Biosynthesis And Characterization Of Copper Nanoparticles From Bauhinia Tomentosa

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Abstract: Plants are one of the richest resources of the world due to a wide range of climate, topology and environments in the country. It is thought that there are over 16000 species of flowering plants, which account for 6% of the total plant species in the world. Over 30% of the geographical area of India, including that in the Himalayas, Western Ghats and Andaman and Nicobar islands, still remain to be floristically explored. Secondary metabolites serve as competitive weapons against bacteria, fungi, amoeba, plants, insects, higher animals and as metal transporting agents. Plants are subjected towards the production of nanoparticles because of its advantages of easily available, safe to handle and broad range of biomolecules such as alkaloids, terpenoids, phenols, flavanoids, tannins, quinones etc. In the present investigation, copper nanoparticles where synthesized from Bauhinia tomentosa leaf extract and subjected for characterization, antioxidant assay, hydrogen peroxide reduction and antibacterial activity studies. The results proved authentically that the copper nanoparticles are efficient in their activities.

Key words: Antibacterial, antioxidant, biomolecules, FTIR, inhibition, phytoconstituents, secondary metabolites, sensitive.

1 INTRODUCTION

Well organized treasures of phytodiversity metabolites (primary and secondary) have indispensable role in various field of medicine [1]. Green synthesis of metallic nanoparticles has attained its significant in the field of optical, sensors, biomedical, biomaterials, drug delivery etc. [2], [3], [4], [5]. Evidences on researches on nanoparticles based therapeutics are employed widely for infection, vaccines and renal diseases [6], [7]. Green synthesis of nanoparticles is conventionally accepted method because of its eco-friendly, cost- effective, single step method which does not involve high pressure, energy, toxic chemicals etc. [8] and it is also proved that these plant constituents produces capping and stabilizes the nanoparticles [9]. Evidences and development of green biosynthesis has been proved with many plant extracts such as Coriandrum sativum [10], Ocimum sanctum [11], Murraya koenigii [12], Petrocelinum crispum [13], Aspergillus orzae [14], Cochlaspermura gossypium [15]. Silver nanoparticles synthesized from Gelidiella acerosa extract revealed spherical nanoparticles with an average size of 23 nm in SEM micrograph [16] and Boswedllia ovalifoliolata ranged from 30-40 nm [17] and 40 nm in Shorea tumbuggaria [18]. Silver nanoparticles from Clitoria ternatea and Solanum nigrum leaf extract proved strong inhibitory effect against Pseudomonas aeruginosa, followed by Staphylococcus aureus, E. coli and Streptococcus viridians [19] and silver nanoparticles from papaya plup proved to be highly toxic against multidrug resistance bacteria [20].

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Bauhinia tomentosa commonly known as yellow bell orchid tree belongs to Fabaceae family and it is best versatile and most commonly used household remedy for many manifestations and they are found in coastal strip from Southern Kwazala- Natal to Maputo land, Mpumalanga, Mozambique, Zimbabwe, Tropical Africa, India and Srilanka.[21]. Bauhinia vahlii commonly known as adattige in Telugu and asamantaka in Sanskrit, which is the largest creeper in India and it showed the presence of sterols, terpenoids, flavonoids, glycosides and tannins [22]. Due to the presence of these phyto constituents in the plant extracts is responsible for antioxidant and antidiabetic activity in lowering of glucose levels [23].

2 MATERIALS AND METHODS

Disease free fresh leaves were collected and washed 3 to 4 times with distilled water and spliced into small pieces. 20g of spliced leaves were weighed and ground with mortar and pestle by adding demineralised water. 1mM concentration of aqueous copper sulphate was prepared and to it equal ratio of aqueous leaves extract was added and the mixtures were incubated for 20 to 30 minutes. The colour change from dark green into light yellowish green indicated the synthesis of copper nanoparticles [24]. The formation of nanoparticles was confirmed by using UV- Visible spectrophotometer of ELICO SL 171 Mini Spec. from the range of 400nm to 700nm and FT-IR with spectral range of 500- 4000 cm^{-1}.

2.1 REDUCING POWER ASSAY

Synthesized copper nanomaterials was subjected to reducing power assay with ascorbic acid as standard [25]. The copper nanomaterials of different concentrations such as 20, 40, 60, 80 and 100 was taken and mixed with 2.5 mL of phosphate buffer (pH 6.6) and 2.5 mL of 1% of potassium ferricyanide solution and the mixture were incubated in water bath at 50^0C for 20 minutes. The reaction was stopped by adding 2.5 mL of 10% trichloroacetic acid and then solution mixture was centrifuged at 3000 rpm for 10 minutes. The upper layer was transferred into sterile test tube and mixed with distilled water and ferric chloride solution. The absorbance at 700nm was measured as the reducing power by using UV spectrophotometer and higher absorbance of the reaction mixture indicated greater reducing power.

2.2 HYDROGEN PEROXIDE SCAVENGING ACTIVITY
The scavenging activity of copper nanomaterials towards hydrogen peroxide radicals was determined [26]. The solution of hydrogen peroxide (40mM) was prepared in phosphate buffer (pH 7.4). The copper nanomaterials of different concentration were taken and hydrogen peroxide solution was prepared with phosphate buffer solution and incubated for 10 minutes. The absorbance was measured at 560 nm using UV spectrophotometer against blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging of copper nanomaterials was calculated by using the formula:

2.3 ANTI-BACTERIAL ACTIVITY STUDIES
A pure culture of Escherichia coli, Salmonella typhi and Staphylococcus aureus were obtained from MTCC, IMTECH, Chandigarh and were cultured in nutrient broth for antibacterial study in the present study. Antibacterial activity study of the organisms was conducted with Muller and Hinton Agar (Hi-Media Pvt. Ltd. Mumbai) by Well Diffusion method. The test organisms were swabbed onto the duplicate petri plates, and four wells were made with the help of sterile cork borer. Different concentrations of green synthesized copper nanoparticles were loaded into four wells in petri plate, respectively and antibiotic kanamycin was used as positive control. Then the plates were incubated for 24 hours at optimum temperature. The zone of inhibition (in mm diameter) were read after 24 hours and recorded.

% Scaveng = Absorbance Control – Absorbance Sample * 100
Absorbance Control

3 RESULTS AND DISCUSSION
In the present study, the leaf extract of Bauhinia tomentosa was subjected to produce copper nanoparticles. When the plant extract was added to copper sulphate solution Cu^+ reduced to Cu^0 followed by colour change from green into dark green which indicated the formation of copper nanomaterials. It is due to surface Plasmon reasonance singularity which is the proved and significant indicator of the formation of nanoparticles. The synthesized nanomaterials were characterized under UV- Visible Spectrophotometer in the range of wavelength from 400 to 700nm and under the Fourirer Transformer Infrared Spectroscopy with spectral range from 500 to 4000 cm^{-1} (Fig.1). The FT- IR spectra for copper nanoparticles showed vibration bands at 3267. 41 cm^{-1} which indicated phenol group stretching and the peak at 2918.30cm^{-1} indicated alkyl C-H stretch. The peak at 1602.85cm^{-1} indicated aromatic C=C bending and at 1408.04 cm^{-1} indicated C-H stretch. The C-O stretching and C-H bending were observed at 1047.35 cm^{-1} and 596.00 cm^{-1}, respectively . The Nerium oleander synthesized copper nanoparticles subjected to FT-IR studies revealed the presence of different functional groups like Alcohol (OH stretch H-bonded,free), Alkane (C-H stretch, -C-H bending) Alkene (=C-H bending, C=C stretch) Amine(C-N, stretch) Nitro compounds (N-O stretch) Acid (OH,stretch) Ester (C-O, stretch) and these functional group plays an very important role in these copper nanoparticles synthesis was reported [27].

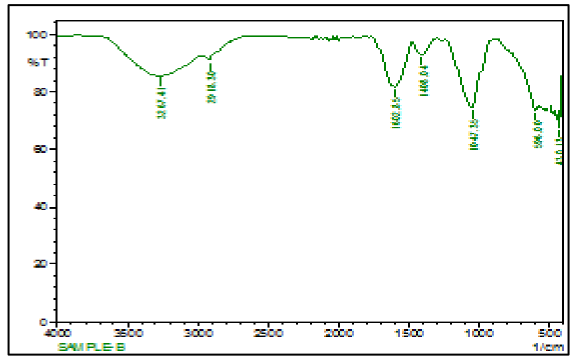

Fig.1 *FT-IR result for Bauhinia tomentosa copper nanoparticles*

Characterization of synthesized copper nanoparticles at different proportions (1:1, 2:2, 3:3, 4:4, 5:5) and the results are graphically represented in the Fig 2 - 6 and at different durations (30 mins, 1 h, 2 hrs, 3 hrs, 4 hrs) were carried out to prove their properties and factors affecting the yields of copper nanoparticles during the period of study and the results were recorded under UV-VIS spectrophotometer and graphically represented in Fig. 7. The copper nanoparticles showed a maximum absorbance between 500 to 550nm. Absorbance in the range of 500–550nm has been used as an indicator to confirm the reduction of copper [28], [29].

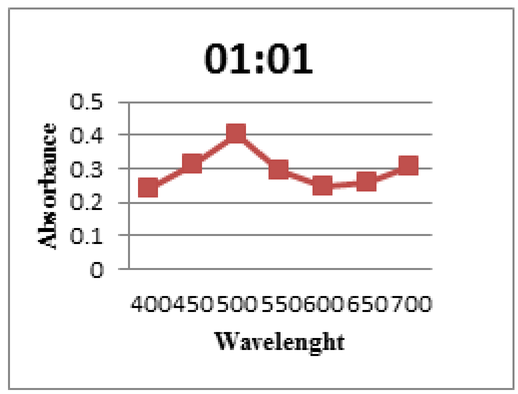

Fig.2 *Copper nanoparticles at 1:1 proportions*

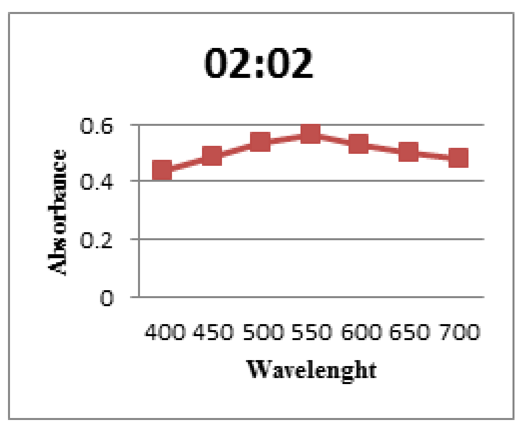

Fig.3 *Copper nanoparticles at 2:2 proportions*

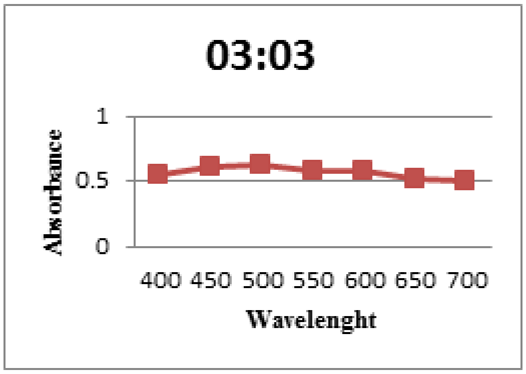

Fig.4 *Copper nanoparticles at 3:3 proportions*

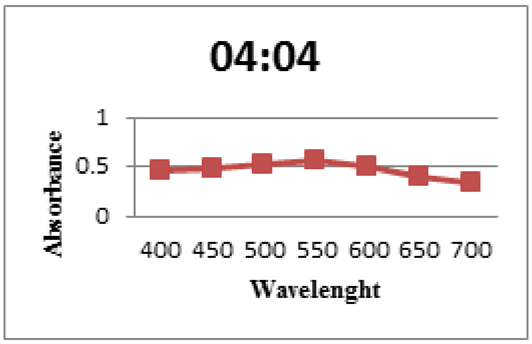

Fig.5 *Copper nanoparticles at 4:4 proportions*

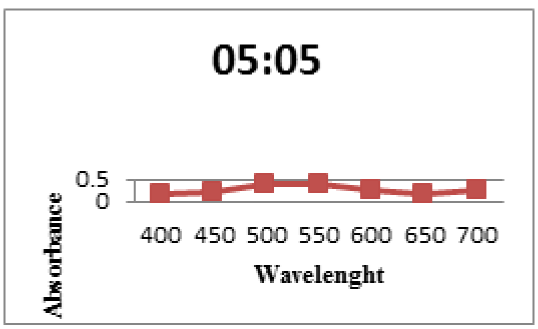

Fig.6 *Copper nanoparticles at 5:5 proportions*

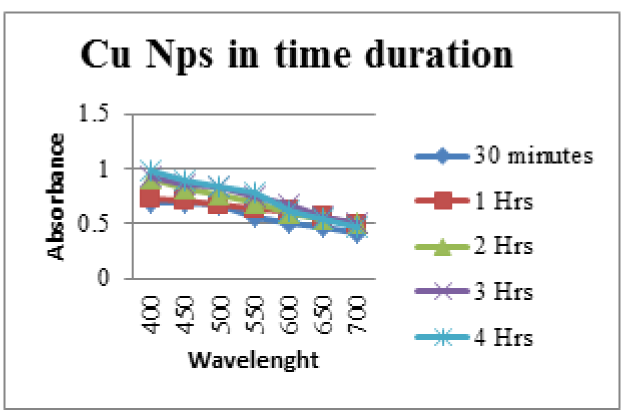

Fig.7 *Copper nanoparticles development at different duration*

3.1 REDUCING POWER ASSAY

The reducing power of copper nanoparticles of Bauhinia tomentosa was investigated by comparing with the reductive ability of ascorbic acid. Reducing power assay is a convenient and rapid screening method for measuring the antioxidant potential. The reducing power of synthesized nanoparticles increased significantly with concentration as compared with ascorbic acid were showed in Fig. 8. The reducing ability of a compound generally depends on the presence of reductants [30] which have been exhibiting antioxidative potential by breaking the free radical chain and donating a hydrogen atom [31]. The Triphala extract showed reducing ability comparatively with increase in concentration. [32]

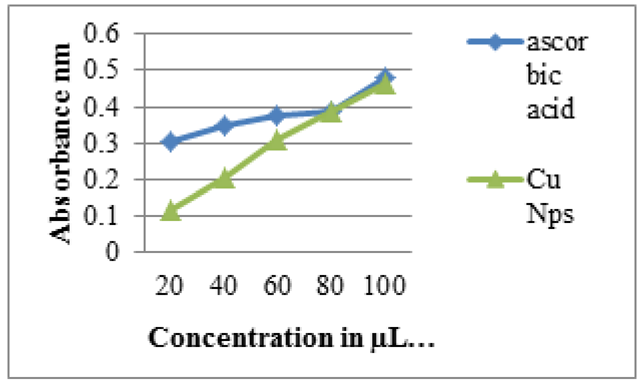

Fig. 8 *Antioxidant property of synthesized copper nanoparticles*

3.2 HYDROGEN PEROXIDE ASSAY

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. It can cross cell membranes rapidly and inside the cell. H_2O_2 probably reacts with Cu2+ ions to form hydroxyl radical which may be the origin of many of its toxic effects [33]. It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate. Therefore, hydrogen peroxide scavenging activity of synthesized nanoparticles was investigated against ascorbic acid as standard. The copper nanoparticles registered gradual increasing antioxidant potential along with ascorbic acid as evident in Fig. 9 during the period of study. The antioxidant studies of T. zeylanicum methanolic extract showed maximum antioxidant potential compared to standard and it is due to extract contains significant amounts of phytoconstituents such as flavonoids, saponins, phenolic compounds [34].

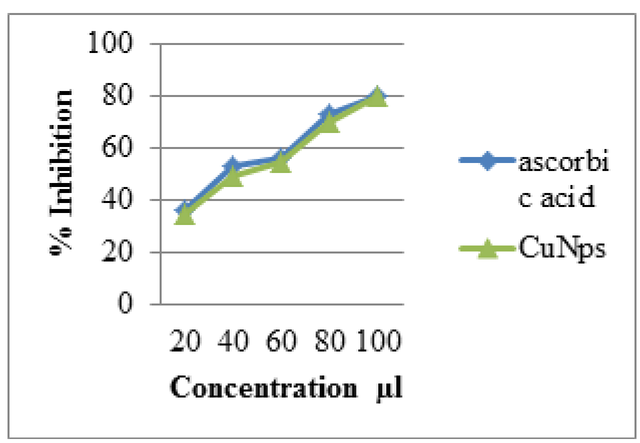

Fig. 9 *Hydrogen peroxide assay of copper nanoparticles*

3.3 ANTIBACTERIAL ACTIVITY STUDIES

The copper nanoparticles of Bauhinia tomentosa were found to be highly sensitive against the three microbes. The maximum zone of inhibition was observed in Escherichia coli followed by Staphylococuus aureus and Salmonella typhi and the results are recorded in the Table 1. The antimicrobial study of Nerium oleander copper nanoparticles showed a maximum zone of inhibition of on Salmonella typhi followed by Bacillus subtilus and Staphylococcus aureus [25].

Table 1. *Antibacterial activity study with synthesized copper nanoparticles*

Microorganism	*Copper nanoparticles*			
	Zone of inhibition in mm diameter			
	30 µL	*60 µL*	*90 µL*	*120 µL*
Escherichia coli	*1.5*	*1.9*	*2.1*	*2.4*
Staphylococuus aureus	*1.1*	*1.4*	*1.8*	*2.1*
Salmonella typhi	*1.9*	*1.6*	*1.8*	*2.1*

4 CONCLUSION

The investigation revealed that the Bauhinia tomentosa plant has the ability to synthesize nanomaterials at different concentration by reduction and capping process in the presence of solution with metal ions and the presence of phytochemicals in the leaf proved to have high therapeutic values to utilize the plant in the medical fields due to its antioxidant and antimicrobial property.

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