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RESEARCH ARTICLE

PHYTOFABRICATION OF COPPER NANOPARTICLES USING RHIZOMES OF *CURCULIGO ORCHIOIDES*(KALI MUSLI)AND ITS ANTIMICROBIAL ACTIVITY.

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Abstract

An eco-friendly and low cost protocol for synthesis of copper nano particles is developed by means of root extract of *Curculigo Orchioides* (Common name Kali Musli, Family Hypoxidaceae) as the reducing and stabilizing agent. The aqueous copper ions, exposed to *Curculigo Orchioides* extract were reduced and in due course turned into copper nanoparticles with the size range of 50 - 80 nm. The green synthesized copper nanoparticles were characterized by UV-VIS, and X-ray diffraction analyses. A probable mechanism for synthesis of copper nanoparticles by the root extract of *CurculigoOrchioides* is also predicted.

The morphology of the particles was confirmed by transmission microscopy (TEM) in the range of 50 - 80nm. The biosynthesized nanoparticles have shown good antimicrobial activity. The present approach is simple, rapid and environmentally benign and may serve to be suitable for large scale commercial production of copper nanoparticles.

Introduction:-

Metal nanoparticles are found to have numerous applications owing to their unique electronic, optical, mechanical and chemical properties that are significantly different from their bulk material(Rao 2004).Copper has an excellent electrical conductivity. Due to relatively low costs, this metal plays a significant role in modern electronic circuit(Schapter et al., 2004). Copper nanoparticles have exceptional electrical conductivity, increased catalytic behaviour, excellent compatibility and surface enhanced Raman scattering activity so they have a bright future in application as essential component of future nano-devices(Pergolese et al., 2006). Cu nanowires used in nanoelectronics have application possibilities for magnetic devices, nanosensors, electron emitters and other electronic applications. Cu nanoparticles have been explored to be used as nanoprobes in medicines and bio-analytical areas(Tomil, 2003). In recent times, Cu nano particles have gained much popularity because of its function in medical field, for dressings of wounds and its antibacterial properties(Borkow et al 2009 and 2010), in preparing gas sensors, as catalyst and solar cells(Li et al 2003and Guo etal 2007).Copper nanoparticles can also be synthesized by and using hydrazine hydrate and starch(Surmawar 2011) electrochemical method (Huang et al 2006), chemical reduction of copper metal salt(Hashemipour2011).Green synthesis of Cu nanoparticles was achieved by using microorganisms(Honary et al 2012), plant extracts such as *Ocimum Sanctum*,Aloe Vera etc.,(Malikarjun et al.2011,Gunalan 2012)

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To enlarge the scope of bio reduction previously unexploited *CurculigoOrchioides*extract was used in the present study to synthesize copper nanoparticles in aqueous solutions without adding any capping agent to protect nano particles from aggregating.

CurculigoOrchioides (family hypoxidaceae) is well known plant in Indian system of medicine. It is herbaceous, tuberous geophillous, perennial widely distributed all over India.Figure 1. (Courtesysource en.neflickrhivemind.org)

This plant is well known as a cure of piles, asthma, jaundice, diarrhoea, colic and gonorrhea. It is also an antioxidant and a tonic for strength, vitality etc. It contains chiefly carbohydrates, alkaloids glycosides, saponins and sterols. Medicinal properties of the herb have been mainly attributed to curculigosides and curculigosaponins. In the present study an attempt was made to synthesize copper nanoparticles after using *CurculigoOrchioides*extract.(Bafna and mishra 2005,Chauhan et al 2010,Rao and Nazar 1978).

Material and Methods:-

Materials:-

Analytical grade $CuSO_4$was purchased from Ranbaxy chemicals limited, India and used without further purification. Double distilled water was used in this experiments. Dried root of *CurculigoOrchioides* (Figure 2)was purchased from local market, identified by the Botany department and a voucher specimen was deposited in the department.

Preparation of extract:-

The dried roots of *CurculigoOrchioides* were crushed to fine powder and then sieved. The sieved powder was then boiled with 50 ml. double distilled water for 10 minutes bath. The extract was cooled, filtered and stored at 4°C for further experiments.

Biosynthesis of copper nanoparticles:-

For preparation of nanoparticles, the prepared root extract was typically spiked to aqueous $CuSO_4$ solution at room temperature. Optimum concentration, 1mM $CuSO_4$ and 10% root extract was selected for present studies. The reductive effect of the root extract on metal ions started too early which was evident by gradual change in light blue coloured solution to light green and finally brown thus indicating presence of copper nanoparticles.(Figure 3 and 4)

Characterisation of nanoparticles:-

UV Visible spectroscopic measurements:-

The bio reductionof copper ions in solution was monitored by sampling of aliquot and measuring the UV-VIS spectra of the solution in quartz cuvettes with a Perkin Elmer, Lambda 900 UV- VIS spectrophotometer.

XRD spectroscopy:-

XRD pattern of synthesized Cu nanoparticles is shown in Figure 5 The XRD pattern shows a high crystallinity of Cu sample level with diffraction angles which correspond to the characteristic face centered cubic (FCC) of copper lines indexed at (111), (200)and (220), respectively.

Transmission Electron Spectroscopy:-

The particles size was characterized by Transmission Electron Spectroscopy and the size was found to be in the range of 50-80 nm.

Antifungal activity of CuNPs:-

The activity of CuNPs was tested in vitro by the agar well diffusion method againstE.Coli. Nutrient agar media plates were prepared and solidified in laminar air flow, after solidification bacterial cultures were swabbed on the plates. Wells were prepared by cutting agar with 1 ml micro tips and filled with the solution containing nanoparticles at a concentration of 100 µg ml^{-1}. The plates were incubated at 37°C for 24 h, after which, the zone of inhibition was measured. The experiments were performed in triplicates (Figure 7)

Result and Discussion:-

Ultra Violet characteristic absorption peak is seen at around 585 nm due to the surface plasmon band of Cu colloids. This has been earlier also reported that Cu NPs synthesized by the chemical reduction method showed absorption peaks at 580 nm.The absorption band is broadened because of wide size distribution of CuNPs additional

absorbance peaks in the region around 300 nm are also seen might be due to the occurrence of some biomolecules in extract such as proteins amino acids flavanoids which were responsible for the reduction in $CuSO_4$.

The potential of plant extracts to reduce various metal ions to their nano particle forms has been well documented. Copper nanoparticles exhibit a brown color in aqueous solution due to the excitation of surface plasmon vibrations in copper nanoparticles. In this direction, reductive potential of root extract of *Curculigoorchioides* has been investigated. Distinct changes in colours from light blue to light green and ultimately to brown depicts the existence of copper nanoparticles in flask.

The curculigosaponins having -OH group in (Orchioside D) and $-OCH_3$ (Xylopyranosyl β-glycopyranoside) have strong reducing property and may reduce copper ions to copper.(Figure 8)

Figure 1:- A healthy plant of *CurculigoOrchioides*

Figure 2:- Dried roots of *CurculigoOrchioides.*

Figure 3:- Initial stage of Visual demostrationof green synthesized copper nanoparticles.

Figure 4:- Transformed copper nanoparticles.

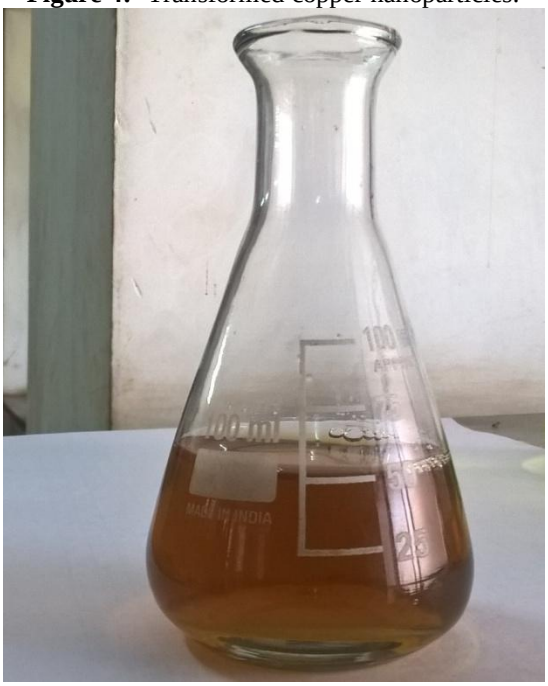


Figure 5:-X Ray Diffraction pattern of copper nanoparticles biosynthesized using *CurculigoOrchioides*

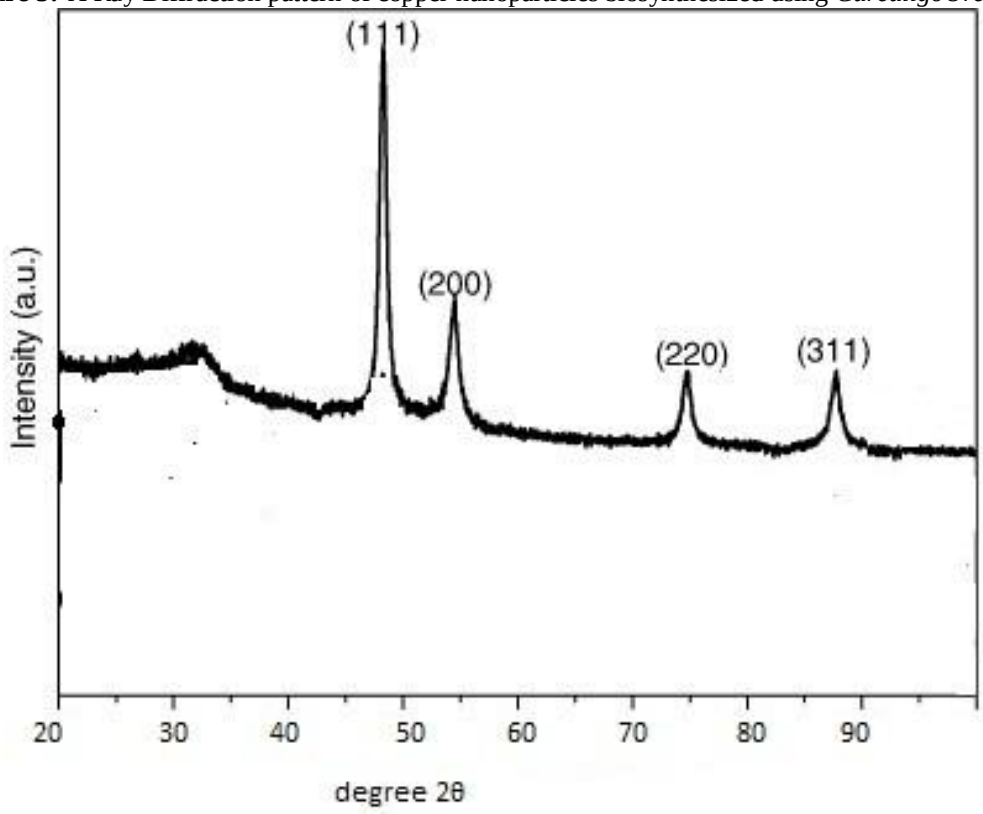


Figure 6:-TEM image of Copper nanoparticles synthesized by *CurculigoOrchioides*

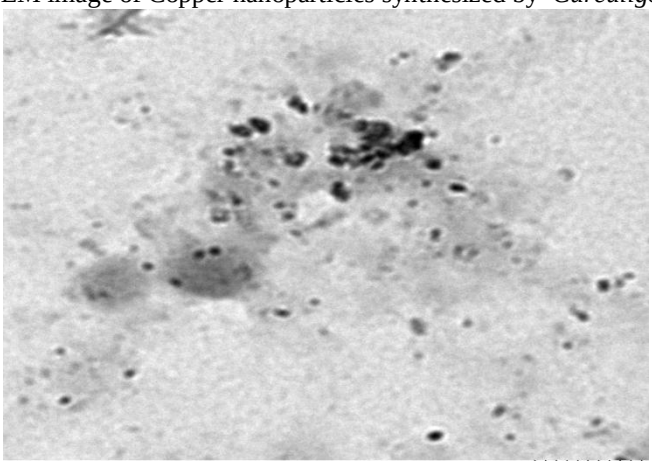
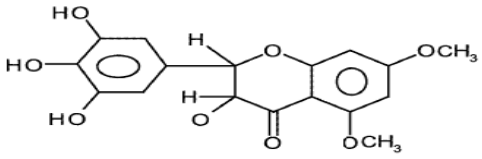
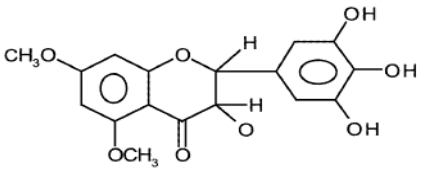


Figure 7:- Antibacterial activity of green synthesized copper nanoparticles against E.Coli.

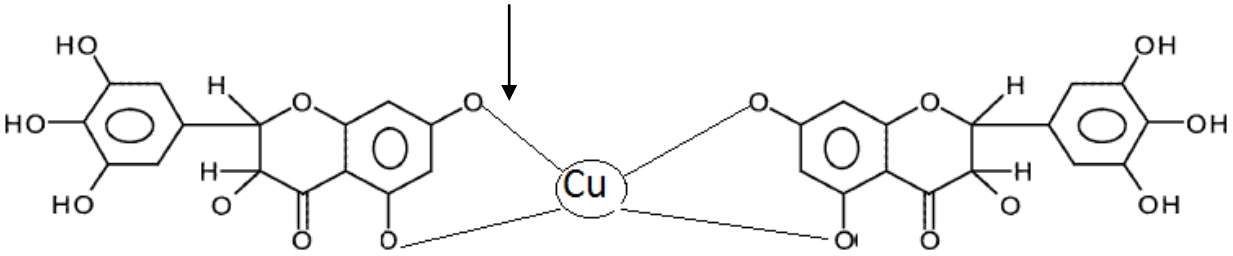
Figure 8:-Proposed mechanism of biosynthesis of copper nanoparticles using *CurculigoOrchioides.*

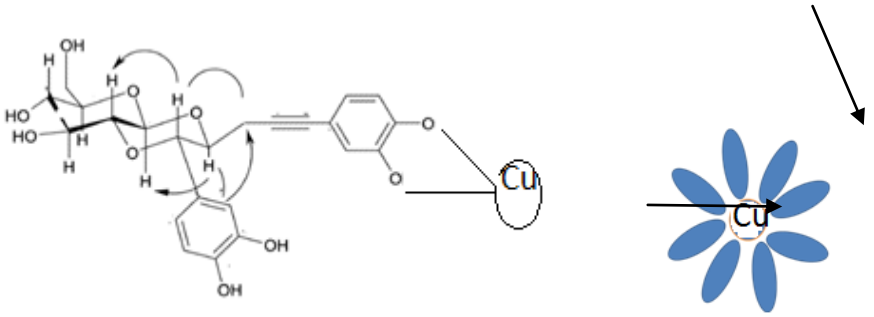




Xylopyranosyl β-glycopyranoside

$CuSO_4$ solution





Orchioside D

Conclusion:-

A facile and eco-friendly protocol for biosynthesis of copper nanoparticles using *CurculigoOrchioides* extract has been developed. The UV-spectroscopic technique indicates that the nanoparticles are well dispersed in the solution without aggregation. The rate of copper nanoparticle synthesis using *CurculigoOrchioides* extract found to be faster than the microbe mediated synthesis. Synthesized nanoparticles can be used in various biomedical, pharmaceutical, biotechnological applications and as well as for large scale commercial applications.

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