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Herbal Extract Mediated Green Synthesis of Copper Nanoparticles, its Characterization and Study of its Property

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ABSTRACT

Ecofriendly green synthesis of nanoparticles of copper was carried out by using extract of Aconitum heterophyllum successfully. The characterization of nanoparticles was done by well-known methods such as UV spectrophotometer and nano-tracking analysis. The size of nanoparticle was found to be around 52nm. Antimicrobial activity was established by Ditch-plate method *against Staphylococcus aureus, B. Subtilis, E. coli, P. aeruginosa.* There was considerable inhibition against tested *Staphylococcus aureus, B. subtilis, E. coli, P. aeruginosa* but lower compared to the synthetically prepared nanoparticles.

KEYWORDS: Green synthesis, A*conitum*, antibacterial activity, copper nanoparticles.

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INTRODUCTION

Nanoparticles are said to be the building blocks of nanotechnology[1]. It can be synthesized using physical, chemical or biological methods. Physical methods include laser ablation, evaporation condensation whereas chemical methods include reductions, micro emulsions, electrochemical processes, etc[2]. Physical and chemical approaches are expensive and also it involves the use oftoxic and hazardous chemicals. Nanoparticles can also be synthesized using bacteria fungi andplants. Biological methods are eco-friendlier and more economical[3]. The use of plant cultures is more preferable than using microorganisms due to the extent of work and labour required in maintaining cell lines. Gold and silver Nanoparticles have been synthesized using various plant extracts including hibiscus (Hibiscus rosasinensis) leaf extract, Neem (Azadirachtaindica) leaf broth etc[4]. Currently, research scientists are focusing on Copper Nanoparticles (Cu-NPs) as they show great potential applications in biocidal properties and wound healing[5,6]. It has also been documented that Copper NPs showed superior antibacterial activity when they were compared against SilverNPs.[7]

Aconitum heterophyllum is one of the herbs used for its medicinal properties. It is used in Indian ayurvedic formulations[8]. Aconitum heterophyllum belongs to the Genus of Aconitum and is generally associated or known for its toxic or poisonous effects. Hunting spears were once coated in aconitum to paralyze or kill prey[9,10]. A. heterophyllum has been reported to have a variety of medicinal uses. It was reported to show anti-diarrhoea activity when it was taken with powdered Ginger, Nutmeg (Jaiphal) or Beel (Bellpetra) [11]. The juice extract of the root when taken with milk, acted as an expectorant while the seeds showed antidiuretic activity. The plant has also been used to treat reproductive ailments, and has also been known to showcase hepatoprotective, anti-atrabilious, antipyretic and analgesic, alexipharmic, antioxidant, anodyne, anti-phlegmatic and carminative properties[14-16].

Phytochemical constituents

A reliable and accurate High Performance Liquid Chromatography (HPLC) method coupledwith Diode-Array Detection(DAD)was developed for simultaneous quantitative determination of six aconitum alkaloids: aconitine, mesaconitine, hypaconitine, benzoylaconine, benzoylmesaconine, and benzoylhypaconine[12].

Table 1: Tabular depiction of phytochemical constituents [8,11]

Class	Presence in root
Alkaloids	+
Carbohydrates	+
Proteins and amino acids	+
Saponins	+
Glycosides	+
Quinones	+
Flavonoids	+
Terpenoids	+

MATERIALS AND METHODS:

Chemicals and reagents:

Biological sample: roots of *Aconitum heterophyllum*, Microbial cultures: *Staphylococcus aureus, B. Subtilis, E. coli, P. aeruginosa.*

Chemicals: Copper Nitrate solution (Merck), nutrient agar [Hi-media], Ampicillin [Hi-media], Methanol

Green synthesis of copper nanoparticles using A. heterophyllum

Theplantsampleiswashedandrinsedwithdoubledistilledwaterfirsttoremoveexternaldustanddried in a muslin cloth for a period of 15 days for removal of moisture. A dry powder is made by well grinding in mortar. The dry powder is boiled in double distilled water till the solution is reduced to half. The sample is subjected to vacuum filtration to obtain the supernatant. 18ml of the supernatant was taken and treated with 50ml of 1mM copper nitrate solution. It was then heated in a microwave for 2 minutes and 9 seconds at 2.55 GHz. The formation of orange red colored solution indicates the presence or formation of CuNPs. The solution is the subjected to centrifugation at 10000 rpm for 10 minutes. The supernatant is discarded and the pellet obtained is washed several times with distilled water. The pellet now obtained is brownish red in color.

Characterization of plant mediated CuNPs

Nanoparticle Tracking Analysis: It was done for visualization and analysis of particles.

UV Spectrometry analysis: The sample obtained was subjected to UV spectrometry analysis for characterization of nanoparticles that were synthesized. For determination of the absorption maxima, a spectrum scan was run in the range of 300-800 nm.

Evaluation of properties of the copper nanoparticles

Antimicrobial activity:

Bacterial maintenance**:** S*taphylococcus aureus, B. Subtilis, E. coli, P. aeruginosa* were maintained on Nutrient agar slants and incubated at 37ºC.

Ditch plate technique: Antimicrobial action of the extracts was tested against the said organisms above by employing the ditch plate technique. The samples were diluted to 50% [v/v] with molten agar. This mixture was poured into a ditch of 1.5cm*0.5cm and allowed to settle. After which the microbes were streaked across. The plates were incubated at 37ºC for 24 hours. The zone of inhibition was measured.

RESULT

1.1 Nanoparticle tracking analysis**:** Experimental studies ascertained that the size of the nano particle is in between 52nm±34nm with a drift velocity of 1674nm/s. The total concentration was found to be 2.01E8particles/ml.

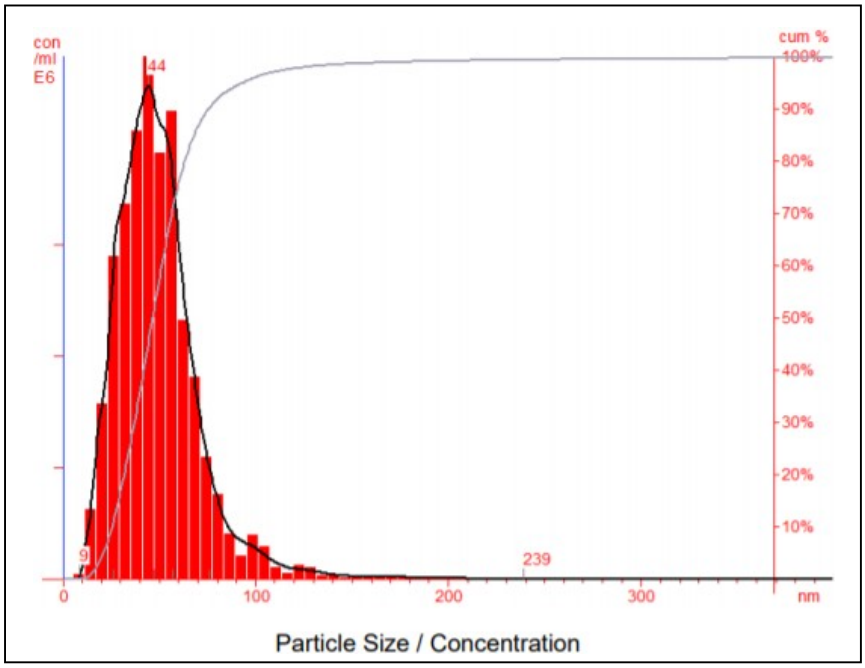

Fig 1: Graphical representation of NTA

UV spectrometry analysis:

The absorption maxima of the green synthesis of CuNPs was found to be at 575nm which is well in agreement with the absorption maxima (synthesis with the help plant leaves) of CuNPs, which is generally found to be between 550nm and 600nm.

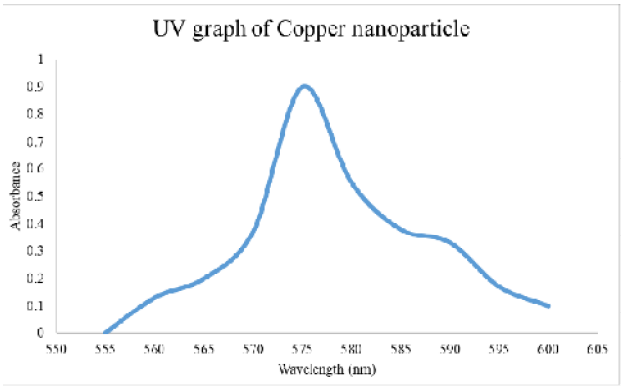

Fig 4. Graphical representation of UV analysis of copper nanoparticle

Antimicrobial Susceptibility testing:

Ecofriendly Green nanoparticles showed weaker inhibition in comparison to synthetically prepared nanoparticles. In the present studies aconitum nanoparticles showed strong inhibition against *S. aureus* and *B. subtilis*show everweak erinhibition against *E.coli* and *Pseudomonasaeruginosa* when *Ampicillin* was used as acontrol.

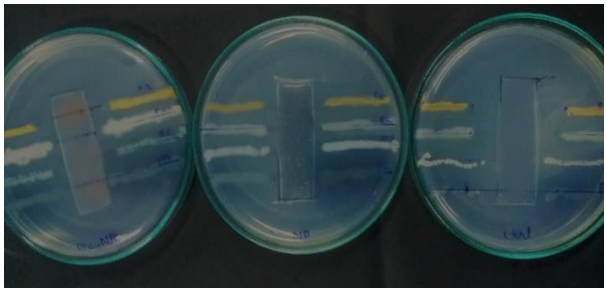

Fig 3. AST plates [left to right] of Green CuNPs, synthetic NPs, and control [Ampicillin]

Table: zone of inhibition exhibited by the samples on the selected bacteria

		Zone of Inhibition								
Sr. no	**Organism**	**Control**			**Copper Nanoparticles**			**Green-CopperNanoparticle**		
		[mm]	[mm]	[mm]	[mm]	[mm]	[mm]	[mm]	[mm]	[mm]
1	*S. aureus*	21	24	23	24	24	25	22	21	22
2	*B. subtilis*	21	22	21	13	14	17	13	14	12
3	*E. coli*	18	16	17	25	23	25	21	21	19
4	*P. aeruginosa*	17	16	18	12	13	15	14	11	14

DISCUSSION:

Ativish is generally linked with the treatment of digestive and intestinal complications. Conventionally, it has found applications in relieving flatulence and promoting optimum bowel health [15]. Aconite also shows diaphoretic activity by acting on the brain stem centers to bring about a reduction in pulse rates. Practitioners of Traditional Chinese methodology use Aconite to relieve pain caused by intercostal and trigeminal neuralgia[19].

Owing to their extensive application sinclinicalaswellasvariousinterconnectedfields, Aconitumand its phytoconstituents have been deeply researched for their characteristic features and methods of extraction. Nanoparticles have been documented in enhancing the activities of plants and their components. For this reason, copper nanoparticles were created to study the antimicrobial activity towards variety of flora, especially those causing enteric ailments[20]. For comparative studies, the desired nanoparticles were created by chemical and green synthesis. This was done to collect relevant data to bring about a comprehensive comparison of the two methods, particularly targeting activity and efficiencyparameters.ItwasfoundthattheantimicrobialactivityofG-CuNPswaslowerbyasignificantlevelof0.05,whenanalyzedstatisticallybyTtest.Hence,whiletheactivitiesofboththemethodsshow negligible difference and are thus comparable, green synthesis is ecologically as well as economically sound. Therefore, it can be said that green synthesis is a better and more preferred option than chemical synthesis.

CONCLUSION:

Using *Aconitum heterophyllum*extract copper nanoparticles were synthesized and their physical characteristics were ascertaine dusing Nano particle tracking system and UV. The size of the nano particle was found to be 52nm. Antimicrobial activities of CuNPs biologically and chemically synthesized were compared and statistically their activities were found to becom parable. There is good activity to wards *S. aureus* and *B. subtilis* and lesser activity towards *E. coli* and *Pseudomonas aeruginosa.* Aconitum there for eprovestobeapotentialcandidatefortreatingenteric in fections and can be further explored for clinicalresearch.

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