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SYNTHESIS OF COPPER NANOPARTICLES FROM BEE PROPOLIS EXTRACT AND ITS ANTIBACTERIAL PROPERTY

***Shubharani Ramnath**

Department of Biological Sciences, Surana College, South End Road, Bangalore-560004, India.

***Corresponding Author: Shubharani Ramnath**

Department of Biological Sciences, Surana College, South End Road, Bangalore-560004, India.

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ABSTRACT

Nature has diverse processes for synthesis of nanoscaled inorganic materials which have contributed to the enhancement of innovative area of research based on biosynthesis of nanoparticles. In the present study, copper nanoparticles were synthesised by using simple method of chemical reduction from ethanol extract of propolis. The biosynthesized copper nanoparticles were characterized with UV-Vis spectroscopy, Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD). The UV-Vis spectra confirmed the peak for synthesized copper nanoparticles at 448 nm. The XRD analysis showed that the copper nanoparticles are crystalline in nature. The FTIR measurement was carried out to identify the possible bio molecules responsible for efficient stabilization of copper nanoparticles. These biologically synthesized copper nanoparticles were effectively utilized for antibacterial activity against human pathogens viz, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Strptococus mutans*, *Bacillus subtilis*, *Klebsella pneumonia*, *Enterococcus faecalis* and *Citrobacter* spp. The antibacterial activity was tested by well diffusion zone inhibition method. *Staphylococcus mutans*, *Klebsella pneumonia* and *Enterococcus faecalis* exhibited the highest zone of inhibition in copper nanoparticles.

KEYWORDS: Antibacterial, biosynthesis, copper nanoparticles, propolis.

INTRODUCTION

Nanotechnology is mainly concerned with synthesis of nanoparticles with desired quality and its potential use in human benefits. The biosynthesis of nanoparticles play an important role in modern research and interconnects nanotechnology and biotechnology, which can be applied in many fields including food, pharmaceuticals, cosmetic, catalyst, bioengineering, biosensors, environmental health and in agriculture.[1,2,3,4] In recent years, the development of efficient green chemistry methods employing natural reducing, capping, and stabilizing agents to prepare nanoparticles with desired morphology and size have become a major focus of researchers. Many scientists have reported the biosynthesis of metal nanoparticle using microorganisms, enzymes, plant extracts and other biological principles as reducing agents with physical or chemical approach to produce solid nanoparticles (1-100 nm) with specific functions.[5,6,7]

Copper and its alloy have wide application in biomedical especially as antimicrobial agent. Due to their high surface-to-volume ratio, helps to interact with other material effectively among various other metals.[8] It is highly toxic to microorganisms and non toxic to animal cells, due to which it is considered safe for human being and used in food package and water treatment industries. In United States, Environmental Protection Agency (EPA) has approved the registration of copper as an antimicrobial agent which is capable of killing harmful bacteria including *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.[9] Thus, biologically synthesized metal nanoparticles exhibiting diverse therapeutic potential are gaining more importance recently.[10]

Propolis is a natural resinous bee hive product collected by honey bees from leaf buds and exudates of various plants, mixed with bee enzyme, pollen and wax. Its complex bio active compounds depend on plant sources in local flora, which are used widely in folk medicine since ancient time. Propolis exhibits a broad spectrum of biological and pharmacological properties such as antioxidant, antimicrobial, anti-inflammatory, antidiabetic, antiulcer, antitumor, cardioprotective, hepatoprotective, immunomodulatory and neuroprotective action.[11,12,13,14,15,16] It has already been reported that the bee propolis can synthesise nanoparticles, due to its broad range of bio molecules like phenols, flavonoids, alkaloids, steroids, terpenoids etc. The natural gums isolated from trees are also used for the production of noble metal nanoparticles like Ag and Au, which acts both as reducing and capping agent.[17] Copper is well known for their antibacterial effect, the synthesis of copper nanoparticles from propolis extract has not been studied. In the present

study, ethanol extract of propolis collected from Karnataka region of India, has been used as reducing agent for the synthesis of copper nanoparticles and evaluation of its antibacterial potential against selected pathogens.

MATERIALS AND METHOD

The fresh propolis sample belonged to *Apis mellifera* colonies from Bijapur region of Karnataka State, India, was collected, cleaned to remove debris and stored in the freezer until further processing.

Preparation of ethanol extract of propolis

The ethanol extract of propolis was prepared by crushing 10 gm of propolis into small pieces and extracted with 100 ml of 70% ethanol and left overnight followed by intermittent shaking at room temperature. The suspension obtained was filtered and the extraction was repeated twice. The extracts were combined, evaporated to dryness under pressure by using rotor evaporator and dried extract was weighed to determine the yield of soluble components.[18]

Synthesis of copper nanoparticles

For the synthesis of copper nanoparticles, 25 ml of ethanol extract of propolis was added drop wise into 25 ml of 1 mM solution of copper sulphate under constant stirring for reduction of copper ions and incubated for 2-3 hours.[19] The reduction of copper sulphate to copper ions was confirmed by change in colour from dark green to straw yellow. Later, the solution was centrifuged for 15 min at 10,000 rpm and dispersed in double distilled water to remove the unwanted biological materials. The formation of copper nanoparticles was confirmed by using UV- Visible spectrometer in the range between 300-600 nm. Fourier transform infrared (FTIR) analysis was carried out to find the bioactive compounds, which is responsible for the reduction of copper ions with the spectral range of 500-4500cm-1. The crystalline structure of the biosynthesized copper nanoparticles was investigated through X-ray diffraction technique using X-ray diffractometer scanned in 2θ between 10° and 100°.

ANTIBACTERIAL ACTIVITY

The Antibacterial assay of copper nanoparticles synthesised from ethanol extract of propolis was studied by agar well diffusion method. The bacterial strain tested were *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Strptococus mutans*, *Bacillus subtilis*, *Klebsella pneumonia*, *Enterococcus faecalis.* and *Citrobacter spp.* The pure cultures of bacteria were swabbed uniformly on the individual plates using sterile cotton swabs on the Nutrient Agar medium. Four wells were made (6 mm in diameter) in the medium plates with the help of gel puncture and 50 µl prepared nanoparticle solution, propolis extract, standard antibiotic (Ampecillin) and 70% ethanol was added to each well.[20] The petri plates were incubated for 24 hrs at 37°C and bactericidal potential of synthesised copper nanoparticles was observed by standard inhibition zone assay.

RESULT AND DISCUSSION

Nanoparticles are refers to spherical particles with diameter in range of 1-100 nm and generally characterised by their size, shape, surface area and disparity.[21] Reduction of copper ions into copper nanoparticles during exposure to propolis extract was observed as a result of the colour change. The colour change is due to the Surface Plasmon Resonance phenomenon. The metal nanoparticles have free electrons, which give the Surface Plasmon Resonance absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with light wave. The ethanol extract of propolis was dark green in colour and after the addition of copper sulphate solution, the colour changed to straw yellow within 3 hours. It indicates the formation of copper nanoparticles. The UV spectrum is the most confirmatory tool for the detection of copper nanoparticles and the result obtained is presented in Fig 1. Absorption bands in the UV-Vis range is exhibited by colloidal dispersion of metal due to the excitation of Plasmon resonance or inter band transition which are characteristic property of the metallic nature of the particle. Light wavelengths in the 300–800 nm are generally used for characterizing various metal nanoparticles in the size range of 2 to 100 nm.[22] In the present study, the UV- Vis analysis from 300-600 nm revealed that maximum absorption spectra of copper nanoparticles formed has absorbance peak at 448 nm.

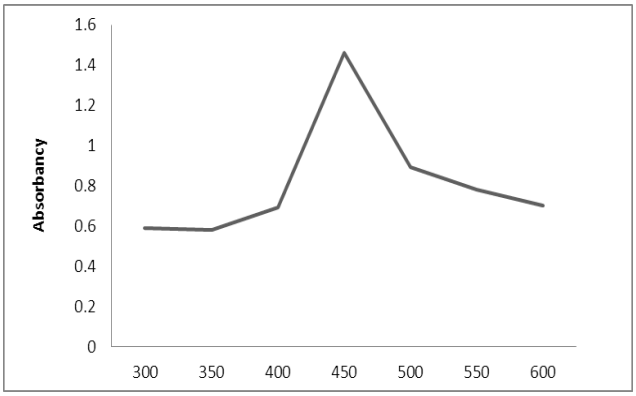

Fig. 1: UV- Vis spectrum of copper nanoparticles.

Fourier transform infrared (FTIR) measurement is an analytical technique, which measures infrared intensity versus wavelength (wave number) of light. It is used to determine the nature of bio molecules and their functional group present in synthesised copper nanoparticle.[23] The FTIR analysis showed the peak value at 3820, 3465, 3018, 2705, 1678, 1240, 728 cm^{-1} (Fig 2) and spectra confirmed the presence of different functional group like alcohol, phenols and acids (O-H), alkane (C-H), amine (C-N), nitro compounds (N-O). The FTIR spectrum of ethanol extract of bee propolis indicates the presence of many fundamental groups

mainly alcohols and phenols are responsible for the their capping and reducing capacity. reduction of copper ions to copper nanoparticles due to

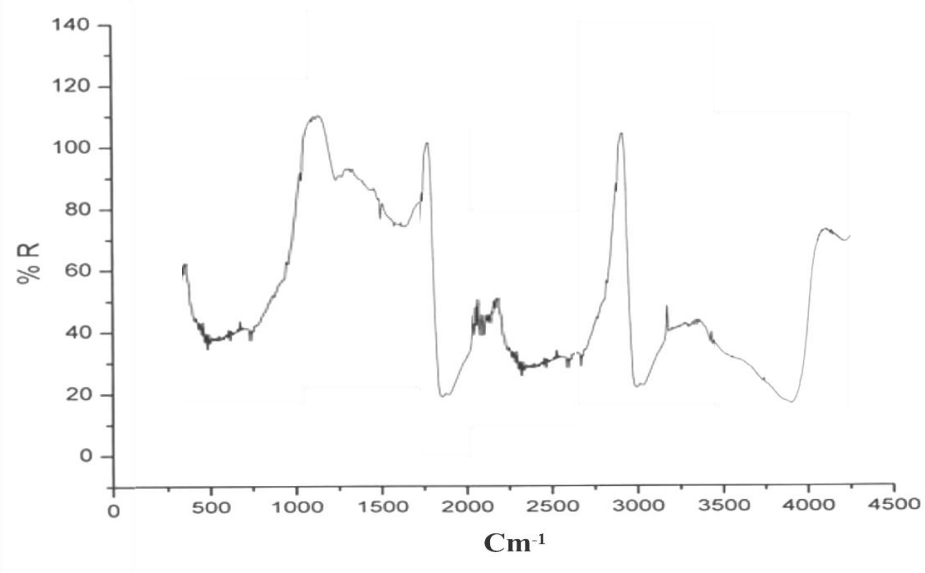

Fig. 2: FTIR spectrum of copper nanoparticles.

XRD is used for the phase identification and characterization of the crystal structure of the nanoparticles.[24] XRD analysis of synthesised copper nanoparticles is shown in Fig 3. The distinct peak at 26^{o} clearly determined the crystal nature of copper nanoparticles obtained by the reduction of copper ions using ethanol extract of bee propolis. This peak might have resulted due to capping agent stabilizing the nanoparticles.

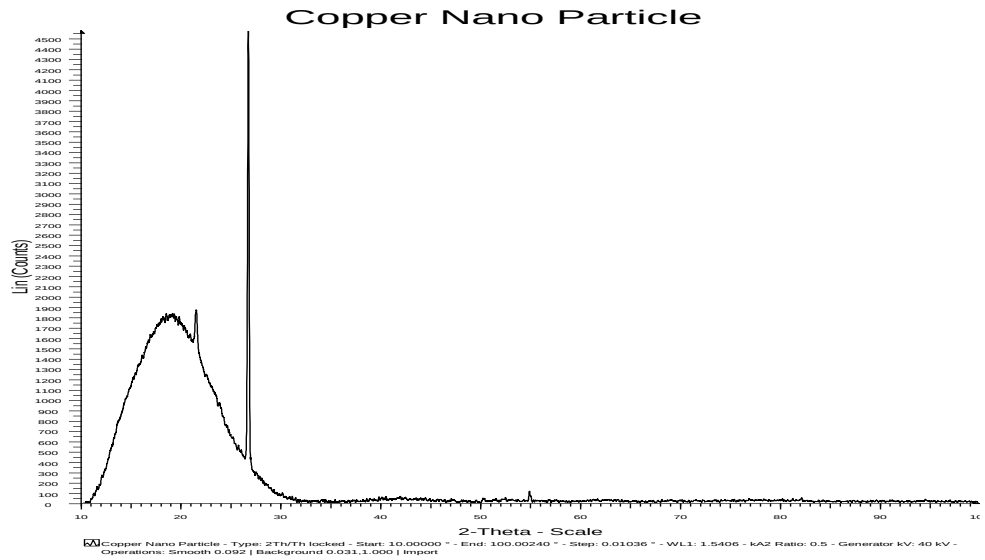

Fig. 3: XRD analysis of copper nanoparticles.

ANTIMICROBIAL ACTIVITY

In medicine, nanoparticles offers many benefits including protecting drug from degradation, enhancing solubility of poorly soluble drugs, increase efficacy of active ingredients and decrease the risk of its side effect and toxicity.[25] According to the present study, the antibacterial activity of synthesised copper nanoparticles from ethanol extract of propolis was studied with selected gram-positive and gram-negative pathogens such as, *Staphylococcus aureus*, *Streptococcus mutans*, *Enterococcus faecalis*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsella pneumoniae*, and *Citrobacter* spp. Antibacterial activity was performed by well diffusion method. The result revealed that the copper nanoparticles of propolis extract was found to be effective against gram-positive bacteria *Staphylococcus*

mutans and *Enterococcus faecalis* followed by *Bacillus subtilis and Staphylococcus aureus* with the inhibition zone of 26mm, 22mm, 18mm and 14mm in diameter respectively. The gram-negative bacteria like *Klebsella pneumonia*, *Escherichia coli* and *Pseudomonas aeruginosa* showed 24mm, 16mm and 14mm in diameter respectively as zone of inhibition, where as *Citrobacter* spp showed minimum inhibition zone. The positive control of Ampecillin showed the highest antibacterial activity than synthesized copper nanoparticles.

Table 1: Evaluation of antibacterial activity of copper nanoparticles.

Sl. No	Microorganisms	Zone of inhibition (mm)			
		Standard Ampecillin	Ethanol (70%)	Ethanol extract of Propolis	Copper nanoparticles
1	*Staphylococcus aureus*	36	8.0	10	14
2	*Staphylococcus mutans*	40	6.4	22	26
3	*Enterococcus faecalis*	32	6.6	20	22
4	*Bacillus subtilis*	32	8.0	14	18
5	*Escherichia coli*	32	7.4	16	16
6	*Pseudomonas aeruginosa*	32	8.2	14	14
7	*Klebsella pneumoniae*	42	8.0	20	24
8	*Citrobacter spp.*	36	6.4	6.8	6.8

CONCLUSION

Biosynthesis of nanoparticles is a kind of bottom up approach where the main reaction occurring is reduction or oxidation. For the first time, the copper nanoparticles were successfully synthesized by using propolis extract for the antibacterial study. This provided cost effective, easy way and efficient synthesis of copper nanoparticles. The functional group present in the ethanol extract of propolis was confirmed by FTIR analysis. These functional groups were mainly responsible for the reduction of copper metal ions into copper nanoparticles. The synthesized copper nanoparticles were analyzed using UV-spectrophotometer, FITR and XRD. Copper nanoparticles were effectively utilized for the antibacterial activity study. The obtained result set a basis for future studies using propolis nanoparticles for different biomedical applications.

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