendly, cost effective method without use of toxic chemicals. The other advantage of this environmental friendly and safe protocol includes a simple reaction setup, mild reaction conditions, use of non-toxic green solvents like water, glycerine and holding compatibility for biomedical and pharmaceutical applications [14,15]. In addition to this, nanoparticles from transition metals play a vital role in organic synthesis as catalysts due to its stability in air, high surface to volume ratio [16]. The use of these catalysts has the advantage of easy separation of products from reaction mixtures and the catalyst is recyclable. Due to this, researchers have paid much attention to the use of nanoparticles as catalysts in organic reactions [17]. Nanoparticles of metals such as copper, silver and gold reported widely in the literature as catalysts in organic reactions [18]. Based on literature survey, the present study has concluded that in most cases, the nanoparticle catalysts have shown much higher catalytic activities than its salts [19].

Vitex negundo belongs to Verbenaceae family with common name 'Five leaved chaste tree' or 'Monk's pepper'. This medicinal plant credited with innumerable commercial and medicinal importance. Though, all parts of *Vitex negundo* are used as medicine in the indigenous system of medicine, the leaves are the most potent for medicinal use. The roots of plant are long woody, cylindrical, tortuous with grey brown colour possessing medicinal values of tonic, febrifuge, diuretic, expectorant anti-rheumatic and useful as a demulcent in dysentery, colic, incephalalgia, uropathy, otalgia, wound and ulcers. The extract of root bark is extensively used as a source of herbal medicine for presence of medicinal phytochemicals [20-22].

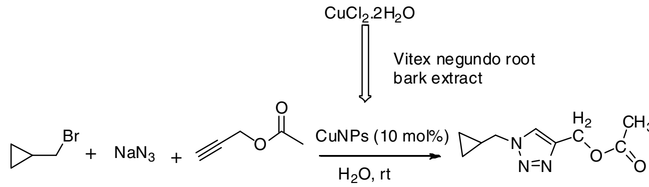
Herein, we wish to report a simple and eco-friendly protocol for synthesis of copper nanoparticles (CuNPs) using aqueous extract of root bark of *Vitex negundo* as reducing agent and stabilizing agent. Also, the catalytic potential of CuNPs was evaluated in Huisgen [3+2] cycloaddition of azides and alkynes for the synthesis of substituted triazole at room temperature. In addition to this, antibacterial profile of CuNPs was also investigated.

EXPERIMENTAL

Preparation of extract of root bark of *Vitex negundo*: The collected root bark was thoroughly washed with double distilled water to remove clay particles. Then *Vitex Negundo* root bark was cut into small pieces and dried at room temperature for 25 days under dust free environment. These dried pieces were converted into powder with the help of mortar and pestle. The dried powder (10 g) was blended with 100 mL of double distilled water in 250 mL round bottomed flask and subjected to reflux at 80 ºC for 30 min with the help of magnetic heating stirrer. Later, the extract was centrifuged at 6500 rpm, filtered and then the filtrate was stored at refrigerator to utilize for further experiments.

Green synthesis of copper nanoparticles: The copper nanoparticles were synthesized by a simple and eco-friendly method. In a 250 mL conical flask, 10 mL solution of $CuCl_2·2H_2O$ (5 mM) was stirred with 100 mL of aqueous root bark extract for 15-20 min. During stirring, the colour changed from dark brown colour to light yellow colour indicating the formation of CuNPs. The formation of CuNPs was monitored by using UV-visible and FT-IR spectrophotometers. The well stirred mixture filtered and centrifuged at 6500 rpm for 20-40 min and the formed precipitation was washed with *n*-hexane and absolute ethanol. In this green synthesis method, biomolecules present in *Vitex negundo* root bark extract can serve as reducing agents.

Preparation of (1-(cyclopropylmethyl)-1*H*-1,2,3-triazol-4-yl)methyl acetate: (Bromomethyl)cyclopropane (1.0 mmol), sodium azide (1.2 mmol), prop-2-ynyl acetate (1.0 mmol) and CuNPs (10 mL) were taken into 50 mL round bottomed flask containing 5 mL of H_2O. The reaction mass was stirred at 80 ºC for 2 h and the progress of the reaction was monitored by TLC. After completion of reaction as checked by TLC, the reaction mass was diluted with water (15 mL) and the solid product, (1-(cyclopropylmethyl)-1*H*-1,2,3-triazol-4-yl)methyl acetate was filtered-off and then washed with brine water (**Scheme-I**). The pure product was dried under vacuum and characterized. Colour: white solid; Yield: 91 %; m.p. 85-87 ºC. ^1H NMR ($CDCl_3$) δ 7.59 (s, 1H, CH, triazole), 5.25 (s, 2H, CH_2), 3.71 (d, J = 6.0 Hz, 2H, CH_2), 2.50 (s, 3H, CH_3), 0.22



Scheme-I: Synthesis of 1,4-disubstituted-1,2,3-triazoles

(m, 1H, CH, cyclopropane), 0.31 (m, 4H, CH_2, cyclopropane). MS (positive) *m/z* (%): 195 $[M+H]^+$ (100 %); Anal. calcd. (found) for $C_9H_{13}N_3O_2$: C, 55.37 (55.36); H, 6.71 (6.65); N, 21.52 (21.50).

Antibacterial activity assay: The antibacterial activity of CuNPs was tested by agar well diffusion method [23] as adopted earlier and with little modifications [24]. Seeded agar was made using nutrient agar medium. After the medium preparation, it was sterilized and allowed to cool so that the medium gets be solidified. Just before solidification, 0.1 mL of diluted inoculum (10^5 cfu/mL) of test organism was added to the medium and then it was poured into sterilized petri dishes under aseptic conditions. Under sterile conditions, wells of 4 mm diameter were punched into the agar medium with the help of sterile cork borer. These wells were filled with 100 µL of root bark extract of 500 µg/mL concentration and solvent DMSO as control. The plates were incubated at 37 ºC for 18 h. The antibacterial activity was evaluated by measuring the zone of inhibition (in mm) against test organisms. The antibiotic erythromycin at 500 µg/mL concentration was used as positive control.

RESULTS AND DISCUSSION

In the present work, we developed an eco-friendly, non-toxic green method for the synthesis of CuNPs using root bark extract of *Vitex negundo* Linn.

Characterization of CuNPs: The biogenic CuNPs were characterized by using UV-visible, FT IR, XRD, SEM and EDX techniques. A single characteristic absorption peak observed at around 550 nm, which confirms the presence of CuNPs and no such peak was observed for pure plant extract. The typical UV-visible absorption spectra is shown in Fig. 1. Creighton and Eadon [25] studied that CuNPs having size around 60 nm can exhibit a surface plasmon peak around 550 nm. The synthesized CuNPs exhibited strong absorption band at 550 nm, which revealed that size of the nanoparticles at around 60 nm. The size also confirmed by XRD (Fig. 2) which showed three intense peaks at around 41.03º (111), 50.26º (200) and 78.50º (220) confirming the crystalline structure of CuNPs. These values are much close to the PCPDF card no. 72-0140. The FTIR analysis for pure extract and synthesized CuNPs were carried out and compared to identify the biomolecules responsible for the stabilization and capping of copper nanoparticles. In present observation, during the formation of CuNPs, the major peaks have shifted from 3314 cm^{-1} (O-H *str.*), 2853 cm^{-1} (C-H *str.*), 1630 cm^{-1} (C=O *str.*), 1442 cm^{-1} (O-H def.) and 1074 cm^{-1} to 3293 cm^{-1}, 2851, 1627, 1414 and 1048 cm^{-1}, respectively. All these changes revealed the presence of hydroxyl and carboxylic acid functional groups which further confirms the involvement of fatty acids and flavanoids in the stabilization and protection of copper nanoparticles [26]. The typical FT IR spectrum is shown in Fig. 3 is the evident that phyto-

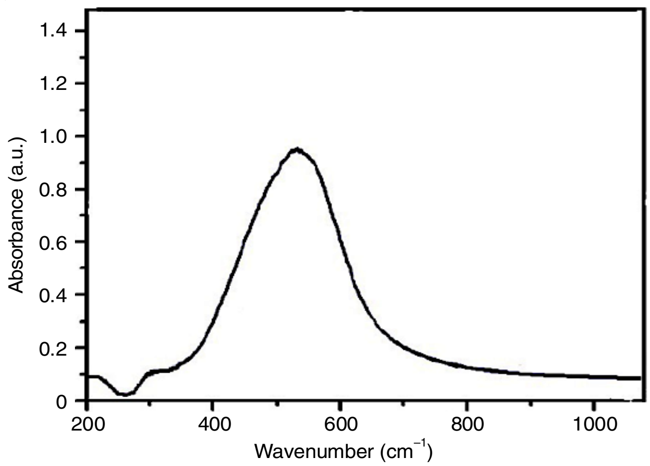


Fig. 1. UV-visible spectra of synthesized copper nanoparticles

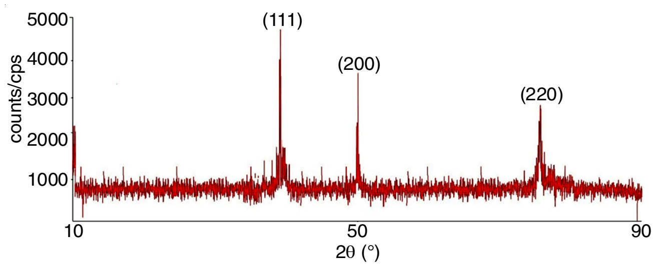
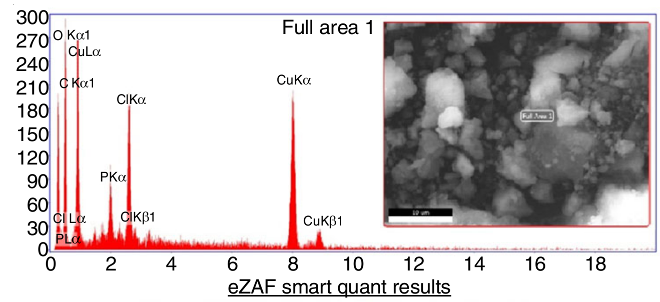


Fig. 2. XRD patterns of synthesized copper nanoparticles

chemicals present in *Vitex negundo* root bark extract in capping of copper nanoparticles. The SEM-EDX analysis confirms the presence of copper nanoparticles. The obtained CuNPs seemed to exhibit spherical, cubic and hexagonal agglomerate morphological structure. The results of EDX analysis shows the distinct peaks of the elements present in the test sample. As shown in Fig. 4, weight percentages of carbon, oxygen, phosphorous, chlorine and copper were 33.18, 38.07, 2.25, 5.14, and 21.26 %, respectively. This data proves the maximum content of organic compounds and minimum content of inorganic compounds *i.e.* metallic copper nanopaticles.



eZAF smart quant results

Element	Weight %	Atomic %	Net Int.	Error %	K ratio
C K	33.18	48.50	35.50	12.74	0.0840
O K	38.07	41.78	62.34	11.93	0.0673
P K	2.25	1.28	20.52	14.15	0.0128
ClK	5.14	2.55	57.35	8.55	0.0377
CuK	21.36	5.90	98.77	4.22	0.1869

Fig. 4. SEM-EDX analysis of synthesized copper nanoparticles

Evaluation of catalytic activity of CuNPs: The catalytical activity of CuNPs was tested for the synthesis of (1-(cyclopropylmethyl)-1*H*-1,2,3-triazol-4-yl)methyl acetate. It was observed that a good yield of product obtained using water as solvent.

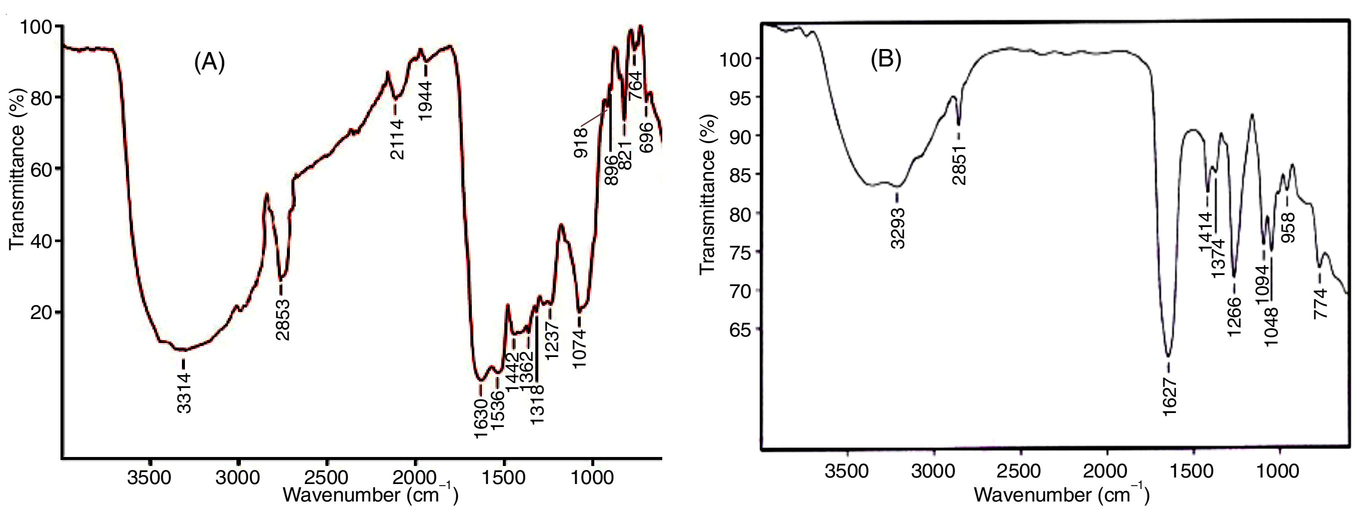


Fig. 3. FT-IR spectra of (A) Pure *Vitex negundo* root bark extract (B) CuNPs capped by *Vitex negundo* root bark extract

TABLE-1
SUSCEPTIBILITY OF TEST BACTERIAL STRAINS TO ROOT BARK EXTRACTS OF *Vitex negundo* AND STANDARD ANTIBIOTICS

CuNPs	Zone of inhibition (mm)				
	Bacillus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Erythromycin*
A	7.0	5.5	4.0	6.0	9.0
B	5.5	4.0	4.5	4.5	8.5
C	6.5	4.0	2.5	4.0	8.0
D	5.0	2.5	3.0	3.5	8.5
Average (mm)	6.0	4.0	3.5	4.5	8.5

TABLE–2
RECENT LITERATURE OF ANTIBACTERIAL ACTIVITY OF CuNPs AGAINST DIFFERENT MICROORGANISMS

S. No.	Targeted microorganism	Zone of inhibition (mm)	Method of synthesis of CuNPs	Ref.
1	*Escherichia coli, Bacillus subtilis, Staphylococcus aureus*	5	Green synthesis method	[27]
2	*Bacillus subtilis*	9	Wet chemical synthesis	[28]
3	*Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa*	6-24	Ultrasound-assisted method	[29]
4	*Staphylococcus aureus, Escherichia coli*	12-16	Green synthesis method	[30]
5	*Staphylococcus aureus*	14	Green synthesis method	[31]
6	*Staphylococcus aureus, Escherichia coli*	1.1-1.7	Green synthesis method	[32]
7	*Escherichia coli*	9.3	Green synthesis method	[33]
8	*Escherichia coli, Staphylococcus aureus*	10-14	Green synthesis method	[34]
9	*Pseudomonas aeruginosa, Escherichia coli*	20-26	Green synthesis method	[35]
10	*Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus*	5-26	Polyol Synthesis	[36]

Antibacterial activity: The antibacterial activity of CuNPs was assayed by agar well diffusion method. The biologically synthesized CuNPs have showed moderate to good range of activity against all the tested organisms such as *Bacillus subtilis*, *Staphylococcus aureaus*, *Escherichia coli* and *Pseudomonas putida*. Zone of inhibition was measured (mm) and compared with standard antibiotic erythromycin. Among all the strains tested *Bacillus subtilis* show very high sensitivity (average of 6.0 mm) against CuNPs. Remaining strains *Staphylococcus aureaus*, *Escherichia coli* and *Pseudomonas aeruginosa* show moderate to less activity and their average zone of inhibition is 4.0, 3.5 and 4.5 mm, respectively (Table-1).

Further, the literature surveyed for antibacterial activity of green synthesized CuNPs to compare the present results (Table-2). This study creates much attention in researchers to focus more investigations of antibacterial profile of CuNPs synthesized by plant mediated green technology.

Conclusion

In the present work, a simple green method for the synthesis of CuNPs using root bark extract of the medicinal plant, *Vitex negundo* Linn is reported. The characterization results were showed that the size of CuNPs is around 60 nm. This green method is more advantageous due to easy purification, use of non-toxic reagents and environmentally benign. The catalytic activity of CuNPs was investigated and it is proved that they possess effective catalytic activity by forming (1-(cyclopropyl-methyl)-1*H*-1,2,3-triazol-4-yl)methyl acetate in good yields. Antibacterial activity data showed that CuNPs worked meritoriously on *Bacillus subtilis* when compared to *Staphylococcus aureaus*, *Escherichia coli* and *Pseudomonas aeruginosa*.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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