RESEARCH PAPER

Green Chemistry for the Synthesis of Ternary Metal Nanoparticles Reify as a Pharmaceutical Drug in Biological Applications and Its Nanotechnological Characterization

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ABSTRACT

Our study provides a brief discussion of the major nano-chemical formulations and its biological effects, as well as the impact of nanotechnology on the future of pharmaceuticals. Effective and eco-friendly strategies of bio conception are also highlighted in this study. The work scrutinizes the structural and antibacterial properties of Ternary metal nanoparticles (Cu-Ag-Fe) formed by a simple ecofriendly chemical method. The X-ray diffraction (XRD) pattern demonstrated the crystalline character of Ternary metal nanoparticles (Cu-Ag-Fe). Field emission scanning electron microscopy (FE-SEM) revealed the morphology. The energy-dispersive X-ray spectroscopy, XRD pattern indicated that the produced Cu, Ag, and Fe were extremely pure. Also Ternary metal nanoparticles (Cu-Ag-Fe) exhibited good biological application especially in antibacterial activity against pathogenic bacterial species such as *S. aureus*, *S.epidermidis*, *E. coli*, and *Klebsiella* sp. Ternary metal nanoparticles (Cu-Ag-Fe) are doped engineered nano composites at nano scale level used to control the experimental drug-resistant pathogens. Disparate traditional antibiotics, they attack microorganisms through numerous concurrent pathways—such as membrane distraction and reactive oxygen species (ROS) generation—making it highly difficult for pathogenic microbe to develop resistance

Keywords: Nano particles, nano scale, pathogens, drug resistance, antibacterial

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1.Introduction

Nanotechnology has revolutionized the biomedical sciences of multipurpose nanomaterials with superior characteristics for Therapeutic and Diagnostics applications. Metal nanoparticles have been shown to possess remarkable physicochemical properties such as large surface-to-volume ratios, quantum confinement effects and tunable optical properties that are absent in bulk material. Within these, copper (Cu), siver (Ag) and iron (Fe) nanoparticles have received interest mainly by their antimicrobial action, cost effectiveness and possibility to magnetically functionalize the particles. By destruction of the membrane, induction of ROS, and inhibition of enzymes, silver nanoparticles show bactericidal activities across a wide spectrum and are effective towards drug-resistant organisms including methicillin resistant S. aureus (MRSA) and multidrug-resistant E. coli [1]. Copper NPs have catalytic activities as well as long-term antibacterial action through the release of Cu^{2+} ions [2, 3], whereas iron NPs show magnetic response, biocompatibility, and structural stability [4]. Due to the global crisis of antimicrobial resistance (AMR), there has been accelerated quest for alternative agents, promoting multimetallic nanoparticles such as prospects for therapies, wound dressing material and anti-microbial coatings [5, 6].

Conventional synthesis methods such as chemical reduction, physical vapor deposition, and sol−gel possess possible perilous resultants high−energy immediate heating solution environment toxic products to kinetics can restrict their applications for the biological systems. Green synthesis by exploiting plant extracts, microbial cultures or biopolymers provides an eco- friendly alternative and here in, Phytochemicals such as polyphenols, flavonoids and terpenoids were reduced and capped to increase their biocompatibility [7, 8]. Synergy effects in this complex bimetallic Ag–Fe, Ag–Cu and Cu–Fe systems on antimicrobial, antioxidant and photo catalytic capability are superior to their monometallic analogues [9]. However, ternary Cu–Ag– Fe NPs are less studied and less than five studies can be reported in literature compared to bimetallic, octahedral, pentad cubes [7,10] despite their increased potential to integrate the antimicrobial properties of silver and copper with the magnetic and stabilizing properties of iron.This deficit is being covered by the current study, which has biosynthesized Cu–Ag–Fe nanoparticles via plant extract-mediated green and simple route. [11,12] The detailed characterization involves UV–Visible spectroscopy for identifying surface Plasmon resonance, FTIR measurement for estimating functional groups, XRD analysis for understanding crystalline phase and crystallite size of particles and finally SEM-EDX is used to study particle morphologies as well as elemental compositions [13]. Biological assessments include antimicrobial (Gram-negative and Gram-positive bacteria), antioxidant (DPPH assays), hemolytic activity for hem compatibility, and cytotoxicity for therapeutic safety [14]. However, this investigation is restricted to the laboratory-scale preparation and in-vitro studies because of the size variation and reproducibility issues associated with natural plant extracts indicating that further animal experiments will be essential. This study attempts to lay a solid foundation for the Cu–Ag–Fe nanoparticles as multifunctional armaments in flaw of biological applications especially AMR, bridging green nanotechnology with critical biomedical hitches.

2.Materials and Methods

2.1. Extract Preparation

Leaves of *Moringa oleifera* were freshly collected from the local botanical gardens in Madurai area Tamil Nadu, India, authenticated and rinsed with deionized water. 50 g of milled leaves were boiled in 500 mL deionized water at 80ºC for 30 min, filtered using Whatman No. 1 paper and the deep green extracts centrifuged at a speed of 5,000 rpm for 10 min to generate clear supernatant form of extract were obtained at pH-6.5-7.0.

2.2. Formation of Nanoparticles

For tri-metallic nanoparticle preparation, 50 mL of mixed precursor solution ($CuSO_4 \cdot 5H_2O$:$AgNO_3$:$FeCl_3 \cdot 6H_2O$ (2:1:1)) at a concentration of 0.01 M was magnetically stirred [800 rpm] with temperature control at 60°C after which time (30 min) the plant extract having volume of about10-20 mL was added drop wise until pH at the end is about pH (9–10) (adjusted with water and NaOH [0.1M]). The reaction was carried out for 2 h and the Progress of the reaction was checked by monitoring color change from blue-green to dark brown, signifying reduction and formation of doped nanoparticles(Figure 1 and 2).

Figure 1. Formation of Nanoparticles

Figure .2. Graphical abstract shows schema of Cu-Ag-Fe Nanoparticles Synthesis

2.3. Observatory analysis

First sign of the biosynthetic production of Cu-Ag-FeNPs was detected by apparent and time-dependent color development in reaction mixture upon addition of *Moringa oleifera* leaf extract into the mixed metal precursor solution. At the beginning of the reaction, the $CuSO_4$– $AgNO_3$–$FeCl_3$ solution had a typical blue-green color caused by hydrated Cu^{2+} ions, which changed successively to yellowish-brown and finally formed a stable dark brown shade (after ca 2 h at elevated pH). This progressive modification corresponds to metal ions (Cu^{2+}, Ag^{+} and Fe^{3+}) being reduced to their equivalent zero valent or oxide-linked nano particulate states similar to the surface plasmon resonance observed in nano-sized metallic systems. Since no further color change was observed following extended stirring, it indicated that the reaction had been completed and that the colloidal stability of the nanoparticle dispersion had been reached. The plant extract functioned as a reducing and stabilizing agent, in which the large amount of polyphenols, flavonoids or other phytochemicals would donate electrons to metal ions promoting the nucleation and growth of tri-metallic nanostructures. Control experiments under extraction-free conditions showed that the precursor solutions were still blue-green and no turbid or sediment formed, thus establishing without question that chemical reduction was not taking place in the absence of bioactive compounds. However, the extract was found to be highly effective in generating not only a significant change in colour but also the formation of a colloidal dispersion that remained uniformly suspended and resulted in dark fine precipitate upon centrifugation and drying, visually confirming successful formation of nanoparticlces. The fact that the dark brown suspensions remained stable for several days with no observable aggregation or sedimentation further confirmed the capping and protection effect of phytochemicals, indicating strong qualitative evidence that the green synthetic route had helped to mediate biosynthesis of Cu-Ag-Fe-nanoparticles suitable for subsequent spectroscopic and biological investigations.

2.4.Nanotechnogical characterization

The prepared Cu–Ag–Fe nanoparticles in nano powder form was encompassed with various characterization techniques including UV-spectroscopic analysis, X-ray diffraction (XRD), SEM and DLS. It divulges and recognizes chemical conformations, crystal edifices, and photoelectric features of nano particles .All the characterization parameters related laboratory works were carried out by assistance of STAR Researchers Academy, Chennai, Tamilnadu, India.

(a) Particles morphology resolve by SEM

The Cu–Ag–Fe nanoparticles surface morphology was detected by using SEM (Scanning Electron Microscope(Hitachi S-4000).The particle nano structure was resolute by electron dispersion at the particle surface .

(b) Particle size Determination by XRD method

The equipped Cu–Ag–Fe nanoparticles were used to determine the particle size using ™ EQUINOX 100 X-ray Diffractometer(Thermo Scientific). Each obtained peak position were interpreted with Scherrer Equation:

$$Dp = (0.94 \times \lambda) / (\beta \times \cos\theta)$$

Whereas, Dp (Average Crystallite size), β (Line broadening in radians), θ (Bragg angle), λ (X-Ray wavelength) and further the equation used in the determination of size of crystallites (grains)s in the form of powder.

(c) Ultraviolet visible spectroscopic analysis

This analysis was carried out by using a UV-Vis spectrometer (Lambda Model: 950,Perkin Elmer, USA) with a range girth of 2 nm, with a 10mm cuvette cell at 37°.

The Cu–Ag–Fe nanoparticles examined underneath the UV visible light source at the wavelength vacillating around 300 to 750 nm to confirm the formation of nanoparticles. The nano particles were mixed with pure distilled water in 1:3 ratio and centrifugated at 6000 rpm enroute for 30 minutes and riddled over Whatman No. 1 filter paper. Some sample filtrates were used for UV-VIS Spectroscopic analysis.

(d)Analysis Dynamic light scattering

The nano sized particle of Cu–Ag–Fe dispersion in liquid form and polydispersity index were evaluated by DLS method (Malvern Nano system).Some limitations working for the analysis comprised a wavelength of 633 nm, RI(refractive index) liquid medium is 1.1.32, temperature 22 °C, viscosity of liquid medium 0.87873 and light scattering angle 173.

2.5.Analysis of phytochemicals

For qualitative phytochemical guesstimate of ***Moringa oleifera*** leaf powder extracts were executed using emblematic procedures, for the finding of important phytochemicals such as flavonoids, tannins, amino acids, steroids, alkaloids, terpenoids, saponin, and polyphenols

2.6. Biological Application

The synthesized Ternary Metal Nanoparticles (Cu–Ag–Fe), pertained in biological applications was first tested against some pathogenic microbes, for the confirmation of its antimicrobial activity.

To prove this, Ternary Metal Nanoparticles (Cu–Ag–Fe), was tested against some human bacterial pathogens together with *Salmonalla Typhi, Staphylococcus aureus, Shigella* and *Klebsiella Pneumonia,* along with the standard antibiotics were assessed using Kirby-Bauer well diffusion assay. For the facilitation of diffusion by the baceterial species were inoculated in culture medium of the plates with 6mm diameter wells were encumbered with 1mg/ml Ternary Metal Nanoparticles (Cu–Ag–Fe), added sideways with positive and negative controls by means of a micro pipette. The positive rheostat delimited 1mg/ml tetracycline while the negative control pure distilled water. All the plates were categorized and allowed nurturing for 24 hrs at *37 °C.* At the end of incubation, the zones of inhibitions were compared to matching the anti microbial activity against tested bacterial pathogens were measured in mm

2.7. Statistical analysis

The research outcome data attained from our work has been articulated by mean (± SD error). Data comprises graphs, plotting all are confirmed by means of Version 5.0, Graph Pad Prism Software (San Diego, CA, USA).

3.Results and Discussion

The green route synthesized Ternary Metal Nanoparticles (Cu–Ag–Fe), were categorized to fix their morphology in addition to size through Scanning Electron Microscope (SEM). Each resultant SEM images shape were gotten which are shown in Fig. 3 and the shape of the nano particles from place to place look like almost all rounded and looks like sphere-shaped. Likewise, the predictable typical particles size were roughly 72 to78 nm.

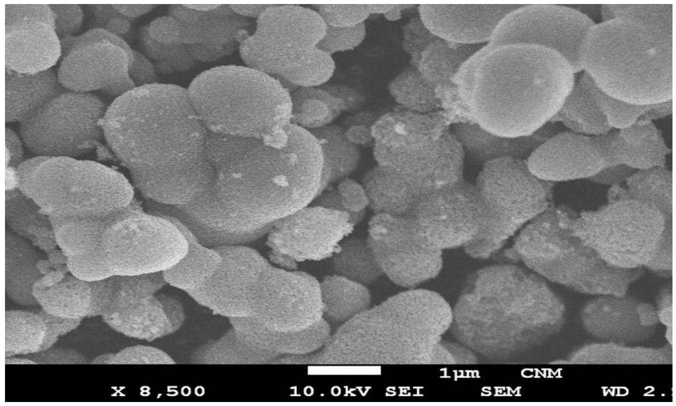


Fig 3. SEM image shows the morphology of Ternary Metal Nanoparticles (Cu–Ag–Fe)

The crystallite of the nanosized Ternary Metal Nanoparticles (Cu–Ag–Fe), using *Moringa oleifera* leaf extract was inspected over X rays diffractions (Fig. 4). Determined nanoparticles size was premeditated by Scherrer equation: ($D = k\lambda/\beta\cos\theta$). The angle of diffraction regarding the (111) lattice planes respectively. The approximate size of the crystallites (grains) were flinch about 76 nanometre range. The lattice limitations for the leaf powder nanoparticles were stubborn to be c = 0.4086 nm.

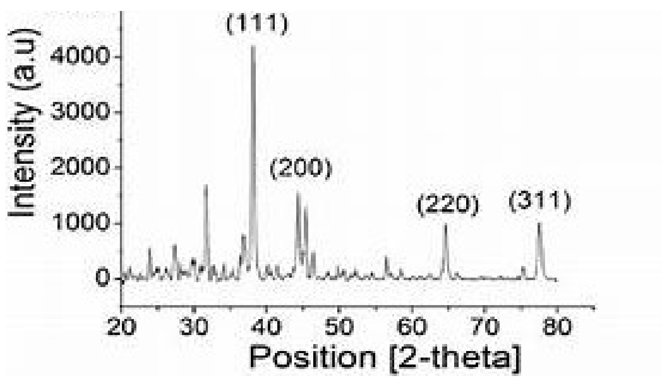


Fig 4.XRD patterns present in Ternary Metal Nanoparticles mixture (Cu–Ag–Fe), shows confirmation peaks, such as 111, 200, 220, and 311are confirmative to crystallite points

UV–Vis spectrophotometric study was approved for the prime examination of **Cu–Ag–Fe** nanoparticles in solution form . The absorbance of brown coloured nano solution was investigated for one week. The absorption spectrum of the nano **Cu–Ag–Fe,** at room temperature showed in Fig. 5 and the spectral range was established from 200 nm to 800 nm. The determined peak wavelength was detected at 376 nm, it confirms that the solution form of **Cu–Ag–Fe** amalgamation consists of polydisperse nano particles.

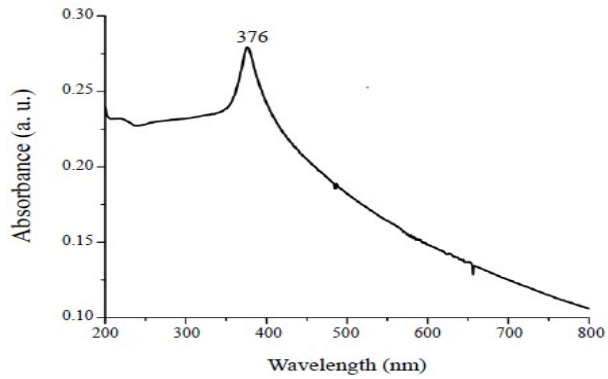


Fig 5. UV-Vis spectra of Ternary Metal Nanoparticles

By using DLS- Dynamic light scattering technique the size of dispersal silhouette of nano particles present in any solution or suspension can be determined. By the way of our study the mean nanoparticles size dispersal as a **Cu–Ag–Fe** suspension , exposed that size liquid form nanoparticles assumed the size range around 81 to 84 nm. The fig. 6, clearly shows, that the **Cu–Ag–Fe** ternary nanoparticles in suspension had assorted sizes of nanoparticles.

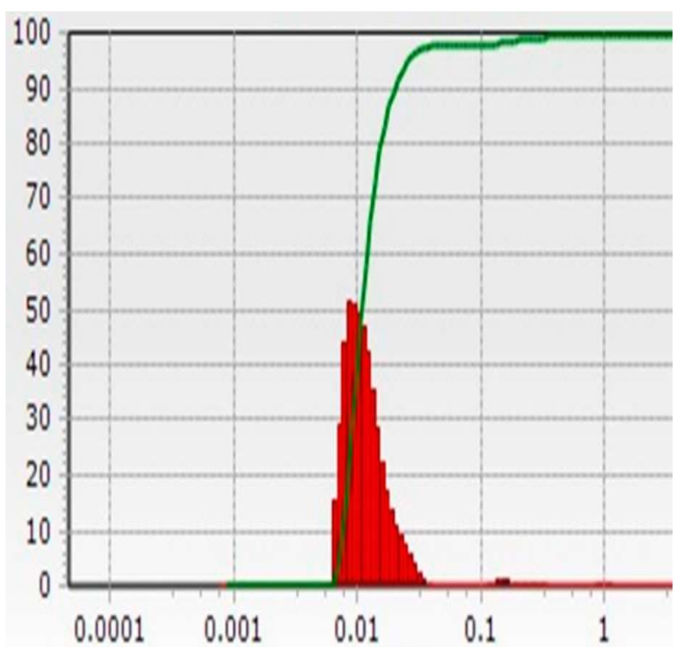
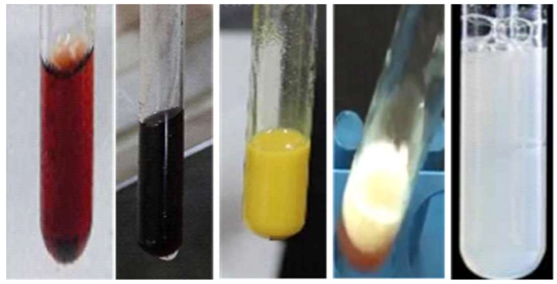


Fig 6. Dynamic light scattering ((DLS) shows nano particle size in solution

In solution or suspension form particle under going Brownian moment are shown on the X- axis (red), characteristic examples of a well-fit autocorrelation curve (green).The intensity of light scattering is taken as a consideration in Y-axis. The nano technology pragmatic preparation of Cu–Ag–Fe shows potent biological applications, and antimicrobial effects. It is due to the presence of valuable phytochemicals. In phytochemical fortitude alkaloid (reddish brown), Tannins (bluish black),flavonoids (yellowish), saponins(foam or froth) and poly phenols derivatives(white precipitate) were identified. The identified phytochemicals exhibited in Table 1 and Fig .7, correspondingly.



(A) (B) (C) (D) (E)

Fig.7- Phytochemical determination (A) Alkaloids (B)Tannins C) Flavonoids (D) Saponins and (E) Polyphenol

Table 1 Phytochemical Analysis

Phytochemicals	**Methods**	**Observation**	**Results**
Alkaloids	Wagner's test	Reddish brown	Presence of alkaloids
Tannins	Ferric chloride test	Blackish blue	Presence of tannins
Flavonoids	Ammonia test	Yellowish	Presence of flavonoids
Saponins	Foam test	Foam formation	Presence of saponins
Polyphenols	Gelatin test	White precipitate	Presence of phenolics

The antimicrobial activity of prepared Ternary Metal Nanoparticles (Cu–Ag–Fe), exhibits constructive result, while equated to tetracycline assayed as standard or control antibiotic. The Double distilled water, used as a negative control did not show the inhibition zone. The inhibition zone of standard antibiotics tetracycline shows average of 32 mm diameter(Table 2; Fig 8).The antimicrobial activities of prepared Ternary Metal Nanoparticles (Cu–Ag–Fe), counter to bacterial pathogenic strains and resulting inhibition zones were paralleled with antibiotic Tetracycline. The Ternary Metal Nanoparticles (Cu–Ag–Fe), shown more active antimicrobial proficiency and killing activity against, *Staphylococcus aureus,* (16mm),*Klebsiella Pneumonia(12mm), Shigella(14mm)* and *Salmonalla Typhi(18mm)* when comparatively analysed to the standard antibiotics.

Table 2

Antimicrobial activity of

Test pathogens	**Negative control (solvent only)**	**Positive Control (tetracycline)**	**Ternary Metal Nanoparticles (Cu–Ag–Fe)**
S.aureus.	Nil	34 mm	16mm
K.pnumoniae	Nil	31mm	12mm
S.typhi	Nil	30mm	14mm
Shigella	Nil	32mm	18mm

Statistical study: Mean frequency of 3 changed evaluates

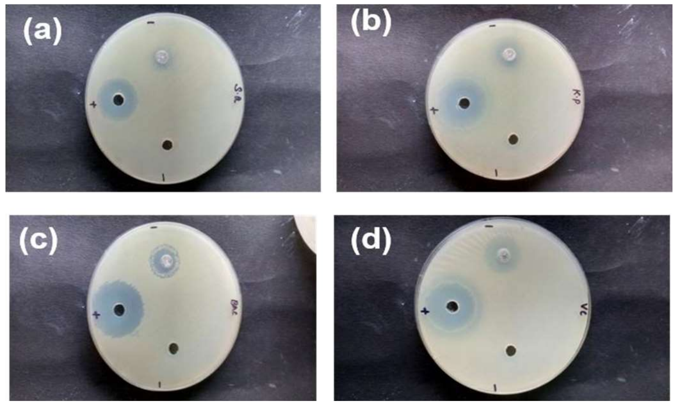


Fig 8. Antimicrobial activity of Ternary Metal Nanoparticles (Cu–Ag–Fe)

Ternary Metal Nanoparticles (Cu–Ag–Fe), treated against selected bacterial species had a significant impact on the degeneration of bacterial species indicates P value = 0.03. Almost, 99 % of experimental bacterial species killed, when Cu–Ag–Fe concentration was increased. The results showing that the Cu–Ag–Fe were found to be significant ($P<0.05$) in the restriction of pathogenic bacterial growth.

Nanotechnology partakes noteworthy progress over the usage of assets built on nanomaterials. Plants have the capability to convert as a nanomaterials, which canister to e changes the composition and structure [15]. Nanoparticle amalgamation by means of many plant portions for example leaf, flower, roots, and fruits [16]. The synthesis of nanoparticles using plant materials into has develop challenging research extent owing to its immense application in numerous fields including medicine and disease control. The preparation of nanomaterials using nanotechnology based green synthesis is to avoiding poisonous chemicals, eco-friendly, and cost efficiency engrossed countless researchers on the way to it [17]. Usage of plant portions and its biotic activities increases the efficacy of nanoparticles. Such plant based nanoparticles advantageous in medicinal ground due its killing activity against bacterial cells and also have antiviral activities. This chattels correspondingly imperative in drug delivery, diagnosis of disease and as a biomarkers [18].

These properties were revealed by the Ternary Metal Nanoparticles (Cu–Ag–Fe), prepared by us using nanotechnology. Apart from the methods above-mentioned, we have used another approach employed to advance the solubility, bioavailability of unwell water-soluble plant material based drugs is green synthesis. This technique useful to reduce size reduction of and the doped nanomaterial made about nanosized particles. Henceforth, is observed as a “top–down” method in the production of fine nano particle [19]. As effectively Ternary Metal Nanoparticles (Cu–Ag–Fe), can be mashed to fine particles irrespective of its soluble properties in liquid based or non-liquid based solvents[20-24]. Similar vicissitudes in colour in the same way been detected in previous studies [25].

The SEM, XRD, and DLS analysis of Ternary Metal Nanoparticles were seen that particles of goblin size and spherical shapes. The phytochemicals such as flavonoid, tannin being act as reducing and capping agents. Mostly plant extracts formed almost spherical nanoparticles[26-28]. In this study, the same results were obtained during the synthesis of Ternary Metal Nanoparticles. The antimicrobial activity in relation to antimicrobial property shown inhibition zones with all-out antibacterial activity of the prepared test Ternary Metal Nanoparticles sample and it also efficiently inhibited the expansion of all tested bacterial strains. The results of our study proved that the Ternary Metal Nanoparticles also have the expediency in biological applications.

4.Conclusion

By using *Moringa oleifera* plant extract Cu–Ag–Fe ternary nanoparticles was successfully synthesized by the green route from the bio reduction of leaf biomaterial. The nanoparticles have been properly characterized by means of UV-vis spectroscopy, SEM, XRD and DLS analysis. Results represented the Cu–Ag–Fe ternary nanoparticles unchanging nature and microbial killing property, these Cu–Ag–Fe ternary nanoparticles may be well exploited in industrial and therapeutic purposes. However, plant based Cu–Ag–Fe ternary nanoparticles approval and utilization necessitate more comprehensive research. This study acquaint with nanotechnology based preparation of Cu–Ag–Fe ternary nanoparticles as an antibacterial agents. The Cu–Ag–Fe ternary nanoparticles highlight their structural stability and potential for enhanced physicochemical and antibacterial properties due to synergistic interactions among the three metals. The biological applications of the Cu–Ag–Fe ternary nanoparticles with nano size can be used as a potential forthcoming antibacterial and antifungal therapeutic.

Therefore, it is suggested that upcoming studies proceeding on such Cu–Ag–Fe ternary nanoparticles should be protracted to embrace in vivo mockups and medicine wellbeing researches.

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