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Chemical and Green Synthesis of Titanium Di Oxide and Copper Nanoparticle using *Zingiber officinale* extract and Determination of their Antibacterial Activity

Hiya Prasad

Abstract: ***The present investigation focused on synthesizing the formation of Titanium Oxide and copper nanoparticles using chemicals as well as green synthesis strategies. The extract was made from the common plant Zingiber officinale (Ginger). Characterization of synthesized nanoparticles of titanium and copper were carried out by UV - VIS spectroscopy which showed a λ_{max} of 190 - 210 nm and 240 - 260 nm, respectively. The absorption spectra of synthesized nanoparticles of both the metals showed similarity with the reported literature. The antibacterial activity of both TiO_2 and Cu nanoparticles was assessed against Escherichia coli bacteria. The MIC of both TiO_2 and Cu nanoparticles was observed in range of 62.5 µg/ml and 125 µg/ml, respectively.***

Keywords: Nanoparticles, Green Synthesis, Titanium oxide, Copper Nanoparticles, UV - visible spectroscopy, Antibacterial activity, Minimum Inhibitory Concentration (MIC)

1. Introduction

Nanoparticles are generally defined as particles in the nanometer range i.e., 10 - 90 nm but most of them are from 1 - 100 nm. They can be both metallic (inorganic) and organic nanoparticles. They can be used in medical and environmental applications, imaging, etc [1] because of their physicochemical characteristics such as volume/surface area ratio, light absorptions, catalytic activity, etc. [2]. Due to their size, NP's can be used for drug delivery as they can transport encapsulated molecules across the membrane and also increase its stability. We can also attach antibodies to the surface of the NPs to elicit an immune response, which could work as a type of vaccine [3].

To reduce the impact of chemicals on environment, here we have reported the comparative synthesis of metal nanoparticles using chemicals and a green approach. Ginger (*Zingiber officinale*) was selected for this investigation. Ginger belongs to the family Zingiberaceae. It originated in South - East Asia and is used in many countries such as India, Pakistan, etc. as a spice and condiment to add flavor to food. Besides this, the rhizome of ginger has also been used in traditional herbal medicine (Ayurveda). The chemical analysis of ginger extract showed that it contains over 400 compounds, majority is carbohydrates (50 - 70%), with the remaining 20 - 30% containing terpenes and phenolic compounds. Gingerol and gingerol - related compounds such as Paradol, Shogaol, and Zingerone, etc. showed antioxidant activity, anti - tumor activity via induction of apoptosis and modulation of genetic activity, anti - inflammatory and anti - analgesic activity, antimicrobial activity, and hepatoprotective activity [4].

1.1. Titanium Dioxide nanoparticles

The Titanium dioxide (TiO2) NP's have many different uses in a wide variety of fields such as photolytic properties, removal of waste from wastewater, pharmaceutical, food industries, solar cells, agriculture, construction etc. and is also conventionally seen as a low toxicity material that will not harm the environment. TiO2 is a very potent oxygen radical generator which it can be excited by UV light to generate oxygen radicals. This system has been used against cancer and other types of treatment to try and control the disease. Synthesis of TiO2 requires high energy, temperature and pressure with help of chemicals which can be either toxic or non - toxic [5]. There have been many studies suggesting that Ti02 has effective antimicrobial activity, but the antimicrobial effect is not alone and works in conjunction with UV. The main mechanism is the production of ROS (reactive oxygen species). Since Ti02 nanoparticle also has high photocatalytic activity, therefore UV light trigger the release of electron which create ROS such as OH*, O_2^- , H_2O_2. ROS can damage the cell membrane, cell wall, and DNA etc. [6]

1.2. Copper Nanoparticle

Copper is required for human survival in a minimal amount that is less than 100 mg per day and can be present in either Cu^+ or Cu^{2+}. Copper nanoparticles have been used in various fields like textiles, agriculture, medicine etc. Even though copper is a widely used mineral, the synthesis of nanoparticle is difficult due to its oxidising ability [7]. They have been used as reaction catalysts since they enable reactions to occur in ambient temperature [8]. Copper Nanoparticles have also been shown to damage biological membranes, DNA etc. therefore it has been quite effective in dealing with viruses that is Covid - 19, H1N1 etc. [9]. They have also been shown to work as nano - biofertilizers i. e., they can increase the length and width of the plant and by optimizing the concentration of the Nano - Cu since it results in enhanced productivity, quality, bioactive compounds, protein content [10].

Cu NPs showed excellent antimicrobial activity against eight bacterial strains. Cu antibacterial action has been related to a sudden decline in cell membrane integrity and the production of reactive oxygen species (ROS). [11] These reactive oxygen species (ROS) are produced due to nanomaterial - membrane interactions, which can cause damage to secondary membranes, hinder the function of proteins, and destroy DNA. [12]. Keeping all these beneficial effects of both metal nanoparticles, we have planned their synthesis using both chemicals and green approach.

2. Materials and Method

2.1. Chemical synthesis of titanium dioxide nanoparticles

Chemicals

a) Titanium tetrachloride ($TiCl_4$)
b) Ammonium hydroxide (NH_4OH)

2.1.1 Synthesis strategy:

1.5 ml of $TiCl_4$ and 20 ml of NH_4OH were first diluted in 10 ml and 80 ml of cold distilled water, respectively. Then, titanium chloride solution was gradually added to ammoniated solution under vigorous stirring, which produced amorphous Titania precipitates. This reaction was carried out for 30 min at 60°C. The precipitates that had formed were collected and washed several times with warm distilled water followed by acetone. This precipitate was then dried in an oven to obtain powder and annealed at 350°C.

2.2. Green synthesis of copper oxide nanoparticles

Materials: Copper sulphate pentahydrate, Sodium potassium tartrate, Sodium hydroxide and *Zingiber officinale* extract

2.2.1. Preparation of extract from *Z. officinale* rhizome

To prepare the extract, Rhizomes of *Z. officinale (*ginger) were collected and were thoroughly washed with distilled water. Then 100 g of rhizome was taken and crushed to form a paste. The paste was boiled with 200 ml of distilled water. The resulting extract was filtered through Whatman's filter paper No.41.

2.3 Synthesis of CuNPs using extract of Ginger

A copper sulphate solution was prepared by dissolving 7 gm of copper sulphate pentahydrate in 100 ml of distilled water. Then, 5 gm. of sodium potassium tartrate and 8 gm. of sodium hydroxide were dissolved in distilled water to prepare another solution. Both solutions were mixed in a 1: 1 ratio and vigorously stirred. Rhizome filtrate was then added to the mixture, and it was heated for 20 min at 50°C. After that, 500 µl of L - ascorbic acid was added to the solution. A red - coloured precipitate was produced after 20 minutes. The precipitate was centrifuged and then washed thrice. To obtain powder, it was dried in the oven. For further studies, it was dissolved in liquid ammonia.

2.4 Antimicrobial Activity:

Nanoparticles dissolved in certain solvents such as water and liquid ammonia are tested against one standard strain of gram - negative bacteria (*Escherichia coli*). The antibacterial activity was checked by determination of the Minimum Inhibitory Concentration by micro broth dilution assay.

2.5 Determination of Minimum Inhibitory Concentration (MIC):

Serial dilution in a 96 - well ELISA plate was used to calculate the MIC. A positive control (bacteria without any drug), a negative control (broth only), a control for kanamycin, and test series with gradients of plant extracts (Zingiber *officinale*) and other samples were taken for their analysis. The bacterial strain of *E. coli* was taken from an exponential culture and incubated for 18 hours at 37 °C with constant shaking. The MIC was measured up to a well where there was no observable bacterial growth and compared to a standard antibiotic.

2.6 UV - Visible Spectroscopy:

The spectrophotometric scanning (190 - 1100 nm) of all the samples were carried out on a Thermo UV - VIS spectrophotometer using a Quartz cuvette.

3. Results and Observation

Copper Nanoparticle: Copper Nanoparticles obtained using green synthesis were subjected to UV - Spectroscopy to study the absorption spectra. The maximum absorption was obtained at **250.6 - 272.82nm** which falls under the UV - C region (Fig.1a). The UV - C region is called the short - wave ultraviolet region, which ranges from 200 - 280nm. The energy band gap of the material has been calculated by the formula: $\mathbf{E = h*c/\lambda}$

Where h is Planck's constant, c - is the velocity of light and λ is the wavelength of maximum absorbance. From the mentioned formula, the band energy is 5.42eV

Titanium Nanoparticles TiO2: Nanoparticles obtained using chemical synthesis were subjected to UV - Spectroscopy to study the absorption spectra. The maximum absorption was obtained at **190 – 210 nm (Fig.1 b),** with the Energy band being 6.42eV

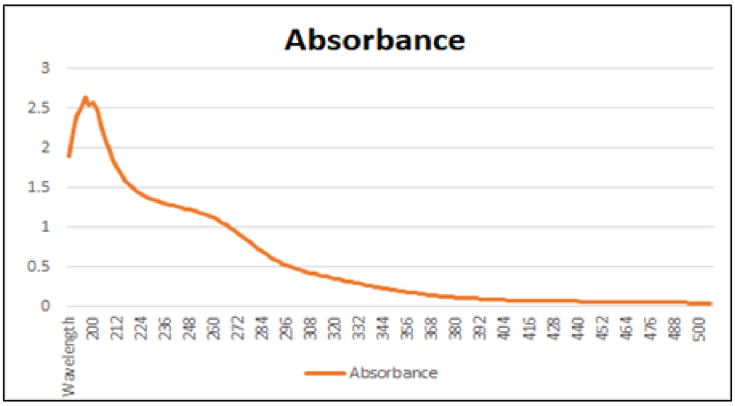

Figure 1: Absorption spectra of copper (a) titanium oxide nanoparticles (b) showing λ max at 250 - 272 and 190 - 210 nm, respectively

Antibacterial Activity:

The antibacterial activity of both metal nanoparticles obtained from different methods was evaluated using a broth dilution assay. The MIC value of each particle has been represented in Table 1. The TiO2 and Cu particle synthesized using ginger extract showed good antibacterial activity against *E. coli* with an MIC value of 62.5 µg/ml and 125 µg/ml, respectively.

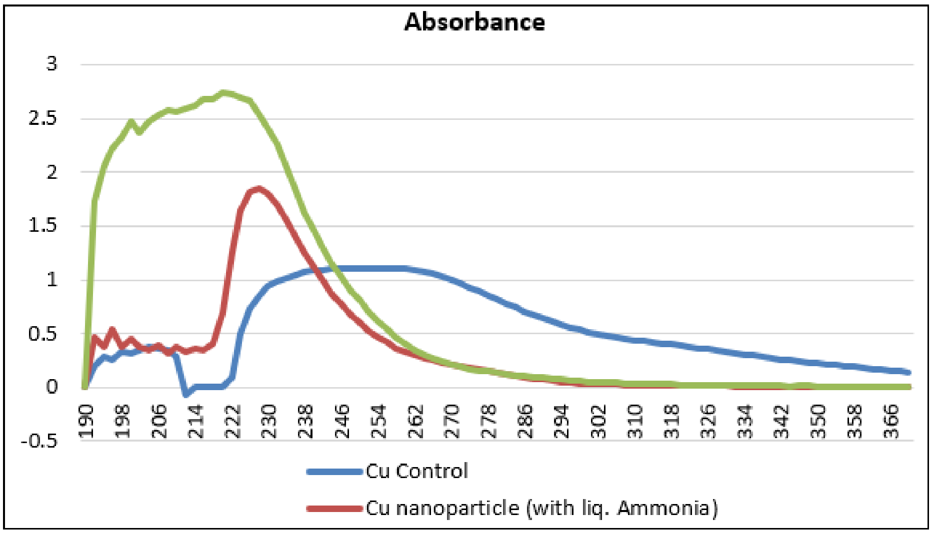

Table 1: The MIC value of metal nanoparticles against the bacterium E. coli

S. N	Nanoparticles	MIC (minimum inhibitory concentration)
1	Titanium Nanoparticle	62.25 ug/ml
2	Copper Nanoparticle	125 ug/ml

4. Conclusion

TiO2 and Cu nanoparticles were effectively synthesized using chemical method and an extract *Zingiber officinale*, respectively. The characterization of synthesized metal particles was carried out by UV - VIS spectrophotometer. Both the particles were evaluated for their antimicrobial activity against the bacterium *E. coli*. Potent antibacterial activity of the particles was observed by microbroth dilution assay.

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