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ANTIOXIDANT AND LARVICIDAL STUDIES OF COPPER OXIDE AND ZINC OXIDE NANOPARTICLES BY MUSA ACUMINATA LEAVES AS REDUCING AGENT

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ABSTRACT

In recent science, nanotechnology is a glowing field for the upcoming researchers. Nanoparticles exhibit better characteristic size, orientation, physical properties and morphology than bulk materials. Nanoparticles can be easily prepared by various physical, chemical and biological methods. The biological approach is the most emerging trend of preparation because this method is eco-friendly, less time consuming and easier than the other methods. Biological methods involve the use of plant extracts, bacteria and fungi. In this present work, plant extract was used to prepare copper oxide and zinc oxide nanoparticles. Among various metallic nanoparticles, copper oxide and zinc oxide nanoparticles display unique characteristics including pharmacological activities that are not observed in metallic state. This study provides useful information regarding the synthesis of copper oxide and zinc oxide nanoparticles using aqueous *Musa Acuminata* leaf (banana leaf) extract. Prepared leaves extract was used to analyse the phytochemicals present in it. These phytochemicals are responsible for the reduction of metal ions to nanoparticles also act as stabilizing agent. Biological studies like antioxidant and larvicidal activity were carried out for the prepared nanoparticles and it was found that prepared nanoparticles exhibits enhanced biological activity than aqueous leaves extract.

KEYWORDS: *Musa Acuminata* leaves, copper oxide nanoparticles, zinc oxide nanoparticles, larvicidal activity and antioxidant activity.

INTRODUCTION

At present, the utilization of waste source of plant extract results, the development of natural production of nanoparticles and this field has been attracted the young researchers.[1,2] Today an interdisciplinary research is gaining considerable interest especially in the field of nanotechnology.[3] Nanotechnology has been fascinated many researchers from different fields such as material science, medicine, chemistry, engineering and physics.[4] The distinctive shape, size, physical, chemical and biological properties of nanoparticles makes superior. Synthesis of nanoparticles (NPs) can be performed using a number of routinely used chemical and physical methods[5] but they show drawbacks like expensive reagent, hazardous reaction condition, longer time and tedious process to isolate nanoparticles.[11] The green synthesis of nanoparticles achieved by plants and their derivatives, microorganisms like bacteria, fungi, algae and yeast.[13] Biologically synthesized nanoparticles are gaining considerable interest in the area of biology because it follows the principles of Green chemistry. Biological method focused on the production of desired products without generation of hazardous intermediate.[6] Physical, chemical and biological applications has been enhanced when the conversion had taken place from metallic state to nano state.[7]

Metal oxide nanoparticles are significant due to their applications in different fields like nanodevices, nanoelectronics, nanosensors, information storage and catalysis.[8] Among various metal oxide NPs, CuO NPs has attracted due to their useful physical properties such as high temperature superconductivity, electron correlation effects and spin dynamics.[8] Applications of CuO NPs include antioxidant, antibacterial, anti fouling, anti-biotic, anti-fungal agent and catalysis.[9-10]

The synthesis of zinc oxide nanoparticles (ZnO NPs) achieved by various methods such as reaction of hydrothermal synthesis, zinc with alcohol, precipitation method[12] but in these methods the generation of toxic chemicals increased as by products. ZnO NPs are used in the elimination of toxic chemicals like arsenic, sulfur from water sources owing to their large surface area by volume ratio than the bulk materials.[14] They also have potential application in the field of medicine like drug delivery, biological activities such as antimicrobial, antioxidant and diagnosis of diseases.[14]

Hence green synthesis is a good choice due to the eco-friendly approach of the synthesis. In our study, we have highlighted the use of environmentally benign leaf extracts of *Musa*

Acuminata for the synthesis of copper oxide and zinc oxide nanoparticles. Synthesized nanoparticles has been used to study the larvicidal and antioxidant activity.

MATERIALS AND METHODS

Materials

All chemicals and reagents used for the research work were of analytical grade (AR) and of the highest purity available. All glassware was washed with sterile distilled water and dried in an hot air oven before use. Fresh *Musa Acuminate* leaves were collected locally in the market. *Culex.quinquefasciatus* larvae were procured from Zonal Entamological Unit, Velapadi, Vellore. The FTIR spectrum was taken using SHIMADZU spectrometer in 4000 - 400 cm^{-1} using KBr pellet.

Preparation of the leaf extract

The fresh *Musa Acuminata* leaves were collected. The *Musa Acuminata* leaves were thoroughly washed several times using normal water and then followed by distilled water to remove impurities, the cleaned leaves were subsequently dried under sunshade to remove moisture completely. leaves were powdered by using mechanical grinder and then stored. 5 g of powdered leaves were taken in beaker along with 75 mL of double distilled water and allowed to boil at 60°C for 20 min, then it was cooled down to room temperature. The extract turned brown. The prepared solution was filtered through Whatman No.1 filter paper to get clear solution and filtrate was stored at 4°C for further works.

"Fig. 1" *Musa Acuminata* leaves extract.

Preparation of Copper sulphate solution

Exact amount of 0.1M copper sulphate solution was prepared by dissolving 2.496 g of copper sulphate in 100 mL of double distilled water.

Preparation of Zinc sulphate solution

An accurate amount of 0.1M zinc sulphate solution was prepared by dissolving 2.875 g of zinc sulphate in 100 mL of double distilled water.

Preparation of copper oxide nanoparticles

Synthesis of CuO NPs was done by the standard procedure.[15,16,17] About 20 mL of leaves extract was added in drops to 80 mL of 0.1M copper sulphate solution under continuous stirring. The colour of the solution changes from blue to green. After the complete addition of the leaves extract, the reaction mixture was kept under stirring for about 3 h. The colour change occurs from brown to dark green colour. This solution was incubated for about 24 h at 4°C. The black residue was settled at the bottom of the beaker, it was carefully collected and dispersed in double distilled water to remove undesirable material. Further the solution was filtered and dried and packed for the characterization purpose.

"Fig. 2" Preparation of copper oxide nanoparticles.

Preparation of Zinc oxide nanoparticles

As per the standard procedure[15,16,17] ZnO NPs were prepared. 80 mL of 0.1M zinc sulphate was mixed with 20 mL of leaves extract under continuous stirring. The colour of the solution changes from colourless to light yellow. Finally the reaction mixture was kept under stirring for about 3 h and colour change takes place from yellow to brown colour. This solution was incubated for about 24 h at 4°C. The colourless residue was settled at the bottom of the beaker, it was carefully collected and dispersed in double distilled water to eliminate unwanted material. The solution was filtered, dried and packed for characterization purpose.

"Fig. 3" Preparation of zinc oxide nanoparticles.

Antioxidant activity

The scavenging activity of the CuO NPs and ZnO NPs was measured by H_2O_2 method. The method was adopted as per the literature with slight modification.[18] An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that can produce free radicals, leading to chain reactions that may damage cells. Hence, an new attempt was made to study antioxidant activity of prepared nanoparticles.

Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activity is one of the best method to study the antioxidant property.[14,15] A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (50 mM, pH 7.4). The concentration of hydrogen peroxide was determined by absorption at 230 nm using a spectrophotometer. Test solutions was prepared by taking different concentrations (0.5 mg, 1 mg, 2 mg and 4 mg) of the sample (CuO NPs and ZnO NPs) in 2 mL ethanol, these were added to 2 mL hydrogen peroxide and shaken vigorously, absorbance at 230 nm was determined after 10 minutes against a blank solution containing phosphate buffer, without hydrogen peroxide act as control. The percentage of hydrogen peroxide scavenging activity is calculated.

Radical scavenging activity = (Ac-As) / Ac x 100

Ac - absorbance of control, As - absorbance of sample

Larvicidal bioassay

The larvicidal activity was done by the procedure of WHO guide lines with some modification.[16] The eggs and egg rafts of *C. quinquefasciatus* were dipped into a plastic bottle containing 500 mL of dechlorinated water for 30-40 min to hatch out larvae. They were maintained in the laboratory as per literature.[17] Mosquito larvae were fed with powdered

nutrient broth once a day. After 4 days the hatched larvae turned into larvae in early fourth stage and were subjected for further experiment. A total of 20 reared mosquito larvae was placed in 200 mL of double distilled sterilized water containing various concentrations (8 mg, 6 mg, 4 mg, 2 mg) of synthesized nanoparticles. A set up with sterile distilled water is considered as negative control. Percentage of mortality was assessed after 24 h of incubation. A number of dead larvae in each batch were counted every hour for 24 h exposure period. The treated larvae was mounted on a slide and examined under a microscope for image capture.

RESULT AND DISCUSSION

Phytochemical Screening Test

Phytochemicals present in the *Musa Acuminata* leaves extract was carried out as per the modern procedure.[19,20] The immiediate reduction takes place between metallic state to nanoparticles can be achieved by phytochemicals present in leaves extract. The chemical reactions, which proceed in the aqueous exract may be as follows.[21]

Metal solution + *Musa Acuminata* leaves extract → $[Metal]^+$

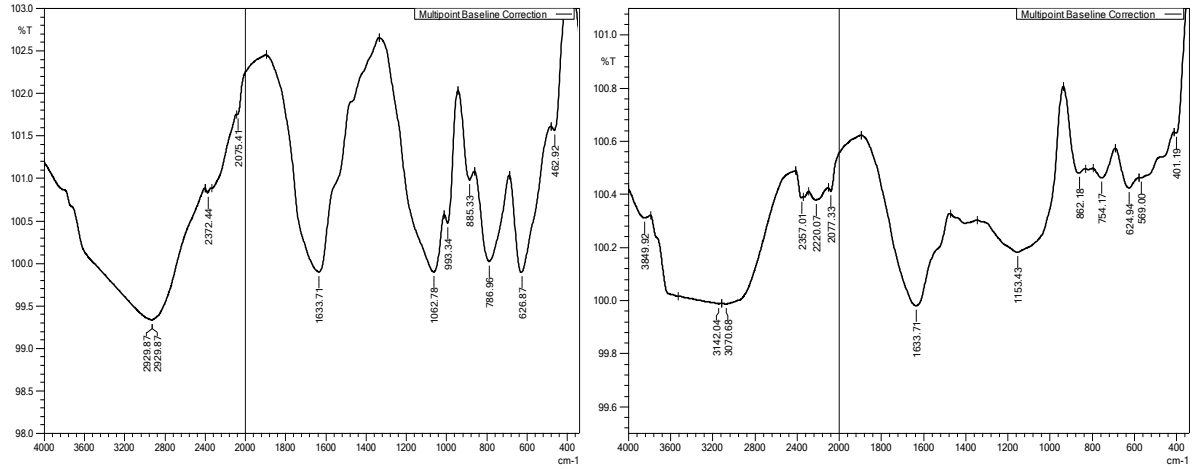
phytochemicals + $[Metal]^+ + e^-$ →[Metal]{ [Reduction]}

Table 1: Phytochemical screening of *Musa Acuminata* leaves extract.

S.No	Test	*Musa Acuminate* leaves	S.No	Test	*Musa Acuminate* leaves
1	Flavonoids	+++	8	Reducing sugars	+
2	Tannins	++	9	Anthocyanins	+
3	Glycosides	+	10	Carbohydrates	++
4	Steroids	+	11	Proteins	++
5	Saponins	+	12	Alkaloids	+++
6	Phenols	+++++	13	Fatty acid	+++
7	Triterphenoids	+++	14	Lignins	+

+ :Present -- : Absence

FT-IR spectra of CuO and ZnO Nanoparticles



"Fig. 4" & "Fig. 5" FTIR Spectra of CuO NPs and ZnO NPs.

The FTIR spectra of CuO nanoparticles (Fig. 4) showed the characteristic absorption bands at 1633 cm^{-1}, 2929 cm^{-1}, 462 cm^{-1}. The strong band observed at 1633 cm^{-1} corresponds to C=O stretching of amides and band at 2929 cm^{-1} represents the O-H stretching of phenolic compounds. The existence of band at 462 cm^{-1} recognized the vibration of CuO NPs and certified the formation of copper oxide nanoparticles.[22,23] The FT-IR analysis of CuO suggested that these particles are surrounded by any of their organic molecules such as polyphenols, terpenoids and alkaloids. Hence, the chemical constituent present in leaf extract such as flavonoids, phenols, alkaloids and fatty acids act as capping and reducing capacity and are responsible for the reduction of copper ion to copper oxide nanoparticles.[24,25]

The FTIR spectra of ZnO nanoparticles (Fig. 5) showed the characteristic absorption bands at 3070 cm^{-1}, 3142 cm^{-1}, 1633 cm^{-1}, 1153 cm^{-1} and 401 cm^{-1}. The strong band appeared around 3100 cm^{-1} corresponds to O-H groups of phenolic compounds. The appearance of band at 1633 cm^{-1} represents C=O group which is present in phytochemicals that are surround by the ZnO NPs. A sharp band obtained at 401 cm^{-1} certified the formation of ZnO NPS.[26,27]

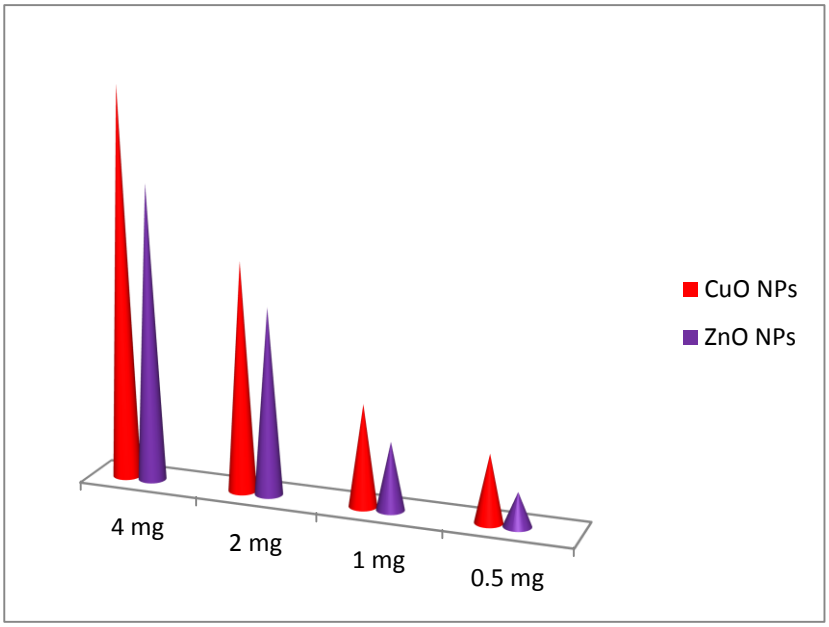
ANTIOXIDANT ACTIVITY

Hydrogen peroxide method

Antioxidant scavenging activity of CuO NPs and ZnO NPs was examined. α-tocopherol with 89.45 % of scavenging activity is used as positive control and ethanol is used as negative control. CuO NPs exhibits better antioxidant scavenging activity than ZnO NPs when compared with standard α-tocopherol. This study proves that scavenging activity of the test solution increased on increasing the concentrations.

Table 2: Antioxidant scavenging activity of nanoparticles.

Concentrations	Scavenging activity %	
	CuO NPs	ZnO NPs
4 mg	46	35
2 mg	27	22
1 mg	12	08
0.5 mg	08	04



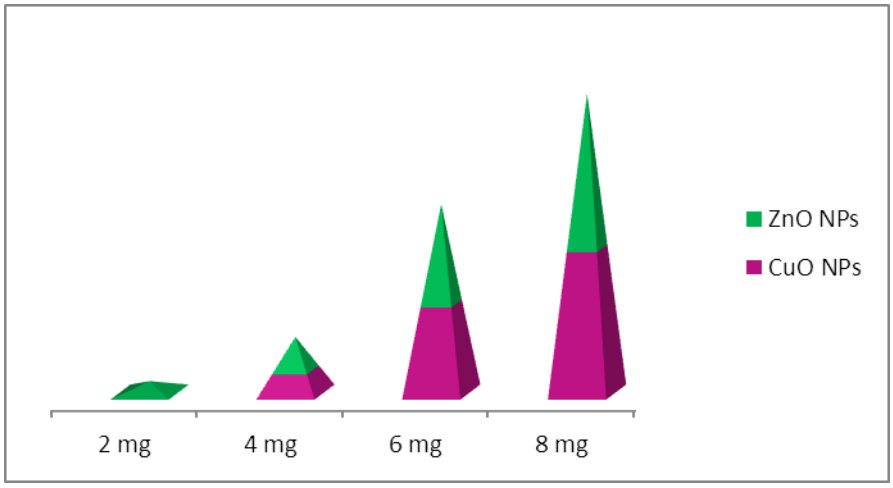
"Fig. 6" Graphical representations of scavenging activity.

Larvicidal activity

The larvicidal activity of synthesized CuO NPs and ZnO NPs was performed against *Culex. quinquefasciatus*. The number of dead larvae was minimum after 24 h of contact period. The highest mortality was found after 48 h of exposure period. Highest concentrations of ZnO NPs and CuO NPs exhibit good larvicidal activity.

Table 3: Larvicidal activity of prepared nanoparticles.

Concentrations	Mortality (out of 20 larvae)	
	CuO NPs	ZnO NPS
2 mg	0	1
4 mg	2	3
6 mg	8	9
8 mg	13	14



"Fig. 7" Graphical representations of larvicidal activity.

CONCLUSION

In the present study CuO NPs and ZnO NPs has been synthesized using *Musa Acuminata* leaf extract and characterized by FTIR. FTIR results shows that reduction of copper and zinc ions to stabilization of CuO NPs and ZnO NPs occur through possible participation of leaf extracts and others metabolites present in the leaf extracts. The prepared nanoparticles were screened to study their biological properties like *in-vitro* antioxidant and larvicidal activity.

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REFERENCES

1. Palanivel V, Sang-Myung L, Mahudunan L, Kui-Jae L, Byung-Taek O. Pine conemediated green synthesis of silver nanoparticles and their antibacterial activity against agricultural pathogens. Appl Microbiol Biotechnol, 2013; 97: 361–368.
2. Savage N, Diallo MS. Nanomaterials and water purification. Opportunities and challenges. Nanopart. Res, 2005; 7: 331–342.
3. Ko SH, Park I, Pan H, Grigoropoulos CP, Pisano AP, Luscombe CK, Frechet JMJ. Direct nanoimprinting of metal nanoparticles for nanoscale electronics fabrication. Nano Let, 2007; 7: 1869-1877.
4. Lanje AS, Sharma SJ, Pode RB, Ningthoujam RS. Synthesis and optical characterization of copper oxide nanoparticles. Advances in Applied Science Research, 2010; 1(2): 36-40.

5. Ershov BG, Janata E, Michaelis M, Henglein A. Reduction of aqueous copper (2+) by carbon dioxide (1−): First steps and the formation of colloidal copper. J Phys Chem, 1991; 95: 8996–8999.
6. Stopic S, Dvorak P, Friedrich B. Synthesis of spherical nanosized copper powder by ultrasonic spray pyrolysis. World Metall, 2005; 58: 195–201.
7. Xiong J, Wang Y, Xue Q, Wu X. Synthesis of highly stable dispersions of nanosized copper particles using L-ascorbic acid. Green Chem, 2011; 13: 900–904.
8. Dhas NA, Raj CP, Gedanken A. Synthesis, characterization, and properties of metallic copper nanoparticles. Chem Mater, 1998; 10: 1446–1452.
9. Sanvicens N, Marco MP. Multifunctional nanoparticles – properties and prospects for their use in human medicine. Trends Biotechnol, 2008; 26(8): 425-433.
10. Sankar R, Manikandan P, Malarvizhi V, Fathima T, Shivashangari KS, Ravikumar V. Green synthesis of colloidal copper oxide nanoparticles using Carica papaya and its application in photocatalytic dye degradation. Spectrochim Acta Mol Biomol Spectrosc, 2014; 121: 746–750.
11. Varshney R, Bhadauria S, Gaur MS, Renu Pasricha R. Characterization of Copper Nanoparticles Synthesized by a Novel Microbiological Method. J Miner Met Mater Soc, 2010; 62: 102-104.
12. Pulit-Prociak J, Chwastowski J, Kucharski A, Banach M. Functionalization of textiles with silver and zinc oxide nanoparticles. Appl Surf Sci, 2016; 385: 543-553.
13. Stankic S, Suman S, Haque F, Vidic J. Pure and multi metal oxide nanoparticles: synthesis, antibacterial and cytotoxic properties J. Nanobiotechnol, 2016; 14: 73.
14. Khan ST, Musarrat J, Khedhairy AL. Countering drug resistance, infectious diseases, and sepsis using metal and metal oxides nanoparticles: Current status Colloids Surf. B, 2016; 146: 70-83.
15. Siddiqui BS, Afshan F, Faizi GS, Naqui SNH, Tariq RM. Two insecticidal tetranortriterpenoids from Azadirachta indica. Phytochemistry, 2000; 53: 371–376.
16. Lalitha A, Subbaiya R, Ponmurugan P. Green synthesis of silver nanoparticles from leaf extract Azhadirachta indica and to study its anti-bacterial and antioxidant property. Int J Curr Microbiol App Sci, 2013; 2: 228–235.
17. Singhal G, Bhavesh R, Kasariya K, Sharma AR, Singh RP. Biosynthesis of silver nanoparticles using *Ocimum sanctum* (Tulsi) leaf extract and screening its antimicrobial activity. J Nanoparticle Res, 2011; 13: 2981–2988.

18. Kumar B, Smita K, Cumbal L, Debut A. Synthesis of silver nanoparticles using sacha inchi (Plukenetia volubilis L.) leaf extracts. Saudi J Biol Sci, 2014.
19. Shankar SS , Rai A, Ahmad A, Sastry M. Rapid synthesis of Au, Ag, and bimetallic Au core-Ag shell nanoparticles using Neem (Azadirachta indica) leaf broth. J Colloid Interface Sci, 2004; 275(2): 496-502.
20. Ren G, Hu D, Cheng EW, Vargas-Reus MA, Reip P, Allaker RP. Characterization of copper oxide nanoparticles for antimicrobial applications. Int J Antimicro Agents, 2009; 33(6): 587–590.
21. Harborne JB. Phytochemical methods: A Guide to Modern Techniques of plant Analysis. 2002.
22. Anita Sagadevan Ethiraj, Dae Joon Kang. Synthesis and characterization of CuO nanowires by a simple wet chemical method. Nanoscale Research Letters, 2012; 20127: 70.
23. Hassan MS, Amna T, Yang OB, E1-Newehy MH, Al-Deyab SS, Khil MS. Smart copper oxide nanocrystals: synthesis, characterization, electrochemical and potent antibacterial activity. Colloids Surf B: Biointerfaces, 2012; 97: 201–206.
24. Xu JF, Ji W, Shen ZX. Preparation and characterization of CuO nanocrystals. J Solid State Chem, 1999; 147(2): 516–519.
25. Huang J, Chen C, He N, Hong J, Lu Y. Biosynthesis of silver and gold nanoparticles by novel sundried Cinnamomum camphora leaf. Nanotechnol, 2007; 18: 105104.
26. Al-Gaashani R, Radiman S, Daud AR, Tabet N, Al-Douri Y. XPS and optical studies of different morphologies of ZnO nanostructures prepared by microwave methods. Ceram Int, 2013; 39: 2283–2292.
27. Jones N, Ray B, Ranjit KT, Manna AC. Antibacterial activity of ZnO nanoparticle suspensions on a broad spectrum of microorganisms. FEMS Microbiol Lett, 2008; 279: 71–76.