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FACILE AND ECO-FRIENDLY METHOD FOR THE SYNTHESIS OF ZINC OXIDE NANOPARTICLES USING *AZADIRACHTA* AND *EMBLICA*

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ABSTRACT: The evolution of green chemistry in the production of nanoparticles has wrapped up an immense consideration because traces of chemicals left unreacted in the chemical synthesis process can be precarious. Owing to copious interest a competent protocol for the production of ZnO NPs without calcinations was developed by green synthesis method using aqueous leaf extracts of *Azadirachta Indica* and *Emblica Officinalis*. The aqueous leaf extract acts as a solvent with manifold roles as promoter, stabilizer and template for the synthesis of nanoparticle. The formed nanoparticles were characterized by SEM, XRD, FTIR, and EDAX for its morphology, size, crystallinity and percentage composition. The results confirmed the formation of nanoflowers of 51 nm for *Azadirachta Indica* and nano flakes of 16 nm for *Emblica Officinalis*. Though both the green synthesis is trendier for nanoparticle synthesis, the biogenic green fabrication of *Azadirachta* is better due to its morphology, particle size and crystallinity. Green synthesis using *Azadirachta* is found to be the best stabilizer for synthesizing nanoparticle. And the study was further initiated and reported that Zinc oxide nanoparticle can be used as an inexpensive and effective adsorbent for the removal of arsenic ions from aqueous solution. This approach offers environmentally beneficial alternatives to more hazardous chemicals and processes and promotes pollution prevention by the production of nanoparticle in their natural environs.

INTRODUCTION: 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.

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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. Nanotechnology has animatedly developed as a research for the synthesis of structure of particles with facet smaller than 100nm.

Nanotechnology is always surrounded by the fact that the particles at nanoscale behave very differently than they do in their original form. Green nanotechnology has been described as the development of clean technologies, to minimize potential environmental and human health risks associated with the manufacture and use of nanotechnology products and to encourage replacement of existing products with new nano-products that are more environmentally friendly throughout their lifecycle [1-4].

Green nanotechnology has goals to produce 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. Green synthesis of nanoparticle is an innovative branch of nanotechnology. Green principle spotlight on choosing reagents that pretense the least risk and spawn only benevolent by products. Green Synthesis of nanoparticle aims to protect the environment not by cleaning up but by inventing new chemical process that do not defile.

Green synthesis of nanoparticle depends on plant source and the organic compound in the crude leaf extract. It has no harmful particles to help in building better products. In the midst of the assorted biosynthetic approaches the use of plant extracts is easily available, safe to handle and cost effective. An economic novel alternative choice for chemical and physical methods of nanoparticles synthesis is green method [5]. Zinc oxide nanoparticles are presently under intensive study for applications in the field of optical devices, sensors, catalysis, biotechnology, DNA labeling, drug delivery, medical, chemical and biological sensors and as catalyst. Nanosized ZnO has been used in sun screen coatings and paints because of its high UV absorption efficiency and transparency to visible light [6].

Increasing awareness towards green chemistry and biological processes has led to the development of an eco-friendly approach for the synthesis of nanoparticles. The plant phytochemical with antioxidant property is accountable for the preparation of Zinc oxide nanoparticle. To pursue a healthy life and space it is imperative to develop a green synthetic approach to obtain nanomaterials targeted on different applications. Dazzling to profuse interest this research opens with a short course on how to synthesize Zinc oxide nanoparticle in a natural scale [7].

Azadirachta indica commonly known as *neem* will achieve a height of 25-30m in a relatively short period of growth. Some of the most cautious researchers are saying that neem deserves to be called a wonder plant because of its antifungal, antidiabetic, antibacterial, antiviral, contraceptive property.

Neem oil is used to improve liver function, detoxify the blood and balance blood sugar levels and is considered to have no side effects. Active constituents of *neem* leaf extract include isomeldenin, nimbin, nimbinene, 6-desacetyllnimbinene, nimbandiol, immobile, nimocinol, quercetin, and beta-sitosterol.

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. 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 pathogens when compared to chemical ZnO nanoparticles [8-10]. *Emblica* belonging to the family Euphorbiaceae possesses anti-viral, antibacterial, anti-cancer and anti-allergy properties. Emblica tree is one of the most celebrated herbs in the Indian traditional medicine system.

The leaves are simple, finely pubescent, 12–18 cm long, light green. The flowers are greenish-yellow. The fruit is light greenish yellow, quite smooth, nearly spherical and hard on appearance. Traditional uses include as eye wash, appetite stimulant and restorative tonic to treat anorexia, indigestion, anemia. *Emblica* tree is becoming increasingly well known for its unusually high levels of vitamin C which is resistant to storage and heat damage during cooking. *Emblica* leaf ash contains zinc 4ppm.

Vitamin C component is actually the more stable and potent anti-oxidant. The use of *Emblica* as an antioxidant has been examined by a number of researchers [11-12]. To the best of our knowledge, the use of *Azadirachta* leaf extract and *Embilica officinalis* 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 and nanoflakes using different precursors like *Azadirachta* and *Embilica* leaf extracts. This way of synthesis is an ability of ours to manipulate green templates to achieve absoluteness in target-oriented green synthesis by way of getting only the desired substances, as far as possible. And the study was further initiated and reported that Zinc oxide nanoparticle can be used as an inexpensive and effective adsorbent for the removal of arsenic ions from aqueous solution [13].

MATERIALS AND METHOD: Leaves of *Azadirachta indica* and *Emblica officinalis* were collected in the month of May 2013 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 [14-15]. The qualitative examination of the aqueous extracts of the leaf sample of *Azadirachta indica* and *Emblica officinalis* showed the presence of phytochemical constituents such as Alkaloid, Carbohydrate, Glycoside, Steroid, Flavonoid, Terpenoid, Tannins and Steroid.

TABLE 1 PHYTOCHEMICAL CONSTITUENTS OF *AZADIRACHTA* AND *EMBLICA* AQUEOUS LEAF EXTRACT

S. No.	Phytoconstituents	Reagents	Aqueous leaf extracts of	
			Azadirachta	***Emblica***
1.	Alkaloids	Mayer's	+	+
		Wagner's	+	+
2.	Carbohydrates	Molisch's	+	+
		Benedict's	+	+
3.	Glycosides	Legal's	+	+
		Borntrager's	+	+
4.	Steroid	Liebermann Burchard's	+	+
5.	Fixed oils	Spot test	–	+
6.	Saponins	Gelatin	–	+
	Tannins	Lead acetate	–	+
		Ferry chloride	+	+
		Wagner's	+	+
7.	Protein	Millon's	–	+
		Biuret	–	+
8.	Flavonoids	Alkaline Reagent	+	+
		Shinoda's	+	+
9.	Terpenoids	Thionyl chloride	+	+

+ Presence; – Absence

EXPERIMENTAL SECTION:

Synthesis of ZnO nanoflowers and nanoflakes using *Azadirachta* and *Emblica*: ZnO nanoflower and nanoflakes were primed by green synthesis method. Zinc acetate dihydrate and sodium hydroxide was used as the preparatory material for nanoparticle synthesis. 0.02M aqueous Zinc acetate dihydrate was added to distilled water under vigorous stirring. After vigorous stirring aqueous leaf extracts of *Azadirachta* and *Emblica Officinalis* was introduced into the different sets of solution followed by the addition of aqueous 2.0M NaOH resulted in a white solutions at pH 12.

This was then separately sited in a magnetic stirrer for 2hr. The precipitates were filtered and washed continually with distilled water followed by ethanol to eliminate the impurities for the final product. Then the white powders of ZnO nanoflower and nanoflakes were obtained after drying at 60˚C in vacuum oven over night. The dried solids were ground in an agate mortar and stored for further use in an air tight container.

Characterization of ZnO nanoparticles: The crystalline phase formation and size of ZnO nanoparticles were analysed by X-Ray Diffraction (XRD) measurements which was carried out using (XRD, PW 3040/60 Philips X'Pert, Holland) with Cu(Kα) radiation (λ =1.5416 A˚) operating at 40 kv and 30 mA with 2θ ranging from 10- 90˚.The average particle size of ZnO nanoparticles was determined from XRD patterns of different ZnO samples according to Scherrer's equation. $D=k\lambda / \beta \cos \theta$ where k is a constant, λ is the X-ray wavelength which equals to 0.15416 nm, β the full width half maximum intensity (FWHM) and θ the half diffraction angle. Fourier Transform Infrared (FT-IR) Spectra was recorded on Jasco FT-IR5300 model spectrophotometer in KBr pellets. The particle size, external morphology of the sample and the elements present were characterized by Scanning Electron Microscope (SEM) (LEO 1530FEGSEM) and Energy Dispersive X-ray Diffractometer (EDX).

RESULTS AND DISCUSSION: X-ray powder diffraction (XRD) is a rapid methodical technique primarily used for phase identification of a crystalline material and provides information on unit cell dimensions. Structure and peak purity of the samples were acknowledged from XRD prototype of the synthesized ZnO nanoflowers and flakes from aqueous leaf extract of *Azadirachta and Emblica* from **Figure 1 and 2.** The peaks obtained for *Azadirachata* are analogous to (100), (002), (101), (102), (110), (103), (200), (112) and (201) planes in the hexagonal phase of ZnO. The XRD patterns of the ZnO nanoparticle are in high-quality accord with the values of standard card (JCPDS NO: 36-1451) [16]. 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 nanoflakes.

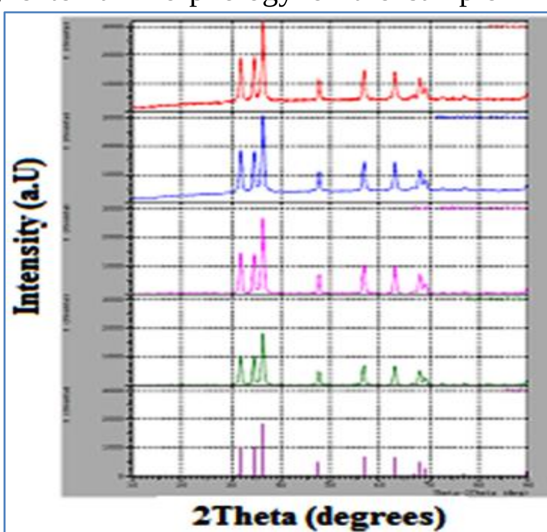

Figure 1 ***Azadirachta***

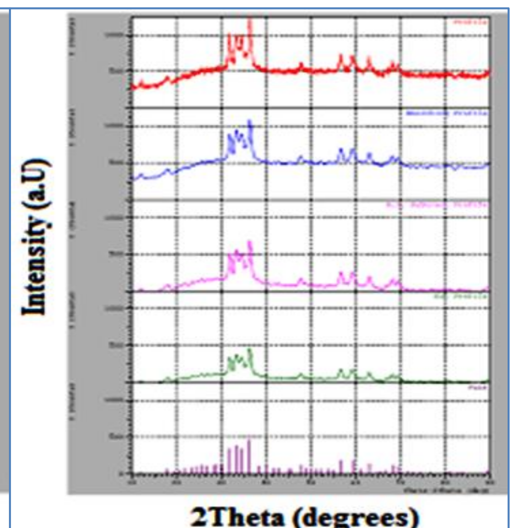

Figure 2 ***Emblica***

Figure 2 exemplify the X-ray powder diffraction patterns of zinc oxide nanoflakes synthesized by reaction of aqueous leaf extract of *Emblica officinalis* with zinc acetate dihydrate and sodium hydroxide. All peaks in the amorphous phase of *Emblica* shows scattered radiations leading to a small disturbance instead of high intensity narrower peak when compared with *Azadirachta* at (100), (002), (101), (102), (110), (103), (200), (112) and (201). The average particle size was about 16 nm which was probable by Debye Scherer's equation ($d=k\lambda /\beta \cos\theta$).

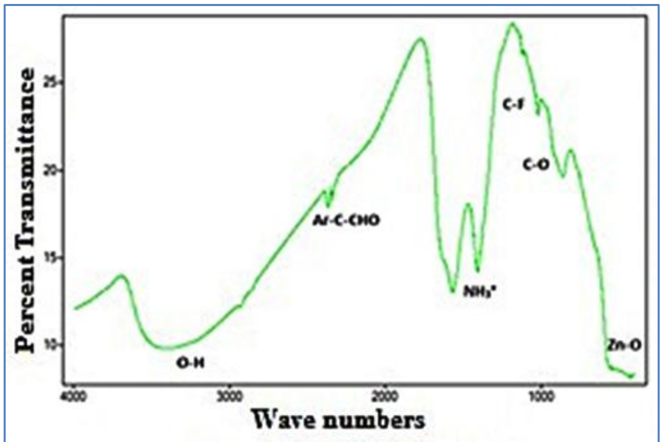

Figure 3 *Azadirachta*

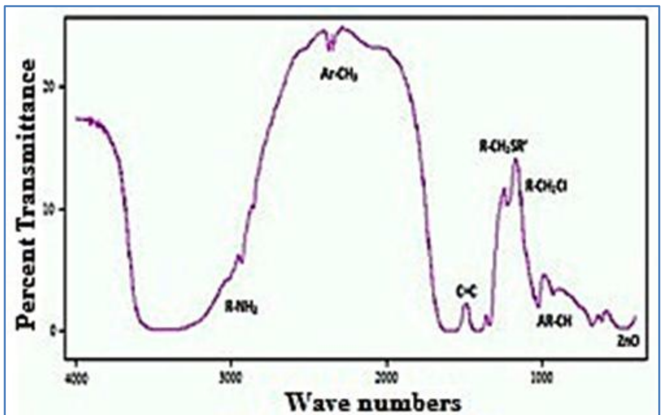

Figure 4 *Emblica*

FIGURE 3 & 4: FT-IR SPECTRUM OF GREEN SYNTHESIS OF ZNO NANO FLOWERS AND ZNO NANO FLAKES OF AQUEOUS LEAF EXTRACT OF *AZADIRACHTA* AND *EMBLICA*

FT-IR measurements were agreed out to identify the biomolecules for capping and proficient stabilization of the metal nanoparticles synthesized by *Azadirachta* leaf extract **(Figure 3)** The FTIR spectrum of Zinc Oxide nanoparticles absorbs at 408-550 cm^{-1}. 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. These bands are indicative of terpenoid group of compounds present in aqueous neem extract. 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).

Therefore the synthesized nanoparticles were surrounded by metabolites such as terpenoids having functional group of alcohols, ketones, aldehyde and carboxylic acids is confirmed. A clear ZnO stretching mode was observed in **Figure 4** at 417- 557 cm^{-1} [9]. The pattern of absorptions at 665 cm^{-1} matches up aromatic C-H. Absorption peak at 930cm^{-1} point out the aromatic stretching (out of plane bending). Primary amine ($R-NH_2$) show two N-H stretching bands in the range 3550-3300 cm^{-1}.

Bands at 1015 cm^{-1} symbolize the aromatic skeletal vibration. The corresponding CH_2 out of plane bending vibration characteristic of group R CH_2 SR' and RCH_2Cl group appears at 1213 cm^{-1} and 1325 cm^{-1} specify the presence of alkane. Absorption at 1617 cm^{-1} is due to C=C stretching vibration. Absorption at 2916 cm^{-1} is assigned to aromatic symmetric CH_3 stretching band. Band absorption near 2373 cm^{-1} corresponds to acid because of the overlapping of C-H.

From FTIR, results can be inferred that the bio-organics [10] like triterpenoids, sterols, alcohols, hydrocarbons, Phenolic compounds flavonoids, lignans, coumarins, tannins, quercetin, alkaloids, cynogenic glycosides from leaves formed a strong capping on the nanoparticles [17-20].

FT-IR study of dry powdered test drug in KBR pellet was investigated to confirm the presence of functional groups.

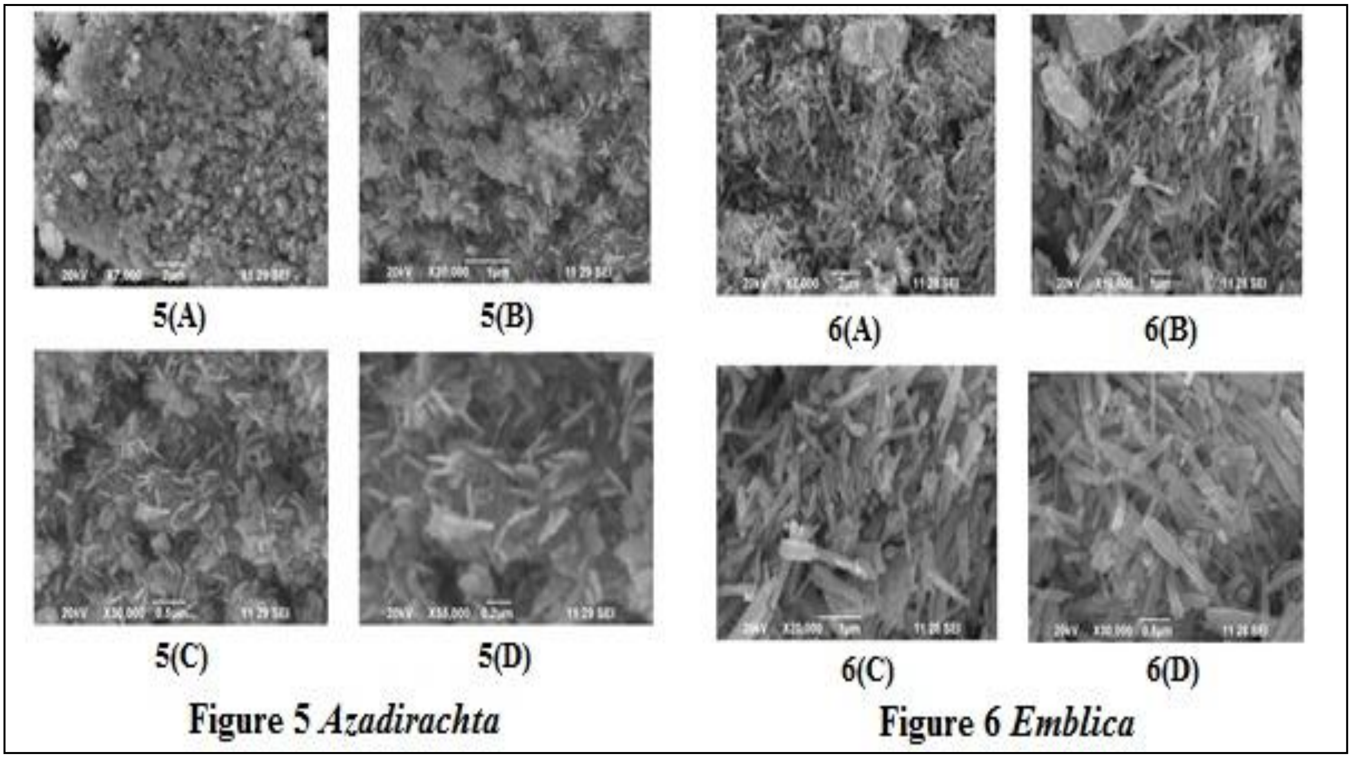

FIGURE 5 & 6: (A), (B), (C) AND (D) REPRESENTATIVE SEM IMAGE OF THE AS-SYNTHESISED ZNO NANOFLOWERS AND NANO FLAKES

SEM image of ZnO nanoparticles synthesized using aqueous leaf extract of *Azadirachta indica* and *emblica officinalis* via green route are shown in the **Figure 5(A), 5(B), 5(C), 5(D) and 6(A), 6(B), 6(C), 6(D).** The low magnified observation **5(A), 5(B) and 6(A), 6(B)** shows that the morphology is hierarchically nano structured flowers and flakes ranging from 0.2-2µm in diameter. Closer observation **5(C), 5(D) and 6(C), 6(D)** shows that the nanoparticles have flower morphology ranging particle size from 100-200 nm. It is obvious that aqueous leaf extract of *Azadirachta* and *Emblica officinalis* acts as a stabilizing agent prevents the nanoflowers and nanoflakes from agglomeration consists of a large quantity of dispersive homo size nano flowers and flakes. The highly magnified SEM image substantiate the approximate flower and flake shape to the nanoparticles and most of the particles exhibit some faceting ranging from 100-200nm [21].

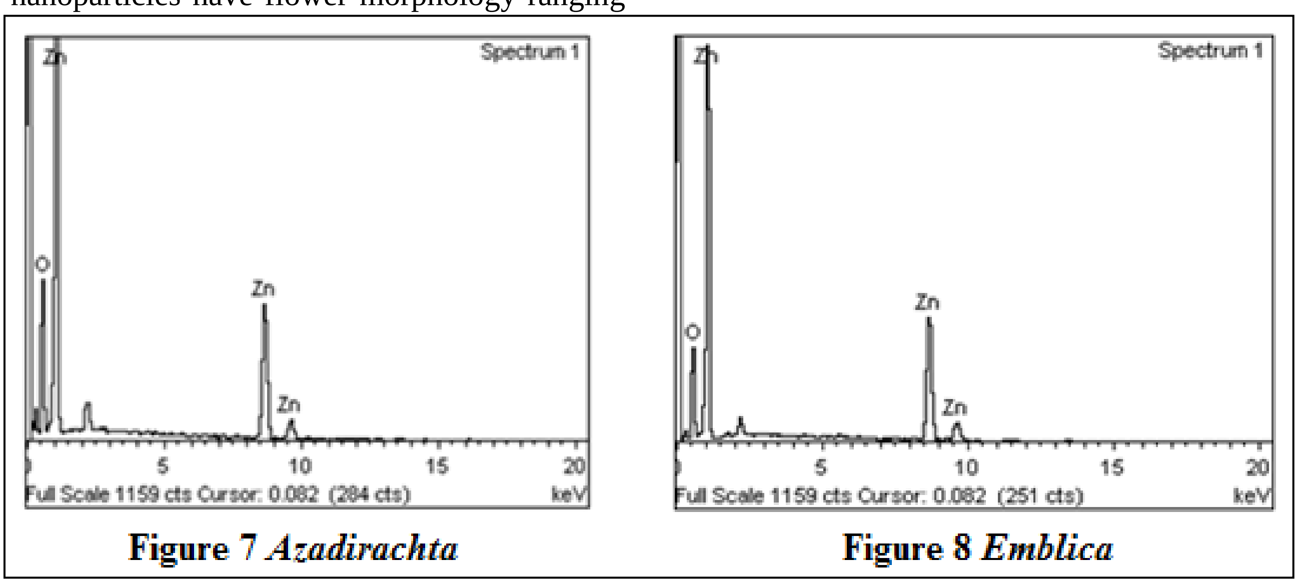

FIGURE 7 AND 8 THE ELEMENTAL SPECTRA OF ZNO NANOFLOWERS AND NANOFLAKES REVEALED BY EDX ANALYSIS OF *AZADIRACHTA AND EMBLICA*

Figure 7 and **8** gives the elemental spectra of ZnO nanoparticles exposed by EDX analysis. It specifies that the ZnO quartzite structure is only composed of two elements Zinc and Oxygen. The XRD, SEM and FT-IR pattern is consistent with the EDX results reported [22-23]. Owing to this reason, the progress of green chemistry with the use of plants in the synthesis of nanoparticles has engrossed a great attention. 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.

CONCLUSION: The activities of leaf extracts of two different plants like *Azadirachta indica* and *emblica officinalis* in the synthesis of Zinc oxide nanoparticles was evaluated. Stable Zinc oxide nanoflowers and nanoflakes were formed by exposing the leaf extract of a foresaid plants to the aqueous zinc acetate dihydrate solution. The prominent doublet absorption at 2921 cm^{-1} indicates C-H stretching vibration of an aromatic aldehyde. These bands are indicative of terpenoids group of compounds present in aqueous neem extract which could act as a stabilizer. Diffraction XRD pattern confirmed that the synthesized ZnO nanoflowers using *Azadirachta indica* shows high-quality accord but peaks in the amorphous phase of *Emblica* shows scattered radiations leading to a small disturbance instead of high intensity narrower peak when compared with *Azadirachta.*

The grain sizes were found to have diameter of 51 and 16 nm respectively for *Azadirachta* and *Emblica.* The scanning electron microscope (SEM) image revealed by uniformly distributed zinc oxide nanoparticles were found on the surface without agglomeration. Further studies are warranted to analyse the applications of synthesized ZnO nanoparticles for water remediation. This way of synthesis is an ability of ours to manipulate green templates to achieve absoluteness in target-oriented green synthesis by way of getting only the desired substances without pollutant.

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