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ORIGINAL ARTICLE

The antibacterial efficacy of synthesized bionanomaterials produced by *Acalypha indica* plant extract

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ABSTRACT
The current study attempted to create bionanomaterials (copper nanoparticles - CuNPs) using Acalypha indica extract with promising antibacterial effects. The obtained bionanomaterials were analyzed by applying the following techniques such as Ultra Violet Visible spectrum, Scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FT-IR). The image of SEM revealed the existence of the aggregation of copper nanoparticles with the size varied from 1to 3 nm. Also, FT-IR spectrum exhibited the active groups found in Acalypha indica involved in oxidizing and stabilizing CuNPs. The well diffusion test was achieved to estimate the antibacterial activity of CuNPs against gram negative bacteria (Escherichia coli, Klebsiella pneumonia), and gram positive bacteria (Staphylococcus aureus, Streptococcus pyogensis, Bacillus subtilis). The present study showed that green synthesized bionanomaterials utilizing Acalypha indica leaf extract may be used for treating bacterial infections.
Keywords: *Acalypha indica, Copper nanoparticles, well diffusion, Bacteria, SEM.*

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INTRODUCTION

The unique properties of bionanomaterials have drawn attention for their use in life applications [1]. The availability and cheapness of copper metal compared to other metals have made copper nanoparticles (CuNPs) produced in various methods a subject of interest to researchers [2,3]. Therefore, copper nanoparticles and their oxides had invested in the purification of drinking water, and the manufacture of solar cells [4]. Also, they have been applied as disinfectants to eliminate a wide range of pathogens [5]. Different techniques have been adopted to obtain nanoparticles with high stability in liquid media including chemical vapour deposition, electrochemical reduction, thermal decomposition, and chemical reduction of copper salts [6]. The chemical approaches, employed to generate copper nanoparticles, are often accompanied by the generation of poisonous and harmful substances, which limit their use as effective drugs in the pharmaceutical and medical fields [7].

Since copper nanoparticles undergo rapid oxidation and accumulation in the air or aquatic conditions, the process of generating pure and ultra-fine copper nanoparticles using biological methods is a challenging task [8]. At present, the process of nanoparticles synthesis using biological methods is better than chemical and physicals methods. The biological methods including green synthesis methods were adopted for their high productivity and environmental friendliness [9]. The synthesis of copper nanoparticles using plant extract is a successful and promising method because it does not cause harm to the environment [10]. Phytochemicals found in plant extract act as oxidizing and capping agents in the bionanomaterials creation [11]. In many culture, people used to cook food and drink the water in copper vessels. The world health organization (WHO) announced the allowed copper content is 177ppb. The metallic copper surfaces have an effective strength to reduce bacterial contamination especially E. coli [12].

Acalypha indica is an annual herbaceous plant belonging to the Euphorbiaceae family. In south India, it is used in traditional medicine to treat various diseases like pneumonia, bronchitis, asthma, and pulmonary tuberculosis. The leaves of *Acalypha indica* is utilized as antiparasitic for children to expel the worms after mixing them with salt and lime juice [13]. Acalyphine is an active alkaloid compound extracted from the leaves of *Acalypha indica*, has an antioxidant effect, so it is adopted to manufacture many pharmaceutical drugs [14]. As a result of the healing properties of *Acalypha indica*, leaves extract was utilized to produce copper nanoparticles and applied against different bacterial species.

MATERIAL AND METHODS

Preparation of the leaf extract

Plant aqueous extracts are a straightforward, cost-effective, and environmentally friendly way to get antibacterial activity. *Acalypha indica* leaves were collected and used to prepare the aqueous extract; 25 g of leaves were washed three times with distilled water, dried in shady places at room temperature, cut into fine pieces, and crushed into 100ml distilled water was added and boiled with 60ºC to 70ºC for about 10mins. Final resulting crude extracts were filtered through No.1 Whatman filter paper (pore size 25µm). This aqueous extract may be kept at 4 ° C and the activity can be maintained for a certain amount of time depending on the plant. Following this procedure, the aqueous extract is ready to be employed in antimicrobial bioassays. A technique for extracting any sort of plant material (leaves) with water using a basic blender to grind the dried leaves with water, followed by centrifuges to remove solid waste, and lastly filtering to produce a sterile aqueous extract is available in the literature [15].

Preparation of Copper solution

For the synthesis of copper nanoparticles, a ten millimoles aqueous solution of copper sulphate ($CuSO4$) was created, and an aqueous extract of *Acalypha indica* leaves was employed. It is maintained at room temperature for 2 hours in a magnetic stirrer.

Synthesis of copper nanoparticles

Acalypha indica leaves were collected from our college campus, cleaned in distilled water, and air dried. The extracted leaves were made by soaking 5 grammes of dried leaves in 100 ml distilled water for 10 minutes at 90 degrees Celsius. To obtain *Acalypha indica* leaf extract, the boiling solution was cooled following centrifugation. 30 mL leaf extract was combined with 100 mL 10 mM copper sulphate solution and heated to 100°C for 15 minutes in a water bath. 1mM NaOH was used to adjust the pH of the solution to 11 before it was boiled for another 30 minutes at 100°C. Copper nanoparticles are formed when the hue of copper sulphate solution changes from blue to reddish-brown. The entire procedure took about 15 hours to complete. Centrifugation at 10,000 rpm for 20 minutes separated the produced copper nanoparticles, proceeded by solution washing with distilled water and twice with ethanol solution before drying at ambient temperature.

UV-Visible spectrophotometer analysis

UV-Visible spectrophotometer (Shimandzu (1700), Double beam, Japan) was used to characterise the produced copper nanoparticles. A UV spectrophotometer with an absorbance range of 350-850 nm was used to measure the decrease of copper nanoparticles.

Fourier Transform Infrared (FTIR) spectroscopy

Copper nanoparticles were made using *Acalypha indica* extract and plant leaves, which were placed onto a Petri-dish and dried in a hot air oven. The dried sample was cleaned, and the powder form of the sample was stored in a sterile eppendorf tube. After that, the powdered sample was used for the FT-IR analysis (Shimadzu, Model 8400S, Tokyo, Japan). The traditional KBr Disc/Pellet technique was used to acquire FT-IR spectra of Copper nanoparticles and oxidised extract of *Acalypha indica*. The sample was made by grinding anhydrous KBr powder into pellets and compressing them. For 50 scans, the FT-IR spectra were measured in the region (4000-400 cm-1) with a resolution of 4 cm-1.

Scanning electron microscopy

Under typical air circumstances, the surface morphology of CuNPs was observed using a scanning electron microscope. At a voltage of 5 kV, a scanning electron microscope (ZEISS Supra 40, Germany) was employed. In a high-vacuum SEM, the material was examined at various magnifications. The material was put onto glass slides, dispersed uniformly, and then vacuum dried for scanning electron microscopy. Different forms observed in SEM are mostly due to frictional characteristics and movement of nano-objects on solid surfaces.

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Determination the antibacterial effect of CuNPs

The antibacterial activity of CuNPs was determined by employing a well diffusion method against the *Streptococcus pyogensi*s, *Bacillus subtilis, Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Eschericha coli* on the Muller-Hinton agar (MHA) plates. The MHA plates were inoculated with bacterial suspension (0.5 McFarland) by a sterile cotton swab. The wells with a diameter 6 mm were prepared to add the serial dilution of CuNPs. The inoculated plates were kept at 37ºC. After incubation, the clear zone appeared around the wells representing the inhibition zone measured by millimetre [16]. Cephalexin was selected as a standard antibiotic for positive control.

RESULTS AND DISCUSSION

UV-visible spectrum of copper nanoparticles

The copper nanoparticles were formed at the moment of adding the *Acalypha indica* leaf extract to the copper solution, which turned into dark brown color. The UV-visible spectrum showed a broad curve ranging from 250 to 350 nm with high absorption at a wavelength of ~280 nm. The majority of the synthesised The typical absorption peak of Cu nanoparticles was stated in the literature to be 590 nm, while our data indicated a curve spanning from 250 to 350 nm [17]. This result is agreed with other reports in the literature [18] and the plant's leaves were found to be used to capture the supremacy of contraceptive effect. The origin extract is said to be able to reduce blood sugar levels by 30%. The leaves are anti-periodic and laxative, and the leaf extract can be used to treat bug stings.

Table 1: Effect of leaf extract of *Acalypha indica* synthesised copper nanoparticles on antibacterial activity

CuNPs Con. (μg/ml)	*E. coli (mm)*	*K.pneumoniae (mm)*	*S. aures (mm)*	*S.pyogensis (mm)*	*B.subtilis (mm)*	Control (mm)
200	10	14	16	18	16	NIL
150	8	19	20	21	20	NIL
100	7	17	19	17	15	NIL
50	-	15	18	10	11	NIL
25	-	11	10	7	9	NIL

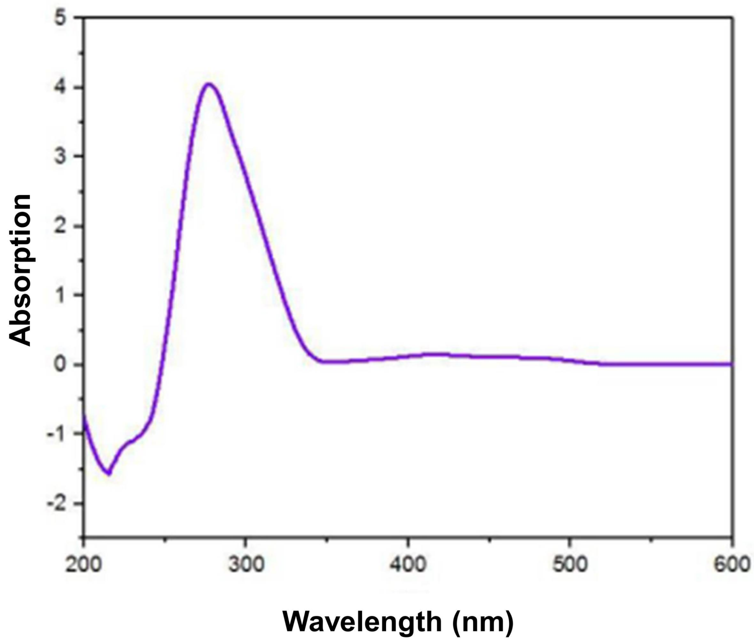

Figure 1. UV spectrum showing the absorbance of synthesized CuNPs Using *Acalypha indica* leaf extract.

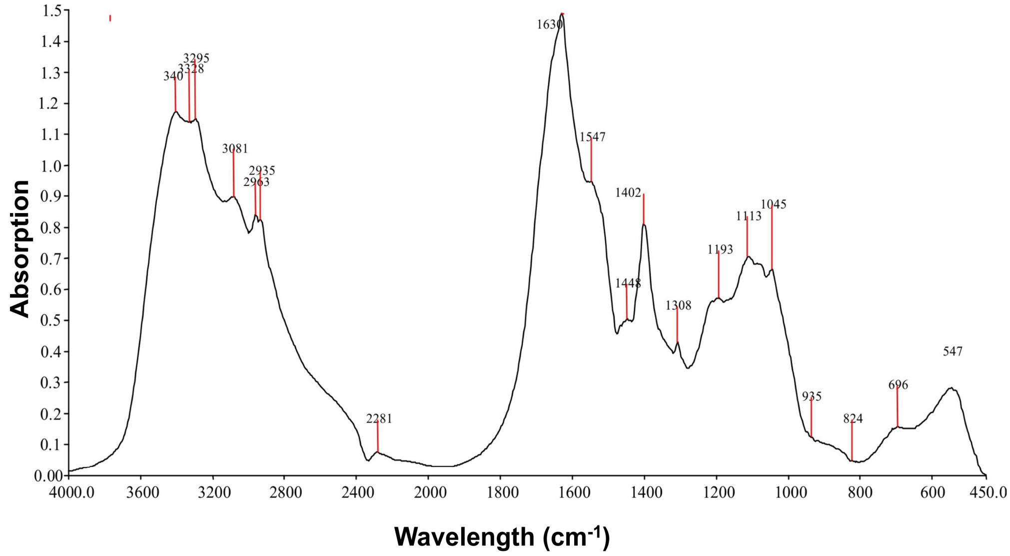

Figure 2. FTIR spectrum of the CuNPs showing different active groups.

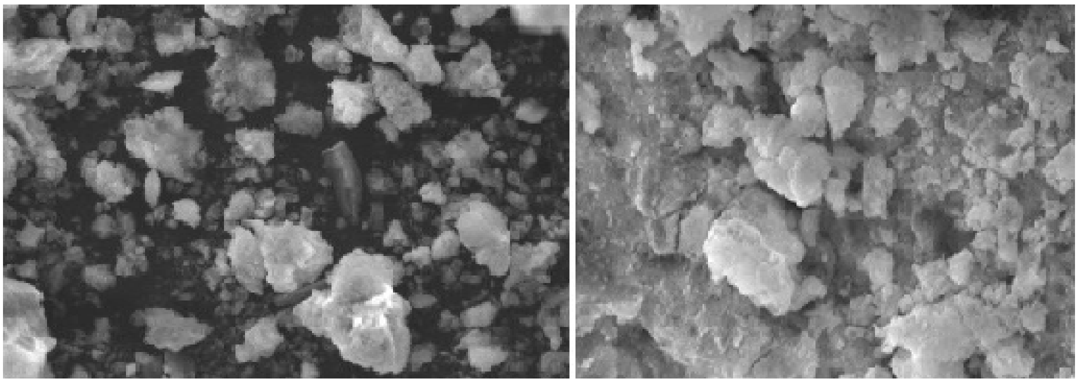

Figure 3. SEM image exhibited the external morphology of CuNPs in 1µm and 3µm.

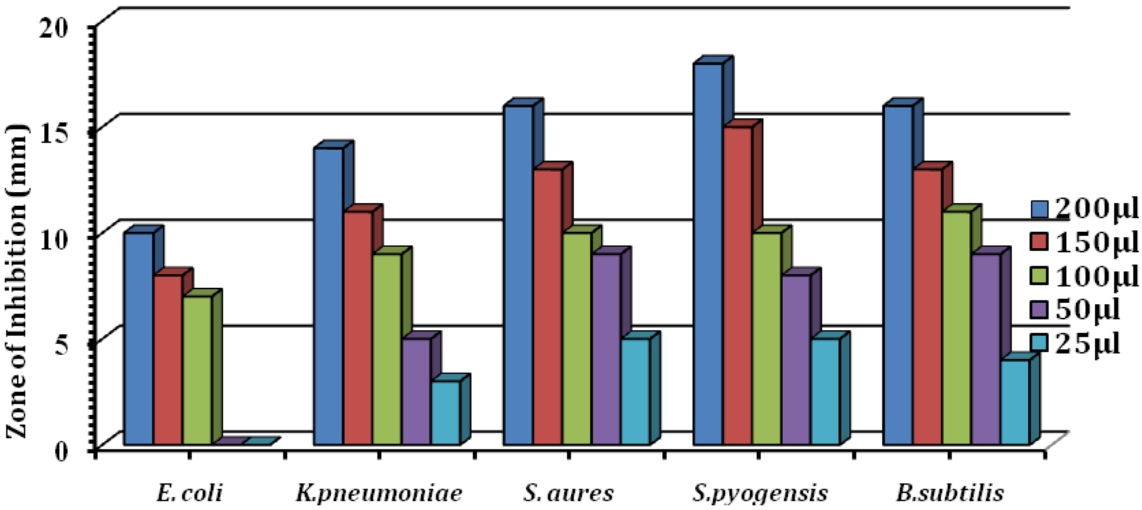

Figure 4. The inhibition zone of copper nanoparticles at different concentrations against various bacteria.

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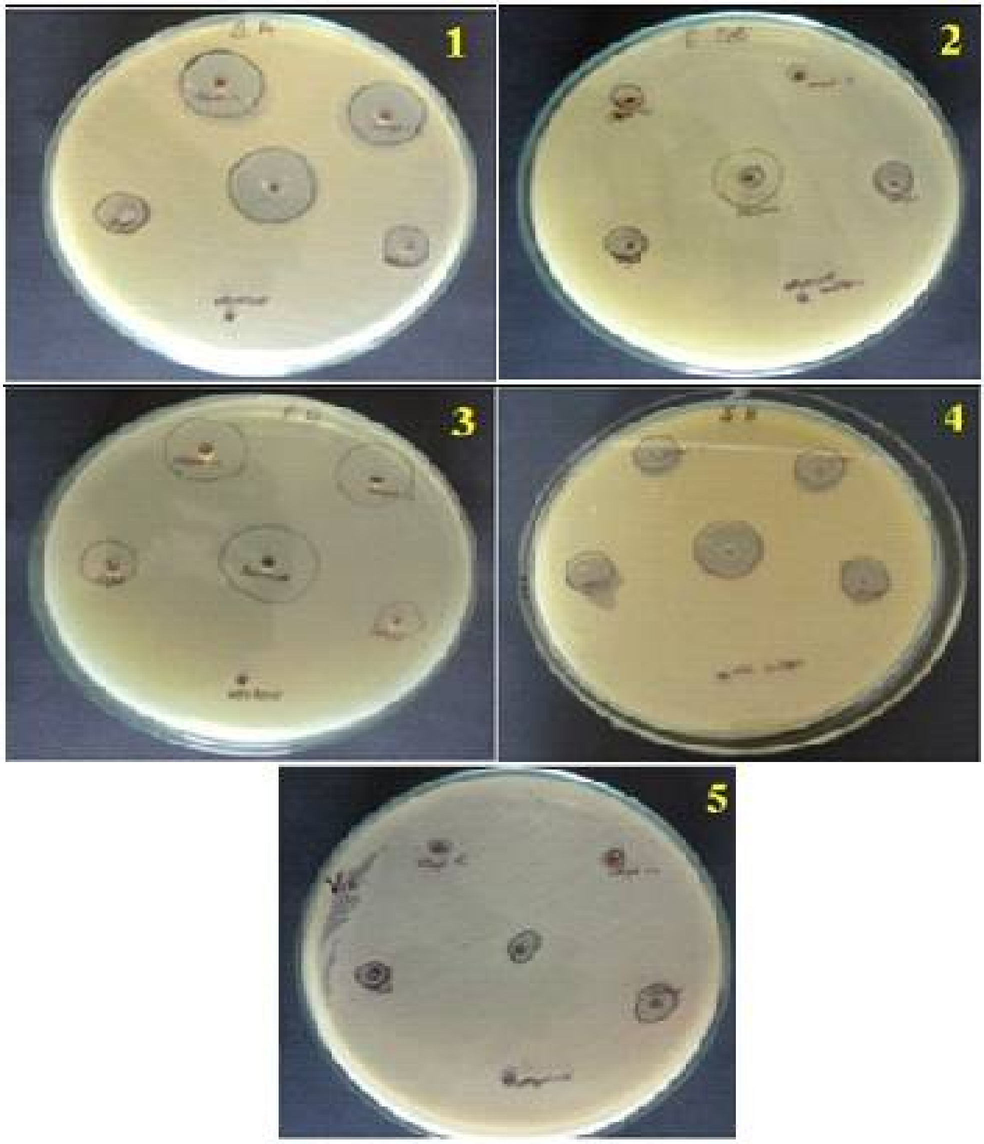

Figure 5. Antibacterial effect of CuNPs upon various bacteria. *(1. E. Coli, 2. K.pneumoniae, 3. S. aures, 4. S.pyogensis and 5. B.subtilis)*

FT-IR Results

The FT-IR spectrum of the CuNPs exhibited many active groups, as shown in Figure 2. The FT-IR spectrum exhibited fifteen peaks in different positions in the curve as following 3400, 3294, 3096, 2957, 2925, 1680, 1546, 1453, 1308, 1278, 1174, 914, 827, 604, 532 cm^{-1}. The FTIR spectra revealed the presence of different functional groups like 1°, 2° amines, amides (N–H stretch); alkynes (terminal) (–C≡C–H: C–H stretch); Alkenes (=C–H stretch); Aldehydes (H–C=O: C–H stretch); Nitriles (C≡N stretch); Alkenes (–C=C– stretch); nitro compounds (N–O asymmetric stretch); alkanes (C–H bend); nitro compounds (N–O symmetric stretch); alkyl halides (C–H wag (–CH2X); aliphatic amines (C–N stretch); carboxylic acids (O–H bend); Aromatics (C–H); alkyl halides (C–Cl stretch); alkyl halides (C–Br stretch). These functional groups exhibited in the FT-IR spectrum endowed the green synthesized copper nanoparticles with biological activities such as antibacterial, antifungal, antiviral, healing agents, anticancer, etc.

SEM analysis

The morphology and size of green synthesized CuNPs were detected by employing scanning electron microscopy (SEM). As a result of the dry sample preparation, the CuNPs appeared in clusters consisting of small spherical nanoparticles, as observed in Figure 3. The shapes of obtained CuNPs were spherical, hexagonal, and cubical with different diameters ranging from 1 to 3nm respectively.

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Determination the antibacterial effect of CuNPs

Agar well diffusion test was implemented to detect the antibacterial activity of CuNPs, against different pathogenic bacteria. As shown in figure 5, the diameter of inhibition zones revealed the potency effect of different concentrations of copper nanoparticles. At 200µg/ml, the maximum inhibition zones of the following bacteria: *Streptococcus pyogensi*s, *Bacillus subtilis, Staphylococcus aureus*, *Klebsiella pneumonia,* and *Eschericha coli* were 18mm, 16mm, 16mm, 14mm, and 10mm respectively, as observed in Table 1. The antibacterial impact of CuNPs on tested pathogenic bacteria increased at high concentrations. The lowest concentration of CuNPs (25µg/ml) showed a minimum inhibition zone in *Streptococcus pyogensi*s, *Bacillus subtilis, Staphylococcus aureus*, and *Klebsiella pneumoniae*. Another study demonstrated the biosynthesis of copper nanoparticles using aqueous *Tilia* extract exhibited high antibacterial activity against pathogenic bacteria like gram-positive and gram-negative bacteria. The emergence of drug-resistant organisms become a health challenge throughout the world and need immediate attentions [19]. The drug resistance in cancers and other microbial strains results from the misuse of antibiotics and related drugs, so there is a need to find drug alternatives capable of meeting the growing market need to find drugs against strains that carry resistance genes [20].

CONCLUSION

In the present study, the leaf extract of *Acalypha indica* was utilized as an oxidizing and capping agent to synthesize to produce bionanomaterials. The FTIR spectrum of green synthesis copper nanoparticles referred to the existence of many phytomolecules such as amines, alkynes, alkenes, and aldehydes related to the antibacterial efficacy of nanoparticles. The SEM image exhibited CuNPs have spherical, hexagonal, and cubical morphology with various sizes ranging from 1 to 3nm. In the present study, the green synthesized bionanomaterials showed antibacterial effect upon tested pathogenic bacteria at 100µg/ml with inhibition zones varied from 7mm to 19 mm. Through our manuscript, we observed that the synthesized copper bionanomaterials showed higher antibacterial activity than the copper particles itself. The results further reveal that the copper particles had negligible activity than copper bionanomaterials.

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