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GREEN SYNTHESIS OF ZINC-OXIDE NANOPARTICLES USING ETHANOLIC EXTRACT OF PELTOPHORUM PTEROCARPUM LEAVES

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ABSTRACT

ZnO is a transparent electrode material that has been employed in emerging applications of transparent electrodes in liquid crystal displays, as well as in heat-absorbing or energy-saving windows. The physical, optical and electrical properties of zinc oxide are unique among transition metal oxides. Pharmaceuticals, electronics, consumer goods, optical and electrical devices, and environmental remediation are among the many fields in which they can be used because of their unique properties. ZnO is also being used in electronics for thin-film transistors and light-emitting diodes

Keywords: Green Synthesis, Zinc-Oxide, Nanoparticles, *Peltophorum Pterocarpum* leaves

INTRODUCTION

Herbal medicine has become an integral part of standard healthcare based on combination of traditional usage and ongoing scientific research. Burgeoning interest in medicinal herbs has increased scientific scrutiny of their therapeutic potential and safety. Natural products and medicinal plants are believed to be an important source of new chemical substances with potential therapeutic efficacy [1].

Zinc oxide has the formula ZnO and is an inorganic compound. White powder, nearly insoluble in water, it is usually found as a white powder. A variety of materials and products use this powder as an additive, including plastics, ceramics, glass, cement, rubber (e.g. car tyres), lubricants, paints, ointments, adhesives, sealants, pigments, batteries, ferrites, fire retardants, etc. ZnO occurs naturally in the Earth's crust as zincite, but is mostly produced synthetically for commercial purposes. A semiconductor with a second group of zinc and a sixth group of oxygen is called ZnO in materials science.

A number of researchers are focusing on the morphology of nano ZnO to achieve their intended purposes. Researchers focus on controlling the size and shape of nanostructures during synthesis. Chemical, physical, and biogenic synthesis techniques are classified into three categories (green route).

Nanoparticles can also be synthesized by controlled precipitation from precursors dissolved in solutions. Through hydrophobic versus hydrophilic forces, a micro emulsion can also be formed between two immiscible liquids, using surfactants. As a result,

nanoparticles form a colloidal suspension. Thermodynamic factors and van der Waal's forces combine to cause particle growth and agglomeration, resulting in larger particles that settle down over time. The stability of colloidal nanoparticles in colloidal suspension is a prerequisite for their use.

The stabilization mechanism of nanoparticles can be classified as either a) electrostatic stabilization, involving the formation of an ion layer on top of the nanoparticles, resulting in coulombic repulsion between adjacent nanoparticles; or b) steric hindrance, involving the adsorption of polymer molecules over the nanoparticles. In the presence of polymer coated nanoparticles, osmotic repulsion feels by polymer molecules due to localized changes in osmotic concentration keeps them (and the nanoparticles) separated [2].

Methodology

Materials And Methods

Pharmacognostical Studies

Morphological characterization of medicinal plants has always been accorded due credentials in the Pharmacognostical studies. Botanical identity of the plants is an essential prerequisite for undertaking the analysis of medicinal properties of any plant. A researcher may succeed in getting a new compound or may find many useful pharmacological active properties in the plant. If the botanical identity of the plant happens to be dubious or erratic, the entire work on the plant becomes invalid [3].

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Macroscopic Evaluation

Various organoleptic characters like colour, odour, taste and nature and thickness were observed.

Physico-Chemical Evaluation

The shade dried powdered plant material of *Peltophorum pterocarpum* was used for the analysis of various physiochemical parameters which is useful in the determination of quality and purity of crude drugs. Ash values, extractive values, loss on drying and foreign organic matters were determined as per the standard WHO guidelines which is very much useful in the determination of quality and purity of the crude drugs [4].

Determination of Ash Values

Ash values are helpful in determining the quality and purity of a crude drug in the powdered form. The residue remaining left after incineration of the crude drug is designated as ash. The residue obtained usually represents the inorganic salts naturally occurring in the drug and adhering to it. An ash value determination furnishes the basis for judging the identity and cleanliness of any drug and gives information relative to its adulteration/contamination with inorganic matter.

Total ash

The determination of ash is useful for detecting low grade products, exhausted drugs & excess of sandy and earthy material. Total ash is useful to exclude drugs which have been coated with chalk, lime or calcium sulphate to improve their appearance. The total ash usually consists of carbonates, phosphates, silicates and silica which include both physiological ash, which is derived from plant tissue itself and non-physiological ash which is the residue of the extraneous matter adhering to the plant surface.

Procedure

Silica crucible was heated to red hot for 30 minutes and it was allowed to cool in desiccators.

About 2g of powdered sample was weighed accurately and evenly distributed in the crucible. Dried at 100 – 105°C for 1 hour and ignited to constant weight at 600±25°C. The crucible was allowed to cool in a desiccator. The percentage yield of ash with reference to the air-dried substance was then calculated.

$$\% \text{ Total ash value} = \frac{\text{Wt. of total ash}}{\text{Wt. of crude drug taken}} \times 100$$

Water soluble ash

The ash was boiled for 5min with 25mL of water. The insoluble matter was then collected in an ash less filter paper. It was washed with hot water and ignited for 15min at a temperature not exceeding 450°C. The weight of the insoluble matter was subtracted from the weight of the ash and the difference in weight represented the water-soluble

ash, the percentage of water soluble ash with reference to the air dried substances was calculated by the formula.

$$\% \text{ Water soluble ash value} = \frac{\text{Wt. of total ash} - \text{Wt. of water insoluble ash}}{\text{Wt. of crude drug taken}} \times 100$$

Acid insoluble ash

Acid insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid, and igniting the remaining insoluble matter. This measures the amount of silica present, especially as sand and siliceous earth. Acid-insoluble ash is useful for detecting the presence of excessive earthy material.

Procedure

To the crucible containing total ash of the sample, 25 mL of dilute hydrochloric acid was added. The insoluble matter is collected on an ashless filter paper (Whatman 41) and washed with hot water until the filtrate is neutral. Filter paper containing the insoluble matter to the original crucible dry on hot plate and ignite to constant weight. The residue is allowed to cool in a suitable desiccator for 30 minutes and weighed without delay. Content of acid insoluble ash with reference to the air dried drug is calculate

$$\% \text{ Acid insoluble ash value} = \frac{\text{Wt. of water insoluble ash}}{\text{Wt. of crude drug taken}} \times 100$$

Sulphated ash

About 3g of air-dried substance was ignited gently at first in a crucible, until the substance was thoroughly charred. Then the residue was cooled, moistened with 1mL of sulphuric acid, heated gently until the white fumes were no longer evolved and ignited at 800 ± 25°C, until all the black particles were disappeared. The crucible was allowed to cool, a few drops of sulphuric acid was added and heated. Then it was ignited as before, cooled and weighed. The percentage of sulphated ash with reference to the air-dried substance was then calculated.

Determination of extractive values

This method is used to determine the number of active constituents in a given amount of plant material when extracted with solvents. Extractive values are useful for the evaluation of phytoconstituent especially when the constituents of a drug cannot be readily estimated by any other means. Further these values indicate the nature of the active constituents present in a crude drug.

Determination of Water-Soluble extractive

About 5g of the powder was weighed and macerated with 100mL of chloroform water (95mL distilled water and 5mL chloroform) in a closed flask for 24 hours. It was shaken frequently for six hours and allowed to stand for eighteen hours. It was then filtered

rapidly, taking precautions against loss of solvent and 25mL of the filtrate was evaporated to dryness in a tarred flat-bottomed shallow dish. 2 mL of alcohol was added to the residue and it was dried at 105°C for 1 hour in the hot air oven and cooled in desiccators for 30min and weighed. The process was repeated till a constant weight was obtained; the percentage of water-soluble extractive value with reference to the air dried drug was calculated.

$$\text{Water soluble extractive value} = \frac{\text{weight of the dried extract}}{\text{weight of the sample taken}} \times 100$$

Determination of alcohol soluble extractive

The alcohol soluble extractive value is also indicative for the same purpose as water soluble extractive value. The solvent strength of alcohol varies from 20-90% v/v. The solvent strength has to be chosen depending upon the strength of alcohol used for the extraction of powdered drug [5].

Procedure

5g of the powder was weighed and macerated with 100mL 90% ethanol in a closed flask for 24 hours. It was shaken frequently for six hours and allowed to stand for eighteen hours. It was then filtered rapidly, taking precautions against loss of solvent and 25mL of the filtrate was evaporated to dryness in a tarred flat-bottomed shallow dish. It was dried at 105°C for 1hour in a hot air oven. The dish was cooled in desiccator and weighed. The process was repeated till the constant weight was obtained. The percentage of alcohol soluble extractive value with reference to the air-dried drug was calculated.

$$\text{Alcohol soluble extractive value} = \frac{\text{weight of the dried extract}}{\text{weight of the sample taken}} \times 100$$

Determination of ether soluble extractive

About 2g of powdered drug was accurately weighed and extracted with anhydrous diethyl ether in a continuous extractive apparatus for 20hours. The ether solution was transferred to tarred porcelain dish and evaporated spontaneously. Then it was dried over phosphorous pentoxide for 18hours and the total ether extract was weighed. The extract was heated gradually and dried at 105°C to constant weight. The loss in weight represents the volatile portion of the extract.

Determination of moisture content (loss on drying)

The crucible with the contents was weighed accurately. The loaded crucible and the lid were placed in the drying chamber (105°C). The substance was heated for a specified period of time to a constant weight. The crucible was covered with the lid and allowed to cool in a desiccator at room temperature before weighing. Finally, the crucible was weighed to calculate the loss on drying with reference to the air-dried substance

$$\% \text{ Loss on drying} = \frac{\text{Loss in weight of the sample}}{\text{Weight of the sample}} \times 100$$

PREPARATION OF ETHANOLIC EXTRACT OF *Peltophorum pterocarpum* LEAVES (EEPPL)

The leaves of *Peltophorum pterocarpum* was collected, shade dried and coarsely powdered. The powdered leaves were defatted with petroleum ether and then the defatted marc was allowed to macerate using 95% ethanol for about 72 hours. The extracts were filtered through whattman filter paper No. 42 (125 mm) to remove all non-extractable matter, including cellular materials and other constitutions that are insoluble in the extraction solvent. The entire extracts were concentrated to dryness using a buchi rotary evaporator under reduced pressure. The final dried samples were stored in an air tight and labelled container [6].

Qualitative estimation

Phytochemical Investigations

Ethanollic extract of *Peltophorum pterocarpum* is subjected to qualitative chemical analysis. The various chemical tests were performed on this extract for the identification of flavonoids, phenolic compounds, alkaloids, glycosides, carbohydrates, carotenoids, proteins, tannin, aminoacids, and sterols as per standard procedure.

ZnO Nanoparticles preparation

Zinc nitrate and glassware was purchased from Merck chemical reagent Co. Ltd. India. All the glassware was washed with sterile distilled water and dried in a hot air oven before use [7].

Synthesis of ZnO nanoparticles

For the synthesis of nanoparticle, 50ml of *Peltophorum pterocarpum* leaves extract was taken and boiled to 60-80°C using a stirrer-heater. 5g of Zinc Nitrate was added to the solution as the temperatures reached 60°C. This mixture is then boiled until it reduced to a deep yellow coloured paste. This paste was then collected in a ceramic crucible and heated in an air heated furnace at 400°C for 2 hours. A light yellow coloured powder was obtained and this was carefully collected and packed for characterization purposes. The material was mashed in a mortar-pestle so as to get a finer nature for characterization [8].

Characterisation of prepared ZnO Nanoparticle UV-Visible spectroscopy

The optical property of synthesized ZnO nanoparticles was observed from the absorption spectra of nanoparticles synthesized at various temperatures and concentrations. This is characterized using an Ultraviolet-Visible Spectrometer (Cary Series, Agilent Technology) with a wavelength in the range of 200 nm–800 nm. UV light for LEDs can also be used for spectroscopy purposes.

They are of three types. UV-A (400 nm–315nm), UV-B (315 nm–280 nm) and UV-C (280 nm – 100 nm). Latter is more environmentally friendly, but the former is better in cost effectiveness and can be used for generic purposes. So, we chose UV–Visible spectroscopy for our analysis as UV-LEDs are application specific.

Fourier Transform Infra-Red spectroscopy

Fourier transform infrared spectroscopy (FTIR) was recorded in the range of 400–4000 cm^{-1} Nicolet IS10 (Thermo Scientific, USA). The various modes of vibrations were identified and assigned to determine the different functional groups present in the *P. pterocarpum* leaves extract.

X-Ray Diffraction (XRD) Analysis

The formation of ZnO NPs was determined by an X-ray diffractometer (PAN analytical BV) operated at a voltage of 40 kV and a current of 30 mA with Cu K α radiation in $\theta - 2\theta$ configurations. The crystallite domain size was calculated from the width of the XRD peaks, assuming that they are free from non-uniform strains, using the Scherrer formula. $D = 0.94 \lambda / \beta \cos \theta$, where D is the average crystallite domain size perpendicular to the reflecting planes, λ is the X-ray wavelength, β is the full width at half maximum (FWHM), and θ is the diffraction angle.

Scanning electron microscopy

Scanning electron microscopy (SEM) analysis was done using Hitachi S-4500 SEM machine. Thin film of sample was prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper, and then the film on a SEM grid was allowed to dry putting it under a mercury lamp for 5 minutes [9].

Results And Discussion

Pharmacognostical Studies

Pharmacognostical studies play a key factor in establishing the authenticity of the plant material. The botanical identity of the plant was established by examining its anatomical features. The results of the pharmacognostical studies are as follows

Organoleptic characterization of *Peltophorum pterocarpum* leaves

Characterization of *Peltophorum pterocarpum* leaves were done.

Assessment of Quality of Plant Material

Determination of Foreign Matter

The powder material was found to be free from contamination by moulds, insects or other animal contamination.

PHYSIOCHEMICAL EVALUATION

Physiochemical parameters are mainly used in judging the purity and quality of the powdered drug. The

physiochemical standards of *Peltophorum pterocarpum* were listed below.

The above results were given that the foreign organic matter of the crude drug material was found to be nil, the percentage of moisture was found to be $9.35 \pm 0.155\%$ w/w, and the percentage of extractive value such as ether, ethanol, aqueous was found to be $1.30 \pm 0.059\%$, $14.30 \pm 0.215\%$, and $12.89 \pm 0.328\%$ respectively.

The determination of the ash value helps to find out where the powder material was adulterated with sand and other organic material. The percentage of ash value such as total ash, water soluble ash, acid insoluble ash and sulphated ash was found to be $12.11 \pm 0.121\%$ w/w, $4.78 \pm 0.047\%$ w/w, $9.21 \pm 0.115\%$ w/w and $14.31 \pm 0.12\%$ w/w, respectively.

Phytochemical Investigations

Preliminary Phytochemical Screening of Ethanolic Extract of *Peltophorum pterocarpum* (EEPPL)

Ethanolic extract of *Peltophorum pterocarpum* was subjected to qualitative chemical analysis. The various chemical tests were performed for EEPPL extract for the identification of phytoconstituent.

The phytochemical screening of the ethanolic extract (95%) of *Peltophorum pterocarpum* (EEPPL) revealed the presence of alkaloids, carbohydrate, proteins, sterols, flavonoids, tannins, saponins, and absence of glycoside, volatile oil, gum and mucilage.

Characterization of prepared Zinc oxide Nanoparticles UV-Visible spectroscopy

A secondary metabolite in plants reduces zinc ions in the solution by converting zinc ions to zinc oxide. Additionally, the plant extract acts as a stabilizing and reducing agent. A UV–visible spectrum analysis was conducted in the range of 280 nm–800 nm to confirm this. ZnO nanoparticles show a peak at 320 nm in the spectrum. In the case of ZnO nanoparticles, the absorbance peak is reported between 310 nm and 360 nm. The higher concentration of plant extract in Sample A results in greater absorption. Sample B corresponds to the graph in fig: 8 that show a smaller absorption peak. It is possible to see two absorption peaks in sample B itself. Initially, sample B was taken for analysis because of its slightly higher absorption peak. As time increases, sample B's lowermost graph shows that the nanoparticles settle down at the bottom, thereby decreasing absorption. We considered sample A further for characterization since it showed a higher absorbance due to its higher concentration. The band gap energy was calculated using $E_g = 1240/\lambda$ eV and found to be 3.8 eV which is comparable to the previously reported values of energy band gap for ZnO nanoparticles.

Fourier Transform Infra-Red spectroscopy

The FTIR spectra of synthetic ZnO NPs are displayed in Fig 9. An absorption band at 3495.01 cm^{-1} was visible in the FTIR spectra and corresponded to the alcohol

group's O-H stretching. Similar to this, Vijaykumar *et al.* observed an absorption peak for ZnO NPs produced by *Plectranthus amboinicus* leaf extract at 3374 cm^{-1} , which matches to the hydroxyl functional group in alcohols and phenolic compounds. The stretching of the alkane group is represented by the absorption bands at 2918.30, 1641.42, and 1384.89 cm^{-1} , respectively. The features of the C=O stretching of the alcohol group can be seen in the absorption band at 1130.29 cm^{-1} . The alkane group's =C-H bending was revealed by the absorption peaks at 918.12, 815.89, and 677.01 cm^{-1} . Similar to this, Nagajyothi *et al.* found an alkynes compound-related absorption maximum at 630.93 cm^{-1} in ZnO NPs made utilising *Coptidis* rhizome. The features of the C-Br bending of the alkyl group were indicated by the absorptions at 651.94, 582.50, and 543.93 cm^{-1} . The reduction of ZnO NPs may be caused by a variety of phytochemicals found in *Peltophorum pterocarpum* leaves extract, including phenolic substances, flavonoid, saponins, steroids, tannins, xanthoproteins, carboxylic acids, coumarins, and carbohydrates. Alkaloids, saponins, tannins, and flavanoids found in *Zingiber officinale* extract are said to be responsible for the stability and decrease of ZnO NPs, according to Janaki *et al.*

X-Ray Diffraction (XRD) Analysis

ZnO NPs' X-ray diffraction pattern. The 100, 002, 101, 102, 110, 103, 112 and 201 planes, respectively, could be attributed to the peak position in the XRD spectrum, which had 2 θ values of 31.827, 34.497, 36.324, 47.611, 56.668, 62.914, 68.026 and 69.153. When compared to the information from JCPDS card No. 36-1451, all of the diffraction peaks supported the hexagonal wurtzite structure of ZnO NPs. The diffraction peaks, which were both strong and narrow, supported the crystalline nature of the produced ZnO NPs. Peaks at 2 values and the associated planes in the current study supported the use of *P. pterocarpum* leaves extract in the synthesis of ZnO NPs. ZnO NPs were produced utilising *Azadirachta indica*, according to Elumalai and Velmurugan, who obtained identical diffraction peaks.

Scanning electron microscopy

Synthesized ZnO NPs observed at different magnifications of 10.05 and 30.25 K. In the SEM images, spherical and irregular nanoparticles were observed. The average size of the ZnO NPs was 69.45 nm. Particle sizes of 50–100 nm were observed in ZnO NPs synthesized from *Punica granatum* peel extract.

Table 1: Organoleptic characterization of *Peltophorum pterocarpum* leaves

S.NO	Characteristics	Observation
1	Colour	Fresh- Green Dried leaf- Yellowish green
2	Shape	Oblong
3	Leaf type	Bipinnate
4	Margin	Entire
5	Apex	Obtuse to retuse
6	Base	Truncate
7	Beneath	Puberulent
8	Midrib	Prominent

Table 2: Ash Values of leaf powder of *Peltophorum pterocarpum*

S.No	Particulars	Ash Value of leaves powder (%w/w)
1	Total ash	12.11±0.121 % w/w
2	Acid insoluble ash	9.21±0.115 % w/w
3	Water soluble ash	4.78±0.047 % w/w
4	Sulphated ash	14.31±0.12 % w/w

Values are expressed as Mean ± SD, n=3

Table 3: Extractive Values of leaf powder of *Peltophorum pterocarpum*

S.No	Extractive value	Results (%w/w)
1	Water soluble extractive	12.89±0.328%
2	Alcohol soluble extractive	14.30±0.215%
3	Ether soluble extractive	1.30±0.059%

Values are expressed as Mean ± SD, n=3

Table 4: Moisture content of leaf powder of *Peltophorum pterocarpum*

S.No	Parameter	Results (%w/w)
1	Loss on drying	9.35±0.155% w/w

Values are expressed as Mean ± SD, n=3

Table 5: Preliminary phytochemical screening of EEPPL

S. NO	TESTS	RESULTS
1	ALKALOIDS	+

2	CARBOHYDRATES	+
3	GLYCOSIDES	-
4	STEROLS	+
5	FLAVONOIDS	+
6	TERPENOIDS	+
7	GUM	+
8	MUCILAGE	+
9	PROTEIN AND AMINO ACIDS	+
10	SAPONINS	+
11	TANNINS	+
12	GELATIN/FAT	-
13	VOLATILE OIL	-

Table 6: Results of XRD analysis at different angles

S. No	2θ	FWHM (β)	Miller Indices	Particle size
1	31.57	0.00230	100	60.30
2	34.24	0.00230	002	60.30
3	36.07	0.00234	101	59.27
4	47.36	0.00244	102	56.84
5	56.42	0.00279	110	49.75
6	62.69	0.00300	103	46.23
7	67.77	0.00326	112	42.55
8	68.91	0.00325	201	42.71

Figure 1: Schematic diagram of ZnO Nanoparticles preparation

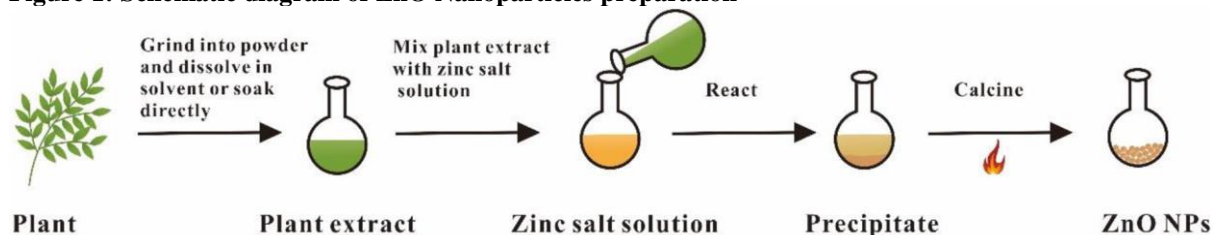


Figure 2: UV-Visible spectra of ZnO nanoparticles at two different concentrations

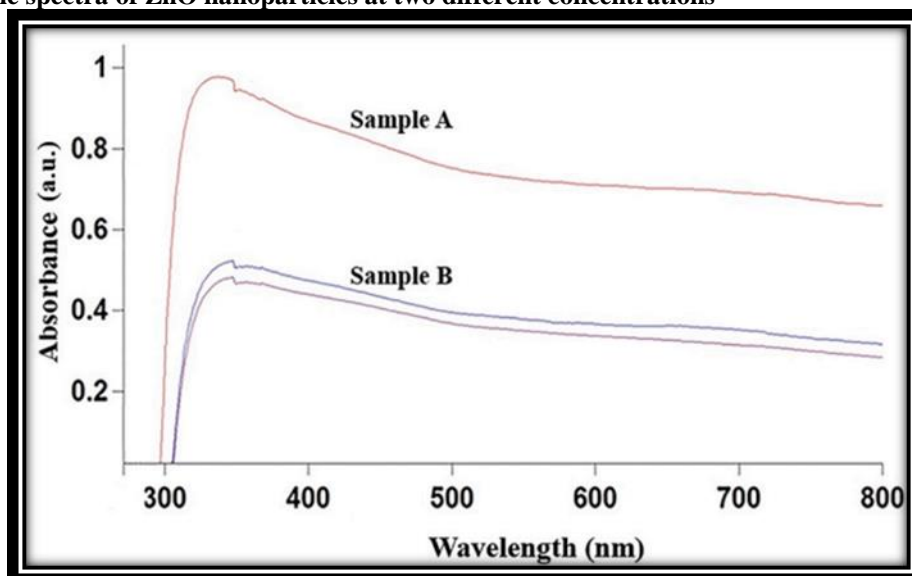


Figure 3: FTIR Spectrum of ZnO Nanoparticles

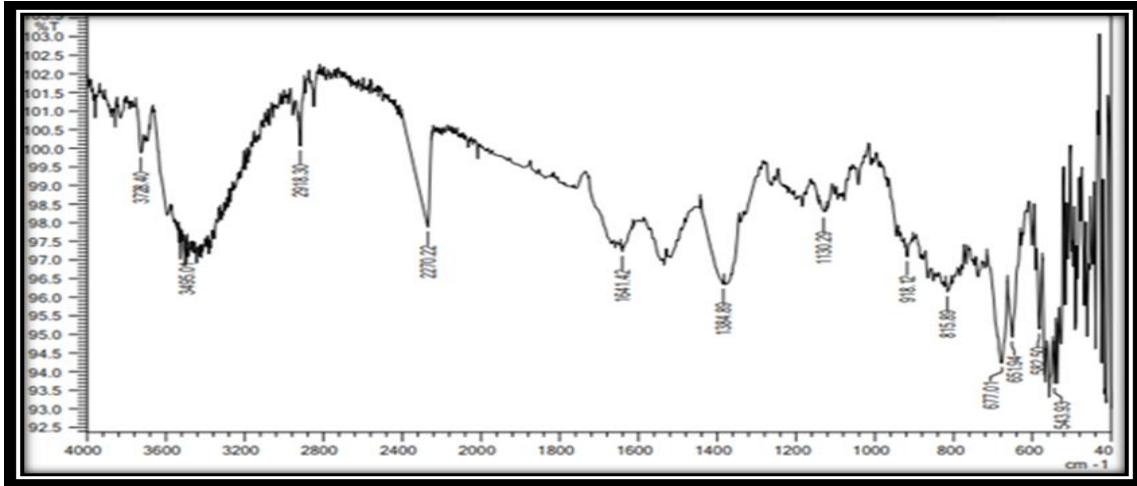


Figure 4: XRD Patterns of ZnO NPs

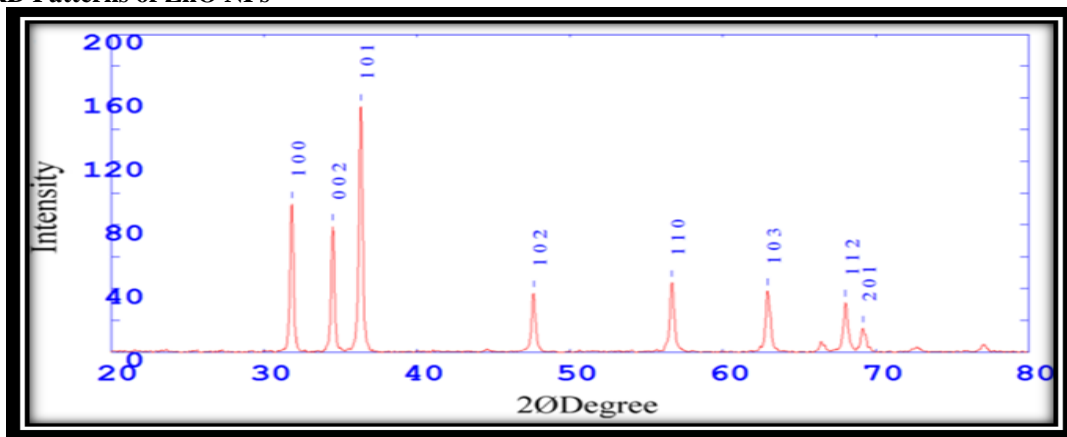
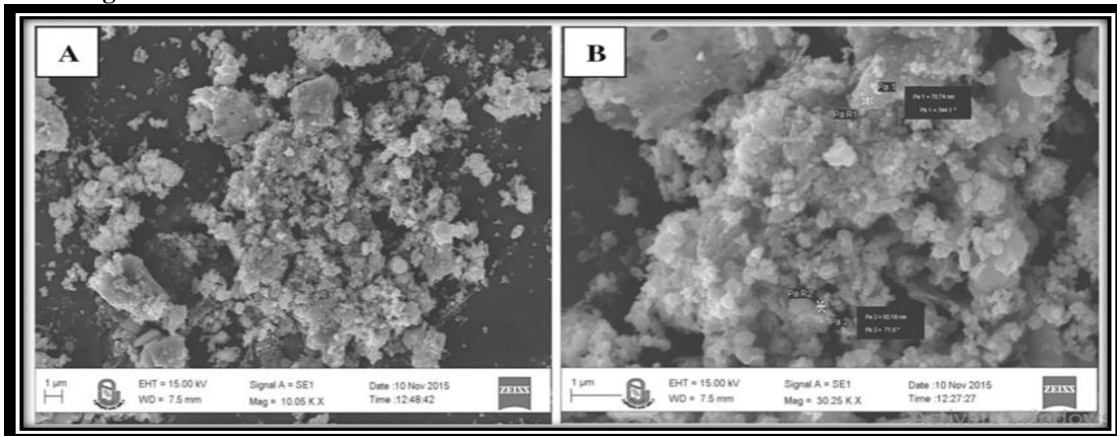


Figure 5: SEM Images of ZnO NPs



CONCLUSION

It is becoming increasingly important for materials scientists, chemists, and biologists to produce metal nanoparticles biologically. In spite of their long history of chemical and physical production, metal nanoparticles have only recently been investigated biologically. Metals have been biologically reduced by plant extracts since the early 1900's; however, the reduction products have not been studied. Plant extracts have recently attracted considerable attention as green methods for synthesizing ZnO NPs due to their

environmental benefits, nontoxicity, and low cost. By using plant extracts instead of harmful and toxic reducing and stabilizing agents, the environment is protected. The presence of strong oxidizing substances is required for the formation of zinc nanoparticles in ions. Nanoparticle stability will be affected by the environmental conditions. There needs to be further research on the mechanism of nanoparticle formation in order to fine tune the synthesis process ultimately leading to nanoparticles whose size and shape parameters can be tightly controlled. The synthesis of ZnO nanoparticles is still in its infancy.

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