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Vernonia Amygdalina Mediated Copper Nanoparticles and its Characterization and Antimicrobial Activity - An In Vitro Study

Research Article

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Abstract

Background: The green approachable metal nanoparticles is treated to be an eco-friendly path and cost-effectiveness. In this present study, nano copper was synthesized profitably by Vernonia amygdalina. Vernonia amygdalina is generally known as sour leaf or bitter leaf which is one among the medically significant plants in African and Mediterranean regions.
Methods: Green synthesis of copper nanoparticles was preliminarily confirmed by color changefrom blue to brown in the reaction mixture. The synthesized nanoparticles were characterized by using ultraviolet double beam spectrophotometer in the wavelength range of 250-750nm.The oral pathogenssuch as Streptococcus mutans, Staphylococcus aureus and Enterococcus faecalis and C. albicans were used to assess the antimicrobial efficacy ofV. amygdalinareinforced Copper nanoparticles. Antimicrobial activity was done according to the Agar well diffusion method atvarious concentrations that ranges from 25 µL, 50 µL and 100 µL.
Results: Zone of inhibition was found to be highest at 100 µL against Streptococcus mutans, Staphylococcus aureus, Enterococcus faecalis and Candida albicans. The mean zone of inhibition was found to be increased as the concentrations of Cu NPs increased.
Conclusion: Green synthesis of copper nanoparticles was initially confirmed by the position of SPR band at 340 nm in UV-Vis spectra. From the findings of the study, we can conclude that V. amygdalinareinforced copper nanoparticles have good antimicrobial efficacy against oral microorganisms.

Keywords: Copper Nanoparticles, Green Synthesis, *Vernonia Amygdalina*, Antimicrobial.

Introduction

Herbal medicines which are otherwise known as phytomedicines, medicinal products of plants or plant parts such as roots, leaves, flowers, barks, seeds and fruits which is widely used to treat various diseases and also to improve the health condition. Herbal medicines which are also called complementary medicines, have been used to ease the pain and to mitigate the diseases and their symptoms [1]. Many plants and fruits which are recognized with abundant sources of phenolic compounds which have many useful properties including antioxidant, antibacterial, anti-inflammatory, hepatoprotective and anticarcinogenic actions [2-4].

*Vernonia amygdalina is a soft woody shrub or tree belonging to the family Asteracea*e and genus Vernonia [5]. It is a perennial plant characterized by its bitter sap from the leaf which has been widely known for its medicinal use. V. amygdalina is commonly used as an antidiabetic [6], antihelminthic [7] and antimalarial medicinal plant [8] and also used to treat digestive disorders. +e main bioactive constituents of the leaves were reported as sesquiterpene lactones [9] and also vernonioside A1, vernonioside A2, vernonioside B1, vernonioside B2 [12, 13], vernodalin, vernolepin, vernomygdin, vernodalol, and vernodalin [10]. Furthermore, V. amygdalina has also been used traditionally to treat toothache which is not studied in detail.

Nanotechnology includes the production, manipulation and use of materials ranging in size from less than a micron to that of individual atoms [11]. Using plants for nanoparticles synthesis can also be advantageous over other biological processes because of

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huge availability of plants materials and also it eradicate the ease of large scale up and the process of culture maintaining, and in turn no use high pressure, energy, temperature and toxic chemical [12]. Plant extracts which have the unique property to act as reducing and capping agent which used in the reduction of metal ions, used in many pharmaceutical preparations [13]. The chapter in nanotechnology which currently provokes the interest of researchers is the noble metal nanoparticles which have the good antibacterial property due to their large surface area to volume ratio [14]. Obviously among the metallic nanoparticles, copper has been probably utilized most due to its stable and catalytic properties. Copper nanoparticles have wide applications as heat transfer systems and also as antimicrobial materials [15, 16]. V. amygdalinamediated silver nanoparticles [17] and zinc oxide nanoparticles [18, 19] was recently reported that it shows good microbial properties against bacteria.

In this study we are using leaf extract of Vernonia amygdalina plants for synthesis of copper nanoparticles at room temperature. Copper nanoparticles synthesis was identified by colour change and UV- visible spectroscopy (UV–vis) and it is confirmed by Transmission electron microscopy. Furthermore, the bacterial effect of Copper nanoparticles was also analyzed with gram positive and gram-negative microorganisms. The main aim of the present study was to synthesize Copper nanoparticles using the leaf extract of Vernonia amygdalina and to evaluate their antimicrobial efficacy against some selected oral microbes.

Materials And Methods

Preparation of aqueous leaf extract

Vernonia amygdalina was bought from Nigerian market. The collected leavesof V. amygdalina were washed 3–4 times using distilled water and shade dried for 7-14 days. The well dried leaves were grinded into fine powder (Figure 1a). The collected powder was stored in an air-tight container. About 1 g of V. amygdalina powder was measured and dissolved in 100mL distilled water and boiled for 5–10 minutes at 60–70°C. The solution was filtered by using Whatman no. 1 filter paper. The filtered extract was collected and stored in 4°C for further use (Figure 1b).

Synthesis of NPs

The copper nanoparticles were synthesized by adding 20mM of copper sulphate(Figure 2a) to 80 ml of distilled water. To that 20 ml of filtered V.amygdalina leaf extract was added and kept under constant stirring using a magnetic stirrer at 45–50 °C for 72 h. The colour change gradually changed from light blue to dark green colour (Figure 2c). At the end, the centrifugation process was carried out to separate synthesized copper nanoparticles from supernatant solution.The obtained pellet after was washed twice with deionized water and dried in a hot air oven at 100°C for 3 h. Finally, the dried powder was stored in properly labeled Eppendorff tube and used for further analysis.

Characterization of copper nanoparticles

The biosynthesis of CuNPs werepreliminary characterized using UV-visible spectrophotometerat 300 to 700 nm wavelength. The results were recorded for the graphical analysis. The aqueous copper nanoparticles and the optical properties were characterized by UV-spectrophotometer (Elico, India). The shape and size of the copper nanoparticles were analysed by using Transmission Electron Microscope (JEOL JEM3100F). The crystalline nature of the nanoparticles were characterized by X-ray diffraction analysis and the Fourier transform infrared spectroscopy was used to detect the functional and chemical group in the range of 4000–400 cm.

Antimicrobial activity of Vernonia amygdalina mediated CuNPs

Thecopper nanoparticles reinforced with Vernonia amygdalinaleaf extract were tested for antimicrobial efficiency by agar well diffusion method. The antibacterial activity of copper nanoparticles wastested against four different oral pathogens like Enterococcus faecalis, Staphylococcus aureus, Streptococcus mutans and the antifungal activity was tested against C. albicans. Different concentrations of NPs (25, 50, and 100 µL) were incorporated into the wells and the plates were incubated at 37°C for 24 h. The antibiotic amoxyrite was used as standard and for C. albicans

Figure 1. (a) Powdered form of V. amygdalina (b) Aqueous extract of *Vernonia amygdalina*.

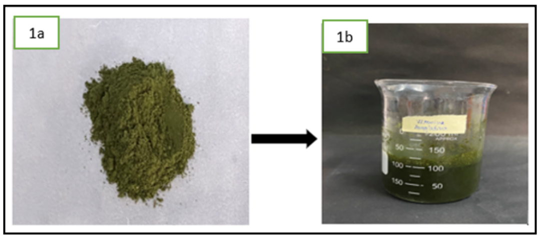


Figure 2. (a) Copper sulphate solution (b) Vernonia amygdalina mediated CuSo4 (c)After 72 hours.

fluconazole was used as standard control. Zone of inhibition was measured for each plate after 24 hours. The experiments were conducted based on our previous studies.

Results

Nanosized materials are having a great interest due to their unique optical properties. Nanoparticles exhibit different arrays of colours during the synthesis process. Plant extract contains several phytochemicals that react with copper sulphate and converts into copper nanoparticles and it was primarily identified by the change of colour from blue to brown in the reaction mixture observed within 1 h (Figure 3a). After 24 h reaction, the colour changing reaction was stopped and precipitation was observed which indicates that the nanoparticle synthesis process was completedAshtaputrey et al. [21]

UV- Visible Spectroscopy

UV-Vis absorption spectra of the green synthesized copper nanoparticles were recorded at a different wavelength from 250-750nm shown in Figure 3b. The copper nanoparticles are synthesized using copper sulphate and Vernonia amygdalina leaf extract as a reducing agent which shows absorption peak at 340 nm. Broadened SPR peak observed in this UV-Vis spectrum confirmed that polydisperse nanosized particles. The peak found in the spectroscopy indicates the formation of V. amygdalina Cu NP.

Transmission electron microscopy

TEM is the most common tool to convict the structure, size, morphology, dispersion, and orientation of biological and physi-

Figure 3 a: Visual Observation of Vernonia amygdalina mediated CuSO4; 3b: UV-Vis spectroscopic analysis of nano copper Vernonia amygdalina mediated CuSO4.

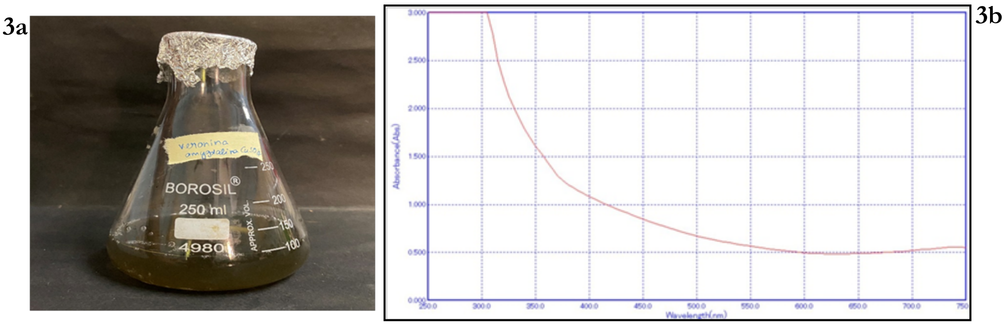


Figure 4: TEM image ofVernoniaamygdalina mediated Copper Nanoparticles.

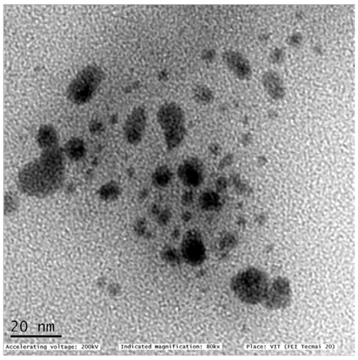


Figure 5: Antimicrobial activity of Vernonia amygdalina mediated Copper nanoparticles against pathogenic microorganisms (5a)Streptococcusmutans (5b)Streptococcus aureus (5c) E.Faecalis (5d) Candida albicans.

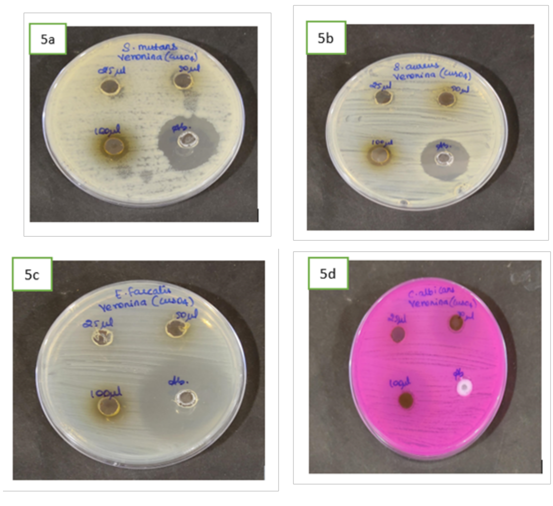
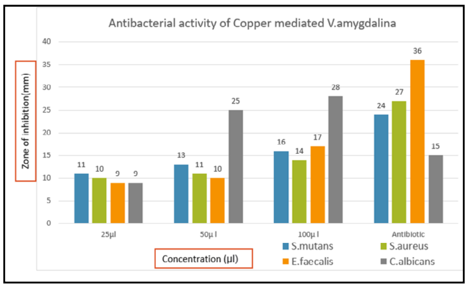


Figure 6: Antibacterial activity of *Vernonia amygdalina* mediated Nanoparticles.



From the above graph in S. aureus, S.mutans, C.albicans, E.faecalis, the zone of inhibition increases with increase in concentration.

cal samples. Figure shows the typical TEM images of nano copper shape was found to be well dispersed, crystalline in nature andthe figure also confirms that shape of copper nanoparticles is spherical in shape and has the broad size distribution between6 to 20 nm. This image explains that the copper nanoparticles arebounded with the phytochemicals of the plant extract.

Antimicrobial Activity

Agar well diffusion methodwas used to determine the antimicrobial activity of Vernonia amygdalina incorporated Copper nanoparticles against *S.mutans, S.aureus, C.albicans, E.faecalis* (Figure 5). The mean zone of inhibition was found to be increased as the concentrations of Cu NPs increased, producing a maximum zone of inhibition for *S.mutans, and E.faecalis* at 100µl concentration.

Discussion

Several investigators had reported that plants contain antibacterial or antimicrobial substances [21, 22]. Vernonia amygdalina used in the preparation of food nutritive seasoning and also in the preparation of food. Apart from its nutritive value, it has been found to be potential in inhibiting the growth of microorganisms. Several research works have reviewed the nutritive, chemical and proximate analysis of *V. amygdalina* [18]. Researchers have also studied the phytochemical and antimicrobial properties of leaf and stem extract. Phytochemicals present in *Vernonia amygdalina* included flavonoids, cardiac glycosides, reducing sugar, terpenoids, saponins, anthraquinones, and alkaloids [23].

The antibacterial activity of *V amygdalina* was found to be dependent on the nature of the solvent used for extraction and the concentration of the extract. Ethanolic extract was observed to possess more antibacterial activities compared to the aqueous extract [24, 25].

Agar well diffusion methods were used to determine the antibacterial activity of different concentrations of Vernonia amygdalina incorporated CuS NPs against *S.mutans, S.aureus, C.albicans, E.faecalis*. Antimicrobial efficacy of E.faecalis was shown in the figure. The mean zone of inhibition was found to be increased as the concentrations of CuS NPs, produced a maximum zone of inhibition for *S.mutans, E.faecalis*, however maximum was found for ampicillin/fluconazole. At 100µl concentration, maximum zone was produced for CuS NPs mediated V. amygdalina compared with fluconazole. Similar results were obtained by the study conducted by : I. I. Anibijuwon et al [26]. UV-Vis absorption spectra of the green synthesized copper nanoparticles were recorded at a different wavelength from 200-600 nm. The copper nanoparticles are synthesized using copper sulphate usingVernonia amygdalina leaf extract as a reducing agent displays an absorption peak at 340 nm. This peak can be assigned to be synthesized copper nanoparticles using plant extract [27]. Antimicrobial activity of copper nanoparticles by green synthesis using V. amygdalina showed promising results [26].

Recommendations

- This product can be given to the patients in the form of a mouthwash.
- In further studies, in vivo studies are recommended with people' acceptance values as well.

Conclusion

This investigation concluded that Vernonia amygdalina extract based green synthesized copper nanoparticles can be used as an alternative to commercially available antimicrobial agents. Green synthesis of copper nanoparticles was initially confirmed by the position of SPR band at 340 nm in UV-Vis spectral.

Ethical Statement: Ethical approval was obtained from Institutional Human Ethical Committee, Saveetha Dental College.

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