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Green Synthesis of Cobalt Nanoparticles from Leaves and Stem Extract Of Morus Indica (Mulberry) And Their Application

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ABSTRACT

The present study an effort has been made to developed nanoparticles used to biological and industrial application. The green synthesis of cobalt nanoparticles using aqueous& methnol extract of morus indica leaves & stem has been demonstrated. The high biological activity of cobalt and its nanoparticles have an extensive range of applications. The healthy morus indica leaves and stem was collected from Echampatti village, Nallampalli in Dharmapuri district of Tamil Nadu in India. The phytochemically isolated components and the water soluble heterocyclic components such as alkaloid and flavones were principally responsible for the reduction of ions and the stabilization of the nanoparticles. The synthesized cobalt nanoparticles are characterized by UV-Visible spectroscopy, FT-IR, SEM, AFM and antibacterial activity. The phytochemical screening test displayed the presence of dynamic phyto constituent of morus indica leaves and stem aqueous & methnol extracts. The synthesized cobalt nanoparticles exhibits spherical shape with in aqueous extracts average diameter range is 54-125 nm& methnol extracts average diameter range is 92-175 nm. Green synthesized cobalt nanoparticles could be a potential antibacterial agent.

Key words*: Nanoparticles, cobalt, antibacterial, UV, FT-IR, SEM and AFM.*

1. Introduction

Nanoparticles are investigated for various therapeutic applications are Carriers of drugs and biological agents,Carriers of gene and DNA, carriers of antigene and vaccines, Controlled and targeted drug delivery, carriers of diagnostic agent, Carriers of MRI contrast. Drug loading is relatively high and drug can be incorporated into the systems without any chemical reaction this is an important factor for [1] preserving the drug activity. Biosynthesis of nanoparticles is a kind of bottom up approach where the main reaction occuring is reduction/oxidation. The need for biosynthesis of nanoparticles rose as the physical and chemical processes were costly. Often, chemical synthesis method leads to presence of some of the toxic chemical absorbed on the surface that may have adverse effect in the medical application [2]. This is not an issue when it comes to biosynthesized nanoparticals via green synthesis route [3]. Green synthesis provides advancement over chemical and physical method as it cost effective, environment friendly, easily scaled up for large scale synthesis and in this method there is no need to used high pressure, energy, temperature and toxic

chemicals. Radioactive ^{60}Co is used in the treatment of cancer.

Cobalt is essential to many living creatures and is a component of vitamin B_{12}. Cobalt is also used in samarium-cobalt permanent magnets. These are used in guitar pickups and high speed motors[4]. *Morus*, a genus of flowering plants in the family Moraceae, comprises 10–16 species of deciduous trees commonly known as mulberries, growing wild and under cultivation in many temperate world regions [5]. The closely related genus *Broussonetia* is also commonly known as mulberry, notably the paper mulberry, *Broussonetiapapyrifera*. Mulberries are fast-growing when young, but soon become slow-growing and rarely exceed 10–15 metres (30–50 ft) tall. The trees can be monoecious or dioecious [6][7]. The mulberry fruit is a multiple fruit approximately 2 to 3 cm (one inch) long. In nature fruits are white, green, or pale yellow. Mulberry leaves, particularly those of the white mulberry, are ecologically important as the sole food source of the silkworm (*Bombyxmori*, named after the mulberry genus *Morus*), the cocoon of which is used to make silk [8][9].

2. Materials and Methods

Cobalt chloride, DMF and ethanol were purchased from Merck and used without further purification. Distilled and deionized water was used in all experimental work.

2.1 Collection and Preparation of plant extract

Healthy Morus Indica leaves was collected from Eachampatti (vill), Nallampalli (via) , Dharmapuri District, Tamil Nadu, India. The plant material were identified and confirmed by Botanical Survey of India (BSI), Coimbatore, TamilNadu, India. The voucher speciman number (BSI/SRC/5/23/2014-15/Tech.1460).

Morus Indica leaf was washed several times with distilled water to remove dust particles and then shade dried. Morus Indica leaf extract was prepared by placing 10 g of dried fine cut in 500 ml glass beaker along with 400 ml of sterile distilled water. The mixture was then boiled for 5 minutes until the colour of aqueous solution colour changed light green colour to dark brownish black colour. Then the mixture was cooled to room temperature and filtered with Whatman No.1 filter paper before centrifuging at 1200 rpm for 2 minutes to remove biomaterials. The extract was stored at room temperature in order to be used for further experiments.

2.2 Synthesis of Cobalt nanoparticles

For a reaction mixture 80ml of 1mM $CoCl_2$ and 20ml of Plant leaves Extract was added. Blank is prepared by addition of 80ml of distilled water to 20ml of plant leaves. The reaction mixture is placed on the dark place since 15 days. The cobalt metal is reduced to co^{2+} ion was indicated by colour change light green colour to dark brownish black colour.

The characterization of synthesized cobalt nanoparticles are characterized by using following parameter techniques such as UV-Visible spectroscopy, FT-IR, AFM and examined the antibacterial activity.

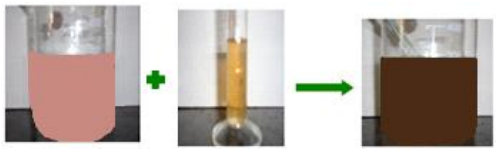

Fig 1 Schematic illustration of synthesized Cobalt nanoparticles

RESULTS AND DISCUSSION

UV-Visible Spectrophotometer Analysis

The intrinsic UV-Visible absorbance properties of many nanomaterials can be used to monitor pertinent properties, such as concentration, size, shape, and aggregation state [16]. silver and gold nanopraticles can be characterized by measuring absorbance in the wavelength ranges of 400-450 nm and 500-550 nm, respectively. The size of nanopraticles has a significant effect on the UV -Vis absorption spectra, i.e. small size particles absorb light of lower wavelength and vice versa[10]. Reduction of cobalt ions into cobalt nanoparticles during exposure to

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plant extracts was observed as a result of the colour change. The colour change is due to the surface plasmon resonance phenomenon (SPR). The metal nanoparticles have free electrons, which give the SPR absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with light wave.

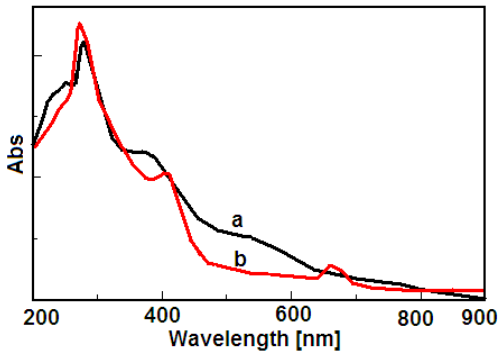

Fig .2. UV-Visible Spectrum of Morus Indica Leaves medicated a) Water extracts used Cobalt nanopaticle b) methanol extract used cobalt nanopaticle

The UV-Vis Spectrophotometer in a range of wavelength from 200-700 nm was observed for Morus India leaves extract medicated cobalt nanoparticles. Previous studies have shown that the spherical Co-NPs contribute to the absorption bands at around 250-650 nm in the UV-visible spectra. e.g, Fesharaki et al.,(2010) reported λ_{max} at 380 nm[11],Lin and Wang (2005) 455 nm[12] Shen et al ., (2008) at 680 nm[13]. The broad peak of cobalt nano particles were appeared at 390 nm. This broad peak is corresponding to the cobalt nanoparticles. The small peak observed in the UV region may be due to the small organic molecules present in reaction mixture .The UV data may support characterization of Morus Indica leaves extract medicated cobalt nanoparticle.

FT-IR Analysis

The major Absorption band appeared at 3425, 2921, 2851, 2284, 1739, 1627, 1423, 1384, 1101, 1022, 880, 628 cm^{-1}. The band at 3425 cm^{-1} corresponds to –OH Streching vibration. The –OH stretching frequency of leaves with metal ion was greater than that of –OH streaching frequency of leaves without metal ion. This is because due to the –OH group co-ordinate with metal ion. The (C=C) double bond peak was appeared at 2921cm-1 and the presence of (C=O) group at 1739cm^{-1}. imine (C=N) group appeared at 1627cm^{-1} The other characteristics peaks appeared at 628cm^{-1} to 1101 cm^{-1}. The band at 2921 cm^{-1} is due to C-H streching H-bonded Alkane. The band at 2851 cm^{-1} due to C-H streching H-bonded Alkane.The band at 2284 cm^{-1} due to C-H streching .The weak band at 880 cm^{-1} is due to C-H bend out of plane bending. The weak band at 628 cm^{-1} is due to C-H out plane bending.

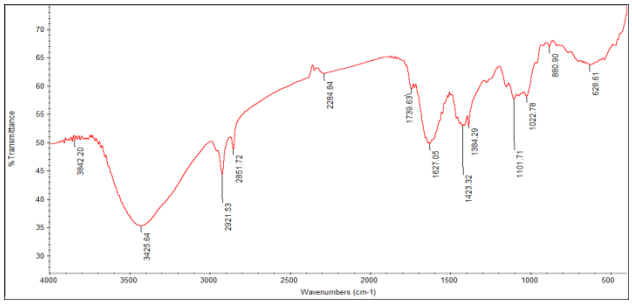

Fig.3. FTIR Spectrum of the *Morus Indica* plant leaves extract for aqueous with metal ion

The strong band at 3430 cm^{-1} is due to O-H stretching H-bonded Alcohols and phenol .The band at 2920 cm^{-1} is due to C-H streching H-bonded Alkane. The band at 2850 cm^{-1} due to C-H streching H-bonded Alkane. The band at 1717cm^{-1} due to overtones. The band at 1628 cm^{-1} due to overtones. The strong band at 1463 cm^{-1} is attributed to the C-H Scissoring in methyl group. The band at 1384 cm^{-1} due to C-H stretching of methyl rock group. The band at 1272 cm^{-1} C-O stretching. The band at 1167 cm^{-1} C-O stretching of ether group. The band at 1074 cm^{-1} is due to in plane C-H stretching bending. The weak band at 880 cm^{-1} is due to C-H bend out of plane bending. The weak band at 720 cm^{-1} is due to C-H out plane bending in long chain methyl rock. The chemical constituents present in Morus Indica leaves aqueous and Methonal extract such as polyphenol components and the water soluble heterocylic components such as alkaloid, flavones imply that the Co-NPs were successfully synthesized and capped with bio-compounds present in the Morus India Leaves Aqueous and Methonal extract by using a green method.

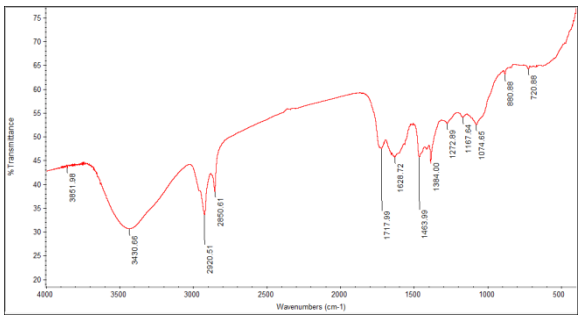

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Fig .4. FT-IR Spectrum of Morus Indica Leaves of Methanol extract with metal ions

Atomic Force Microscopy Analysis

Atomic Force Microscope is employed to analyze the shape of the cobalt nanoparticles synthesized by green method using Morus Indica leaves aqueous extract and methonal extract **fig** 4.1 & 4.2 shows AMF image of cobalt nanoparticle. Majority of the particle were spherical in shape formed with diameter range 54-125 nm & 92-182 nm. These particle were well distributed with aggregation. Therefore from these stuides ,the synthesized Morus Indica leaves aqueous extract mediated cobalt nanoparticles may efficient application in pharmacology.

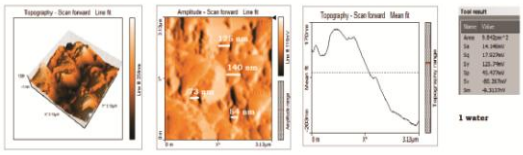


Fig.5. AMF image of Morus Indica leaves aqueous extract mediated Co-NPs

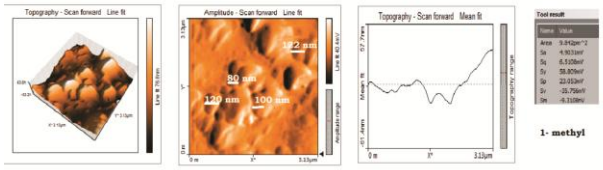


Fig.6. AMF image of Morus Indica leaves Methanol extract mediated Co-NPs

Antibacterial assay

Four gram-positive bacterial strains *Bacillus subtillis(B.subtillis),Enterococcus faedalis (E.Faedalis), Staphylococcus aureus (S.aureus)* and three gram-negative bacterial strains *Salmonella typhi (S.typhi, Klebsiella pneumonia (K. pneumonia), Shigella boydii (S.boydii)* were used. All the bacterial strains were obtained from clinical laboratories, Namakkal District, Tamil Nadu, India. The test organisms were prepared into cultivating a loopfall of culture in a 5 ml of Mueller Hinton broth and incubated (37 º C) for 14 hours.

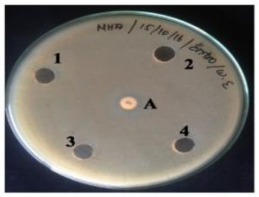
The bacterial activities of the various extracts were evaluated by means of the agar well diffusion assay. The assay was carried out according to the method. Approximately 25 mL of Mueller Hinton Agar (MHA) (HiMedia) were poured into sterile petridish and allowed to solidify. About 100 µL bacterial into columns were poured than swabbed on the MHA media by using sterile cotton swab. In each of these plates four wells (5 nm diameter) were punched in to the agar by using sterile cork borer. Than 50 µL of each extract (50 mg / Ml) was separately added into wells and allowed to diffuse at room temperature. Equal volume of DMSO was served as negative control and standard antibiotic (Ciproflaoxacin) used as positive control. The plates were incubated of 24 hours at 37 º C and the diameter (in nm) of clear zone of growth inhibition was recorded.

Report of Different con. Of products Zone in mm

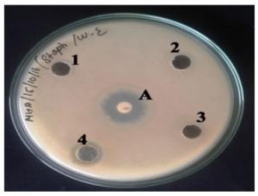
S. No	Name of the isolates	Water extract				Methanol extract				Genta mycin
		1	**2**	**3**	**4**	**1**	**2**	**3**	**4**	
1.	*E.coli*	-	-	-	-	-	-	-	12	13
2.	*P.aeruginosa*	-	-	-	11	-	-	-	-	-
3.	*S.aureus*	-	-	-	12	-	-	-	13	16
4.	*E.faecalis*	-	-	-	-	-	11	13	15	-

Antimicrobial activity of water extract

Enterococcus faecalis

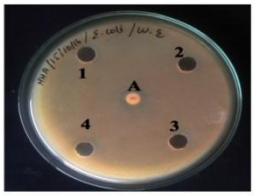


Staphylococcus aureus

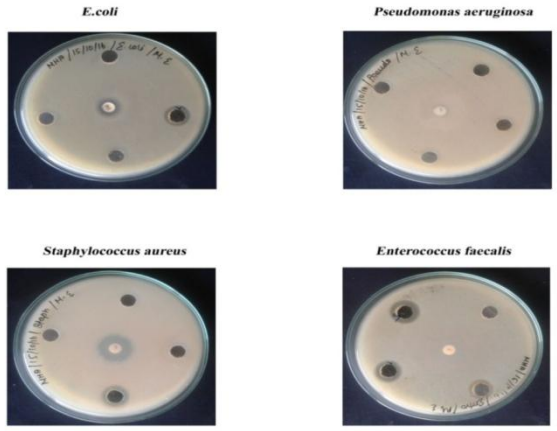


Pseudomonas aeruginosa

E.coli



1- 1mg, 2-2mg, 3-3mg, 4-4mg, A- Ampicillin



1- 1mg, 2-mg, 3-3mg, 4-4mg, A- Ampicillin

Antimicrobial activity of methanol extract

CONCLUSION

Green synthesis of cobalt nanoparticles using aqueous & methanol extract of morus india leaves has been demonstrated. The polyphenol components and the water soluble heterocyclic components such as alkaloid and flavones were principally responsible for the reduction of cobalt ions and the stabilization of the nanoparticles. Qualitative tests confirmed the presence of bioactive phyto constituents. Se colloidal solution showed absorption maximum at 293 nm. FT-IR spectra revealed the presence of reducing group in the extract responsible for CoNPs synthesis. The synthesized cobalt nanoparticles of methanol & water extract were near spherical shape with average diameter range is 92-182 nm & 54-125 nm. Green synthesized cobalt nanoparticles could be a potential antibacterial agent to treat diseases caused by bacteria.

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