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Biogenesis of Metal Nanoparticles and its Potential Targeted Drug Delivery Systems for Urolithiasis

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Abstract: Urinary stones are a biggest issue that people experience worldwide. Urinary tract infections that lead to struvite urinary stones are the result of improper organ function, particularly kidney. The present study concentrated on an economical and environmentally friendly method for producing copper nanoparticles utilizing *Eclipta prostrata* leaves extract as a reducing and capping agent. UV-Visible spectroscopy shows SPR band at 256 nm which was used to validate the synthesis of copper nanoparticles. According to FT-IR Spectroscopic investigations, phytoconstituents have a major role in the reduction and capping of copper nanoparticles. *E. prostrata* mediated copper nanoparticles were crystallized and had a spherical shape, according to XRD and SEM. The EDX analysis showed the elemental composition and the amount of copper nanoparticles. The copper nanoparticles mediated by *E. prostrata* were found to have an inhibitory efficiency of 88.3% showing that they were an effective inhibitor for the struvite crystals. According to FT-IR spectra the shifting of the band confirmed that the phytocomponents present in *E. prostrata* leaves extract is responsible for anti-urolithiatic activity.

Keywords: *Eclipta prostrata,* UV-visible, FT-IR, SEM, EDAX, Anti-urolithiatic activity, Phytoconstituents, Nanoparticles

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Introduction

Nanotechnology is most preferable technology in the field of science (Singh *et al*., 2016). The nanoparticles are also known as nanocrystals (Dinh *et al*., 2015). It exhibits various properties like physical, biological and chemical etc. The nanoparticles can be synthesized in various methods-- Physical and chemical etc. (Ealia and Saravanakumar, 2017). In chemical synthesis the nanoparticles may exhibit some poisonous chemical for medicinal purpose which affect the human health.

So, to reduce the side effects we prefer green synthesis (Parveen *et al*., 2016). Because of the small size the nanoparticles have lot of applications in medicinal field (Luo *et al*., 2006). It can also cure kidney stones, diagnosis and drug delivery (Fathabad *et al*., 2020). The modern medicine is also known as allopathic medicine. This allopathic medicine is used to radiation therapy, antibiotics and surgery. The modern medicine also had the defect, it does not concentrate the root of the disease but it treats only for the symptoms of the disease. It also had side effects (Das *et al*., 2011).

Nearly 5% of the population is affected by the kidney stone disease. They are passing nearly 8 to 10% of their life time (Randal, 1937). Whenever the urine concentration is high then it leads to the formation of kidney stone. The kidney stones are made up of crystals and organic compounds. The oxalate, calcium and uric acid are able to form kidney stones (Qiu *et al*., 2005). Lack of magnesium also leads to kidney stone formation (Mohammadinejad and Mansoori, 2020). *Moringa oleifera*, *Asparagus racemosus*, *Rotula aquatica* are the natural inhibitors. It can inhibit the growth of the crystal formation (Akthar *et al*., 2013). The aim of the present study was to prepare and analyze the copper nanoparticles (CuNPs) and to investigate anti-urolithiatic activity of produced copper nanoparticles using herbal plant leaves of *Eclipta prostrata.* This research revealed the characterization of struvite crystals and it prevents the growth of struvite crystals. Therefore, the synthesis of copper nanoparticles by using *E. prostrata* leaves act as an inhibitor for the formation of renal stone.

Materials and Methods

Aqueous Leaves Extract of E. prostrate:

E. prostrata leaves were collected, cut into tiny pieces and washed with running water two to three times. The samples were dried under the sunshade. After that the leaves were finely crushed by using the morta. The powder was stored in an air tight container and kept in cool and dark place. 50 g of powder was poured into 50 ml of water and soaked for 24 h before use. The extract was prepared by hot percolation method (Ulaeto *et al*., 2020).

Qualitative and Quantitative Analysis of E. prostrata Leaves Extract:

The qualitative analysis of phytochemical of *E. prostrata* leaves were done by utilizing standard procedure. The quantitative analysis of phytochemical constituents revealed that Flavonoids, Terpenoids, Saponins, Phenols, Alkaloids, Tannins (mg/g) were present in *E. prostrata* leaves.

Synthesis and Characterization of Copper Nanoparticles:

To the 5 ml of leaf extract added 50 ml of 3 mM copper sulphate. The mixed solution was kept undisturbed. After a few min the color changed from dark green to reddish brown. This showed the development of CuNPs. The reduced CuO^+ was subjected to UV-spectrum analysis. The characterization of the developed CuNPs was used to identify by their functional group and the wavelength of the particle by FT-IR and UV-spectroscopy. The size and morphologies were identified by using SEM analysis. Crystalline structure and size can be identified by XRD, elemental composition can be identified by EDAX analysis.

Crystals Magnification:

0.5 ml of Ammonium dihydrogen orthophosphate was taken and dissolved in 100 ml of water. Sodium Meta silicate is dissolved in 20 ml of water. This mixture is poured into ammonium dihydrogen orthophosphate by using side of the test tube. The pH was 9.4. The pH range was adjusted to 6. The coagulated substance was produced and preserved with sealed stoppers and stored for 4-5 days. After 4 to 5 days, the gelation process occurred. The magnesium acetate was added to the mixture at a molarity of one without tearing the gel. The gel was kept at room temperature (37^{0}C). The components of crystals were determined by FTIR analysis (Feng *et al*., 2019).

Formation of Struvite Crystal Growth and the Classification of Various Additives Solutions:

It was revealed that *E. prostrata* leaf extract promotes the formation of struvite crystals. After adding magnesium acetate solution to the gel, the growth of the struvite crystals was explored using various concentrations of 1-5%. Copper nanoparticles were generated and added in varied amounts, along with an equal amount of supernatant solution. The crystal growth was identified by the standard mass and the inhibition efficacy is computed using calculations.

Results and Discussion

Phytochemical investigation:

E. prostrata leaves extract was analyzed for phytochemical using various test standards. *E. prostrata* leaves extract contained coumarins and anthraquinone in moderate amount. Emodin was found in trace amount. Terpenoids, Flavonoids, Protein, Anthocyanin, Carbohydrate, Phenols, Xanthoprotein were strongly present. The plant extract prevents Anti HIV. Figure 1 shows the occurrence of phytochemical components in the leaves extract of *E. prostrata* (Yu *et al*., 2020).

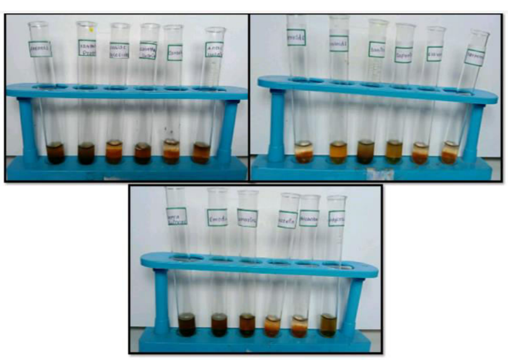

Fig. 1: Qualitative analysis of *Eclipta prostrata* leaves extract.

Quantitative Investigation of E. prostrata Leaves Extract:

Phytoconstituents found in *E. prostrata* leaves in various amount have been reported based on quantitative investigation. The aqueous leaves extract has the highest concentration of tannins followed by saponins, alkaloids, terpenoids, flavonoids and phenol (Fig. 2). Tannin is employed for hypolipidemic action, saponins for anticancer cytotoxic activity and alkaloids for Anti-inflammatory function.

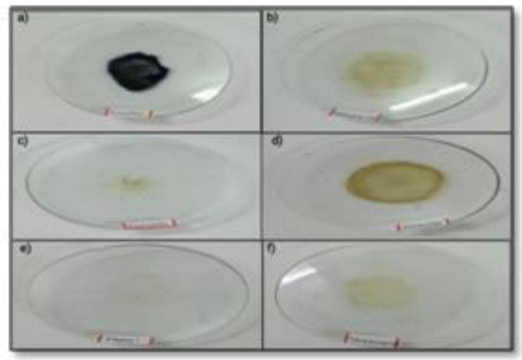

Fig. 2: Quantitative analysis of Eclipta prostrata leaves extract

UV-Visible Spectroscopy:

In this study, the aqueous leaves extract of *E.*

prostrata was used to generate absorption spectra of copper nanoparticle. The extract of *Eclipta prostrata* leaves was combined with copper sulphate solution which turned the solution from green to dark brown (Fig. 3). As a result of this process, copper ions turned into CuNPs. The presence of CuNPs is confirmed by colour change. Under the UV-visible spectroscopy it showed an absorbance peak about 256.18 nm (Fig. 4).

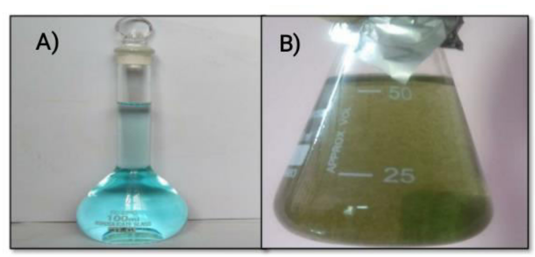

Fig. 3: Visual observations of synthesized CuNPs.

FT-IR Spectroscopy:

The functional groups were identified by using FT-IR analysis. Figure 5 shows the peak and

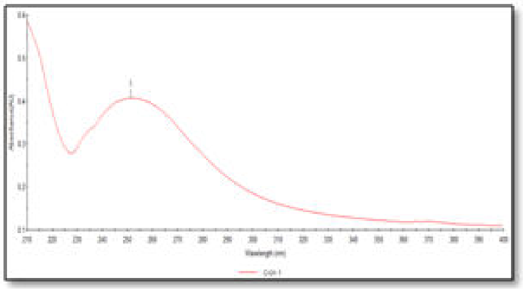

Fig: 4 UV-Visible spectrum of synthesized CuNPs

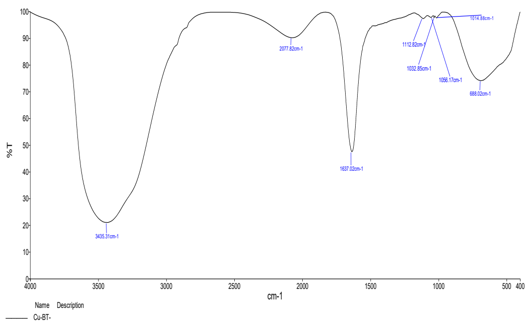

Fig. 5: FTIR peak and stretching of the synthesized copper nanoparticles.

stretching of aqueous leaves extract of *E. prostrata.* O-H stretching was responsible for the prominent broad peak at 3435 cm^{-1}. The peak at 2077 cm^{-1} showed C=C=C stretching and suggested the presence of phenolic group. The peak at 1637cm^{-1} was due to C=C stretching, 112 cm^{-1} was related to C-N stretching, 1032 cm^{-1} was attributed to S=O stretching, 1014 cm^{-1} was attributed to C-F stretching and 688 cm^{-1} was attributed to C-Br stretching.

SEM Analysis:

The scanning electron microscopy of the green synthesized CuNPs is shown in the Figure 6. The size and morphology of nanoparticles were determined using an environmentally safe technique. The formed nanoparticles were determined using SEM analysis. Copper nanoparticles have a diameter of 65.59 nm and an average diameter of 80.06 nm, indicating that they are spherical in shape.

EDAX Analysis:

The EDAX analysis shows the elements outline as well as the amount of biosynthesized CuNPs

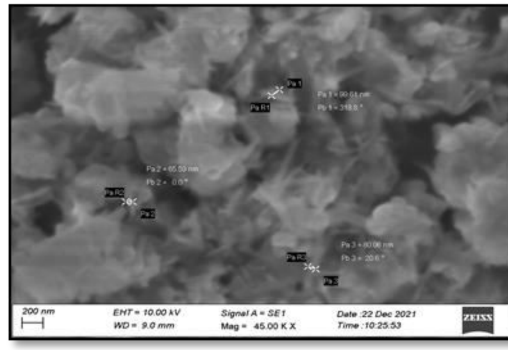

Fig. 6: SEM Analysis of synthesized copper nanoparticles.

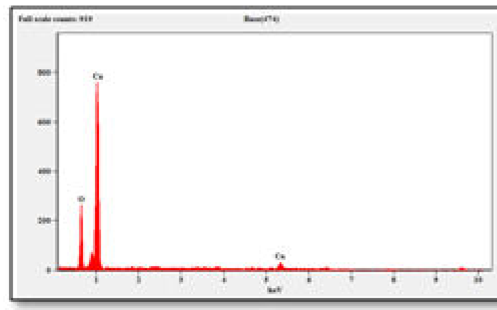

Fig: 7 EDAX Analysis of synthesized copper nanoparticles.

present. The EDAX analysis clearly shows the occurrence of elemental copper and oxide present in the sample. The amount of synthesized copper nano in the sample is 69.88%. While oxygen present in the sample is 27.65%. It shows the sample contains more purity and less impurity. Copper oxide nanoparticles are formed (Fig. 7) (Das *et al.*, 2018).

XRD Analysis;

XRD was used to characterize the biosynthesized CuNPs. The crystalline nature of the CuNPs can be identified. The strong peak shows the XRD pattern of synthesized CuNPs which is crystalline in nature. The highest value of the sample is shown in Figure 8. 33.40, 39.40, 47.30, 55.90, 58.60 and 68.10 were the highest points recorded. 43.33 nm, 17.63 nm, 45.31 nm, 46.99 nm, 95.19 nm, 100.20 nm and 50.43 nm were the crystalline diameters of the corresponding peaks. Figure 8 shows X-Ray diffraction peaks values of synthesized copper nanoparticles.

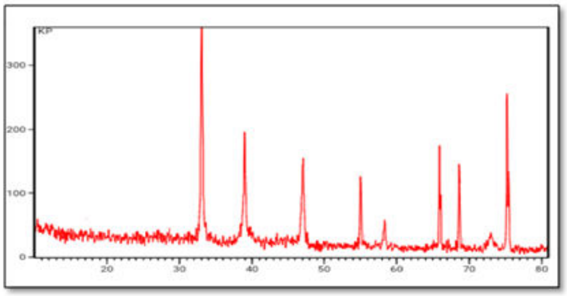

Fig. 8: X-Ray diffraction peaks values of synthesized copper nanoparticles.

Antiurolithiatic Activity of Leaves Extract of E. prostrsta:

The addition of an inhibitor revealed the morphology of the struvite crystal. It was determined by its standard mass and perceived measurement lengthwise. In this study, we used the gel technique, in which clean $Mg(CH_3COO)_2$ was used. It acts as a control. The crystallization process progresses within a day, and checked on regular basis to assess the crystals obtained (Table 3; Fig. 9).

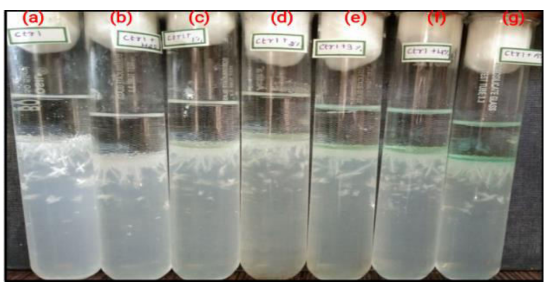

Fig. 9: Growth of struvite crystals.

In the this study, struvite development was decreased owing to the inhibitory impact of leaf extract on *E. prostrata* in an *in vitro* context. As a result the distilled water has no harmful impact on crystal growth. Because of bioorganic molecules and phytochemical substances identified in the leaves of *E. prostrata,* the inhibitory activity of generated copper nanoparticles was discovered (Das *et al*., 2017).

The Inhibitory activity of produced copper nanoparticles against *E. prostrata* is shown in Figure 10. The size of the crystals reduced over time, as illustrated in the image the crystal average weight is computed.

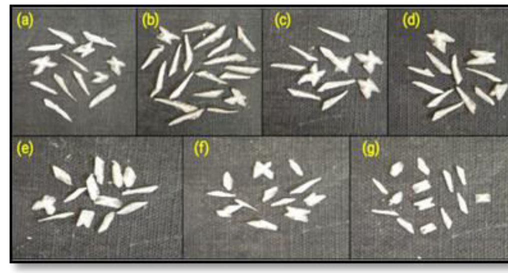

Fig: 10 Harvested struvite Crystal Morphology.

The inhibitory effect of synthesized copper nanoparticles was reduced from 1.89 g to 0.22 g. The inhibitory effect of synthesized cu nanoparticles was 88.3 per cent. The weight of the obtained crystals was measured using a measuring scale. It depicts the struvite crystals length decreasing over time (Fig. 11).

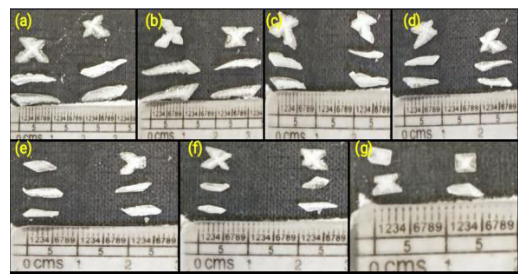

Fig. 11: Harvested Struvite crystal on scale measurement.

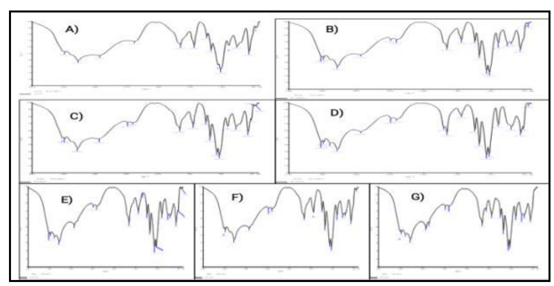

Fig: 12 The FTIR spectra if struvite crystals obtained using copper nanoparticles obtained from *E.prostrata* leaves extract.

Investigation of FTIR Analysis of Struvite Crystals:

The FTIR spectral analyses of crystals with different percentage composition of synthesized copper nanoparticles are synthesized by *E. prostrata* leaves extract (Fig. 12). The band values that represent various Phytoconstituents were summarized. As a result, the peak for HNH deformation modes of NH_4 units shifts from 2375 cm^{-1} to 2398 cm^{-1} and from 1434 cm^{-1} to 1432 cm^{-1}.

This demonstrated the inhibitory activity of copper nanoparticles that were created by using an extract of *Eclipta prostrata* leaves.

Conclusion

The major goal of green synthesis of copper nanoparticles is to provide an alternate route for limiting the negative impacts brought on by physical processes and chemical techniques. The synthesized copper nanoparticles were examined using UV-visible Spectroscopy, and peak intensity at 256 nm was noted. FT-IR analysis showed functional groups and characteristics of synthesized copper nanoparticles. According to SEM analysis, the synthesized copper nanoparticles have a spherical form. By using EDAX analysis the elemental signals were located. XRD analysis revealed crystalline nature of the synthesized copper nanoparticles. The appropriate length and weight of the crystals were calculated. The average weight and size of the developed struvite crystals gradually lowered by gradually increasing the proportion of cu nanoparticles mediated via *E. prostrata* leaves extract. The highest level of inhabitation was discovered to be 88.3%. According to an analysis of FT-IR spectra, shifting of the band revealed the inhibitory activity of synthesized copper nanoparticles.

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