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PLANT-MEDIATED SYNTHESIS OF COPPER NANOPARTICLES OF POLY HERBAL SIDDHA MEDICINE VALLARAI CHOORANAM AND ITS ANTIBACTERIAL AND BIOMEDICAL APPLICATIONS

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ABSTRACT

The nanoparticles have various benefits in the biomedical field. The present research aimed to synthesize the copper nanoparticles from siddha therapeutics poly-phyto Vallarai Chooranam and its biomedical applications of antibacterial and anticancer properties. A copper nanoparticles (Cu NPs) process was carried out utilizing vallarai chooranam (VC) as a biological reducing substance as well as the UV–visible spectra at 432 nm were used to confirm the Cu NPs. Proteins as well as other ligands were found to be responsible for the capping of produced Cu NPs in UV-Visible spectroscopy (UV). Further, X-ray Diffraction (XRD) and Transmission Electron Microscopy (TEM) explored the crystalline nature of spherical morphological particles with a size range of 40 and 50 nm. An average diameter of Cu NPs was identified as 204.6 nm utilizing Dynamic Light Scattering (DLS) analysis as well as the zeta potential was – 141.9 mV, indicating that VC-Cu NPs are stable. The antimicrobial activity of VC-Cu NPs against *Bacillus subtilis* demonstrated a maximum zone of inhibition (14 mm). Cu NPs strongly reduced MCF-7 cell growth, by an IC_{50} score of 3.44088+/-230.7(6703 percent) µg/ml of the concentration in the minimum tested

concentration (10µg/ml), Cu NPs were able to suppress the cell line's development by lesser than 10%. The inclusion of 50µg/ml Cu NPs, on the other hand, dramatically slowed the cell line's development (by >93%). We showed that a simple, effective, and fast synthesis process of Cu NPs can be utilized to create vast quantities of Cu NPs for use in biomedical applications.
Keywords: Vallarai chooranam, Cu NPs, anti-bacterial, anti-cancer activity.

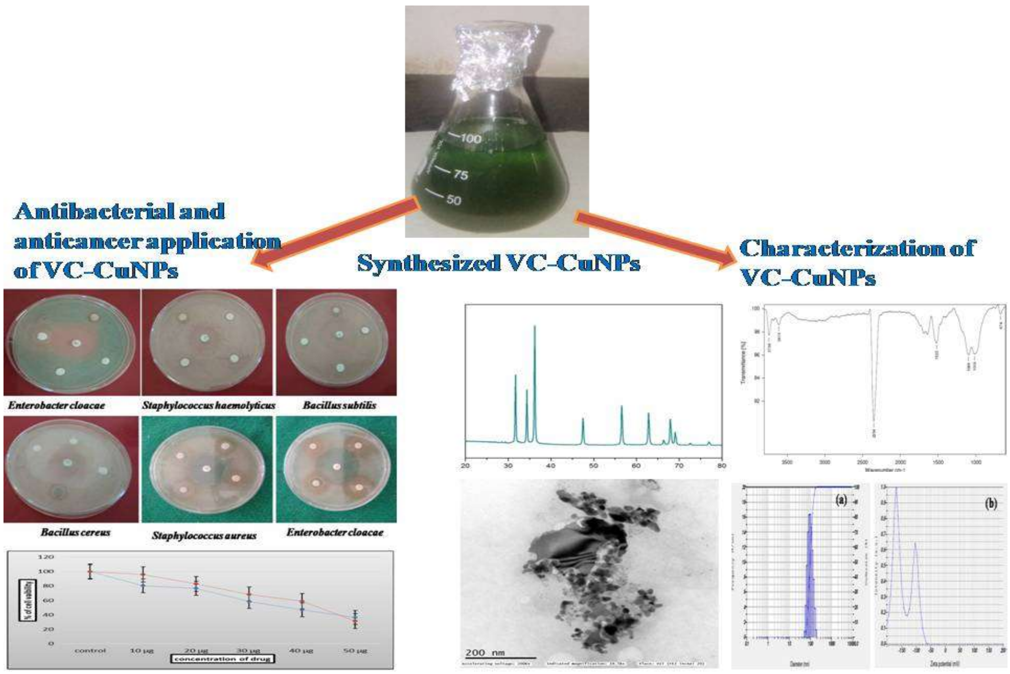


Figure 1. Graphical Abstract of synthesized VC-CuNPs and their biomedical applications

INTRODUCTION

Plants are well-known for their increased flavonoid nutritional supplies for humans, their assistance in preventing heart disease, their higher free radical scavenging action, cytotoxic effects, followed by anti-HIV capabilities, chemotaxonomic indicators, and antibacterial substances (1-4). It serves as an important part of the water cycle, ecosystem balancing, providing oxygen for environmental preservation, producing compounds for medicine finding, and providing wood, timber for home, and furniture. Plants are giving closer attention to the use of phytochemicals in nanotechnology (NT) these days (5-8). NT is a progressive field and the elements with diameters of 10^7 to 10^9 m. Ecological sciences, bio-NT, imposed microbiology, drug, and drug–gene distribution methods, quantum dots, chemistry, as well as chemical sector, and optoelectronics are some of the applications of NT (9). The biosynthesis method of nanoparticles is a greener approach, environment-friendly, and economically feasible (10-11). The biosynthesis of NPs by employing simple prokaryotic microbial cells and eukaryotic plants. Since it does not require energy, temperature, or pressure and therefore can easily cope with increased output. The creation of nanoparticles via chemical, physical, and biological processes (12), on the other hand, the chemical approach may adsorb hazardous substances on the surface, resulting in severe medical effects. The green synthesis also obtains several crucial characteristics for creating stable and well-characterized nanoparticles, like the best organisms to use, reaction conditions, and characterization techniques (Fig. 2). To choose the appropriate flora for greener synthesis, one must first understand its detoxifying and heavy metal accretion capabilities, as well as reaction parameters like pH, temperature, etc. (9).

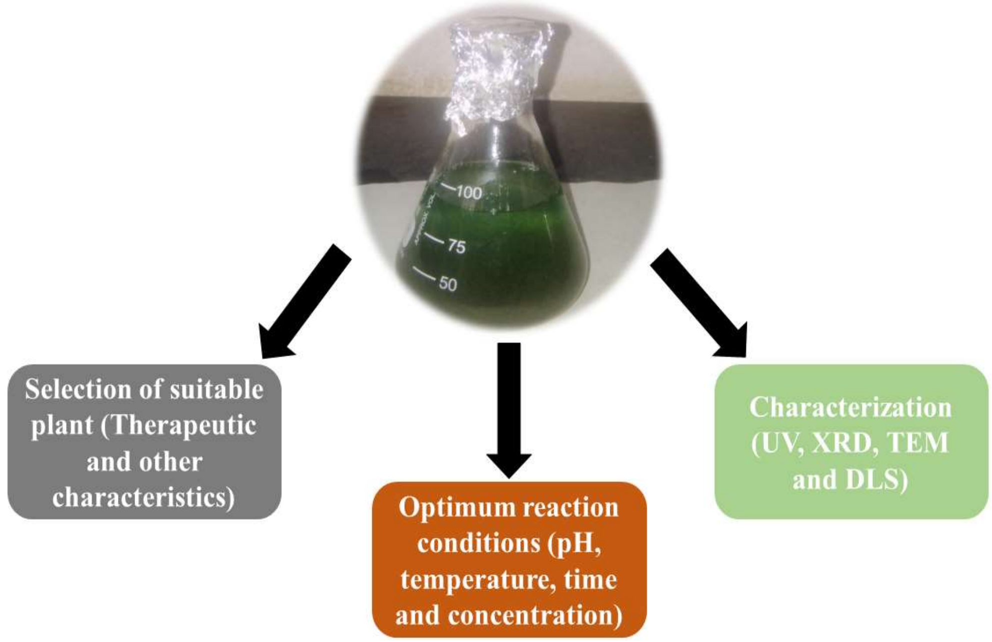


Figure 2. Significant features for the development of greener NPs.

Siddha is an ancient Indian medical system regarded as the mother medicine of the ancient Tamils and Dravidians of South India. Siddhars were those who were involved in the establishment of this Siddha school of thought. Siddhars were mystics or upright people who gained magical abilities. Because of their cultural acceptance, tolerance with the human body, and less adverse effects, medicinal herbs produced from plant extracts were rapidly being used to treat a wider range of medical conditions, primarily in poor nations primary care (13). Ayurveda is renowned as the "science of longevity" since it gives a complete strategy for living a long and healthy life. It provides diet and nutrition programs that help the body rejuvenate itself. It provides treatment options for a variety of common ailments, including food allergies, which currently have few therapy options. Three ancient Indian medical systems are Ayurveda, Siddha, and Unani. The Vedas, and other ancient religions, mention health systems (14). Colloids, precipitates, spherical NPs, fullerenes, or dendrimers are instances of three-dimensional nanostructures that display all nanoscale parameters. Nanomaterials could be employed as a lubricant in the future. They're used in everything from paints and coatings to ceramics, batteries, clays, and sometimes even fuel cells. Metal NPs were particularly essential owing to their vast uses in various domains (15).

VC is a polyherbal made up of ten distinct herbs, including *Centella asiatica*, *Myristica fragrans* (Jaadhikai, Jaadhipathri), *Eletteria cardamomum, Syzgium aromaticum*, *Quercus infectoris, Emblica officinalis*, *Terminalia chebula*, *T. belerica* and *Taxus beccata* in a required quantity. VC is a traditional medicine that's been used to treat diabetes, urinary tract infections, leucorrhoea, as well as venereal disease. It's also recognized for improving memory, purifying the blood, and having good antibacterial properties (16). We established this as an innovative study to investigate the VC in the realm of nanoscience based on an accurate literature review. The precipitation method is a simple one that has piqued the interest of industry because of its lower energy and temperature requirements, as well as its low cost and cost-effective method for large-scale productivity and increased yield. The initial precursors copper nitrate and copper chloride were used to examine the precipitates as they were formed. The present research aimed to synthesize the copper nanoparticles from siddha therapeutics poly-phyto Vallarai Chooranam and its biomedical applications of antibacterial and anticancer properties.

MATERIALS AND METHODS

Copper nitrate (greater than 99 percent pure) and other necessary chemicals were acquired from Sigma Aldrich and Hi-media, India.

Collection and processing of the sample

Vallarai chooranam was procured from the Central Council for Research in Siddha (CCRS), Chennai.

Preparation of Vallarai chooranam

Vallarai chooranam (VC) is a polyherb composed of 10 different herbs such as *Centella asiatica* (50 g), *Myristica fragrans* (Jaadhikai-10 g), *M. fragrans* (Jaadhipathiri-10 g), *Syzgium aromaticum* (10 g), *Eletteria cardamomum* (10 g), *Taxus beccata* (10 g), *Quercus infectoris* (10 g), *Emblica officinalis* (10 g), *Terminalia chebula* (10 g), and *T. belerica* (10 g).

Preparation of Vallarai chooranam extract

Vallarai chooranam (5g) was combined with 100ml distilled water and left at room temperature for 1 hour after boiling at 80°C with regular shaking. The solution was then filtered via Whatmann No1 filter paper. For later usage, the filtrate was obtained and kept back at 4°C.

Production of copper nanoparticles from Vallarai chooranam

Under constant stirring, a 25 ml solution of chooranam extract was dropped into 100 ml of a 1mM copper nitrate solution. The solution was incubated for 24 hours after the chooranam extract was completely added. The green colour solution turned straw yellow after a certain amount of time, indicating the creation of Cu NPs. The sample was then agitated at 10,000 rpm for 15 minutes to eliminate any undesired biological elements before being distributed in double distilled water.

Isolation and Identification of pathogenic bacteria

Wound pathogens were collected from Anand Hospital, Manali, Chennai, Tamil Nadu. The samples were aseptically inoculated on blood agar (with 5% sheep blood) and MacConkey agar plates, incubated aerobically at 35°C–37°C for 24–48 h. The pathogens were identified by standard microbiological and biochemical profiling (17-22).

Antimicrobial activity of VC-CuNPs

The antimicrobial action of CuNPs was assessed towards the following pathogenic microbes *Enterobacter cloacae*, *Staphylococcus haemolyticus, Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, and *Enterobacter cloacae*. These cultures were grown on a suitable medium and nurtured overnight at 37°C before being stowed at 4°C in the refrigerator. For the nutrient agar medium, a 6 mm-sized disc was created, and each disc was immersed in varying concentrations (100, 50, 25, and 12.5µg) of manufactured CuNPs. Pure microbial pathogen cultures were subcultured on a suitable medium. Ciprofloxacin (10µg) was utilized as a reference for comparability. The zones of microbial inhibition were evaluated after a 24-hour incubation period at 37°C. The tests were carried out thrice (23-24).

Anti-tumor activity (Cell Viability Assay)

MCF-7 cells

The National Centre for Cell Sciences (NCCS) in Pune, India, provided MCF-7 cells. The MCF-7 cells were grown up in DMEM with 10 percent Fetal bovine serum at 37°C in a humid chamber with 5% CO_2 and 95% air (FBS). MCF-7 cells are plates at a density of 0.2x106 cells per well in 96-well plates. In a pressurized CO_2 incubator, MCF-7 cells were then treated with compounds for 24 hours. After that, 20µL of 5 mg/mL MTT (3-(4, 5-dimethylthiozol-2-yl)-2,5-diphenyl tetrazolium bromide) was introduced to the wells then overnight at 4 hours in a humid environment, after which 200µL of DMSO was applied to disintegrate the MTT formazan crystals. Cultures were incubated in the same conditions without the test chemicals in a control sample. The absorbance was measured at 570 nm shortly after the purple tint appeared. The formazan produced in the control cells was assumed to have a 100% viability rate. The amount of MTT transformed into insoluble formazan salt was used to determine relative cell viability. The mean of three independent tests was determined and reported as (percent) of viable cells vs concentration (µM) (25-26).

RESULTS AND DISCUSSION

Production of VC-Cu NPs

The colour change indicates the presence of VC-CuNPs. The synthesized VC-CuNPs were shown in Fig. 3.

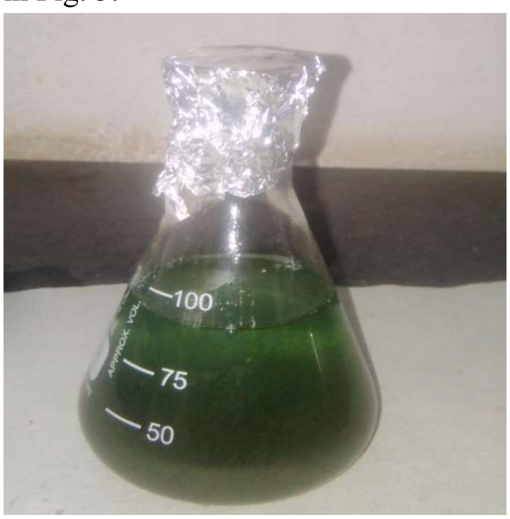


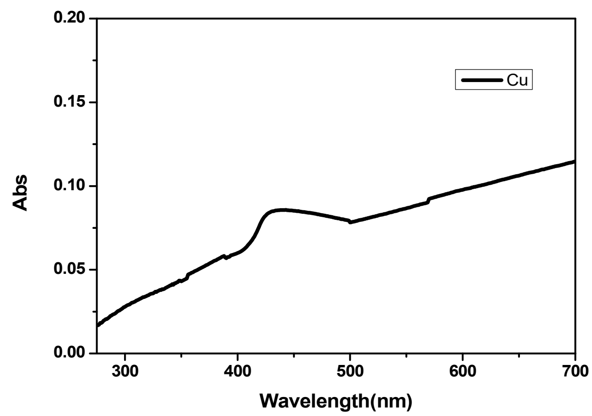
Figure 3. VC-Cu NPs production.

Characterization study of VC-Cu NPs

The produced NPs considered for instrumental studies like UV, TEM, XRD, and FTIR, and the Particle size of Nanoparticles and their Zeta potential which are followed with their scanning images.

UV-visible spectroscopy

Metal colloidal dispersion has an absorbance peak in the UV–visible range (Fig. 4). This is owing to an excitation of the Plasmon resonance or interband shift, which is a characteristic of the metallurgical nature of the particles. Copper nanoparticles with a diameter of 200 nm usually have a surface Plasmon (SP) peak at 560–570 nm. The absorbance peak of the produced Cu nanoparticles was 590 nm, indicating that the copper nanoparticles are roughly 200 nm in size (27).



FFigure 4. UV Visible study of Cu NP.

XRD

XRD patterns were used to confirm the crystalline structure as well as the shape of NPs. The XRD form of produced NPs peaks detected at 43.39°, 50.49°, and 74.18° correlate to the metallic Cu planes (111), (200), and (220). These 3 peaks are very identical to those in the standard JCPDS Card No. 04-0836 for the pure fcc (facial centred cubic) metal Cu standard spectrometer. Several more diffraction peaks emerged at 29.63°, 36.54°, 42.44°, 61.57°, 73.58°, and 77.49°, respectively, matching the (110), (111), (200), (220), (311), and (222) planes of cuprite, representing the development of cubic Cu (I) oxide nanocrystals. A cuprite XRD peaks are nicely corresponding to the conservative powder diffraction card of bcc (body-centered cubic) cuprite. This suggested that the Cu material was very pure, crystal, and well-ordered in a certain orientation when it was created. Impurity caused no other peaks to appear (Fig. 5). The particle size was determined using the Debye–Scherer formula.

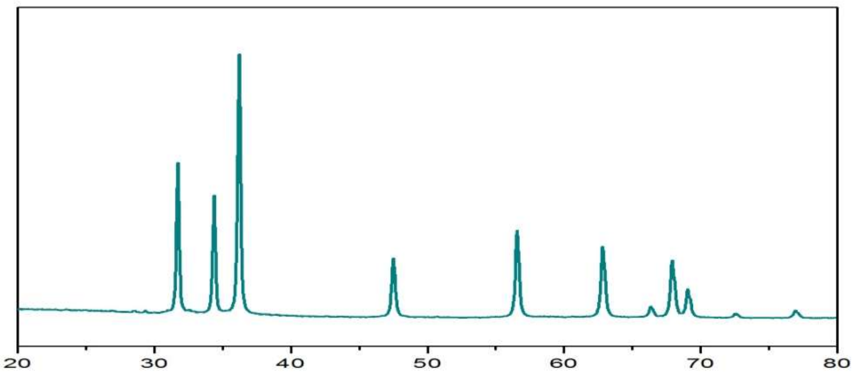


Figure 5. XRD pattern of Cu NPs.

FTIR

An FTIR spectrometer was used to record FTIR spectra. The pellets form of the samples was evaluated with FTIR equipment after a predetermined amount of sample was crushed with KBr. The copper nanoparticles produced using Vallarai churanam extract stabilizer were subjected to FTIR analysis to recognize the probable molecules responsible for capping and reducing agent (Fig. 6). FTIR spectrum of Cu NPs made with various copper salts stabilized with the extract. The broader peaks detected at around 3738 cm-1 [N-H stretching, Amines] and 674 cm-1 [C-H "OOP", aromatics] demonstrate the stretched frequency of the hydroxyl group (OH group) that exists in the surface of the Cu NPs. The FTIR spectra of Cu NPs extract show band at 3610cm-1[O-H Stretch, free hydroxyl, alcohol, phenol], 2354cm-1[H-C=O: C-H stretch, Alhehydes], 1522cm-1[N-O Asymmetric stretch, Nitro substances], 1099cm-1[C-N Stretch, Aliphatic amines], 1019cm-1[C-N Stretch, Aliphatic amines] resembles O-H Stretch H-bonding alcohols as well as phenols, carbonyl stretch, N-H bending primary amines, resembles C-N stretch of the aromatic amino group and C-O stretch alcohols, ethers. Cu nanoparticles were found to be accompanied by organic compounds such as terpenoids, alcohols, ketones, aldehydes, and carboxylic acid, according to their FTIR spectra (28).

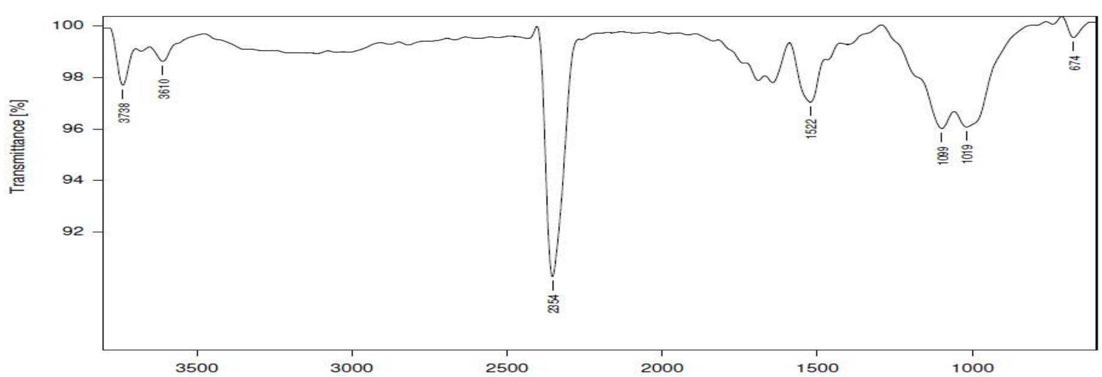


Figure 6. FTIR spectra of Cu NPs.

Copper particle size and Zeta potential

The diameter (nm) vs frequency (percent /nm) spectrum for the nanoparticles was collected, by diameter in nm on the x-axis with frequency (percent /nm) on the y-axis (Fig. 7). The Cu NPs zeta potential spectrum was obtained as zeta potential vs. intensity spectrum, by zeta potential as the x-axis and intensity as the y-axis. The hydrodynamic diameter of the hydrosol was measured using a dynamic light scattering approach (particle suspension). CuNPs were identified as 204.6nm the documented value of zeta -141.9mV which leads to a clustered state of the molded CuNPs (28).

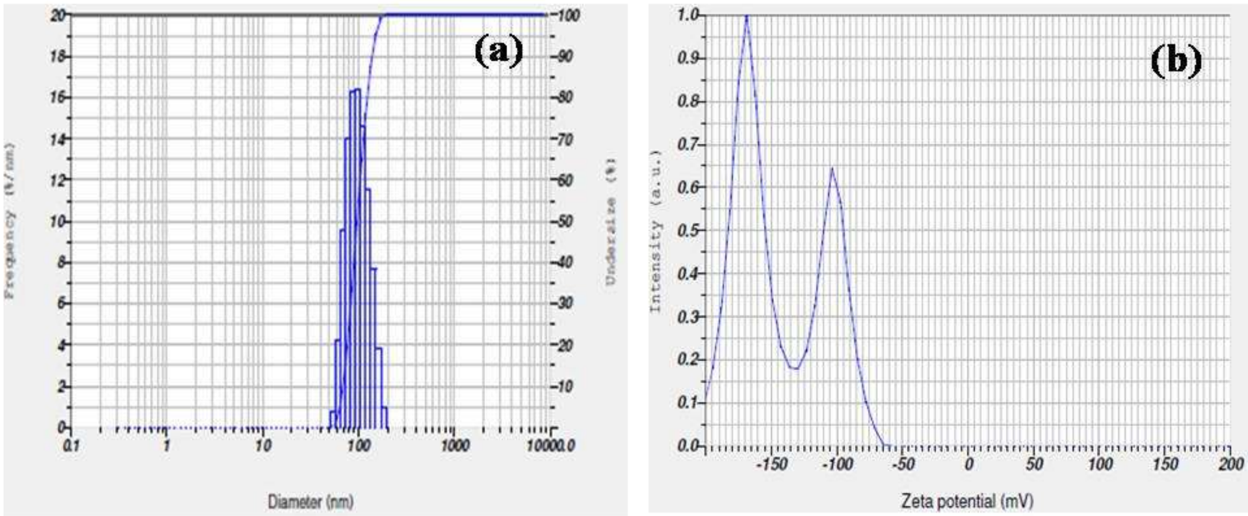


Figure 7. (a) Particle size and (b) Zeta potential of Cu NPs.

TEM Cu NPs

TEM pictures confirmed that Cu NPs are approximately spherical and irregular in shape (Fig. 8). Average particle size was 200 nm through quasi-elastic light scattering data and there is a slight accumulation of NPs are perceived it was owing to the existence of proteins exist in the leaf (28).

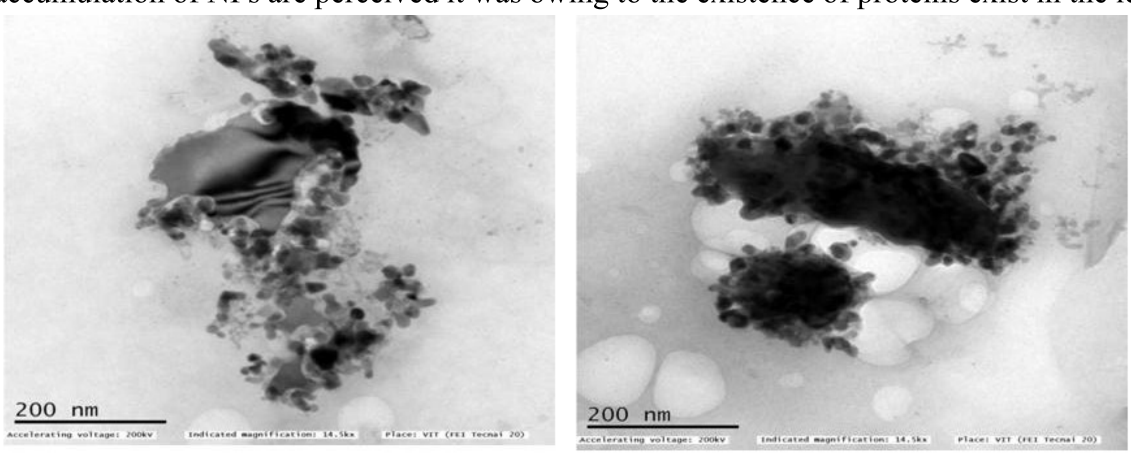


Figure 8. TEM pictures of Cu NPs

Antimicrobial action of Cu NPs

The gram-positive and gram-negative bacteria were identified by microscopic and biochemical characterization. It was familiar that Cu NPs display a light blue color, rising owing to the excitation of SP vibrations in the Cu NPs. Cu NPs attained from Vallarai chooranam revealed very stronger suppressing action towards Gram +ve and Gram -ve microbes. Five concentrations of Cu NPs (100, 50, 25, and 12.5µg) were processed and applied towards an array of microbial species *viz., Enterobacter cloacae*, *Staphylococcus haemolyticus*, *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, and *Enterobacter cloacae*. The greater concentration (100µg) of Cu NPs exhibited an important antimicrobial impact compared with other concentrations (100, 50, 25 and 12.5 µg) (Fig. 9 and Table 1).

The process whereby copper nanoparticles permeate microbes is unknown, but research suggests that when microbes were handled with copper nanoparticles, changes in their morphologies occurred, leading to a rise in their permeability, impacting proper transport through all the plasma membrane, having left microbial cells ineffectual of correctly controlling transport it through plasma membrane, going to result in apoptosis (28). Copper nanoparticles have been found to have infiltrated the bacterium and caused harm by reacting with phosphorus and sulfur-containing substances like DNA. Moreover, when compared to Vallarai chooranam CuNPs showed good antibacterial activity. More size and shape-reliant uptake of Cu NPs into MCF7 cells were noted, highlighting the need for further research into the size and shape-reliant antibacterial and cytotoxic properties of nanofluids. Cu NPs have well-grown surface chemistry, chemical permanency as well a small shape, giving them the ability to communicate with microbes (28). Furthermore, the particles interact with the outer membrane's structural constituents, potentially causing structural alterations, deterioration, and inducing apoptosis. The results of this research could lead to the creation of new antibacterial systems that rely on CuNPs for clinical uses.

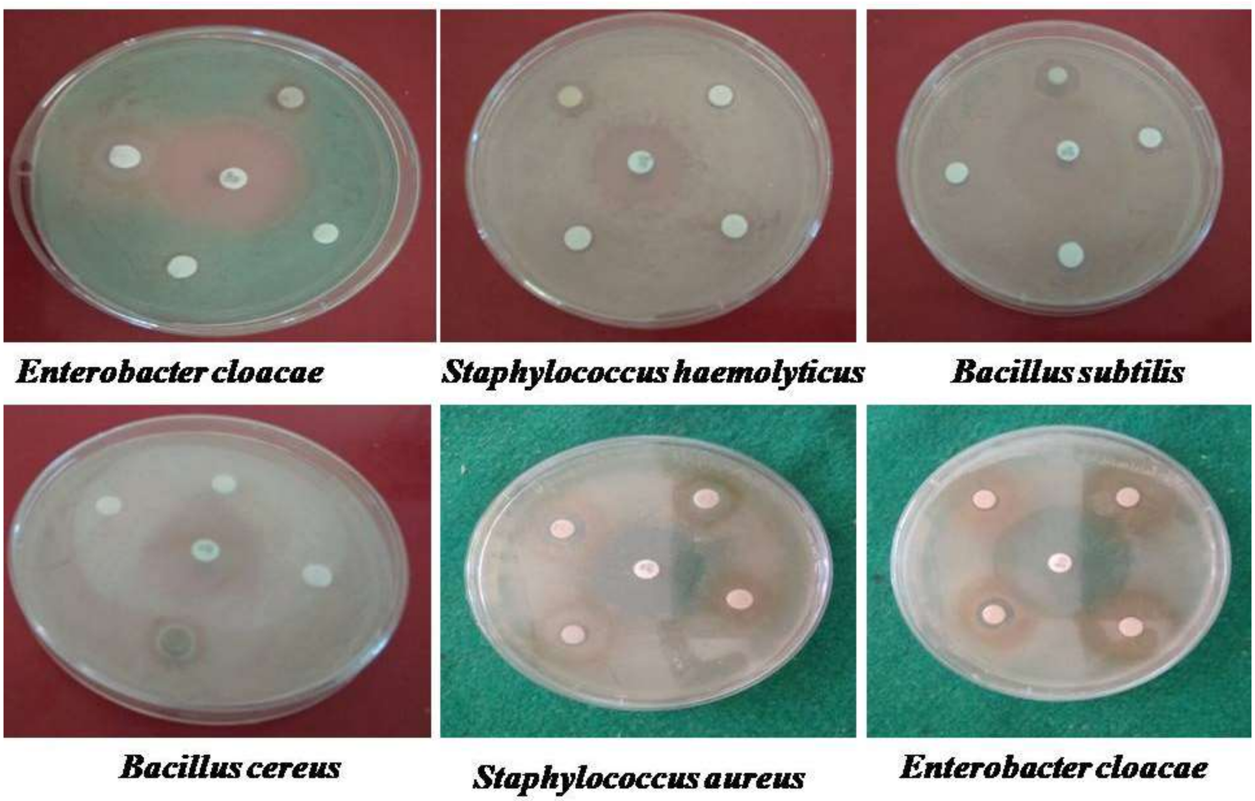


Figure 9. Anti-microbial activity of VC-Cu NPs.

Table 1. Anti-microbial activity of Cu NPs.

Isolated pathogens	Different concentrations of VC-CUNPs				Positive Control (Tetracycline 10µg)
	12.5 µg	25 µg	50 µg	100µg	

Enterobacter cloacae	-	-	10mm	11mm	28mm
Staphylococcus haemolyticus	-	9mm	10mm	11mm	21mm
Bacillus subtilis	-	9mm	10mm	14mm	26mm
Bacillus cereus	-	9mm	10mm	12mm	20mm
Staphylococcus aureus	-	9mm	10mm	13mm	28mm
Enterobacter cloacae	-	-	10mm	12mm	30mm

Anticancer activity

CuNPs cytotoxicity was tested in vitro on MCF-7 cell lines at various doses (10, 20, 30, 40, and 50 µg). The pattern's cytotoxicity examination revealed a direct dosages relation, with cytotoxicity increasing as concentrations climbed (Fig. 10). The IC50 value was calculated by plotting the CuNPs concentration on the X-axis against the percent of cell viability on the Y-axis. The specimens had a high level of cytotoxicity toward MCF-7 cell lines. CuNPs strongly suppressed MCF-7 cell growth, by an IC_{50} score of 3.44088+/-230.7(6703 percent) g/ml of the concentration in the minimum tested concentration (10g/ml), CuNPs were able to limit the cell line's development by lesser than 10%. The inclusion of 50µg/ml CuNPs, on the other hand, greatly slowed the cell line's development (by >93%). But when compared drugs showed a high cytotoxicity effect when compared to CuNPs. Earlier research displays that phyto-chemicals deplete intracellular antioxidants thus persuading tumor cell death (29).

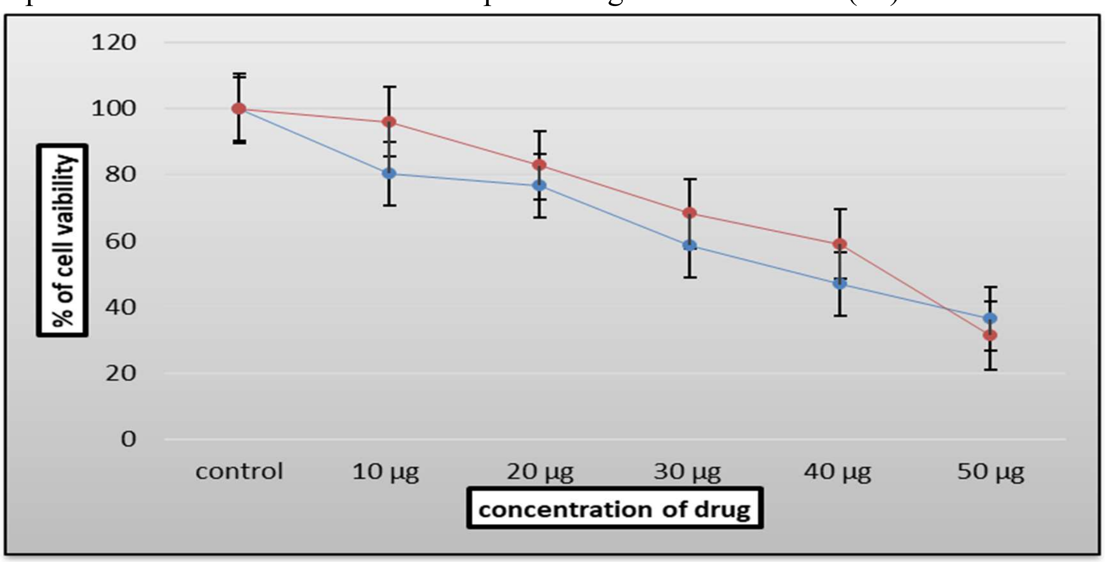


Figure 10. Anti-cancer study of VC-Cu NPs

CONCLUSIONS

It can be concluded that the Cu NPs made from Vallarai chooranam were cost-effective, nontoxic, and environmentally friendly. The medicinal phytocompounds found in the aqueous extract have a reducing and capping character. VC-CUNPs possess antibacterial and cytotoxic actions towards MCF7 cancer cells. Vallarai chooranam may act as a reference for prospective antitumor medications, as the extraction and biofunctionalized CuNPs. Vallarai chooranam extract will be used for even further studies in holistic therapies due to its multi-functional therapeutic applications. This simple, effective, and quick green synthesis of CuNPs could be utilized to generate larger-scale production of Cu NPs for usage in different biomedical and experimental purposes.

The authors report no financial or any other conflicts of interest in this work.

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Competing interests

Authors declare no competing interest.

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