Biosynthesis of Copper Nanoparticles and Their Antimicrobial Activity

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Abstract

Development of green nanotechnology is generating interest of researchers toward ecofriendly biosynthesis of nanoparticles. Biomolecules present in plant extracts can be used to reduce metal ions to nanoparticles in a single-step green synthesis process. This biogenic reduction of metal ion to base metal is quite rapid, readily conducted at room temperature and pressure, and easily scaled up. In this study, biosynthesis of stable copper nanoparticles were done using datura meta leaf extract. These biosynthesized Cu nanoparticles were characterized by UV/Vis-spectroscopy, Particle size analyzer (PSA), Transmission electron mictroscopy (TEM), Energy dispersive X-ray Analysis (EDX),Fourier transform infrared spectroscopy (FTIR). It was observed that the datura meta leaf extract can reduce copper ions into copper nanoparticles within 8 to 10 min of reaction time. Thus, this method can be used for rapid and ecofriendly biosynthesis of stable copper nanoparticles.Synthesis mediated by plant extracts is environmentally benign. The reducing agents involved include the various water soluble plant metabolites (e.g. alkaloids, phenolic compounds, terpenoids) and co-enzymes. Extracts of a diverse range of plant species have been successfully used in making nanoparticles. In addition to plant extracts, live plants can be used for the synthesis. It was found that copper nanoparticles were also found to exhibit reasonably good antimicrobial activity when compared with standard Chloramphenicol, which suggests its potential use as antimicrobial agent. Hence, there is scope to develop new methods for the synthesis of nanoparticles which should be required inexpensive reagent, less drastic reaction condition and eco-friendly.

Keywords: Copper Nanoparticle, CuNps, Antimicrobial, Green Synthesis

1. Introduction

Nanotechnology is a collective definition referring to every technology and science which operates on a nanoscale. Nanoparticles have different properties than larger particles and these properties can be utilized in wide spectra of areas such as in medicine, information technologies, energy production and storage, materials, manufacturing and environmental applications. Although nano-derived applications have great potentials, there are some concerns about the potential nanoparticles have to cause adverse effects on human health and the environment. The different properties that make nanoparticles so promising are at the same time properties that are likely to have impact on ecosystems and organisms [1, 2].

Nanoscience is about the phenomena that occur in systems with nanometer dimensions. Some of the unique aspects of nanosystems arise solely from the tiny size of the systems. Nano is about as small as it gets in the world of regular chemistry, materials science, and biology. The diameter of a hydrogen atom is about one-tenth of a nanometer, so the nanometer scale is the very smallest scale on which we might consider building machines on the basis of the principles we learn from everyday mechanics, using the 1000 or so hydrogen atoms we could pack into a cube of size 1 nm×1 nm×1 nm. If this is all that there was to nanoscience, it would still be remarkable because of the incredible difference in scale between the nano world and the regular macroscopic world around us. In 1959, Richard Feynman gave a talk to the American Physical Society in which he laid out some of the consequences of measuring and manipulating materials at the nanoscale. This talk, "There is plenty of room at the bottom," is reproduced in its entirety in Appendix B. It does a far better job than ever I could of laying out the consequences of a technology that allows us to carry out routine manipulations of materials at the nanoscale and if you have not already read it, you should interrupt this introduction to read it now.

The remarkable technological implications laid out in Feynman's talk form the basis of most people's impression of Nanoscience. But there is more to Nanoscience than technology. Nanoscience is where atomic physics converges with the physics and chemistry of complex systems. Quantum mechanics dominates the world of the atom, but typical nanosystems may contain from hundreds to tens of thousands of atoms. In nanostructures, we have, layered on top of quantum mechanics, the statistical behavior of a large collection of interacting atoms. From this mixture of quantum behavior and statistical complexity, many phenomena emerge. They span the gamut from nanoscale physics to chemical reactions to biological processes. The value of this rich behavior is enhanced when one realizes that the total number of atoms in the systems is still small enough that many problems in Nanoscience are amenable to modern computational techniques. Thus studies at the nanometer scale have much in common, whether they are carried out in physics, materials science, chemistry, or biology. Just as important as the technological implications, in my view, is the unifying core of scientific ideas at the heart of Nanoscience. The field of nanoscience has been established recently as a new interdisciplinary science which can be defined as a whole knowledge on fundamental properties of nano-size objects [3]. Size and shape of nanoparticles provide an efficient control over many of their physical and chemical properties [4, 5], and their potential application in optoelectronics [6, 7], recording media [8, 9], sensing devices [10, 11], medicine [12-14] and catalysis [15].

When we bring materials down to the nanoscale, the properties change and nanoparticles have

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other optical, magnetic or electrical properties than larger particles. These properties are and will be utilized in a wide spectrum of areas as in medical applications, information technologies, energy production and storage, materials, manufacturing, instrumentation, environmental applications and security. There are few industries that will escape the influence of nanotechnology and consequently will it also affect our daily life in the future [1, 16, 17].

Copper nanoparticles, due to their excellent physical often do with antibiotics [2, 3]. The emergence of and chemical properties and low cost of preparation, nanoscience and nanotechnology in the last decade have been of great interest. Copper nanoparticles have presents opportunities for exploring the bactericidal effect wide applications as heat transfer systems, antimicrobial of metal nanoparticles. The bactericidal effect of metal materials, super strong materials, sensors and catalysts. nanoparticles has been attributed to their small size and Copper nanoparticles are very reactive because of high surface to volume ratio, which allows them to interact their high surface-to-volume ratio and can easily interact closely with microbial membranes and is not merely due to with other particles[18] and increase their antimicrobial the release of metal ions in solution efficiency. Colloidal copper has been used as an antimicrobial agent for decades. Copper nanoparticles (2-5 nm) have revealed a strong antibacterial activity and were able to decrease the microorganism concentration by 99.9%. Due to the stability of copper nanoparticles supported on a matrix and their disinfecting properties, copper nanoparticles can be used as a bactericide agent to coat hospital equipment [19, 20].

In recent years, Cu nano particles have attracted much attention of researchers due to its application in wound dressings and biocidal properties, potential industrial use such as gas sensors,catalytic process, high temperature superconductors and solar cells. In literature, the Cu nanoparticles are synthesized from (a) vapor deposition [21], (b) electrochemical reduction[22],(c) radiolysis reduction,(d) thermal decomposition[23], (e) chemical reduction of copper metal salt[24] and (f) room temperature synthesis using hydrazine hydrate and starch[24]. In recent, green synthesis of Cu nanoparticles was achieved by using microorganisms [25], plant extract

2. Synthesis of Copper Nanoparticles

2.1 Materials and methods

2.1.1 Materials

All the reagents and metal salts of AR grade were purchased from Sigma-Aldrich and used without further purification. Solvents used for spectroscopic studies were purified and dried before use. All aqueous solutions were prepared from quartz distilled deionized water, which was further purified by a Millipore Milli-Q water purification system (Millipack 20, Pack name: Simpak 1, Synergy). Melting points (uncorrected) were taken in a single capillary tube using a VEEGO (Model No: VMP-DS, India) melting point apparatus. The colloidal solutions were centrifuged in REMI, Model No. R-8C laboratory centrifuge. FT-IR spectra were recorded on Bruker, tensor 27Infrared spectro-photometer as KBr pellets. The spectra were recorded at room temperature. Absorption spectra were studied on a Jasco V-570 UV-Vis recording spectrophotometer. pH of the solutions was measured using pH analyzer LI 614- Elico. The particle size and zeta potential were determined by using the Malvern Zetasizer (Model; ZEN3600) as such without dilution. TEM images were recorded in MACK/model JEOL, JEM 2100 at an accelerated voltage of 200 kV. A drop of dilute solution of a sample in water on carbon coated copper grid was dried in vacuum and directly observed in the TEM. Fluorescence

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spectra were recorded on Jasco FP-6500 spectrofluorimeter. The antimicrobial susceptibility of nanoparticles was evaluated using the disc diffusion or Kirby-Bauer method and zones of inhibition were measured after 24 hours of incubation at 35 °C.

2.1.2 Methods

Datura metal plants were collected from in and aroundSarva Vidyalaya campus, Kadi,Gujarat. 100 gram of leaves were washed with tap water, ground and boiled with 500 ml of de-ionized water for 10 min. Finally the product was filtered and stored in freezer for further investigations. A 50% of leaf extract was made up to 250 ml.Synthesis of Cu nanoparticles using Datura metal leaf extracts, 1 ml of Datura metal leaf extract was added to 100 ml of 1mM aqueous CuSO4.5H2O solution in a 250 ml Erlenmeyer flask. The flask was then kept overnight at room temperature. The Cu nanoparticles solution thus obtained was purified by repeated centrifugation at 12,000 RPM for 15 min followed by re-dispersion of the pellet in de-ionized water. Then the Cu nanoparticles were dried in oven at 100 °C. The solid product obtained was washed twice with de-ionized water and dried at 80°C for 8 h. Finally the dried powder was stored in properly labelled containers and used for further studies.

2.2 Antibacterial activity of -CuNps

The antibacterial activity of Copper nanoparticles was evaluated using the disc diffusion and Kirby-Bauer method [26]. The antibacterial assays were done on bacterial organisms like Escherichia coli, Bacillus megaterium, Staphylococcus aureus, Bacillus subtilis, by Muller Hinton Agar (MHA) plates. Sterile paper disc of 10 mm diameter containing Copper nanoparticles and standard antibiotic chloramphenicol (100 µg/ml) containing discs were placed on each plate as control. The plates were incubated at 35°C overnight, and the inhibition zones around the discs were measured.

About Datura metal plant

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Scientific classification	
Kingdom:	Plantae
(unranked):	Angiosperms
(unranked):	Eudicots
(unranked):	Asterids
Order:	Solanales
Family:	Solanaceae
Genus:	Datura
Species:	D. metel
Binomial name	
Datura metel	
L.	

History and Traditions & Folklore

Datura has been employed as both a medicinal and ceremonial plant in many diverse cultures including Chinese, Zuni Indian, Mexican and Native Americans of the Southwest. Recorded use can be traced back to early Sanskrit, Chinese and Arabian writings. The noted eleventh century Arabian physician mentioned the herb as "metel nut", and the Greek Dioscordies wrote of it as well. The name datura was adapted to Latin by Linnaeus from the Sanskrit Dhatura. Datura was used as a surgical anesthesia by the Chinese, who imported the plant from India between A.D. 960 and 1644. In the Americas datura has played a major role in religious rites and medicine and is detailed in the earliest herbal of the New World, the Codex Berberina Latina, circa 1542[27].

Also known as angel's trumpet or devil's trumpet, datura metel is an medicinal plant whose use dates back as far as 3000 years. Today, it is mainly used in traditional Chinese medicine as a treatment for asthma, chronic bronchitis, chronic pain, seizures, and coma. This plant has also been used for its anesthetic, or pain-killing, properties. Datura metel is known as an anticholinergic, meaning it reduces spasms by blocking the transmission of nerve impulses. Additionally, it is a well-known plant with deliriant properties, or capable of causing hallucinations or delirium. It has been the choice of many cultures over the years for astral or hamanistic journeys, or as a rite of passage.

Its ability to control spasms has led to datura metel being used frequently in Chinese herbology to treat the wheezing of asthma, and in Vietnam, it is added to asthmatic cigarettes. Great care must be taken when using this herb, as the toxic dose is very close to the medicinal dose. The wrong dosage can induce hallucinations, severe intoxication, and even death. Use of this plant should be closely monitored by an experienced practitioner trained in herbology.

The alkanoids contained within the plant is what makes it effective as a anesthetic. Alkanoids are chemical compounds produced by a large number of organisms, and many have pharmaceutical effects. The alkanoids in datura are hycosamine, hycosine, and atropine.

The region of origin is somewhat of a mystery, but today datura metel is cultivated world-wide for its medicinal purposes, as well as for the pretty flowers. It grows to a height of approximately 3 feet (0.914 m), and does not do well in the shade. It prefers sandy, loamy, and well-drained soil. It flowers from June to July.

Datura metel is unregulated and uncontrolled by the federal government in the United States, although some states do have laws that make possessing or using it illegal. Federal law does not approve of human consumption. Indeed, there are many dangers associated with the recreational use

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of datura metel, including death, and it should be used for medicinal purposes only, and under the care and supervision of a healthcare provider. In the past, dautra was considered a sacred herb and was used in religious ceremonies, initiation rituals, and even in ritual sacrifices. Its medicinal properties were well-known, and was used in poultices, plasters, and ointments. Today, its medicinal effects can still be a valuable part of the medical field.

TRADITIONAL USES: Datura metel was first documented in Sanskrit literature. Somewhat later, the Arabic physician Avicenna touted the importance of its medicinal applications and provided the exact appropriate dosage to the Arabs, who categorized the plant as a narcotic. Ingesting too much Datura metel is very dangerous and can lead to insanity or even death, so great care must be taken with its consumption.

Indian Thorn Apple flowers are often depicted in Hindu Tantric art, usually in connection with incarnations of Shiva. The thorn apple also appears in ancient Tibetan and Mongolian texts, which demonstrates that Datura metel was indigenous to Asia prior to the fifteenth century. It is not known when the Indian Thorn Apple was introduced to Africa. Today, Datura metel remains a psychoactive plant of great enthnopharmacological significance, especially in India, Southeast Asia, and Africa.

According to the Vamana Purana, the thorn apple grew from the chest of the Hindu god Shiva, the lord of inebriants. In the Garuda Purana, it is said that Datura flowers were offered to the god Yogashwara (a.k.a. Shiva), on the thirteenth day of the waxing moon in January. In Nepal the plant is considered sacred to Shiva. Thorn apple flowers and fruits are among the most important offering gifts of the Newari tribe of Nepal. At every puja, (offering service or ceremony), Shiva is offered Datura fruits in order to gain his favor.

In Varanasi, Shiva's sacred city, D. metel fruits and rose flowers are made into sacrificial ceremonial garlands for the lord of inebriation and sold to pilgrims, then left as offering at the entryways to his temples. These Datura chains are devoutly placed around the lingam, the deity's phallic-shaped image, as fresh flowers are tossed over the top of it[28].

In northern India, it is widely known that Datura metel can be used for inebriating purposes. Smoking the plant is regarded as pleasurable and not dangerous, whereas eating or drinking it is considered dangerous and is generally avoided. Yogis and sadhus in particular smoke thorn apple seeds and leaves together with Cannabis indica and other herbs such as Aconitum ferox and Nicotiana tabacum [28].

In Tibet and Mongolia, the thorn apple is used as incense in Vajramabhairava Tantra rituals intended to make the wealthy poor and to drive out certain spirits and energies.The fruits or seeds are also used to induce insanity[29].

In the Philippines, the Ingorot, a Malayan tribe from Luzon, boil the leaves to make an inebriating soup that is eaten communally in a ritual circle. In China, the white blossomed variation of Datura metel, alba, is considered sacred, as it is believed that glistening dew drops rained down from the heavens onto its flowers while the Buddha was giving a sermon. In ancient China, it appears that it was a popular practice to steep the aromatic flowers of D. metel in wine before consumption. Stories say that if someone laughs while the flowers are being packed for use with wine, the wine evokes laughter in all those who drank it. If the flowers are picked while someone dances, all those who drink cannot help but dance[28].

In Africa, Datura metel is used for criminal activities and in initiations. The seeds are used to poison victims so that they can be robbed. Seeds are added to the locally brewed beer to potentiate its

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effects[28].

In Tsongaland, which stretches from Mozambique to the Transvaal, a variant of Datura metel known as fastuosa is utilized as an entheogenic ritual drug in the initiation of girls as they pass into womanhood. The girls are painted with red ocher (a symbol of menstrual blood). One after the other, they are made to lie down in the fetal position on a mat made from palm fronds while others dance around them holding onto their hips. Special songs are sung. Afterwards, the girls are tied to a tree while others beat the tree with sticks until the white sap – which symbolizes sperm – starts to flow from its bark [30].

The next stage is a water ritual, through which the initiates are cleansed, as a symbol of casting aside childhood. Before ingesting the thorn apple, the girls are required to stretch an animal skin over a vessel of water. Older women perforate the skin with sticks and stir the water. Following this symbolic defloration, a "school mother" covered entirely in Datura leaves, toad skins, and dog teeth bursts out from behind the bushes. She approaches the girls, spits on them, and tells them repeatedly that they will soon hear the voice of the fertility god[30].

The thorn apple drink, made by boiling the herbage in water and rumored to contain powdered human bones and/or human fat, is then carried around in a ceremonial seashell by the school mother and given to each girl to drink from. They experience visions that are shaped and influenced by ritual music and the singing of the school mother. The path into womanhood is channeled through the ceremonial phase by the shaving off of the pubic hair pre-initiation, and by the placement of clay cubes with pieces of straw protruding from them in between each girl's legs. These symbolize the fact that when their pubic hair grows back in, it will belong to a woman, not a girl. At the end of the initiation, the girls are freed from their ceremonial restraints and coverings, dressed in new clothes adorned with ornaments, and they dance and sing, now ready for marriage. The related species Datura wrightii is used to pass boys into manhood[30].

MEDICINAL USES: There is evidence that Datura metel seeds have been used in ancient Indian medicine, modern Indian folk medicine, and Ayurvedic medical practices. The most common medicinal uses for Datura in these systems are for skin conditions, anxiety disorders, and respiratory ailments, along with a litany of other conditions. The seeds are also sometimes used as a substitute for opium [30].

In Java the seeds are inserted into cavities or chewed to relieve dental pain. The plant is also used to treat skin diseases, colds, and anxiety in TCM. The plant is used to treat asthma in all regions of the world, either as a smoke or an incense.

Medicinal Uses: Chinese

Properties: Analgesic, Anodyne, AntiCancer, Antirheumatic, Demulcent, Poison, Psychedelic

Parts Used: Seeds, leaves, flowers

Constituents: alkaloids, atropine, ahyoscyamine, scopolamine, ascorbic-acid, allantoin

Datura Side Effects: Seeds are extremely toxic, the leaves less so,the whole plant contains powerful alkaloids. This is not a plant to be taken lightly. Can be fatal and cause permanent mental imbalances if abused.

3. Result & Discussion

3.1 Characterization of copper nanoparticles

The most common technique for characterization of metal nanoparticle is Uv -Vis spectroscopy,

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which is used for analysis of intensely coloured colloidal dispersions having surface Plasmon absorption. Certain metal colloids like Au, Ag and Cu, exhibit strong absorption bands in the visible region and are therefore intensely coloured. The plasmon bandwidth increases with decreasing size in the intrinsic size region (mean diameter smaller than 25 nm) and also increases with increasing size in the extrinsic

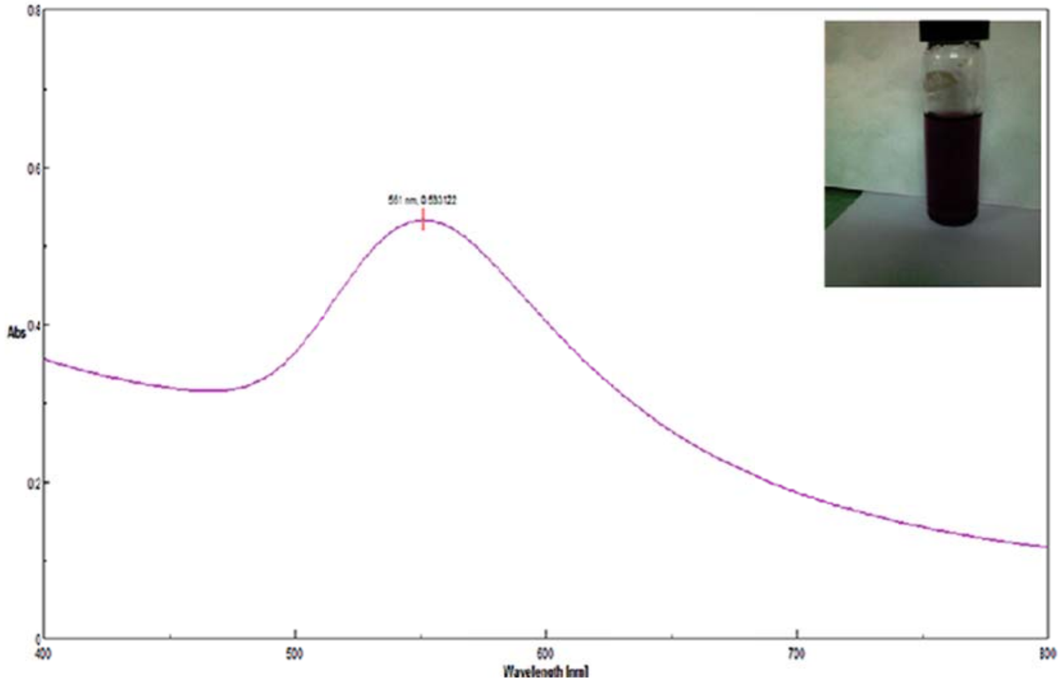

Fig. 1 Absorption spectra of the copper nanoparticles.

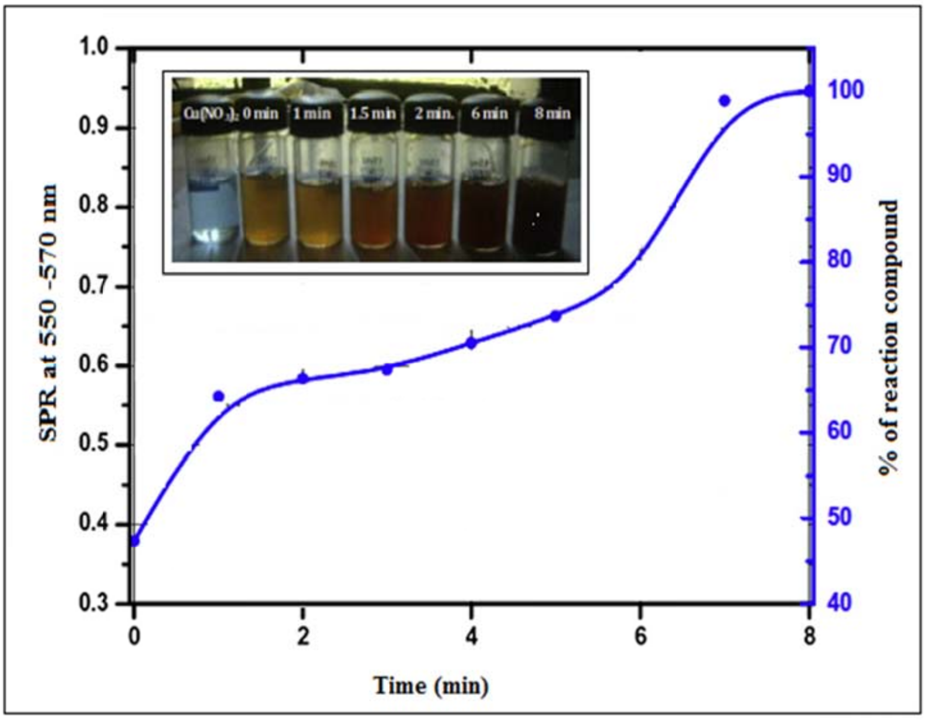

Fig. 2 SPR change of the reaction mixture as a function of time. Inset Figure shows the color of the reaction mixture.

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size region (mean diameter larger than 25 nm)[31]. The surface plasmon resonance shows a red- shift in the extrinsic size region and as the particle size increases, the copper nanoparticles colour changes from blue to yellow and finally purple-black. The characteristic surface plasmon band for 15-20 nm gold nanoparticles at 551 nm peak wavelength was observed (Fig.1) in the UV-Visible spectrum, confirming the presence of metal nanoparticles and inset of figure shows its purple black colour.

The intensity of SPR increased with irradiation time as shown in (Fig. 2). The % conversion of Cu2+ to Cu0 was calculated based on the absorption value. The inset in Fig.2 shows the corresponding colors of the mixtures. It is clear that 100% reduction occurred in about 8 min. The formation of CuNP is rapid especially in the beginning (>50% in 2 min) followed by a slow kinetics and finally reaching a plateau region.

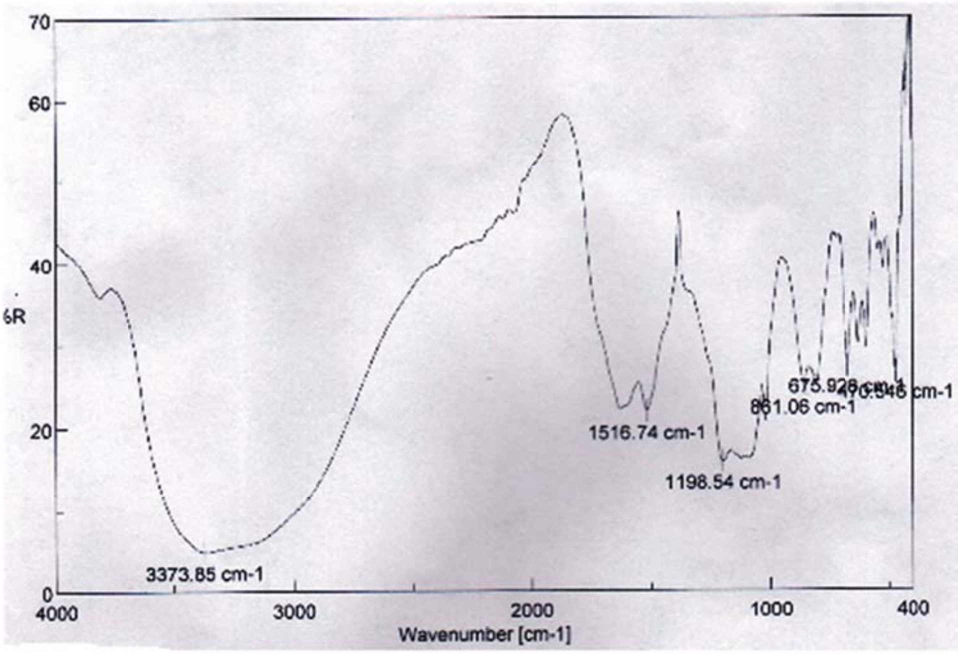
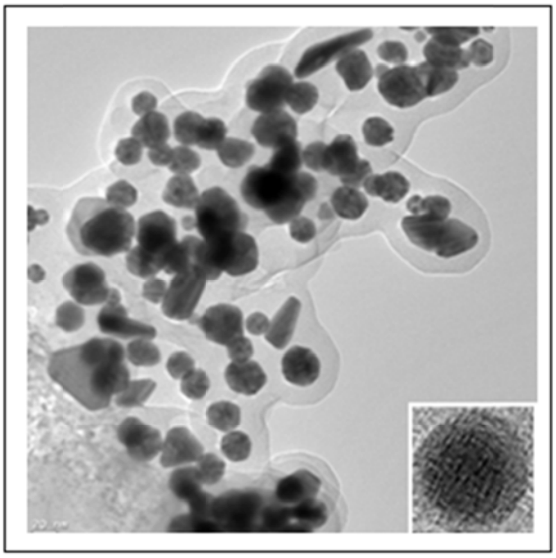


Fig. 3 FTIR spectra of CuNps



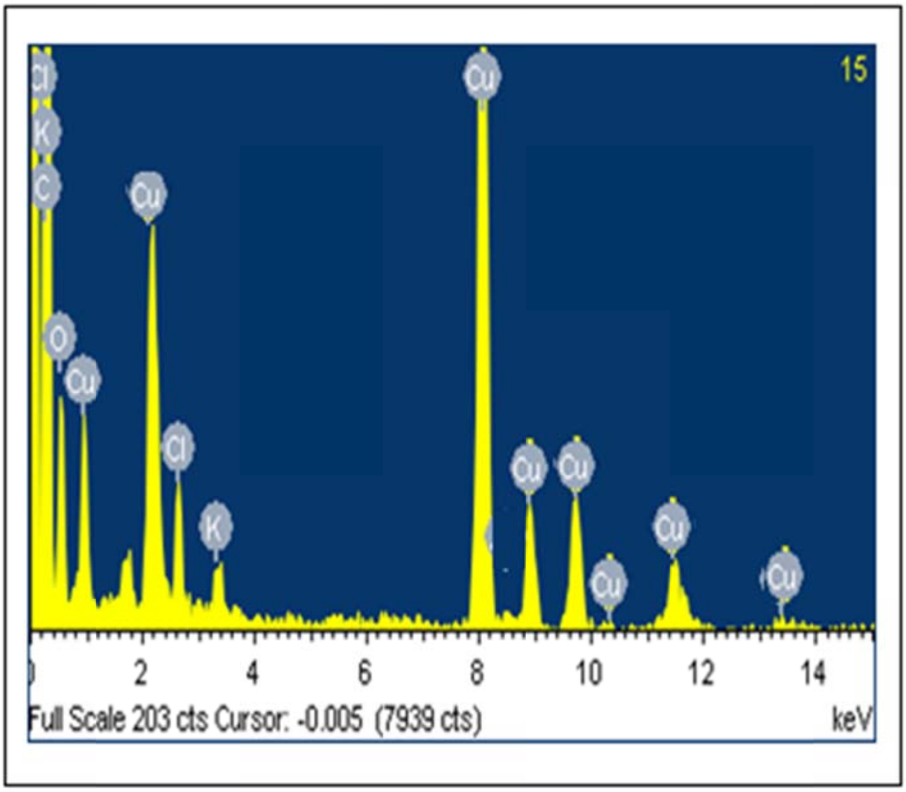


Fig.4 Transmission electron micrographs (TEM) & Energy –Dispersive X-ray spectra EDX spectrum of copper nanoparticles.

The FTIR spectrum of Cu nanoparticles is shown in (Fig.3). The IR spectrum of Cu nanoparticles shows band at 3373 cm-1, 1635 cm-1, 1516 cm-1, 1376 cm-1 , 1198 cm-1 corresponds to O-H Stretching H-bonded alcohols and phenols, carbonyl stretching, N-H bend primary amines, corresponds to C-N stretching of the aromatic amino group and C-O stretching alcohols, ethers respectively. FTIR spectrum of Cu nanoparticles suggested that Cu nanoparticles were surrounded by different organic molecules such as terpenoids, alcohols, ketones, aldehydes and carboxylic acid.

The size and shape of green synthesised copper nanoparticles were analysed using TEM, Particles are spherical and particle size is in the range of 15–20 nm (Fig.4). Particles are well dispersed, which was confirmed by TEM studies. This is very similar to previous literature [17]. Energy dispersive X-ray analysis (EDAX) The shape and size of particles were determined recording Transmission Electron Microscopy (TEM) image of CuNps are shown in Fig.4 which display a morphology of particles. We can see the various shape of particle like spherical, hexagonal, roads etc with an average size of 20 nm. The presence of a band in EDX spectrum at 3 KeV is conformation of composition of >99 % of Cu metal atom[32].

Fig.4 of green synthesized copper nanoparticles, which denotes that the strong signal in the copper and confirmed the formation of nanoparticles. Hence, it is worth studying the antibacterial behaviour of synthesised CuNps of 15 nm particle size and with +21 mV zeta potential value (Fig. 5-6). The zeta potential is related to the surface charge density and high magnitude of zeta potential denotes stability of the nanoparticles in suspension. For copper nanoparticles, the positive zeta potential results from the presence of protonated amine groups are present in alkaloid,steroid etc.

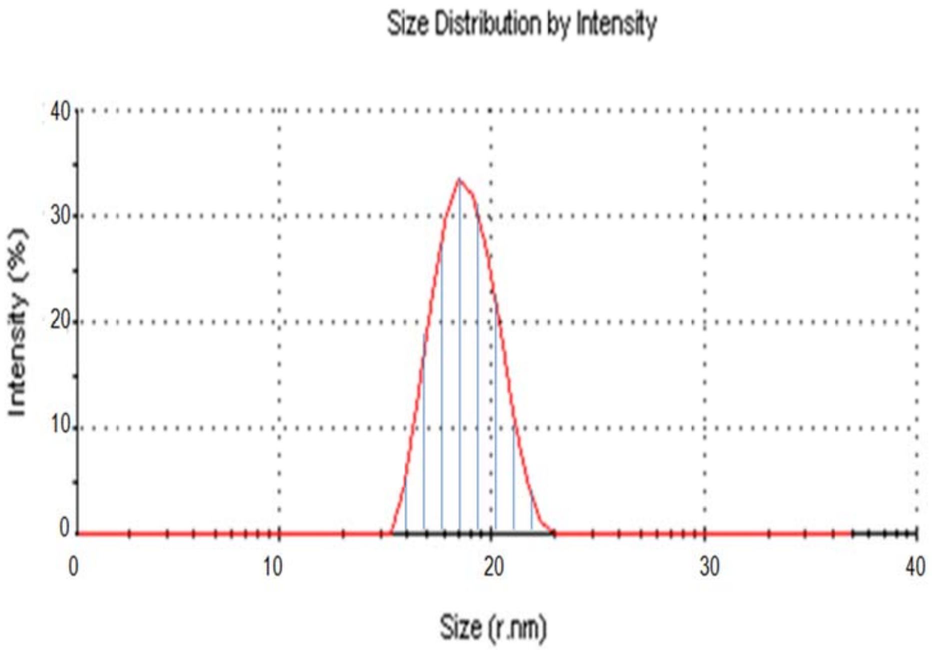


Fig.5 Size distribution of Copper nanoparticles by particles size analyzer

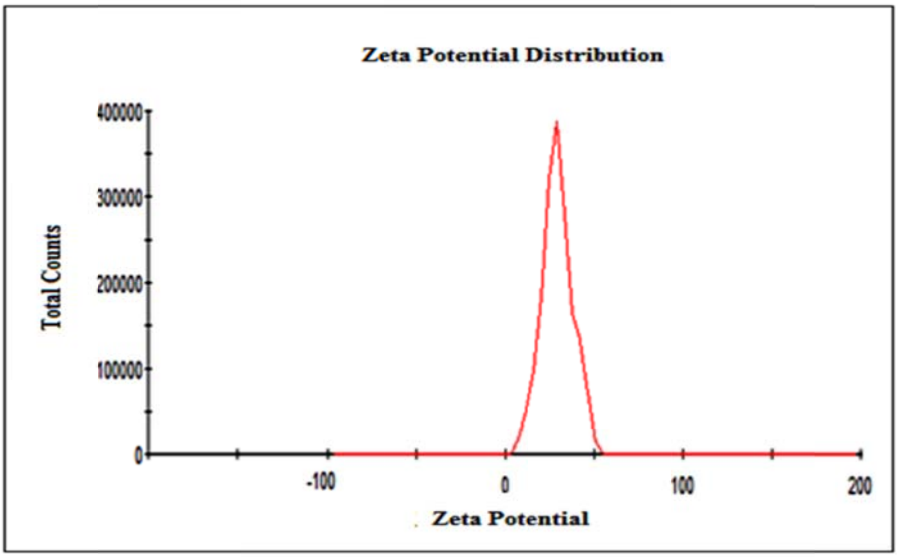


Fig.6 Zeta potential of Copper nanoparticles by zetz size analyzer

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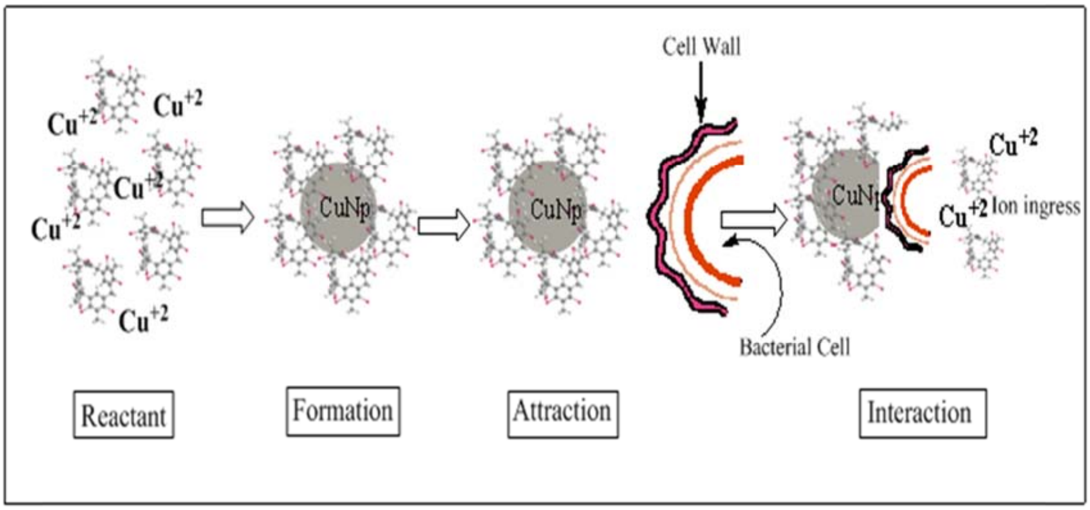

Fig.7 Mechanism action of Copper on the microbes

Although the exact mechanism of action of Copper on the microbes is still not known but the suggested possible mechanism of action of metallic Copper, Copper ions and Copper nanoparticles is according to their morphological and structural changes found in the bacterial cells. Some studies have reported that the positive charge of Copper ions is crucial for its antimicrobial activity through the electrostatic attraction between negative charge cell membrane of microorganisms and positive charge nanoparticles [26, 33]. The nanoparticles get attached to cell membrane and also penetrate inside the bacteria. The bacterial membrane contains sulfur-containing proteins, and the Copper nanoparticles interact with these proteins in the cell as well as with the phosphorus containing compounds like DNA. When Copper nanoparticles enter the bacterial cell, they form a low molecular weight region in the centre of the bacteria to which the bacteria conglomerate thus, protecting the DNA from the Copper ions (Fig.7). The nanoparticles preferably attack the respiratory chain and inhibit cell division finally leading to cell death. The nanoparticles release Copper ions in the bacterial cells, which enhance their bactericidal activity [34, 35].

3.2 The antimicrobial effect of CuNps

CuNps on bacteria such as Staphylococcus aureus, Escherichia coli, Bacillus megaterium and Bacillus subtilis were compared with standard antibiotics such as chloramphenicol. Zone of inhibition is the only criterion which has been used to compare the antimicrobial activity. On this basis, it was observed that the antimicrobial activity of CuNps is generally more than the plant extract of datura metal except with s. aureus. This observation has led us to conclude that antimicrobial activity of not been compromised with the formation of CuNps rather it has been enhanced marginally. Although the antimicrobial activity of CuNps is slightly less than that of standard chloramphenicol therefore, it is reasonable to propose that CPH-AgNps hold the potential of their use as a good antibacterial agent. (Table 1)

Table 1 Antimicrobial activity (Zone of inhibition in mm) of only plant extract and CuNps

Name of compound	**Zone of inhibition (mm)**							
	E.coli		*B.subtilis*		*S.aureus*		*B.megaterium*	
	50 ppm	100 ppm	50 ppm	100 ppm	50 ppm	100 ppm	50 ppm	100 ppm
A*	9	8	10	10	9	8	9	7
Datura plant Extract	6	4	7	4	8	8	6	5
CuNps	7	8	9	8	6	5	8	7

A* = Chloramphinicol (Antibiotic control)

4. Conclusions

In conclusion, an efficient, eco-friendly and simple method has been developed for the preparation of CuNps, where Datura plant extract acted as both reducing as well as stabilizing agent. Nanoparticles were found to be stable at room temperature, It was also found that CuNps were with 5 nm size and +21 mV zeta potential, exhibited reasonably good antimicrobial activity, when compared with standard Chloramphenicol, through the electrostatic attraction between positively charged nanoparticles and negatively charged cell membrane of microorganisms. This suggests their potential use as an antimicrobial agent. This method have merits over other reported methods are easily available starting materials, inexpensive and procedure is easy to carry out any laboratory, use of toxic reagent is avoided and pollution free.

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