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Green synthesis and characterisation of biocompatible zinc oxide nanoflowers using *azadirachta indica*

Gnanasangeetha.D[1,*] and SaralaThambavani.D[2]

[1]Department of Chemistry, PSNA College of Engineering and Technology, Dindigul, Tamilnadu.
[2]Bharathiar University, Coimbatore, Tamilnadu.

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ABSTRACT

Green synthesis of Zinc Oxide nanoflowers by plant extract is currently under utilization. This research account on the exploit of aqueous leaf extract of *Azadirachta indica* in the biosynthesis of bioactive zinc oxide nanoflowers with aqueous solutions of zinc acetate and sodium hydroxide at ambient temperature. The aqueous extract of *Azadirachta Indica* can be used as a template to control particle size and stabilize ZnO nanoparticles. The ZnO nanoflowers prepared by the green synthesis exhibited a hexagonal wurtzite structure with a crystalline size of 51 nm and particle size of 100 nm. To make the process environmentally viable the reaction was carried out under solvent free conditions. Formation of zinc oxide nanoflowers was confirmed by Fourier Transform Infrared Spectroscopy (FT-IR), X-ray diffraction patterns and scanning electron microscopy (SEM) with Energy dispersive X-ray (EDX) patterns. The results confirmed that aqueous leaf extract of *Azadirachta indica* is a suitable green template to prepare heterogeneous ZnO nanoflowers. Green methods are be good competent for the chemical procedures, which are environment friendly and convenient.

Introduction

Green synthesis of nanoparticles has two goals of producing nanomaterials and products without harming the environment or human health and producing nano-products that provide solutions to environmental problems. It uses existing principles of green chemistry and green engineering to make nanomaterials and nano-products without toxic ingredients at low temperatures using less energy and renewable inputs wherever possible and using lifecycle thinking in all design and engineering stages. In addition to making nanomaterials and products with less impact to the environment green synthesis of nanoparticles also means using nanotechnology to make current manufacturing processes for non-nano materials and products more environmentally friendly. The synthesis of zinc oxide nanoflowers has gained a great significance due to its remarkable physical and chemical properties. In particular, zinc oxide nanoparticles can be used in many fields such as biosensors, cosmetics, electronics, catalysis semiconductors, drug delivery and tumour imaging as they possess size and shape dependent unique properties [1-2]. Different physical and chemical methods have been used to synthesis ZnO NPs including Chemical precipitation method, Co-precipation method, vapour deposition, reduction in micro-emulsion, Sol-gel and electro and photo chemical process [3-11]. In these conventional methods toxic synthetic and chemicals are used as reducing and stabilizing agents. Synthetic reducing agents are not favoured for producing ZnO NPs for biomedical applications as traces of such chemicals left unreacted in this process can be harmful [12]. Therefore there is considerable interest in developing new procedure for ZnO NPs which are simple rapid and environmentally benign. In this context aqueous leaf extract of *Azadirachta indica* plants have been used to synthesis and stabilize ZnO NPs without the involvement of synthetic chemicals. Nanotechnology is an up-and-coming and hasty mounting field of science which is being exploited in an extensive spectrum of disciplines such as electronics, energy, environment and health sectors. Nanoscience has revolutionized these fields in achieving the processes and products that are hardly possible to evolve through conservative systems. Nanotechnology is the creation and consumption of functional materials devices and systems with novel properties and functions that are achieved through the control and reformation of matter at the atomic, molecular and macromolecular levels [13]. During the last few years novel structures phenomena and process have been observed at the nanoscale and new experimental, theoretical and simulation tools have been developed for investigating them. Far-reaching outcomes for the twenty first century and envisioned in both scientific knowledge and a wide range of technologies in most industries, health care, biology, environmental and conservation of materials and energy [14]. Despite large unproven track record in the environmental arena, nano meter sized materials and technology offer great promise for delivering new improved environmental technologies maintaining and improving the soil, water and air quality represent some of the most formidable challenges facing global society in the twenty first century. Pollutant from such diverse sources as oil and chemical spills, pesticides and fertilizer run off, abandoned [15]. Nanotechnology is always surrounded by the fact that the particles at nanoscale behave very differently than they do in their original form. It also raises the point about how good is it for environment and for human life. These are some of the issues that nanotechnology poses even its growth. Towards some extent, this is trying to be addressed by green nanotechnology. This means have non harmful particles to help

Tele:
E-mail addresses: sangithprakash@yahoo.co.in

in building better products. This is today being adopted in different areas like medicines, automobiles, defenses etc. With this has the theme for this research we present interesting green method explaining various challenges and opportunities that green nanotechnology throws open for researches[16]. From the very beginning of civilization mankind has depended upon medicinal herbs to treat a myriad of diseases, disorders and injuries [17]. Even today more than half of all modern medicines ranging from aspirin to the newest treatment for breast cancer are based upon ingredients from plants. *Azadirachta indica* commonly known as *neem*, is a species of tree indigenous India, Burma (Mynamar), Bangladesh, Pakistan and Africa. A standard *neem* will achieve a height of 25-30m in a relatively short period of growth. *Azadirachta* is a genus of two species of trees in the mahogany (Meliaceae) family. Numerous species have been proposed for the genus but only two are currently recognized, *Azadirachta excelsa* and the economically important tree *Azadirachta indica*. The latter is informally called the *neem* tree. To those millions in India *neem* has miraculous powers and now scientists around the world are beginning to think they may be right. Two decades of research have revealed promising results in so many disciplines that this obscure species may be of enormous benefit to countries both rich and poor[18-19]. Even some of the most cautious researchers are saying that *neem* deserves to be called a wonder plant. *Neem* still called the village pharmacy in its native India is one of the most ancient and widely used herbs in the world. In fact herbalists in ancient India had documented the healing qualities of this remarkable tree long before western civilization discovered the analgesic qualities of the willow tree from which aspirin is derived [20-21]. In India *Azadirachta indica* is variously known as Sacred Tree, Heal All, Nature's Drugstore, Village Pharmacy and Panacea for all diseases. Products made from *neem* trees have been used in India for over two millennia for their medicinal properties *neem* products are believed to be anthelmintic, antifungal, antidiabetic, antibacterial, antiviral, contraceptive and sedative *neem* oil is used to improve liver function, detoxify the blood and balance blood sugar levels and is considered to have no side effects [22-23]. *Neem* is a key ingredient in non-pesticidal management (NPM), providing a natural alternative to synthetic pesticides. *Neem* seeds are ground into a powder that is soaked overnight in water and sprayed onto the crop. *Neem* does not directly kill insects on the crop. It acts as an anti-feedant, repellent, egg-laying deterrent, protecting the crop from damage. The insects starve and die within a few days. *Neem* also suppresses the hatching of pest insects from their eggs. *Neem* cake is often sold as a fertilizer. The resin from the trees have been attributed with medical benefits [24-25]. A component in the resin is an effective insecticide azadirachtin. *Neem* gum is used as a bulking agent and for the preparation of special purpose food. A mixture of *neem* flowers and bella (jaggery or unrefined brown sugar) is prepared and offered to friends and relatives, symbolic of sweet and bitter events in the upcoming new year [26]. *Neem* oil is non drying and it resists degradation better than most vegetable oils. In rural India it is commonly used to grease cart wheels as lubricants. An exudate can be tapped from the trunk by wounding the bark. This high protein material is not a substitute for polysaccharide gum, such as gum Arabic [27]. It may however, have a potential as a food additive, and it is widely used in South Asia as *neem* glue. In parts of Asia *neem* honey commands premium prices and people promote apiculture / apiary by planting *neem* trees. Active constituents of *neem* leaf extract include isomeldenin, nimbin, nimbinene, 6-desacetyllnimbinene, nimbandiol, immobile, nimocinol, quercetin, and beta-sitosterol. Two additional tetracyclic triterpenoids zafaral [24,25,26,27-tetranorapotirucalla-(apoeupha)-6alpha-methoxy-7alpha-acetoxy-1,14-dien-3,16-dione-21-al] (1) and meliacinanhydride [24,25,26,27-tetranorapotirucalla-(apoeupha)-6alpha-hydroxy,11alpha-methoxy-7alpha,12alpha-diacetoxy,1,14,20(22)-trien-3-one] (2) have been isolated from the methanolic extract of *neem* leaves. Researchers have found that azadirachtin and selected semi-synthetic derivatives block the development of the motile male malarial gamete in vitro. NIM-76, a spermicidal fraction obtained from *neem* oil, may directly inactivate a virus versus preventing viral replication, as it did not inhibit viral multiplication once the infection was present. NIM-76 stimulated cellular mediated immunity and lymphocyte proliferation, which may contribute to its antimicrobial effects [28-30]. Azadiractin is a tetranortriterpinoid constituent of *neem* that interrupts metamorphosis in insects, causing pesticidal effects. Results prove that green ZnO nanoparticles show more enhanced biocidal activity against various pathlogens when compared to chemical ZnO nanoparticles [31]. To the best of our knowledge, the use of *Azadirachta* leaf extract at room temperature for the green synthesis of zinc oxide nanoparticles has not been reported. Hence the present study was carried out to synthesis and characterizes the zinc oxide nanoflowers using *Azadirachta* leaf extract.

Experimental Section

Leaves of *Azadirachta indica* were collected in the month of May from its natural habitat from nearby Seelapadi village, Dindigul district, Tamil Nadu. The plant was authenticated by Dr. D. Sarala Thambavani. The leaves were cleaned and washed with double distilled water, finely grinded and filtered. The different qualitative chemical tests were performed for establishing profile of given extract for its chemical composition. Qualitative phytochemical analysis was done using the standard procedures [32], [33]. The qualitative examination of the aqueous extracts of the leaf sample of *Azadirachta indica* showed the presence of phytochemical constituents such as Alkaloid, Carbohydrate, Glycoside, Steroid, Flavonoid, Terpenoid, Tannins and Steroid.

Table 1. Phytochemical constituents of *Azadirachta indica* aqueous leaf extract

SL.No	Phytoconsitituents	Reagents	Aqueous
1.	**Alkaloids**	Mayer's	+
		Wagner's	+
2.	**Carbohydrates**	Molisch's	+
		Benedict's	+
3.	**Glycosides**	Legal's	+
		Borntrager's	+
4.	**Steroid**	Libermann burchard's	+
5.	**Fixed oils**	Spot test	-
6.	**Saponins**	Gelatin	-
		Lead acetate	-
7.	**Tannins**	Ferry chloride	+
		Wagner's	+
8.	**Protein**	Millon's	-
		Biuret	-
9.	**Flavonoids**	Alkaline Reagent	+
		Shinoda's	+
10.	**Terpenoids**	Thionyl chloride	+

+ Presence

– Absence

ZnO nano flowers were prepared by Green synthesis method. In this synthesis 0.02M aqueous Zinc acetate dihydrate was added to 50 ml of distilled water under vigorous stirring. After one hour stirring aqueous leaf extract of *Azadirachta indica* at different sets (0.25, 0.5, 1ml) were introduced into the above. At room temperature, aqueous 2.0M NaOH was added drop by drop to reach pH 12. This was then placed in a magnetic stirrer for 2hrs. After completion of reaction the white precipitate formed was washed continually with distilled water followed by ethanol to get rid of the impurities. The obtained precipitate was dried in a hot air oven at 60˚C for overnight. During drying, complete conversion of Zn $(OH)_2$ into ZnO took place.

The particle size and external morphology of the sample were characterized by Scanning Electron Microscope (SEM) (LEO 1530FEGSEM). Fourier Transform Infrared Spectrometer (FT-IR) spectra were recorded on Jasco FT-IR5300 model spectrophotometer in KBr pellets in the range of 4000-400 cm^{-1}. The samples were characterized for crystal phase identification by powder X-Ray Diffractometer (XRD, PW 3040/60 Philips X'Pert, Holland) with Cu (Kα) radiation (ʎ =0.15416 nm) operating at 40 kv and 30 mA with 2θ ranging from 10- 90˚.

Results and discussion

FT-IR measurements were agreed out to identify the biomolecules for capping and proficient stabilization of the metal nanoparticles synthesized by *Azadirachta* leaf extract (Fig.1.). The FTIR spectrum of Zinc Oxide nanoparticles absorbs at 408-550 cm^{-1} [34], [35]. The O-H stretch appears in the spectrum as a very broad band extending from 3400 cm^{-1}. This very broad O-H sretch band is seen along with a C=O peak, it almost certainly indicates the compound is a aliphatic carboxylic acid. Two peaks attributed to C-F stretching at 1105 cm^{-1} constitutes mono and poly fluorinated compounds. Medium absorption in the region 1581-1415 cm^{-1} implies the presence of aromatic ring. The absorption peak at 1015 cm^{-1} corresponds to C-O stretching of saturated primary alcohol. The prominent doublet absorption at 2921 cm^{-1} indicates C-H stretching vibration of an aromatic aldehyde .The presence of this doublet allows aldehydes to be distinguished from other carbonyl containing compounds[36], [37]. These bands are indicative of terpenoid group of compounds present in aqueous neem extract [38]. Some of the major chemical constituents present in neem leaves have been identified through detailed studies using NMR, FTIR as quercetin rhamnoside (0.45%) a flavonoid quertcetin (0.257%) and nimbin (0.19%). A few other constituents are also present are nimbocinone (250 ppm), nimbandiol (130 ppm) [39]. Therefore the synthesized nanoparticles were surrounded by metabolites such as terpenoids having functional group of alcohols, ketones, aldehyde and carboxylic acids is confirmed.

Structure and peak purity of the samples were acknowledged from XRD patterns. Fig.2. shows the XRD prototype of the as- synthesized ZnO nanoparticle from aqueous leaf extract of *Azadirachta Indica*. The peaks obtained are analogous to (100), (002), (101), (102), (110), (103), (200), (112) and (201) planes in the hexagonal phase of ZnO [40]. The XRD patterns of the ZnO nanoparticle are in high-quality accord with the values of standard card (JCPDS NO: 36-1451), [41].

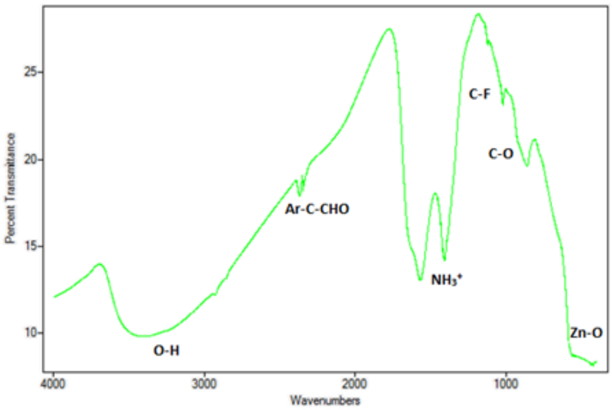

Fig.1.FT-IR Spectrum of green synthesis of *ZnO NPs* using *Azadirachta indica*

More over predominantly broad peaks at about 31º and 36º are indicative of nano-crystalline nature of the ZnO phase. No other contamination peaks are observed. From the XRD peaks the average grain size was estimated using Debye Scherer's equation as 51nm for the as prepared ZnO nanoparticles.

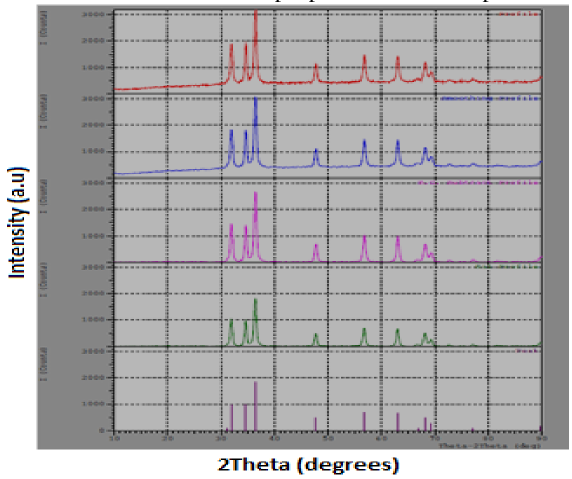

Fig.2 XRD spectra of ZnO NPs of aqueous leaf extract of *Azadirachta indica*

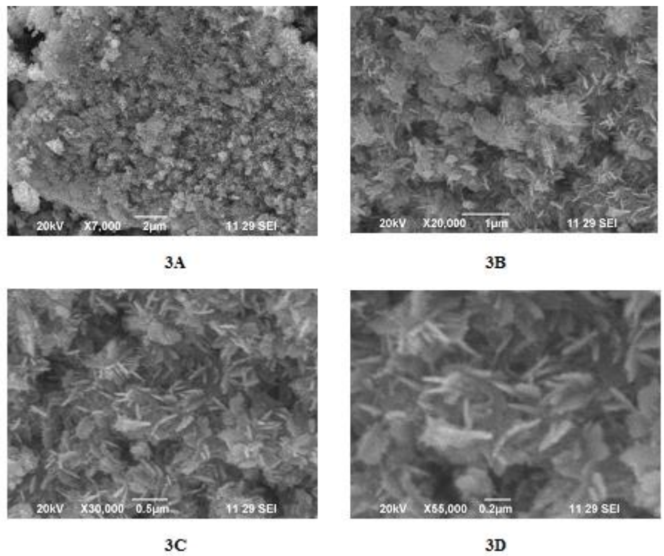

Fig. 3 A, B, C, D Representative SEM image of the as-synthesised ZnO nanoflowers

SEM image of ZnO nanoparticles synthesized using aqueous leaf extract of *Azadirachta indica* via green route are shown in the Figure3A,B,C,D. The low magnified observation 3(A) and 3(B) shows that the morphology is hierarchically nano structured flowers ranging from 0.2-2μm in diameter. Closer observation 3(C) and 3(D) shows that the nanoparticles have flower morphology ranging from 0.2-2µm of particle size from 100-200 nm. It is obvious that the sample consists of a large quantity of dispersive homo size nano flowers 3(A), 3(B), 3(C), 3(D). The highly magnified SEM image substantiate the approximate flower shape to the nanoparticles and most of the particles exhibit some faceting ranging from 100-200nm [42].

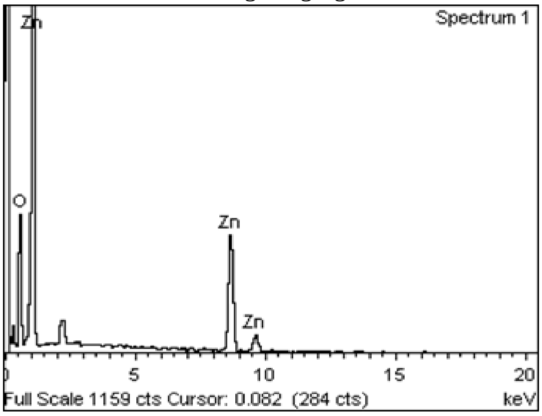

Fig.4 The elemental spectra of ZnO NPs revealed by EDX analysis

Fig.4 gives the elemental spectra of ZnO nanoparticles exposed by EDX analysis. It specifies that the ZnO wurtzite structure is only composed of two elements Zinc and Oxygen. The XRD, SEM and FT-IR pattern is consistent with the EDX results reported. In biological field the potential utility of zinc oxide nanoparticles in the treatment of cancer have been reported by scores of researchers. Owing to bountiful advantages associated with this eco friendly nature it has been explored as a powerful catalyst for several organic transformations. This research opens with a short course on how to synthesize Zinc oxide nanoparticle in a natural scale.

Conclusions

A novel biological approach for the formation of Zinc Oxide nanoflowers using aqueous leaf extract of *Azadirachta* at room temperature is invented. (EDX) authenticate the formation of Zinc Oxide nanoflowers. X-ray diffraction spectroscopy (XRD) shows characteristic peak at (100), (002), (101), (102), (110), (103),(200),(112) and (201) which confirms that the as-synthesized material possess wurtzite hexagonal phase of ZnO nanoflowers. The morphology and the image of ZnO NPs was characterized by SEM and their typical images. The synthesized nanoflowers were surrounded by metabolites such as terpenoids having functional group of alcohols, ketones, aldehyde and carboxylic acids is confirmed by FT-IR studies. The plant phytochemicals like terpenoids, flavonoids, alkaloids present in the aqueous leaf extract with antioxidant property were accountable for the preparation of Zinc oxide nanoflowers. As ZnO NPs is non toxic, it can be used as photocatalytic degradation materials of environmental pollutants. So the exploration of the plant systems as the potential nanofactories has heightened interest in the biological synthesis of nanoflowers. Synthetic reducing agents are not favoured for producing ZnO NPs for biomedical applications as traces of such chemicals left unreacted in this process can be harmful. Therefore there is considerable interest in developing new procedure for ZnO NPs which are simple rapid and environmentally benign. In this context aqueous leaf extract of *Azadirachta Indica* plants have been used to synthesis and stabilize ZnO NPs without the involvement of synthetic chemicals.

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