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Greener Synthesized Copper Nanoparticles incorporated in Polyethylene Glycol/ Polyvinyl Alcohol Nanocomposite for Food Package Applications

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ABSTRACT

Green Synthesis of nanoparticles enables for medical and Food package applications. Copper nanoparticles (CuNPs) are having antimicrobial and anti-viral properties that provides the cushion in drug delivery applications. Green Synthesis of copper nanoparticle from Capparis zeylanica plant and solution casting it with Polyethylene Glycol/Polyvinyl Alcohol can be utilized for food packaging, drug delivery and cancer therapy, etc. Capparis zeylanica plant extract act as both reducing and stabilizing agent on the outermost layer of copper nanoparticles. The synthesized nanoparticles and nanocomposite were characterized and found to be of 65 nm of particle size using scanning electron microscopy. Fourier transform infrared spectroscopy shows the presence of various bio-active materials that were responsible for the stabilization of CuNPs. Differential Scanning Calorimentric studies on the composites shows that there a significant increment in the glass transition and melting temperature of the composites of +10 °C with that of the polymer blend. Moisture absorption and Anti-microbial analysis were carried out to understand the stability of the nanocomposites. Polyethylene Glycol/Polyvinyl Alcohol + Copper nanoparticles nanocomposite shows an excellent characteristic in most aspect that able to fit for bio-medical and food packaging applications.

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INTRODUCTION

In the past decade, research in bio-medical, food industries, pharmacy, engineering and various technologies were highly influenced by nano due to the novel properties showed by the materials. The popularity of nanotechnologies has addicted researchers to explore unknown depths of their fields of new materials for various critical problems. The controlled restructuring of the matter at nano level allows enhancing the properties of material [1-6]. Nanoparticles have wide range of applications in Food Industry, Biomedical, Electronics, Automobiles, and in fields of Agriculture [2-7].

The nanoparticle preparation method can be adapted based on the application such as physical, chemical and biological. The Green synthesis technique is uses for synthesising nanoparticles for biomedical applications, food and pharmaceutical applications such as bio-

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sensing, bio-imaging, targeted drug delivers and gene therapy applications [8-12].

In nano green synthesised, particles have a coating on the surface by the biomolecules from the plant extract provides an extra protection for the nanoparticles that enables its application towards medical without any alteration. Green synthesis processed nanoparticles are widely used in food processing and packaging, Biomedicine, targeted drug delivery for cancer therapy, Tumour treatment and pharmacy industry [13-18].

Different variety of plants were employed for the preparation of nanoparticles for biomedical and pharmaceutical applications. *Capparis zeylanica* is a rare species, indigenous, herbaceous and perennial plant native to E. Asia, India, Myanmar, Thailand, Cambodia, Laos, Vietnam, and Malaysia. It is the most well-known member of the Capparidaceae family [19-20]. In traditional Siddha, *Capparis zeylanica* has been used as a drug for many illnesses. It was testified to possess antioxidant, antipyretic, analgesic, anti-inflammatory, antimicrobial and immunostimulant activity [21]. Fatty acids, flavonoids, tannins, alkaloids, E-octadec-7-en-5-ynoic acid, saponins glycosides, terpenoids, saponin, p-hydroxybenzoic, syringic, vanillic, ferulic, and p-coumanic acid were found in phytochemical screening of the plant [19-22]. The copper nano particles are very good antimicrobial agents, it toxic to virus and fungi, because of this features the copper nanoparticles are widely uses in bio medicinal application, food packaging to reduce the food borne diseases and targeting drug deliver [23-26].

Nanocomposites are used for the targeted drug delivery in various illness treatments [27-44]. Polyethylene Glycol (PEG) was used to bio-conjugate itself to the target, by coupling with the target molecule to optimize the process of pharmacokinetic properties of the drug treatment. PEG is an inactive substance that acts as the vehicle for drug and can be used as surface coating on nanoparticles [30-32]. Polyvinyl alcohol (PVA) is a derived from polyvinyl acetate is commonly used in textiles, medical, food packaging industries due to its strength, thermal stability, transparency, biocompatibility, water solubility and low manufacturing cost. PVA is a type of biodegradable resin derived from petroleum it can be used for in soft contact lenses, eye drops, coating and finishing agents [33-43].

In this present research investigation, copper nanoparticles were synthesized using *Capparis zeylanica* plant extract and the resultant nanoparticles were blended with PEG/PVA to form nanocomposites. The CuNPs were analyzed using UV-spectrometer, FITR, SEM with EDAX, TEM and XRD and PEG/PVA + CuNPs composite are analyzed using FTIR, Moisture Absorption, DSC, Antimicrobial Study.

MATERIALS AND METHODS

Materials

Indian caper is the scientific name for *Capparis zeylanica*. A climbing scandant shrub that can be found all over India. *Capparis zeylanica* is a member of the capparidaceae family. Plants are 2-3 m tall, have 3-6 mm long recurved thorns, are

Fig. 1. *Capparis zeylanica* Plant [4]

branched and have elliptic or narrowly lanceolate leaves with a rounded base and mucronate apex. PEG (Sigma, average molecular weight of 400), PVA (average molecular weight of 17 000) [4,5].

Preparation of Capparis zeylanica leaf extract and Copper Nanoparticles

Capparis zeylanica leaves are washed thoroughly using tap running water and followed by double distilled water to remove the dust and other waste particles. Clean leaves were dried for 4 h using hot air oven at room temperature. The dried leaves were grinded using mechanical grinder and the obtained powdered particles were filtered and fine powder were weighed of 5 g and added to 50 ml of double distilled water and boiled for 3 h under Soxhlet apparatus. The plant extract is then filtered and stored for further nanoparticles preparation [4,5].

The copper nanoparticles preparation process involves 0.001 mM copper sulphate solution is introduced in 25 ml of leaf extract solution and the mixture kept in incubation for 36 hr. After 24 h the color changes from green to straw yellow color that indicates the copper nanoparticle formation.

Synthesis of PVA/PEG blend with Nanoparticles

PVA (50 %)/PEG (50 %) films were prepared as follows: For each film, 2 g of PVA/PEG is dissolved in 50 ml double distilled water under stirring at 90 °C for 2 h until PVA/PEG dissolution completely and cooled to room temperature. The mixed solution stirred for another 1 h at room temperature then the copper nanoparticles were measured and added into blend and stirred and it has been casted into glass petri dishes and placed in an oven at 50 °C for 36 h in air [38].

Characterization

Characterization of CuNPs

Crystallization of CuNPs can be observed using X-ray diffraction analysis using Rigaku instrument. UV-Spectrometer has used for analysis of CuNPs size range is between 300-700 nm. Structure and size of CuNPs is analyzed using SEM and TEM. EDX analysis gives the elemental composition in the reaction mixture.

FTIR Analysis for PEG/PVA+ CuNPs Nanocomposites

When salts, plasticizers, and fillers are applied to the polymer host, the matrix interacts and/or complexes. This has an impact on the local structure of polymer backbones and has a direct impact on immobility. Infrared spectroscopy studies will provide proof of potential complexation and interactions in this context. A Nicolet 5 DXC spectrometer was used to capture infrared spectra. The solution casting process was used to produce thin films. To extract the residual solvent and complete the polymer blend mixing, all of the samples were annealed at 140° C for 2 hours. At 150° C, a minimum of 32 scans with a resolution of 2 cm^{-1} were signal averaged.

Moisture Absorption Analysis of PEG/PVA+ CuNPs Nanocomposites

The Moisture Absorption Test is used to assess the material's moisture absorptivity. To determine

Fig. 2. (a) PVA/PEG and (b) PVA/PEG blended with CuNPs

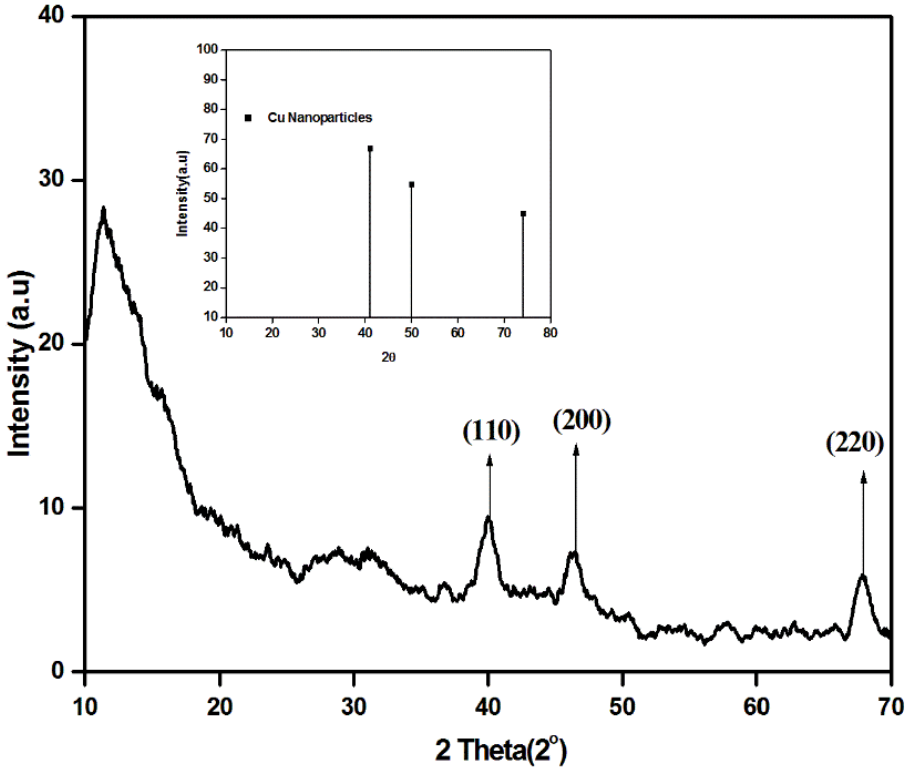

Fig. 3. XRD of copper Nanoparticles and JCPDS data of CuNPs (insert).

moisture absorption, square samples of polymer films (2 cm diameter) were placed on a desiccator's tray, which was initially filled with $CaCl_2$. The setup was left for a total of 24 h. The weight of the samples was measured after 24 h and used as the initial weight. Then, instead of $CaCl_2$, $Ca(NO_3)_2$ was used to complete the moisture absorption of the biopolymer composites. At the time interval t, the weights of the samples were calculated. The films were easily reinserted into the desiccator's tray. After a certain amount of time, the procedure was repeated until the films reached a constant weight. The following equation was used to measure the percentage of moisture absorption.

$$MA = \frac{(M_F - M_I)}{M_I} \times 100 \quad (1)$$

Whereas, MA is moisture absorption in %, M_F is final weight of the polymer in g, M_I is initial weight of the polymer in gram.

RESULTS AND DISCUSSION

XRD Analysis of CuNPs

The plant extract mediated synthesized Cu nanostructure was confirmed by the characteristic peak in the XRD image which was shown in the Fig 3. All diffraction peaks correspond to the characteristic face centered nanoparticles. These diffraction lines observed at 2θ angle for the copper nanoparticle at 40.23, 47.45 and 67.84° have been indexed as 111, 200 and 220 [38]. The XRD patterns were analyzed by Scherer formula. Usually, 3 peaks were observed for Copper nanoparticles such as 2θ values of 41, 50 and 74° respectively which corresponds to (111), (200), and (220) planes from JCPDS, copper nanoparticles file No. 04–0836. The presence of bio-stable materials in the CuNPs leads to the variation of 2θ. The above mentioned 2θ values of three peaks are in correspondence with that of the standard JCPDS, hence confirms that the resultant particles are (FCC) Copper in nanoparticles.

UV – Visible Spectroscopy of CuNPs

UV Visible spectroscopy is one of the most important techniques to confirmation of nanoparticle preparation. The greener synthesized of CuNPs using *Capparis zeylanica* leaf extract was found to have the values in the range between 400 – 500 nm shown in Fig 4. This initially confirms

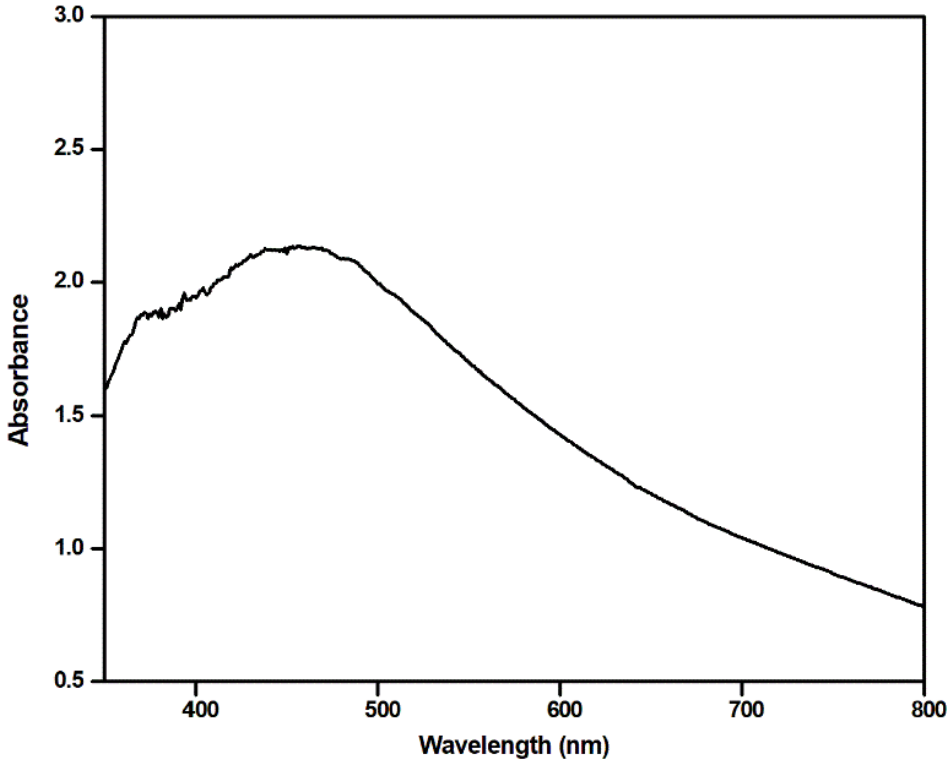

Fig. 4. UV –copper Nanoparticles

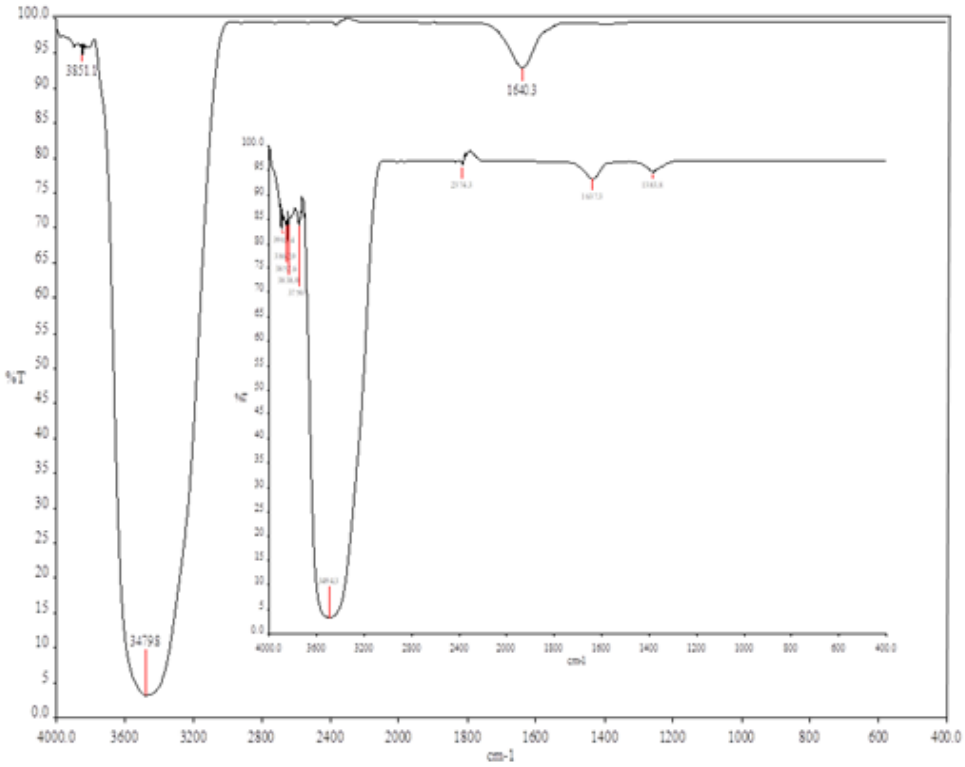

Fig. 5. FTIR analysis of leaf extract and insert copper Nanoparticles [4].

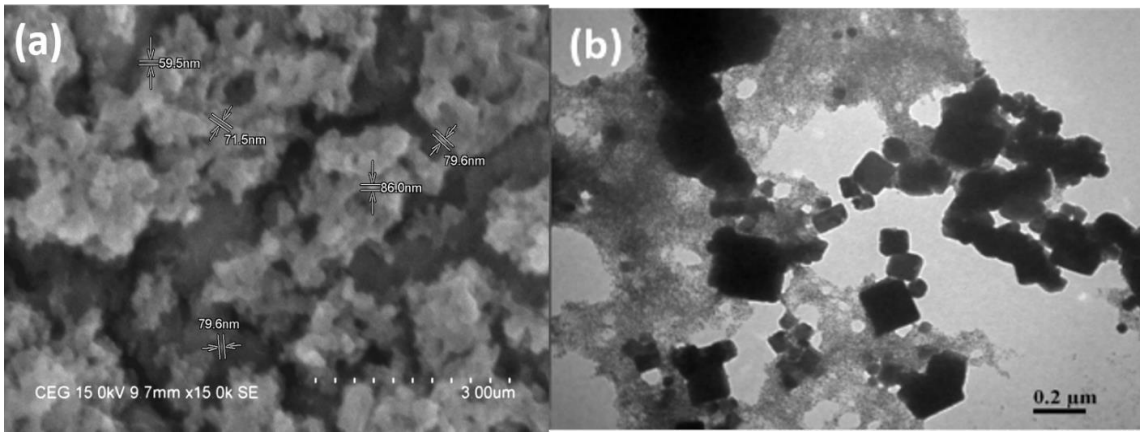

Fig. 6. (a) SEM and (b) TEM micrograph of CuNPs.

the formation of CuNPs from the leaf extract of *Capparis zeylanica* and the stabilization of the particles to have very small size that also confirmed from SEM and TEM.

FTIR – Spectroscopy Analysis of Plant extract and CuNPs

After drying properly, a drop of extract was combined with KBr powder and pelletized. The pellets were then analysed using FTIR Spectroscopy. In order to investigate the functional group and adsorbents the plant extraction shows the FT-IR spectral peaks are recorded at 3851.1, 3479.8 and 1640.3 cm^{-1} as shown in Fig. 5. Particularly the plant leaves contained the chemical constituents of phenolic compounds, Alkaloids, flavonoids,

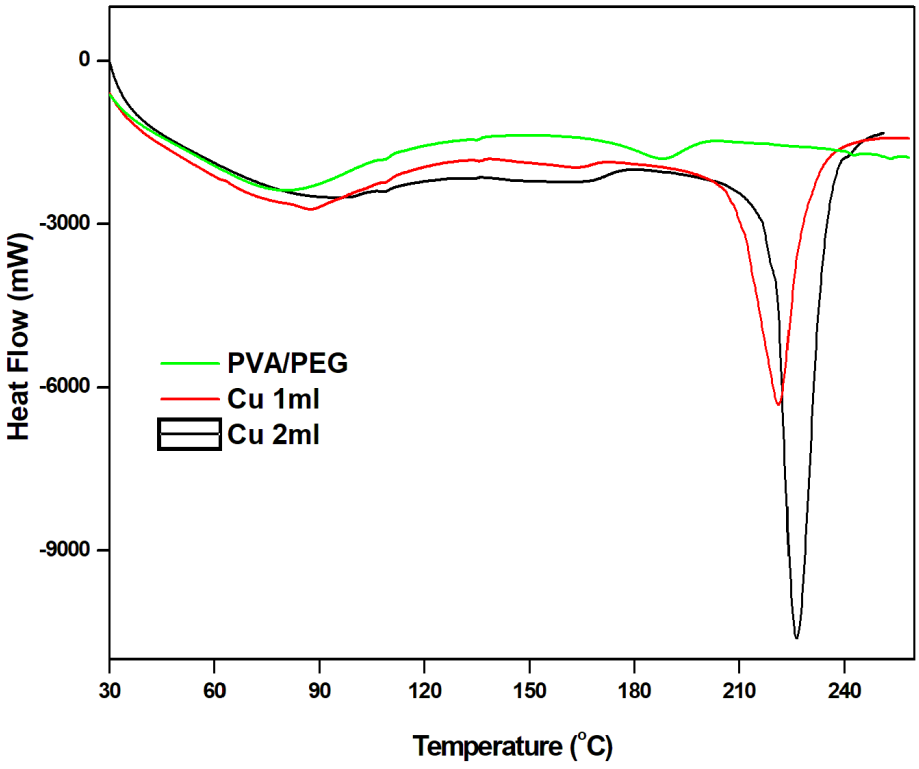

Fig. 7. DSC analysis of CuNPs + Blend

and fatty acids, such as thioglycoside, β-carotene, glycocapparin, α-amyrin are acting as a capping and reducing agents. For copper Nanoparticles FTIR spectral are at 3902.4, 3962.4, 3852.6, 3838.0, 3750.1, 3494.3, 2374.3, 1637.3 and 1383.8 cm^{-1} reduction agent spectral shown Fig 5 insert [4].

Morphological analysis of CuNPs

Scanning Electron Microscopy provided further insight into the morphology and size details of the copper nanoparticles. The SEM analysis of the sample after reduction from the Fig 6 (a) shows that the presence of the CuNPs in the sample with the average size of about 60 - 85 nm.

The TEM analysis was carried out to determine the mean particle size and morphology of the nanoparticles. The particle size of synthesized copper nanoparticles is highly subjective by the concentration of leaf extract. The CuNPs TEM analysis confirmed that the particle size is

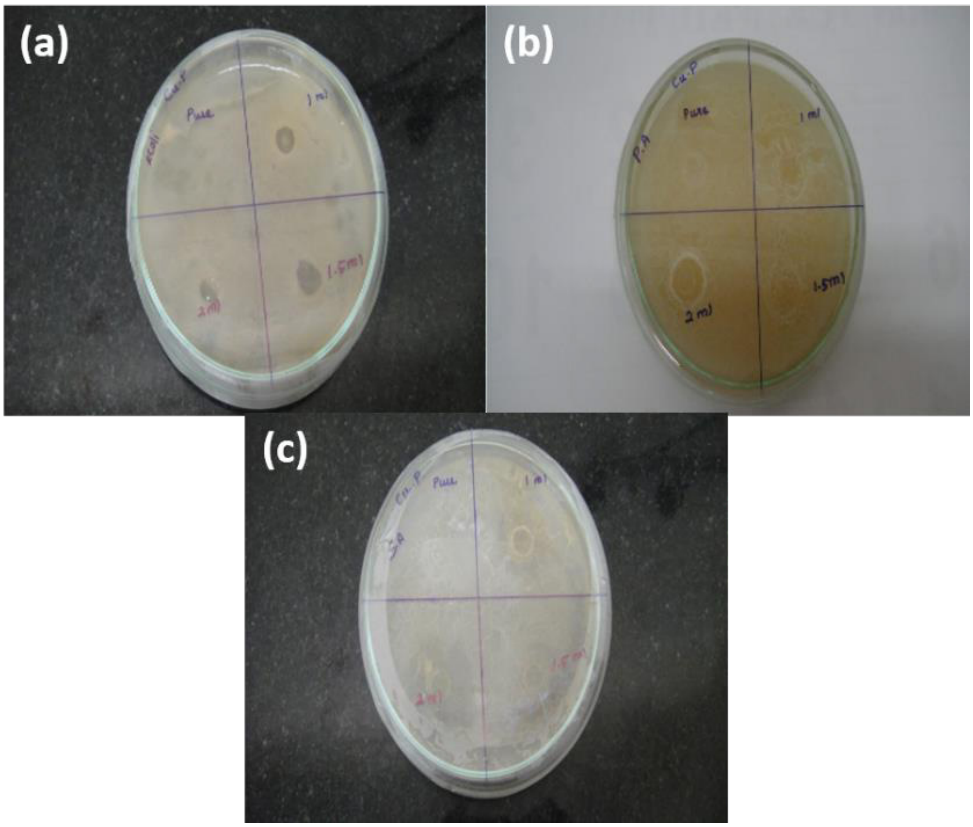

Fig. 8. Antimicrobial activity of PEG/PVA + CuNPs - (a) *Escherichia coli*, (b) *Pseudomonas Aeruginosa* and (c) *Staphylococcus Aureus*.

Table 1. Zone of inhibition of PEG/PVA + CuNPs

Species	PEG/PVA	1ml	1.5ml	2ml
Escherichia coli	-	±1 mm	±1.5 mm	±2 mm
Pseudomonas Aeruginosa	±0.5 mm	±1.5 mm	±2 mm	±2.5 mm
Staphylococcus aureus	-	±0.5 mm	±1 mm	± 1.5 mm

decreased when the concentration of leaf extract is increased in the reaction mixture and the nanoparticles are in cubical structure shown in Fig 6(b).

DSC Analysis of PEG/PVA+ CuNPs Composites

A Differential Scanning Calorimeter was used to determine the glass transition temperature (Tg) of the polymer blend films. Within a temperature range of -70 to 250 °C, the scan rate was 20 °C/ min. Following the first scan, the specimens were rapidly cooled to -70 °C and measurements were taken with 3mg samples on DSC sample plates. This technique was used to ensure that the polymer blends were thoroughly blended and that any residual solvent or water in the specimen was completely extracted. The PEG/PVA blend's glass transition and melting temperature (Tm) were 78.8 and 187.6°C, respectively, as shown in Fig 7. The glass transition and melting point values were observed to be increased when the CuNPs nanoparticles are added into it. In order to understand the thermal property of the PEG/PVA polymer after the addition of copper nanoparticles 1 ml and 2 ml of CuNPs were added to PEG/PVA blend [38-44].

Antimicrobial Study of PEG/PVA+ CuNPs Composites

The Disk diffusion method is widely used to determine bacterial antibiotic sensitivity. Antimicrobial activity was tested on *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, among other pathogenic bacteria. Bacteria were grown on a nutrient agar medium. On nutrient agar medium, pure bacteria cultures were sub-cultured. The species are scattered over the medium using L-rod after the medium has solidified. The discs were then positioned on the medium. Copper bio composite samples were freshly prepared and inserted into the seeded plate discs. At 37°C, the samples were incubated for 24 hours. After the incubation time, a region of inhibition was found around the disc, indicating a positive test result. Figure 8 shows the zone of inhibition values for the blend with Copper nanoparticles [43,44]. With a 2ml concentration of CuNPs, the maximum zone of inhibition value was obtained for Pseudomonas Aeruginosa, and the maximum zone of inhibition value was obtained [4,5,38].

FTIR Analysis of PEG/PVA + CuNPs Composites

FTIR was used to characterize the presence of specific chemical groups in the biopolymer composites which contains PEG/PVA and nanoparticles. FTIR spectra were obtained with the range between 4000 – 400 cm^{-1}. An FTIR spectrum of PEG/PEVA + CuNPs in blend is shown in fig 9. The peak at 3200 – 3650 cm^{-1} indicates the

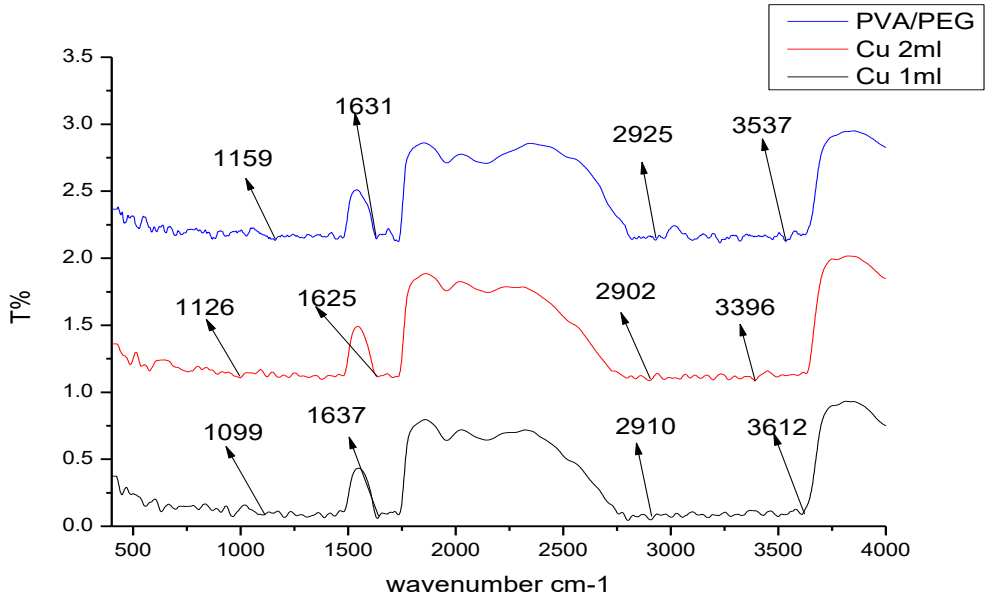

Fig. 9. FTIR analysis of PEG/PVA + CuNPs nanocomposite

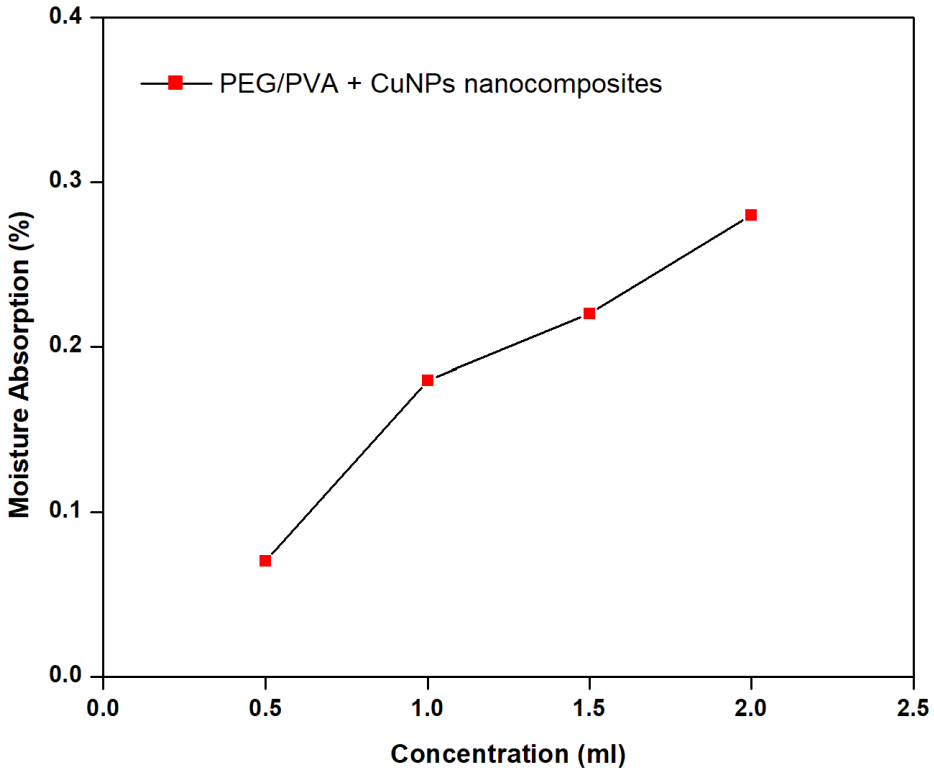

Fig. 10. Moisture absorption analysis of PEG/PVA + CuNPs nanocomposite.

present of OH group is engaged in the formation of hydrogen bond or complex with metal particles. The presents of C-H and CH_2 bonds in Alkanes that were observed in the PVA structure was confirmed with the intense bonding peaks around 2700 – 3000 cm^{-1} and 1550 - 1650 cm^{-1} respectively. The C=O stretching of the Ester group of the (blend) PEG/PVA is observed at peaks around 1050 – 1300 cm^{-1} [38].

Moisture Absorption Analysis

This is performed to decide the moisture absorptivity of the nanocomposite material. From Fig. 10 the moisture absorption value of the PEG/PVA + CuNPs nanocomposite found to be increased with increasing concentration of the Cu nanoparticles in the nanocomposite. The obtained outcome clearly shows that the nanocomposite is thicker when the CuNPs are added into the biopolymer blend which specifies that the percentage of moisture absorption be governed by the concentration of nanoparticles [38, 41].

When the concentration of copper nanoparticles increases the moisture absorption content initially increases. The moisture absorbed in the composite materials is dependent upon many factors like type of climatic exposure, temperature and severity of exposure humidity [33-37]. It can be observed that initially, there is almost a linear increase in the equilibrium moisture content, then after gradually increases. Typically, when the moisture content increases, the permeability of microbes on the bio-polymers that allows the degradation of the polymers usually. However, in case of PEG/PVA + CuNPs nanocomposite the antimicrobial activity also increases. Therefore, very less degradation might have occurred, that leads to increases in the self-life of PEG/PVA biopolymers [37-41].

CONCLUSION

Cu NPs were successfully synthesized using green synthesis method and incorporated in PEG/PVA polymer blend. The obtained Cu NPs were confirmed to be in the average of 65 nm of particle size. The characterizations of FTIR, DSC, Moisture absorption, and Anti-microbial analysis indicated the synthesized nanoparticles were mixed with PEG/PVA biopolymer blend. Food packaging applications are ideally suited to the PEG/PVA+ CuNPs composite. Antimicrobial research was

conducted on a variety of pathogenic bacteria. A thin layer of proteins and metabolites surrounded these reduced nanoparticles. From a technical standpoint, the obtained copper nanoparticles could be used in biomedical applications, and this simple technique has many advantages, including cost-effectiveness and compatibility with medical, food, and pharmaceutical applications.

CONFLICT OF INTEREST

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

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