ISSN 0974-3618 (Print)
0974-360X (Online)

www.rjptonline.org

RESEARCH ARTICLE

Synthesis of Zinc Oxide Nanoparticle using *Cocos nucifera* male Flower Extract and Analysis their Antimicrobial Activity

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ABSTRACT:
Nanotechnology deals with the invention and practice of material in the nanoscale measurement. The large bandwidth and high exiciton binding results in peculiar potential applications like antibacterial, anti-inflammatory, antioxidant property. In recent studies Zinc oxide nanoparticles (ZnO NPs) had been synthesized frequently for the antibacterial, antifungal studies. Since the physical and chemical production of these nano particals, from the plants extract is economic and ecofriendly, the environment is protected from the usage of toxic chemicals. Thus the current study is the synthesis and characterization methods to ZnO nano particals using different biological sources. The objective of this study is to evaluate the potential of *Cocos nucifera* male flowers extracts for the synthesis of zinc oxide nanoparticles and their antimicrobial properties.

KEYWORDS: Green synthesis, Zinc oxide nanoparticles, Antimicrobial activity.

1. INTRODUCTION:

Now a days natural product have been used for the treatment of human diseases. Large proportion of current drugs in modern medicine has been developed from natural molecules (1). The modernization of new biologically active natural products continues to high intense in the research field. Infact, the high natural biodiversity represents a wide range of diverse chemical structures with potentially new molecules which posses biological activities. Such new natural molecules can serve as chemical templates for the synthesis and design of novel drugs.

The crude plant extract contains secondary metabolites such as flavonoids, alkaloids, terpenoids and phenolic acid and these compounds are mainly responsible for the reduction of ionic into bulk metallic nanoparticles formation (9).

Received on 05.08.2019 Modified on 20.09.2019
Accepted on 16.11.2019

Research J. Pharm. and Tech 2020; 13(5): 2151-2154.
DOI: 10.5958/0974-360X.2020.00386.8

The primary and secondary metabolic activities involved in the redox reaction for the synthesize of nano particles. Many of the previous reports exhibit that the biosynthesized nanoparticle control genotoxicity, apoptosis related changes and oxidative stress effectively (10). Based on that, the present study is focused on the biosynthesizes of metallic nanoparticles from the plant extract and involved in the study of biological activities of such metallic nanoparticles.

Nanoparticle has multifunctional properties and has wide applications in various fields such as nutrition, medicine and energy (5,6). Biosynthesis methods posses more benefits over other classical synthesis procedures. This is due to the availability of more biological entities and eco-friendly procedures (7). Various chemical methods have been projected for the synthesis of zinc oxide nanoparticles (ZnO NPs). Some of the methods such as reaction of zinc with alcohol, precipitation method, hydrothermal synthesis, vapor transport, etc [1–4]. In order to reduce the usage of toxic chemicals, the researchers are concentrated in the usage of biosynthesis method which is eco-friendly and a one step process in the synthesis of nanoparticles [5]. The biological system involved in the synthesis of nanoparticles are plants and their derivatives, microorganisms such as bacteria, fungi, algae, yeast etc., [6–9]. A medicinal plant, inhabitant of South America is *P. caerulea L.*

(Passifloraceae), used against various pathogens which causes diseases such as diarrhea and their medicinal activity reported in many animal models [10]. Biosynthesis of ZnO NPS from the extract of plants such as *Sargassum muticum, Aloe vera, Eichhornia crassipes, Borassus flabellifer* fruit, and also some other bacterial and fungal species such as *Escherichia coli* and *Bacillus subtilis*, , *Lactobacillus plantarum* Ureolytic bacteria have been reported [11]. For the medicinal purpose metal oxide nanoparticles have been widely used for in the past decades [12]. Some of the metal oxide nanoparticles which posses various biological activity are Fe_3O_4, TiO_2, CuO and ZnO [13,14,15]. Antimicrobial activity of ZnO NPs against various pathogens such as *B. subtilis*, *Salmonella*, *Listeria monocytogenes*, *Staphylococcus aureus* and *E. coli* using disc diffusion method has been reported [16,17]. Also Xie et al reported similar results by *Punica granatum* mediated synthesized ZnO NPs [19]. It was also reported that ZnO NPs has showed high antibacterial activity against urinary infection disease [20]. The aim of the present study is to synthesis ZnO NPs from the plant *Cocos nucifera* and their antibacterial activity against pathogens causing various infections.

2. MATERIALS AND METHODS:

2.1. Plant collection and preparation of the plant extract:

The male flower of *Cocos nucifera* was collected from the northern part of Chennai. 5g of freshly collected *Cocos nucifera* male flowers were washed with running tap water followed by Milli-Q water and soaked in a 250 mL Erlenmeyer flask containing 100 mL Milli-Q. Then the solution was boiled for 8 minutes at 70°C. The extract obtained was allowed to cool at room temperature then filtered through Whatman number-1 filter paper, and the filtrate was stored for further experimental use.

2.3. Synthesis of ZnO NPs:

1 mM Zinc acetate $[Zn(O_2\ CCH_3)_2\ (H_2O)_2]$ was dissolved in 50ml Milli-Q water and stirred for 1 h [4] . Then add 20mL of NaOH solution slowly added into the Zinc acetate solution and add 25mL of plant extract to the same. After 1 hour the color of the reaction mixture was changed. The solution was left in stirrer for 3hours the appeared yellow color appeared confirms the synthesis of ZnO NPs. The precipitate formed was separated from the reaction solution by centrifugation at 8000 rpm at 60°C for 15 min and pellet was collected. The pellet was dried using a hot air oven operating at 80 °C for 2 h and preserved in air-tight bottles for further studies.

2.4. Characterization of biosynthesized ZnO NPs:

a. FT-IR studies:

Fourier transform infrared (FT-IR) spectra for the plant extract and plant extract zinc nanoparticles were recorded using Bruker FT-IR model-Tensor 27 at a frequency range of 400 to 4000 cm^{-1}.

b. Scanning electron microscopy (SEM) studies:

Surface morphology studies were carried out by recording the SEM photographs of zinc nanoparticles powder were recorded using Hitachi S-3000H model scanning electron microscope.

c. Antimicrobioal activity of synthesized ZnO NPs:

The antibacterial activity of synthesized ZnO NPs was performed in E. coli, and Streptococcus sp. Fresh overnight culture of each strain was swabbed uniformly onto the individual plates. The 25µl, 50µl and 75µl of ZnO NPs solution impregnated disc were placed onto the plates and incubated for 24 h at 37°C. Commercial antibiotic discs were placed as control. After incubation period got over, different levels of zonation formed around the disc was measured.

3. RESULTS AND DISCUSSION:

3.1. Visual observation:

Visual color change is the preliminary test for nanoparticle synthesis of ZnO NPs. (Fig. 2) represents the synthesis of ZnO NPs synthesized using freshly prepared *Cocos nucifera* fresh flower extract. Color change from half white to pale yellow represents the synthesis of ZnO NPs.

3.2. UV-visible analysis:

UV-vis spectroscopy is frequently conducted to confirm the synthesis of ZnO NPs. Conducting electrons starts oscillation at certain wavelength range due to the surface plasmon resonance (SPR) effect. Fig 1 represents the UV-visible spectra of freshly prepared ZnO NPs. The peak obtained at 350nm clearly shows the presence of ZnO NPs in the reaction mixture. Initial peak obtained at the range of 370nm got further raised due to the oscillation of more electrons after 4 hours which depicts the continuous synthesis of ZnO NPs.

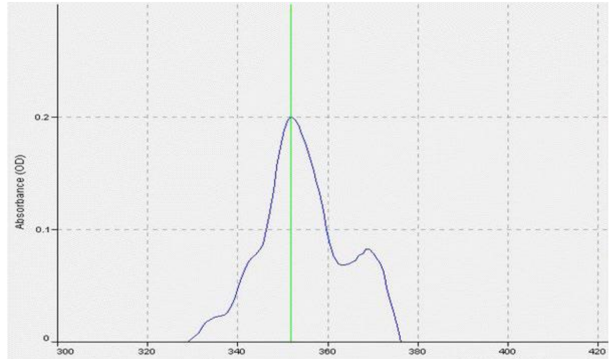

Fig: 1 UV-vis spectrum of ZnO NPs synthesized by *Cocos nucifera*

3.3. FTIR:

Substance specific vibrations of the molecules lead to the specific signals obtained by FT-IR spectroscopy. FT-IR spectra and functional group involved in ZnO NPs synthesis illustrated the peak in the range of 500–4000 cm^{-1}. Broad peak obtained at 3223 cm^{-1} corresponded to OH stretching vibrations, peak in the range of 1598 cm^{-1} and 1423 cm^{-1} corresponded to C = C stretch in aromatic ring and C = O stretch in polyphenols and C–N stretch of amide-I in protein. Weak peaks obtained at (1068 to 1070), (1024 to1008) and (877 to 879) demonstrated the presence of C –O stretching in amino acid, C –N stretching and C –H bending respectively. A very ignorable peak obtained at 644.22 and 567.07 demonstrated the probable presence of C- Alkyl chloride and Hexagonal phase ZnO. Plant extract itself had shown a broad range at 3265.49 corresponding to N–H stretch of amide group, sharp peaks obtained at 1598.99and 1394.54 corresponded to C = C stretch of alkane group and -C –H bending alkane group respectively. Weak peaks obtained at 1238.30 and 526.57 strongly depicted the presence of C-N stretch amine group and C-X stretch alkyl Halide (59).

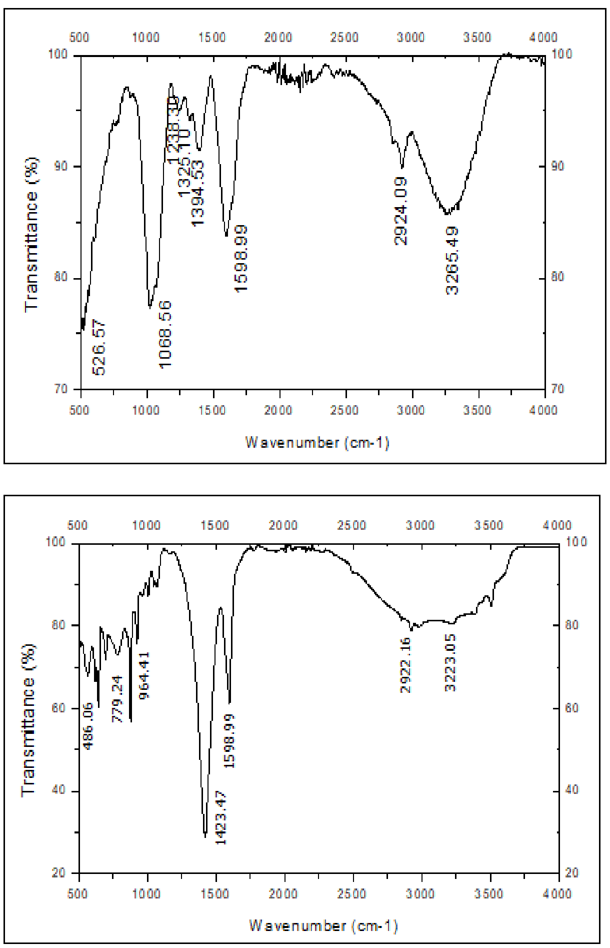

Fig 2: FT-IR Spectrum of ZnO NPs (a) Plant extract (b) ZnO NPs with plant extract.

3.4. SEM:

SEM analysis is done to visualize shape and size of nanoparticle. Scanning electron microscope was used to determine the shape of *Cocos nucifera* plant extract capped ZnO NPs. SEM images were seen in different magnification ranges like 2µm –50µm which clearly demonstrated the presence of spherical shaped nanoparticle

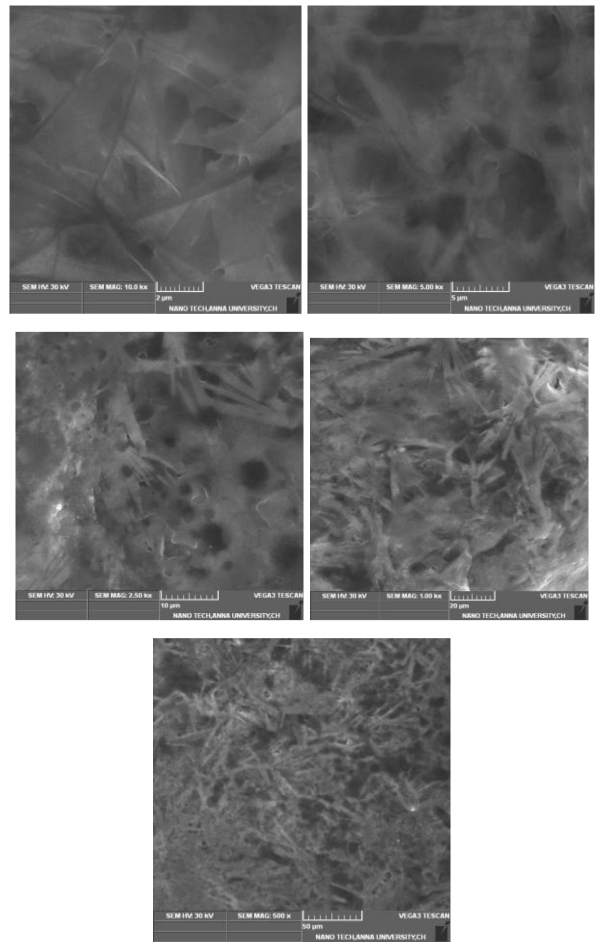

Fig 4: SEM Images of ZnO NPs in different magnification ranges (a)10µm (b)20µm.(c)50µm

4. Antimicrobial activity:

Antimicrobial activity of synthesised ZnO NPs were investigated against gram positive bacteria such *Staphylo-coccus aureus* and gram negative *Escherichia Coli*. Maximum zone of inhibition (15mm) was observed for both the microbes. It was observed that nanoparticles exhibited higher antibacterial activity against gram-negative bacteria as compared to gram-positive bacteria with a maximum activity. The results exhibit that *Cocos nucifera* can be used for the synthesis of nanoparticles, providing a new platform to this noxious plant and making it a value added weed for the synthesis of nanoparticles.

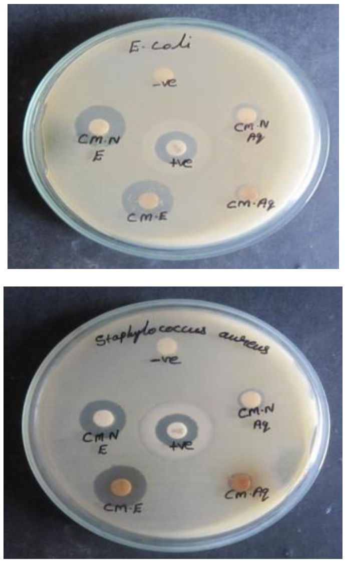

Fig 5: Antibacterial activity of ZnO NPs against (a) Streptococcus sp. (b) E.coli

5. CONCLUSION:

The nanoparticle synthesized in this experiment is non-toxic and eco-friendly. The phytochemicals present in the plant extract inducing oxidation and reduction reaction to synthesis metal oxide nanoparticle. The functional groups present in the phytochemicals induce the synthesis of nanoparticle were amines and alkanes that are widely seen in secondary metabolites such as terpenoids, flavonoids, alkaloids, etc. As a preliminary confirmation, the rapid synthesis of ZnO NPs was measured using the UV- Vis spectroscopy at a maximum absorbance of 350 nm. The SEM analysis of ZnO NPs demonstrated the size approximately in the range of 30–50 nm. Also the anti-bacterial activity of ZnO NPs has proved that these can be used as potent anti-bacterial agent.

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