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Biosynthesis and Characterization of Copper Nanoparticles from Tulasi (*Ocimum sanctum* L.) Leaves

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A B S T R A C T

Nanotechnology is mainly concerned with synthesis of nanoparticles of variable sizes, shapes, chemical compositions and controlled dispersity with their potential use for human benefits. The subject nanotechnology deals with manufacturing, study and manipulation of matter at nano scale in the size range of 1-100 nm which may be called as nanoparticles. Development of green nanotechnology is creating interest of researchers towards eco-friendly biosynthesis of nanoparticles. Biomolecules present in plant extracts can be used to reduce metal ions into nanoparticles in a single-step green synthesis process. Tulasi (*Ocimum sanctum* L.) is an aromatic plant belongs to family *Lamiaceae*. Tulasi is a traditional medicinal plant of India, having good source of bio-reduction and stabilizers. The constituent of tulasi are alkaloids, glycosides, tannins, saponins and aromatic compounds and also it contains minerals like Ca, Mn, Cu, Zn, P, K, Na, and Mg where the concentration of Cu is more in tulasi leaves than other leaves. It constitutes 12.31 mg/kg of Cu. Recently *Ocimum sanctum* L. leaf extracts have been used in the synthesis of silver nanoparticles and gold nanoparticles. Tulasi is a source of bio-reduction and stabilizers. The copper is highly toxic to microorganisms such as bacteria. copper nanoparticles were synthesized from various plant extracts such as *Hibicus rosasinensi*, *Ocimum santanum* leaf extract, *Syzygium aromaticum* (Cloves), Lemon fruit extract, *Vitis vinifira* extract, *Eucalyptus*, *Cassia alata*, *Centellaasiatica*, *Malva sylvestris* etc. Various instrumental techniques were adopted to characterize the synthesized Cu NPs, viz., Dynamic light scattering analyzer (Zetasizer), UV–Vis spectroscopy, FTIR, SEM, TEM and XRD.

Introduction

The term nanotechnology, buzzword of present day science owes its origin from the Greek word ‘nano’ literally meaning ‘dwarf’. When it is expressed in terms of dimension one nanometer equals to one billionth of a meter ($1nm=10^{-9}m$). The subject nanotechnology deals with manufacturing, study and manipulation of matter at nano scale in the size range of 1-100 nm which may be called as nanoparticles (Rajan, 2004). Nanotechnology represents the design, production and application of materials at atomic, molecular and macromolecular scales in order to produce new nanosized materials

(Hahens *et al.,* 2007). Nantechnology is mainly concerned with synthesis of nanoparticles of variable sizes, shapes, chemical compositions and controlled dispersity with their potential use for human benifits (Elumalai *et al.,* 2010).

Another way that nanotechnology can be defined is by differentiating between the production processes of 'top-down' and 'bottom-up'. Top-down refers to the fabrication of nanostructures by miniaturising present methods, such as machining and etching techniques. The other approach is bottom-up, sometimes labelled as molecular nanotechnology, whereby nano-sized objects are constructed from smaller units, even down to the manipulation of individual atoms (Albrecht *et al.,* 2006).

The increased surfaces of nanoparticles are responsible for their different chemical, optical, mechanical, magnetic properties as compared to bulk materials (Mazur, 2004). Physical and chemical methods of synthesis of nanoparticles (NPs) are expensive, time consuming, labour intensive and also requires more energy. These methods are potentially hazardous to the environment and living organisms due to use of toxic reducing and stabilizing agents (Mittal *et al.,* 2013).

Therefore, there is a need to develop cost effective, non-toxic and eco-friendly method for synthesis of nanoparticles. Biological methods of synthesis would help to remove harsh processing conditions by enabling the synthesis at physiological pH, temperature, pressure, and at the same time at lower cost. Large number of micro-organisms have been found to be capable of synthesizing inorganic nanoparticles composite either intra or extracellularly (Vithiya and Sen, 2011).

Development of green nanotechnology is creating interest of researchers towards eco-friendly biosynthesis of nanoparticles. Biomolecules present in plant extracts can be used to reduce metal ions into nanoparticles in a single-step green synthesis process. This biogenic reduction of metal ion is quite rapid, readily conducted at room temperature and pressure and easily scaled up (Parikh *et al.,* 2014). It is cost effective and main advantage is eco-friendly compared to other methods like Laser ablation, arc discharge etc., (Gopinath *et al.,* 2014). These are some of the leaves *viz.*, neem (*Azadirachta indica*), sajna (*Moringa oleifera*), arjun (*Terminalia arjuna*), tulsi (*Ocimum sanctum*), turmeric (*Curcuma longa*); rhizomes of ginger (*Zingiber officinale*) and turmeric; fruits of amla (*Emblica officinalis*), haritaki (*Terminalia chebula*), bohera (*Terminalia belerica)* and bulbs of garlic (*Allium sativum*) which contains minerals like Cu, P, Mg, K Na, P, Zn and Mn (Bhowmil *et al.,* 2008).

Tulasi (*Ocimum sanctum*) is an aromatic plant belongs to family *Lamiaceae* (Kashif and Ullah, 2013). Tulasi is a traditional medicinal plant of India, having good source of bio-reduction and stabilizers. The constituent of tulasi are alkaloids, glycosides, tannins, saponins and aromatic compounds and also it contains minerals like Ca, Mn, Cu, Zn, P, K, Na, and Mg where the concentration of Cu is more in tulasi leaves than other leaves. It constitutes 12.31 mg/kg of Cu (Bhowmil *et al.,* 2008). Recently *Ocimum sanctum* leaf extracts have been used in the synthesis of silver nanoparticles and gold nanoparticles. Tulasi is a source of bio-reduction and stabilizers (Vennila and Nithya, 2016). The copper is highly toxic to microorganisms such as bacteria. copper nanoparticles were synthesized from various plant extracts such as *Hibicus rosasinensi, Ocimum santanum* leaf extract, *Syzygium aromaticum* (Cloves), Lemon fruit extract, *Vitis vinifira* extract, *Eucalyptus, Cassia alata, Centellaasiatica, Malva sylvestris* etc. (Hariprasad *et al.,* 2016).

Various instrumental techniques were adopted to characterize the synthesized Cu NPs, viz., Dynamic light scattering analyzer (Zetasizer), UV–Vis spectroscopy, FTIR, SEM, TEM and XRD. Synthesis of colloidal Cu NPs was monitored by using UV-Visible spectroscopy (Joseph *et al.*, 2016). Nanoparticles are generally characterized by their size, morphology and surface charge by using advanced microscopic techniques such as scanning electron microscopy (SEM), transmission electron microscopy (TEM) and atomic force microscopy (AFM). The average particle diameter, their size distribution and the charge they carry affect the physical stability and *in-vivo* distribution of nanoparticles. Electron microscopy techniques are very useful in ascertaining the overall shape of polymeric nanoparticles, which may determine their toxicity. The surface charge of nanoparticles affects the physical stability and redispersibility of the polymer dispersion as well as their in-vivo performance (Pal *et al.*, 2011). Nanoparticles can serve as "magic bullets" containing herbicidal, nano-pesticidal and as fertilizers or genes, effect which target specific cellular organelles in plant and release their contents (Genady *et al.*, 2016).

Materials and Methods

The biosynthesis of copper nanoparticles from tulasi (*Ocimum sanctum* L.) leaves was carried out as described below.

Preparation of plant extract

The leaves were cleaned and washed thoroughly with distilled water and subsequently dried in solar tunnel dryer at 40 ˚C for 2 days to remove moisture completely. Dried leaves were ground to make into a fine powder. The obtained powder was passed through a 20 mesh sieve (840 µm) to get uniform size. 10 g of uniformly sized powder was taken in a beaker along with 100 ml of deionised water and it is allowed to boil at 60° C for 30 min. under reflux condition and cooled down to room temperature. The prepared solution was double filtered through Whatman No.1 filter paper there by powdered leafy materials were filtered out and clear solution was obtained. The filtrate was stored at 4° C for further experiments (Mekal *et al.*, 2016).

Biosynthesis of copper nanoparticles

The plant extract of tulasi leaves (25 ml) was mixed with 100 ml of 1mM aqueous copper sulphate pentahydrate ($CuSO_4.5H_2O$) solution under continuous string. After complete mixing of leaf extract with precursor the mixture was kept for incubation at 31˚ C for 24 h. A change in the colour from light green to dark green was observed and this indicated the formation of copper nanoparticles. The solution was then centrifuged at 6000 rpm for 30 min. followed by re-dispersion of the pellet in deionised water to remove any unwanted biological materials (Mekal *et al.*, 2016). The details are presented in Figure 1.

Characterization of biosynthesized copper nanoparticles

Synthesized copper nanoparticles were subjected to various characterization studies for identification of size and morphology.

Size analysis using zetasizer

Zetasizer was used (dynamic light scattering) to study the average particle diameter (nm) of biosynthesized copper nanoparticles. 1 ml copper nanoparticles were mixed in 1 ml of distilled water. The suspension of Cu NPs was sonicated at 25 °C using the digital ultrasonication bath for 15 min. After sonication, the sample was centrifuged using

high speed centrifuge at 10000 rpm for 10 min. The prepared sample of Cu NPs suspension filled in disposable cuvette upto ¾th of volume and cuvette was placed in dynamic light scattering chamber. During the analysis, settings were made in Malvern software as given in Table 1.

The average particle diameter (nm) was recorded for all the three samples from size distribution by intensity graph (Das *et al.,* 2014).

Absorbance analysis using UV-Visible spectrophotometer

UV-Visible spectrophotometer measures the extinction (scatter + absorption) of light passing through a sample. Nanoparticles have unique optical properties that are sensitive to the size, shape, concentration, agglomeration state and refractive index near the nanoparticles surface, which makes UV-Visible for identifying, characterizing and studying the nanoparticles.

Biosynthesized copper nanoparticles were characterized by using UV- Visible spectrophotometer. The sample was prepared by diluting of 1 ml of Cu NPs into 2 ml distilled water and measuring the UV-Visible spectrum of solutions. The absorbance of the sample recorded in wavelength ranged between 400-600 nm (Mekal *et al.,* 2016).

Surface morphology analysis using scanning electron microscope (SEM)

The scanning electron microscope (SEM) image of the test sample surface is obtained by scanning it with a high energy beam of electrons in vacuum chamber. When the beam of electrons strikes the surface of the specimen and interacts with atoms of sample, signals in the form of secondary electrons and back scattered electrons are generated that contain information about sample's surface morphology.

The morphological features of copper nanoparticles were studied by using SEM. The aluminum stub (~1 cm dia.) was employed on sample holder and cleaned to remove surface oils or dirt by using acetone and blowing with compressed gas. The double coated conductive carbon tape was used as adhesives and pasted on stub. Thin layer of dried sample (~0.2 ml) placed on adhesive surface, then it was coated with palladium to make the samples conductive using sputter coater for about 90 s. Sample holder was removed from the sputter coater and placed in vacuum chamber of SEM and magnification was (1 to 30,000 times) carried out to get clear morphology of copper nanoparticles at the accelerating voltage of 1 to 20 kV with working distance of the sample at 10 mm (Joseph *et al.,* 2016).

Phase identification using X-ray diffraction (XRD)

X-ray diffraction (XRD) is a rapid analytical technique primarily used for phase identification of a crystalline material present in copper nanoparticles.

Powder diffraction pattern of copper nanoparticles was recorded in the high angle of 2 theta range (0°-80°). Copper nanoparticles (~1 ml) were placed uniformly spread on glass sample holder and placed in scanner chamber. The set scan speed and step size 0.3 °/min and 0.001 s, respectively were fixed (Djangang *et al.,* 2015). The XRD pattern was recorded to phase identification of copper nanoparticles.

Statistical analysis

Completely randomized design was used to analyse the data. After proper analysis, data

were accommodated in the tables as per the needs of objectives for interpretation of results. The Microsoft Excel was used for analysis and interpretation. The statistical procedures for agricultural research given by Gomez and Gomez (1976) were referred.

Results and Discussion

Biosynthesis of copper nanoparticles from tulasi (*Ocimum sanctum* L.) leaves

The biosynthesis of copper nanoparticles was carried out using copper sulphate pentahydrate ($CuSO_4.5H_2O$) solution and tulasi leaf extract.

The reaction of nanoparticles synthesis started after the tulasi leaf extract was added into 1mM aqueous copper sulphate pentahydrate ($CuSO_4.5H_2O$) solution. After 24 h of incubation, the colour of the mixture turned into dark green from light green which indicated the formation of copper nanoparicles.

The colour change was due to active molecules present in tulasi leaf extract which acted as a reducing and capping agent. The tulasi leaf extract reduced the copper metal ions into copper nanoparticles. The average size of biosynthesized copper nanoparticles obtained from 1 mM aqueous ($CuSO_4.5H_2O$) solution was found to be 37.61 nm.

The biosynthesis of copper nanoparticles was carried out using tulasi leaf extract and $CuSO_4.5H_2O$. The nanoparticles synthesis reaction would initiates by addition of tulasi leaf extract in 100 ml of 1 mM aqueous $CuSO_4.5H_2O$ solution. After 24 h of incubation, the colour of light green mixture was turned into dark green, which indicates the formation of copper nanoparticles. The colour change was due to active molecules present in the extract which reduced the $CuSO_4.5H_2O$ metal ions into copper nanoparticles.

According to Mekal *et al.,* (2016) the synthesized copper nanoparticles were confirmed by the change of colour after addition of tulasi leaf extract into the copper sulphate solution. The leaf extract acts as both reducing and capping agent.

Characterization of biosynthesized copper nanoaprticles

The characterization of copper nanoparticles *for identification of its size and morphology are given as below,*

Dynamic light scattering (Zetasizer) analysis

The characterization of copper nanoparticles in terms of average particle diameter was recorded in nm from the intensity distribution analysis by using Zetasizer and shown in Table 2. It revealed that three biosynthesized samples with average particles diameter were 37.61 nm.

The average particle diameter 37.61 nm of copper nanoparticles was used for the further characterization and application.

The results of Zetasizer revealed that the average size of biosynthesized copper nanparticles was found to be 37.61 d.nm as shown in Figure 1. This is in agreement with previous findings which suggested that, as the reaction temperature increases, both synthesis rate and conversion of copper nanoparticles increased.

The average particle size decreased from 110 nm at 25 ˚C to 45 nm at 95 ˚C (Lee *et al.,* 2011). The variation in particle size was probably due to change in climatic conditions during biosynthesis (Zainala *et al.,* 2013).

Fig.1 Process flow chart for biosynthesis of copper nanoparticles from tulasi (*Ocimum sanctum* L.) leaves

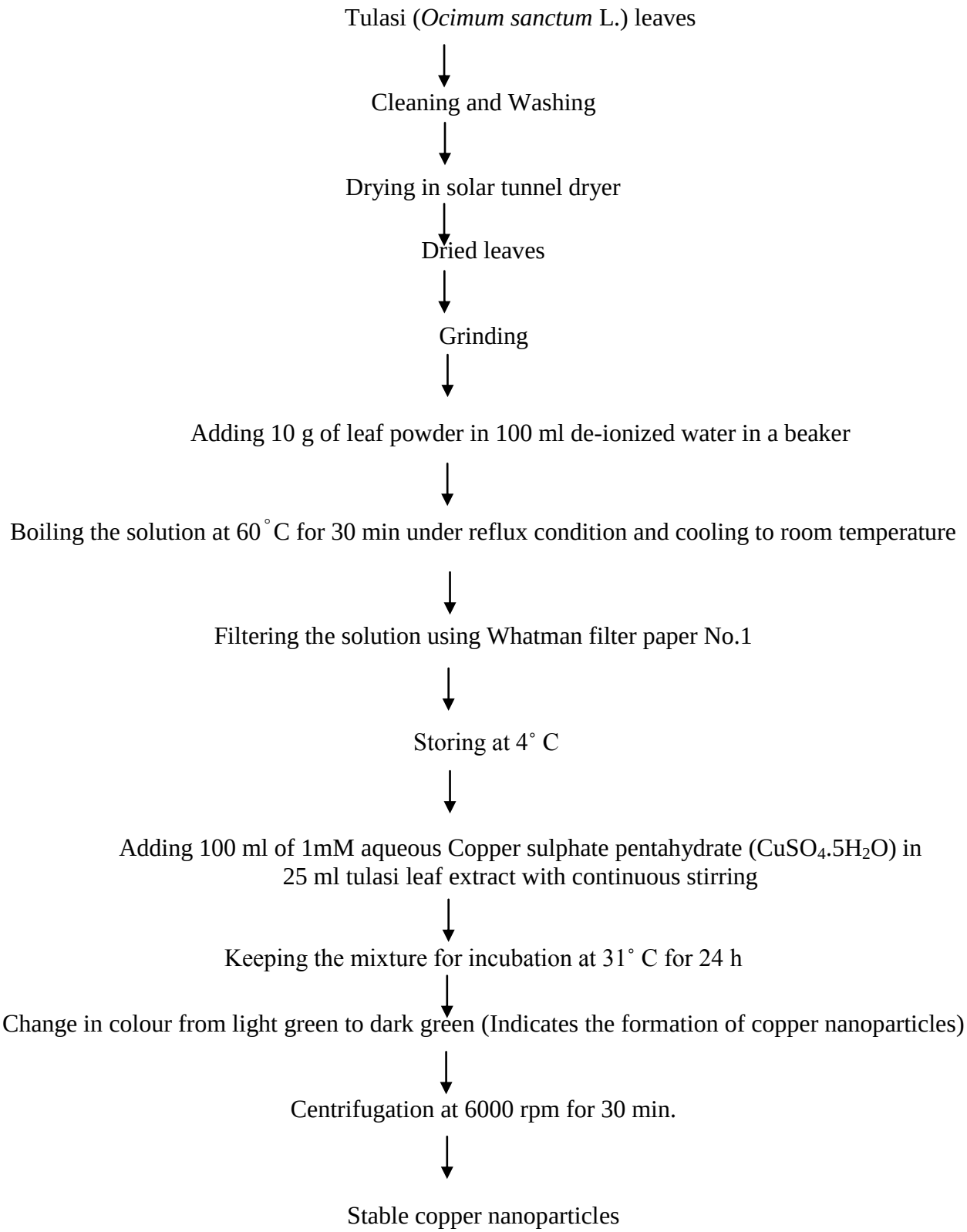

Fig.1 Z-average (d.nm) of copper nanoparticles (Cu NPs)

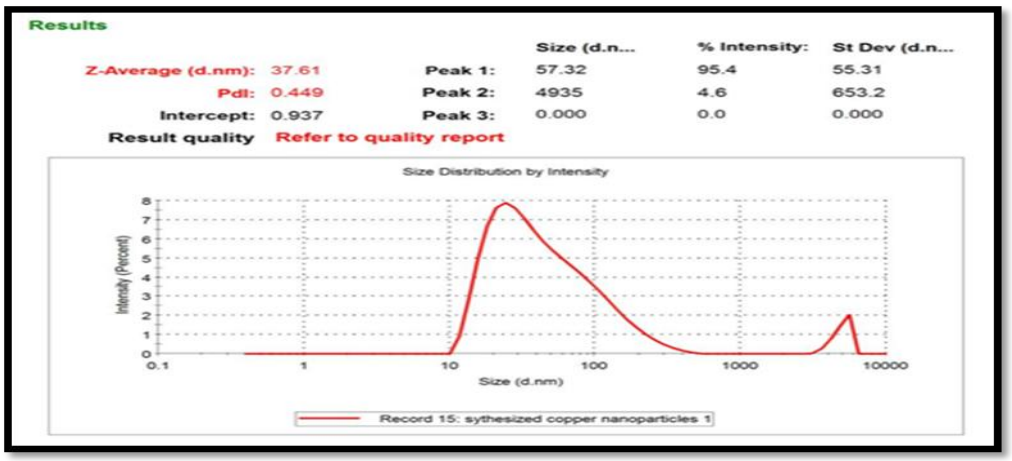

Fig.2 Absorbance value of copper nanoparticles (Cu NPs)

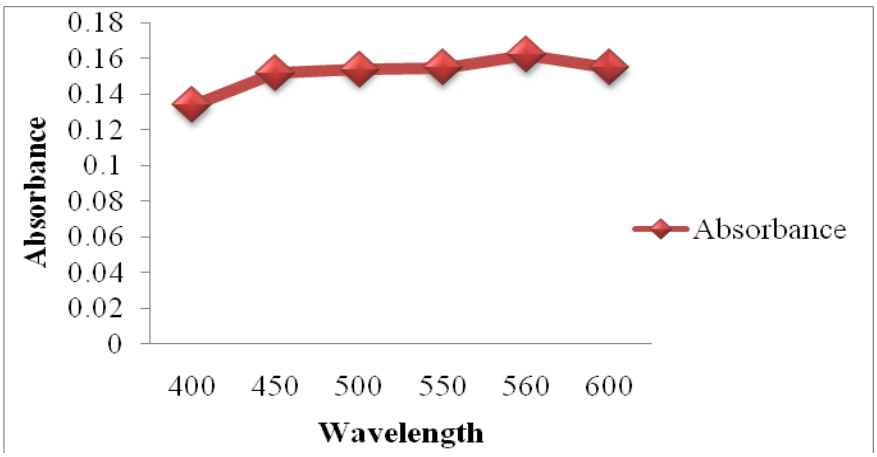

Fig.3 SEM image of copper nanoparticles (Cu NPs)

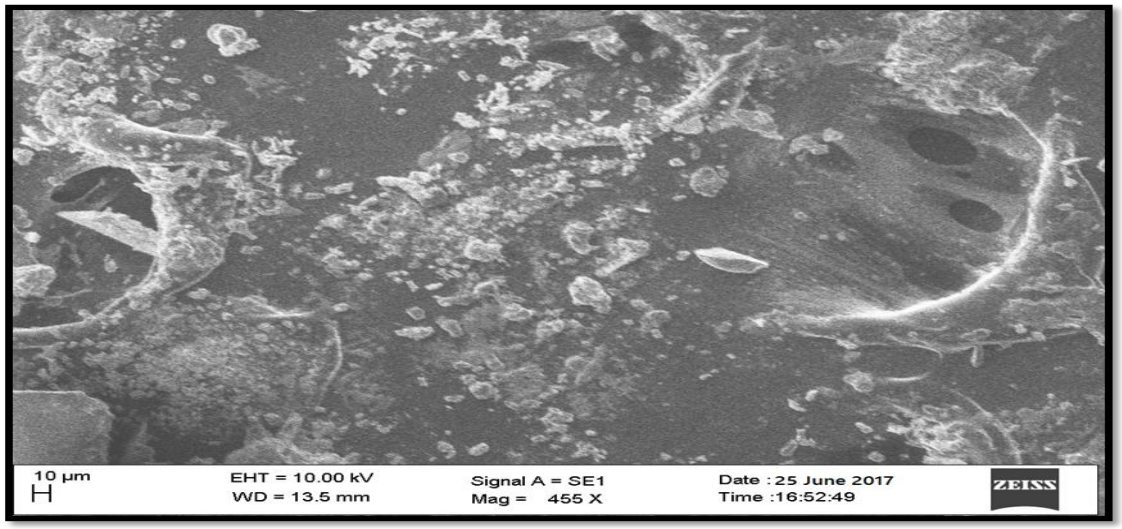

Fig.4 XRD pattern of copper nanoparticles (Cu NPs)

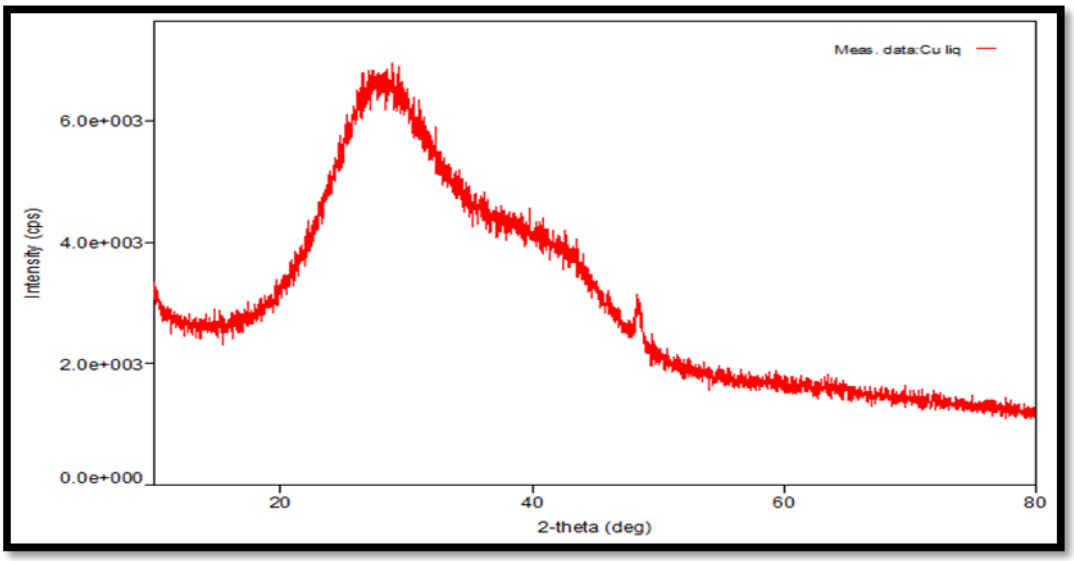

Table.1 Malvern software setting for Zetasizer analysis

Parameters	Distilled water	Copper nanoparticles
Viscosity	1 cP	-
Refractive index	1.36	1.54
Temperature	20 °C	20 °C

Table.2 Z-average (d. nm) of copper nanoparticles

Sample No.	Z-average (d. nm)	Peak	Size (d. nm)	Intensity (%)	St. Dev (d. nm)
1	37.61	Peak 1	57.32	95.4	55.31
		Peak 2	4935	4.6	653.2
		Peak 3	0.000	0.0	0.000

Table.3 UV-Visible spectrophotometer analysis of copper nanoparticles

Sl. No.	Wavelength (nm)	Absorbance
1	400	0.134
2	450	0.152
3	500	0.154
4	550	0.155
5	560	0.162
6	600	0.155

UV-Vis spectrophotometer analysis

The UV-Visible spectrophotometer analysis shown in Table 3 revealed that different absorbance values were observed corresponding to wavelength, when wavelength band between 400-600 nm. The sharp bands of copper nanoparticles were observed at 560 nm (λ_{max}). The UV- Visible spectrum of Cu NPs recorded maximum absorption band edge at 560 nm as

shown in Figure 2. Saranyaadevi *et al.,* (2014) found that the Cu NPs formation was confirmed from the peak at 531 nm with the UV range of 560- 640 nm, and hence it is evident with current result. The peak value was found to be gradually decreased with the increase in particle size. The surface plasmon vibrations of copper nanoparticles produced a peak at near 562 nm (Hariprasad *et al.,* 2016).

Scanning electron microscope (SEM) analysis

The clear magnified SEM image at the accelerating voltage of 10 kV with working distance of the sample at 13.5 mm, showed that nanoparticles were in spherical with uniform shape distribution. SEM analysis was showed that the copper nanoparticles were spherical in shape as shown in Figure 3. Similar results were observed by Saranyaadevi *et al.,* (2014) and Hariprasad *et al.,* (2016) i.e. spherical in shape of copper nanoparticles.

X-ray diffraction (XRD) analysis

X-ray diffraction pattern of the copper nanoparticles showed broad halo at about 2θ = 20 - 40° region which confirms the amorphous nature of Cu NPs.

Figure 4 showed the X-ray diffraction pattern of the copper nanoparticles and it showed the broad halo at about 2θ = 20-40° region which confirms the amorphous structure of Cu NPs. Saranyaadevi *et al.,* (2014) was found that the XRD spectrum at two different diffraction peaks at 39.1°and 68.3° region and diffraction peaks obtained at 2θ angle. Peaks observed at 2θ values of 42.47° by Fatma *et al.,* (2017).

Copper nanoparticles were synthesized from tulasi leaves and characterized by various analytical techniques. Dynamic light scattering (Zetasizer) analysis data showed that the average particles diameter of 37.61 nm were used for the further characterization and applications. The UV- Visible spectrum of Cu NPs recorded maximum absorption sharp band edge at 560 nm. SEM analysis data showed that the copper nanoparticles were in spherical shape. XRD pattern of biosynthesized copper nanoparticles revealed that, copper nanoparticles were in the amorphous form.

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