

EXPLORING THE POTENTIALS OF MARINE RESOURCES FOR THE SYNTHESIS OF NANOPARTICLES**R. Kanchana*¹ and Bhargavi Bhat²**

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ABSTRACT

In the present investigation, we report the green synthesis of multifunctional Cobalt oxide nanoparticles using the green seaweed, *Cladophora prolifera*, a good alternative to the electrochemical methods. Biosynthesised nanoparticles were characterized by UV-visible spectrophotometry, Scanning electron microscopy and Energy Dispersion spectral analysis (EDS). UV-visible absorption scan of Cobalt oxide nanoparticles revealed a peak at 490nm indicative of Cobalt oxide nanoparticle synthesis. The synthesized Cobalt oxide nanoparticles were of size 32.7nm and EDS analysis revealed Cobalt as the major element. Further the synthesized nanoparticles were investigated to evaluate the antimicrobial activity, hemocompatibility and in reduction of dyes used in textile industries. The nanoparticles showed promising compatibility in all these applications which would transform it to a multifunctional nanoparticle potential for industrial applications.

KEYWORDS: antimicrobial; hemocompatibility; multifunctional; nanoparticles; seaweed.**INTRODUCTION**

Green synthesis of nanoparticles provides an advancement over chemical and physical methods as it is cost effective, environment friendly, easily scaled up for large scale synthesis and further there is no need to use high pressure, energy, temperature and toxic chemicals. Biosynthetic methods employing either biological microorganism or plant extracts have emerged as simple and viable alternative to chemical methods but most of the methods are still in developmental stages. The marine ecosystem has captured a major attention in recent years, as they contain valuable resources that are yet to be explored much for the beneficial aspects of human life.^[1]

The sea weeds are rich in biologically active substances that may reduce the metal salts and hence biosynthesis of nanoparticles using sea weeds has turned much attention towards the utilization of nanoparticles are limited.^[2] The present study is therefore aimed to design eco-friendly synthesis renewable marine resources. However no reports on the usage of sea weeds in the green synthesis of Cobalt oxide nanoparticles using sea weed is available. The present study paves the way for future therapeutic applications of nanoparticles in biology and medicine as there is involvement of neither toxic chemicals nor reducing agents. Moreover, this process could be easily scaled up for the industrial applications to

increase the yield of the nanoparticles significantly, which undoubtedly would establish its commercial viability.

MATERIALS AND METHODS**Synthesis and characterization of Cobalt oxide nanoparticles**

Fresh and healthy thallus of green sea weed, *Cladophora prolifera* was collected from the coastal area of Goa during post monsoon and the algal extract was prepared by boiling 10 g in water and filtering the suspension. Cobalt oxide nanoparticle synthesis was carried out by incubating the extract with aqueous solution of Cobalt (II) nitrate hexahydrate. The synthesised nanoparticles were characterized by UV-visible spectrophotometry, Scanning electron microscopy (SEM) and Energy Dispersion spectral analysis (EDS).

Applications of Cobalt oxide nanoparticles**Evaluation of antimicrobial activity**

The antibacterial activity of Cobalt oxide nanoparticle was carried out using the well diffusion assay against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Proteus vulgaris* and the antifungal activity against *Rhizopus sp*, *Penicillium sp*, *Fusarium sp* and *Aspergillus niger*.^[3]

Hemocompatibility activity

A simple procedure was followed to check the hemolytic activity of the synthesised nanoparticles by the well diffusion method using blood agar plate and the appearance of zone of clearance is taken as an indication of haemolytic activity. Algal extract (C1) and metal salt solution (C2) were used as controls.^[4]

Dye decolorization activity

Azo dyes are a major class of synthetic, colored organic compounds that account for about half of the textile dyestuffs used today which are difficult to degrade and result in the threat of environmental pollution. Nanoscale particles are recently gaining great interest in environmental remediation circles. In the current study, applicability of Cobalt oxide nanoparticles in the removal of cationic (Methylene blue) dye was investigated. Control set without the addition of nanoparticles was carried out in parallel.^[5]

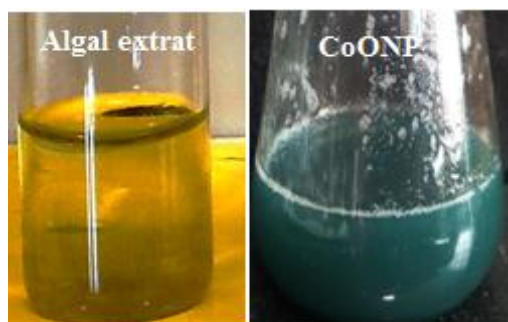


Fig 1: Synthesis of Cobalt oxide NP.

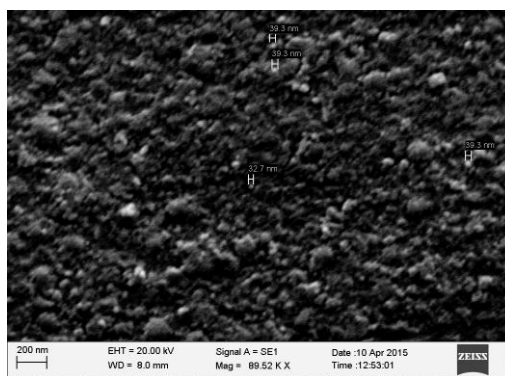


Fig 3: SEM analysis.

Applications of Cobalt oxide nanoparticles

The green synthesized Cobalt oxide nanoparticles exhibited excellent antimicrobial activity against test pathogens (Table 1; Fig. 5). Several mechanisms have been proposed to explain the inhibitory effect of nanoparticles on bacteria. It is assumed that the high affinity of nanoparticles towards sulfur and phosphorus is the key element of the antimicrobial effect. Due to the abundance of sulphur containing proteins on the bacterial cell membrane, nanoparticles can react with sulfur-containing amino acids inside or outside the cell membrane, which in turn affects bacterial cell viability. It has been hypothesized that Co oxide nanoparticles

RESULTS AND DISCUSSION

Synthesis and characterization of Cobalt oxide nanoparticles

The reduction of Cobalt (II) nitrate hexahydrate to Cobalt oxide nanoparticles was identified by change in colour of the reaction mixture to greenish-grey from yellowish green colour after 24 h, which indicated the formation of CoO nanoparticles (Fig. 1). The UV-Vis spectrum (Fig. 2) showed maximum absorbance at 490 nm is a characteristic of this noble metal particles which is due to the surface plasmon resonance of Cobalt oxide nanoparticles.^[6] SEM analysis shows the synthesized nanoparticles are of size 32.7nm (Fig. 3). Cobalt is revealed as the major constituent element from EDS analysis (Fig. 4). Other minor weak signals indicates the presence of capping material which is another advantage of nanoparticles synthesized using biological extracts over those synthesized using chemical method.^[7]

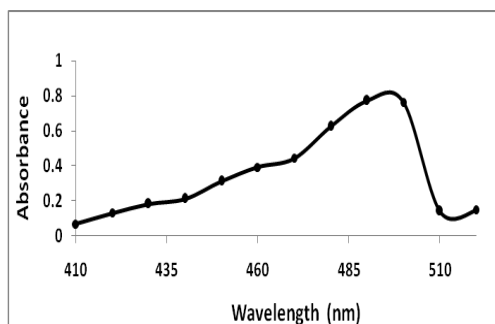


Fig 2: UV-Vis spectrum.

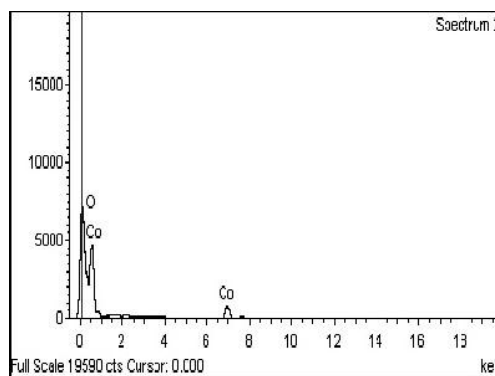


Fig 4: EDS analysis.

primarily affect the functions of membrane bound enzymes resulting in the loss of cellular integrity and osmotic culminating in acute toxicity to fungal cells.^[8]

Evaluation of hemocompatibility of nanoparticles should be considered as one of the factors of assessing systemic toxicity. Haemolytic properties and interactions with RBC are main parameters for the biocompatibility of nanoparticles.^[5] The inhibition of hemolysis of mammalian erythrocytes in the presence of Cobalt oxide nanoparticles substantiates its biocompatibility (Fig. 6).

Cobalt oxide nanoparticles have successfully shown decolorisation of Methylene blue instantaneously with more than 50% of the dye being removed within the 30 min of the experiment (Fig. 7 & 8). The experimental conditions need to be optimized for complete reduction

of the dyes. Thus the results of this pilot scale experiment extend applications of this nanoparticle in the remediation of cationic and anionic dyes in textile industries.

Table 1: Antifungal activity of CoO NP.

Concentration ($\mu\text{g/mL}$)	<i>Rhizopus sp</i>	<i>Penicillium sp</i>	<i>Fusarium sp</i>	<i>Aspergillus niger</i>
0.13	10.6 ± 0.5	11.3 ± 0.5	11.3 ± 0.5	11 ± 1
0.18	13 ± 1	12.6 ± 0.5	12	12.3 ± 0.5
0.20	13.3 ± 0.5	13.3 ± 0.5	11.6 ± 0.5	12.6 ± 0.5
0.26	14.3 ± 0.5	13.6 ± 0.5	11.3 ± 0.5	14 ± 1
$\text{Co}(\text{NO}_3)_2(\text{H}_2\text{O})_6$	0	0	4.0	0
Algal Extract	0	0	0	0

*Well diffusion method on PDA plate for antifungal activity and the zone of clearance was measured after the incubation period. Values are presented as the mean \pm SD of the three triplicates of the experiments.



Fig 5: Antibacterial activity.

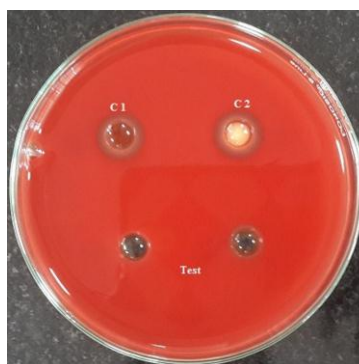


Fig 6: Haemolytic activity.

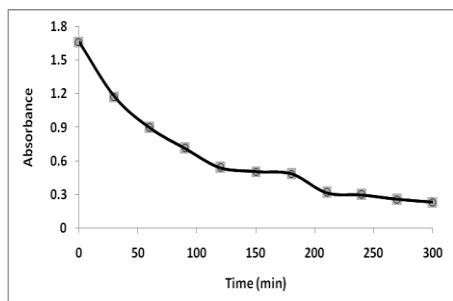


Fig 7: UV-Vis spectrum of reduction of Methylene blue dye treated with CoO NP



Fig 8: Reduction of Methylene blue dye

CONCLUSION

In conclusion, it has been demonstrated that the extract of the green seaweed is capable of producing Cobalt oxide nanoparticles. The antifungal activity of the nanoparticles is a promising alternative technique against the traditional use of conventional fungicides and can be used in the preparations of new formulations like pesticides and fungicides for applications in the management of plant diseases. The biosynthesized nanoparticles showed hemocompatibility and in reduction of dyes used in textile industries. Thus the biologically synthesized Cobalt oxide nanoparticles could be of immense use in medical field and in textile industries. Moreover, this process could be easily scaled up for the industrial applications to increase the yield of

the nanoparticles significantly, which undoubtedly would establish its commercial viability. Further studies are needed to fully characterize the toxicity and the mechanisms involved with the antimicrobial activity of these particles. More investigations about field applications and *in vivo* are needed.

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REFERENCES

1. Mohandass C, Vijayaraj AS, Rajasabapathy R, Satheeshbabu S, Rao SV. Biosynthesis of silver nanoparticles from marine seaweed *Sargassum cinereum* and their antibacterial activity. *Indian J Pharm Sci*, 2013; 75: 606-10.
2. Kumar P, Senthamil Selvi S, Lakshmi Prabha A, Prem Kumar K, Ganeshkumar RS. Synthesis of silver nanoparticles from *Sargassum tenerrimum* and screening phytochemicals for its antibacterial activity. *Nano Biomed Eng*, 2012; 4: 12-6.
3. Singh K, Panghal M, Kadyan S, Chaudary U, Yadav PJ. Antibacterial Activity of Synthesized Silver Nanoparticles from *Tinospora cordifolia* against multi drug resistant strains of *Pseudomonas aeruginosa* isolated from burn patients. *J Nanomedicine Nanotechnol*, 2014; 5: 192-98.
4. Laloy J, Minet V, Alpan L, Mullier F, Beken S, Toussaint O, Lucas S, Dogné J.M. Impact of silver nanoparticles on haemolysis, platelet function and coagulation. *Nanobiomedicine*, 2014; 1: 1-9.
5. Kutwin M, Ewa S, Sławomir J, Natalia K, Barbara S, André C. Structural damage of chicken red blood cells exposed to platinum nanoparticles and cisplatin, *Nanoscale Research Letters*, 2014; 9(257): 1-6.
6. Seshadri S, Prakash A, Kowshik M. Biosynthesis of silver nanoparticles by marine bacterium *Idiomarina sp. PR58-8*. *Bull Mater Sci*, 2012; 35: 1201-05.
7. Shankar SS, Rai A, Ahmad A, Sastry M. Rapid synthesis of Au, Ag, and bimetallic Au core Ag shell nanoparticles using Neem (*Azadirachta indica*) leaf broth. *J Colloid Interface Sci*, 2004; 275: 496-02.
8. Sulaiman GM, Mohammed WH, Marzoog TR, Al-Amiery AA, Kadhum AAH. Green synthesis, antimicrobial and cytotoxic effects of silver nanoparticles using *Eucalyptus chapmaniana* leaves extract. *Asian Pac J Trop Biomed*, 2013; 3: 58-63.