SYNTHESIS OF ZINC OXIDE NANOPARTICLES USING *MORINGA OLIEFERA* FLOWER EXTRACTS AND EVALUTION OF ITS ANTIMICROBIAL ACTIVITY

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Abstract

Nanotechnology is most dynamic fields of in material science it deals with matter having at least one dimension sized from 1-100nm. Plant mediated synthesis of nanoparticles is called green synthesis of nanoparticles. Nanoparticles were synthesized by various Biological and chemical method. In Biological method nanoparticle are synthesized by using plant extracts, enzymes and agricultural waste. Zinc Oxide is an organic compound with the formula Zno.. Widely used in materials and products rubber, Plastics, glass, food, ointments, etc. Moringa oleifera is a medium sized evergreen tree that is native Africa and Asia. Moringa oleifera belongs to the family moringaceae. Moringa have incrediable medicinal properties. It contains proteins, vitamins and minerals. It act as an antioxidant. Moringa is used for "tired blood" (anemia) arthritis and other joint pain (rheumatism) , diarrhea, epilepsy, fungal, viral and parasitic infections. The plant is endowed with immense secondary metabolites and astonishing nutritive properties. The different parts of the plants have already been exploited for the synthesis of various metal nanopaticles with valuble bioactive mechanisms. Silver nanoparticles from M.Oleifera have been reported to induce apoptosis of human cervical carcinoma cells. Phytochemicals present in the Moringa Oleifera plant. Phytochemicals were involved in the Anti fungal and Anti Bacterial activity. To study the characterization of synthesized Zinc oxide nanoparticles using FTIR, XRD and SEM. Structure and phase purity of the sample was identified from X Ray Diffraction patterns. The Scanning Electron Microscope analysis was used to determine the structure and particle size of the reaction products that were formed. Energy-Dispersive X-ray spectroscopy is an analytical technique used or the elemental analysis or chemical characterization of a sample. The green synthesis of nanoparticles were reported to had advancement over chemical and physical methods. Our proposed study aimed on synthesis of silver nanoparticles using extracts of Moringa Oleifera flower.
KEY WORDS: Moringa Oleifera, zinc oxide, phytochemical, green synthesis

INTRODUCTION

An important aspect of nanotechnology concerns the development of experimental processes for the synthesis of nanoparticles of different sizes, shapes and controlled dispersity. With the development of new chemical or physical methods, the concern for environmental contaminations are also heightened as the chemical procedures involved in the synthesis of nanomaterials generate a large amount of hazardous by products. Thus, there is a need for 'green chemistry' that includes a clean, nontoxic and environment-friendly method of nanoparticle synthesis [1]. "Green nano-synthesis" which is based on using ecofriendly reagents or biogenic processes, reduces the pollution risk at source level and avoids waste rather than treat or clean it up after it is formed. More accurately, green synthesis processes whereby the precursor of the nano-material to be synthesized is reduced effectively via a biochemical interaction with active compounds of the natural extracts, is gaining momentum [2,3]. oxide NPs revealed antimicrobial activities even at minor concentrations, appropriate for thin coating applications [4]. Chemical techniques of synthesis of NPs are also excessive in the atmosphere [5]. So, for many causes, mainly for eco-friendly benefits, the green synthesis techniques or green preparation of NPs has been deliberated by scholars [6]. *Moringa oleifera* is filled in nourishment due to the presence of essential phytochemicals. The phytochemical profile of its leaves exhibited the presence of essential minerals, vitamins, sterols, anthraquinones, alkaloids, terpenoids, flavonoids, tannins, and saponins [7]. These phytoconstituents lead to anti-inflammatory, antiulcer, antidiabetic [8], anticancer [9], antimicrobial, antioxidant, and antifungal properties [10]. The present study brought a biomimetic approach for the green synthesis of ecofriendly zinc

oxide nanoparticles from *Moringa oleifera* through bioreduction, UV-visible spectroscopy, scanning electron microscopy, X-ray diffraction characterization, and antimicrobial studies.

MATERIALS AND METHOD

Preparation of flower extract

Moringa oleifera plant flowers were collected from the surroundings of kangeyam. The flowers washed several times with water to remove the dust particles and then dried light. The extract used for the reduction of zinc ions (Zn2+) to zinc nanoparitcles (Zno) was prepared by placing 50g of washed dried flowers in 500ml of glass beaker along with 200ml of double distilled water. The flower until was then boiled for 60,minute until the colour of the aqueous solution changes from watery to light yellow by using sodium hydroxide magnetic stirrer. The extract was cooled to room temperature and filtering using filter paper. The extract was stored in a refrigerator in order to be used for further experiments.

Preparation of Zinc Nanoparticles

For the synthesis of nanoparticles, 10ml of *Moringa oleifera* flower extract was taken and boiled to 60-80 degree of Celsius using a stirrer-heater. 0.4 grams of zinc acetate dehydrate salt was added to the solution as the temperatures reached 60 degree Celsius. This mixture is then boiled until is reduced to deep yellow coloured paste. This paste was then collected in a ceramic crucible and heated in an air heated micro wave oven at 400 degree Celsius for 2 hours. A light yellow coloured powered 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.

Characterization Techniques of ZnO Nanoparticle:

Fourier Transform Infrared Spectroscopy

FTIR relies on the fact that the most molecules absorb light in the infra red region of the electromagnetic spectrum. This absorption corresponds specifically to the bonds present in the molecules. The frequency range are measured as wave numbers typically over the range 4000-600 cm^{-1}.

X-Ray Diffraction

X-Ray Diffraction is based on the constructive interference of monochromatic X-Rays and crystalline sample. These X-rays are generated by a cathode ray tube, filtered to produce monochromatic radiation, collimated t concentrate, and directed toward the sample.

Scanning Electron Microscope

A scanning electron microscope is a type of electron microscope that produces images of a sample by scanning it with a focused beam of electrons. The electrons interact with atoms in the sample, producing various signals that contain information about the sample's surface topography and composition.

These films of the sample were 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 the SEM grid was allowed to dry by putting it under a mercury lamp for 5 minutes.

Energy Dispersive X-Ray Spectroscopy

The principle of EDAX is a qualitative and quantitative X-Ray micro analytical technique that provides information on the chemical composition of a sample for elements with atomic number. $(Z) \geq 3$.

ANTIBACTERIAL ACTIVITY

The standardized inoculums is inoculated in the plates prepared earlier (aseptically) by dipping a sterile in the inoculums removing the excess of inoculums by passing by pressing and rotating the swab firmly against the side of the culture tube above the level of the liquid and finally streaking the swab all over the surface of the medium 3 times rotating the plate through an angle of 60^{oo} C after each application. Finally pass the swab round the edge of the agar surface. Leave the inoculums to dry at room temperature with the lid closed.

Each Petri dish is divided into 2 part, in one part sample disc such as MFE (100µg) disc (discs are soaked overnight in SAMPLE solution) and Std Ciprofloxacin 10µg, are placed in another part of the plate with the help of sterile forceps. Then Petri dishes are placed in the refrigerator at 4º C or at room temperature for 1 hour for diffusion. Incubate at 37 º C for 24 hours. Observe the zone of inhibition produced by different samples. Measure it using a scale and record the average of two diameters of each zone of inhibition.

ANTI FUNGAL ACTIVITY

EXPERIMENT

The standardized inoculums is inoculated in the plates prepared earlier (aseptically) by dipping a sterile in the inoculums removing the excess of inoculums by passing by pressing and rotating the swab firmly against the side of the culture tube above the level of the liquid and finally streaking the swab all over the surface of the medium 3 times rotating the plate through an angle of 60 ºafter each application. Finally pass the swab round the edge of the agar surface. Leave the inoculums to dry at room temperature with the lid closed.

Each Petri dish is divided into 2 parts, in one part sample disc such as MFE(100µg) disc (discs are soaked overnight in sample solution) and Std Fluccanazole 10µg, is placed in the another part of the plate with the help of sterile forceps. Then Petri dishes are placed in the refrigerator at 4º C or at room temperature for 1 hour for diffusion. Incubate at 28 º C for 48hours. Observe the zone of inhibition produced by different samples. Measure it using a scale and record the average of two diameters of each zone of inhibition.

RESULT AND DISCUSSION

The present study involves various parts of medicinal plant species *M. Oleifera* for the synthesis of ZnO nanoparticles. The plant is endowed with immense secondary metabolites and astonishing nutritive properties. The different parts of the plants have already been exploited for the synthesis of various metal nanoparticles with valuable bioactive mechanisms. This report aimed on synthesis of ZnO nanoparticles using extracts of *Moringa oleifera* flower.

The results of the phytochemical analysis of the flower extract in various solvents has shown a remarkable variation in the presence the above studied phytochemical compounds in the studied taxa. The detailed investigations of qualitative analysis of phytochemicals results are shown in Table 1.0.

Table 1.0 shows the Qualitative Phytochemical analysis of ZnO NPs in *Moringa Oleifera* flower extracts

S. No	Phytochemicals	*Moringa Olelfera*
1.	Flavonoids	Present
2.	Saponins	Present
3.	Tannins	Present
4.	Glycosides	Present
5.	Proteins	Present
6.	Carboydrates	Present
7.	Quinones	Present
8.	Carotenoids	Present
9.	Phlobatannins	Absent
10.	Phenols	Absent
11.	Terpenoids	Absent

12.	Alkaloids	Present

[11] reported the analysis of Phytochemicals present in the *Moringa Oleifera* Plant. They reported presence of alkaloids, proteins, glycosides, proteins, saponins, tannins and terpenoids. Pinal et al revealed that these Phytochemicals were involved in the anti fungal and Anti Bacterial activity

[12] reported the synthesis of silver nanoparticles (AgNPs) from aqueous leaf extract of *M.Oleifera*. [13] proposed that the leaf mediated Ag nanoparticles from *M.Oleifera* are used in optical limiting. The AgNPs from M.Oleifera leaves have been reported to possess significant antifungal activity against candida albicans [14] Silver nanoparticels from *M.Oleifera* have neen reported to induce apoptosis of human cervical carcinoma cells[15]. The silver nanoparticels reduced by the gum of *M.Oleifera* was reported to exhibit antibacterial activity against Stapylococcus aureus, E.Coli and Peudomonas aeruginosa [16]. Silver nanoparticles from the seeds of *M.Oleifera* are reported to control major dengue vector Aedes aegypti and against dengue serotype DEN-2 [17].

The present study synthesized ZnO nanoparticles from the flower of *M.Oleifera*, and these nanoparticles at the concentration of 100µg/ml exhibited antibacterial activity against gram positive and gram negative bacteria such as *Staphylococcus aureus and Escherichia Coli* and it also exhibited its zone of the anti fungal activity of the organisms of *candida albicans* in the same concentration. ZnO nanoparticle antibacterial activity and zone of inhibition is shown in the Table 2.0. Anti fungal activity of *M.Oleifera* flower extract is shown in the table 3.0.

Table 2.0 shows the Anti Bacterial activity of *Moringa Oleifera* Flower extract and its zone of inhibition aganist the oraganisms.

S. No.	ORGANISMS	Zone of Inhibition(mm)	
		STD CIPROFLOXACIN (10µg/disc)	SAMPLE- MFE (100µg/disc)
1.	*Staphylococcus aureus*	38	22
2.	*Escherichia coli*	40	21

Table 3.0 Shows the Anti fungal activity of *M.Oleifera* flower extract and its zone of inhibition.

S. No.	ORGANISM	Zone of Inhibition(mm)	
		STD Fluccanazole (10µg/disc)	SAMPLE- MFE (100µg/disc)
1.	*Candida albicans*	42	21

4.0 X-Ray Diffraction Analysis

Structure and phase purity of the sample was identified from XRD patterns. Figure shows the typical XRD pattern of ZnO nanoparticles prepared by green synthesis method using powered form of flower extract of *Moringa Oleifera*.

From the broadening of the XRD peaks the crystalline size (d) of the nanoparticles was calculated using Debye Scherrer's formula.

$d = k\lambda / \beta\cos\theta$

Where,

λ = is the wavelength of x-Rays (1.5406Å)

β = is the full width in radiations at half maximum (FWHM) of diffraction peaks

θ = is the diffracted angle of X-Rays pattern

The average crystalline size of ZnO nanoparticle is 27.85nm.

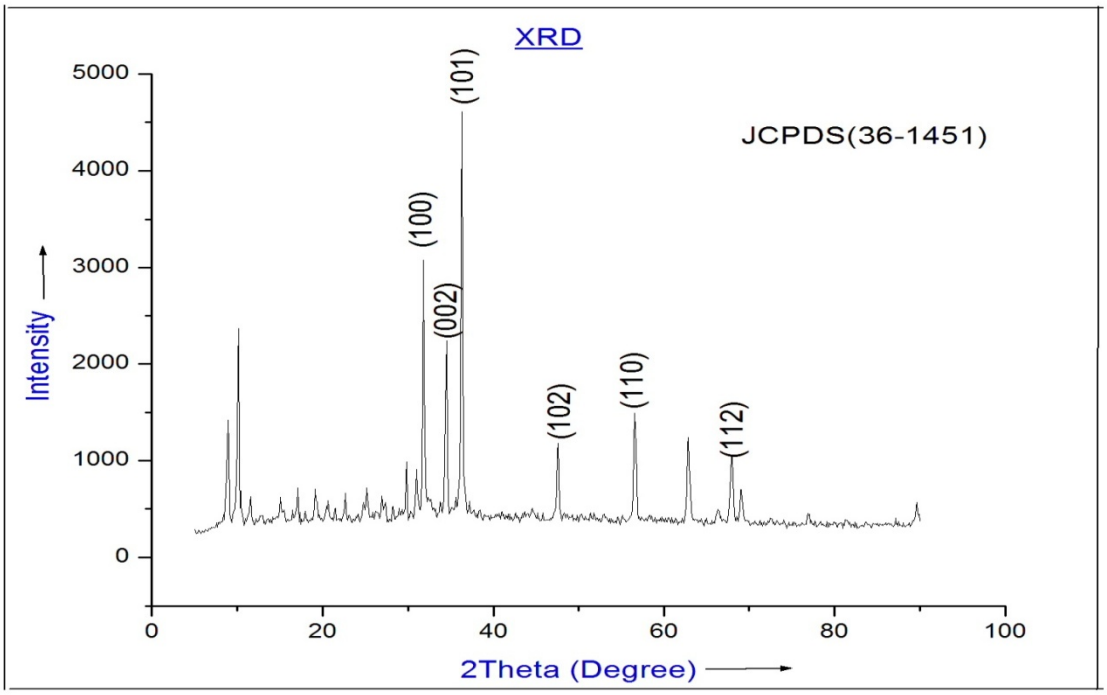


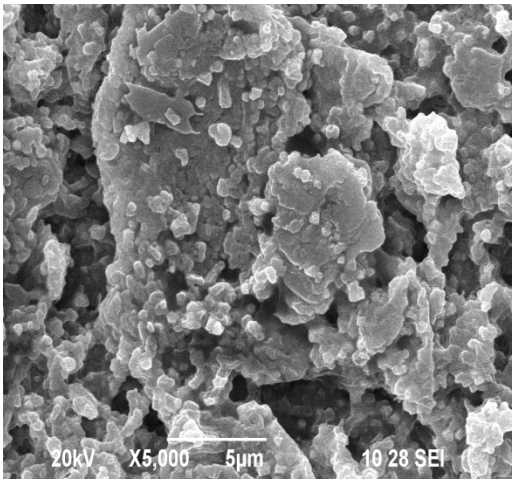
Figure 4.0 shows XRD graph of ZnO Nanoparticles

5.0 Scanning Electron Microscope(SEM):

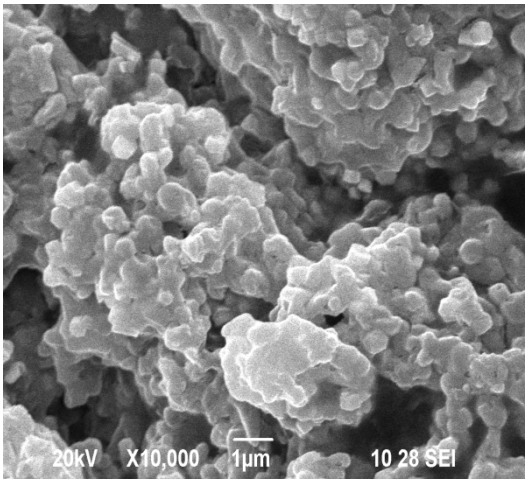
The SEM analysis was used to determine the structure and particle size of the reaction products that were formed.

SEM image has showed individual zinc oxide particles as well as a number of aggregates. The SEM images of the prepared sample shows the Spherical with Rod like Nanostructure. The morphological information and confirmation of SEM image of the prepared samples of Zno nanoparticles shown in the Figure 5.0 a), b), c) and d)

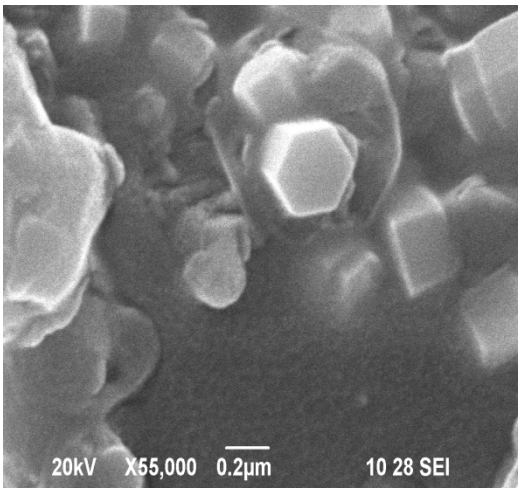
Figure 5.0 shows SEM images of ZnO NPs (flower extract)



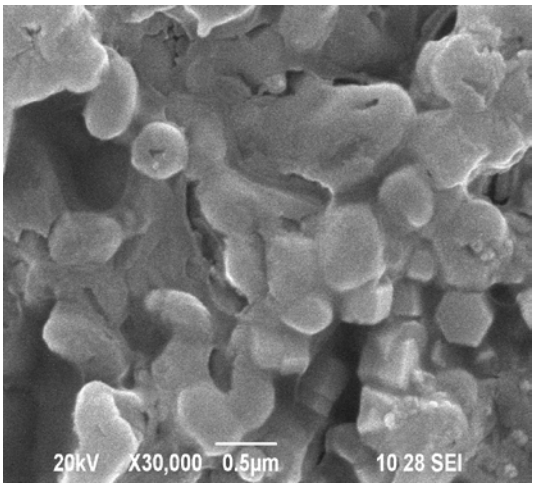
a)



b)



c)



d)

6.0 Fourier Transform Infra Red Spectroscopy

To specify the functional groups in the ZnO particles prepared from the flower extract of Moringa Oleifera. FTIR- spectra is recorded in the wavelength from 400cm^{-1} to 4000cm^{-1}.

The figure 6.0 shows FTIR analysis for ZnO nanoparticles using Moringa Oleifera flower extract and measured absorption band is carried out in the wave number range from 400 cm^{-1} and 4000 cm^{-1}. The mode of vibrations range at 649- 3418 gave the O-H Stretching, N-H Stretching, NH_2 Bending, C-H out of plane depending and C-S Stretching. It reveals the presence of Oxygen and Nitrogen groups.

Figure 6.0 Shows FTIR of ZnO Nanoparticles

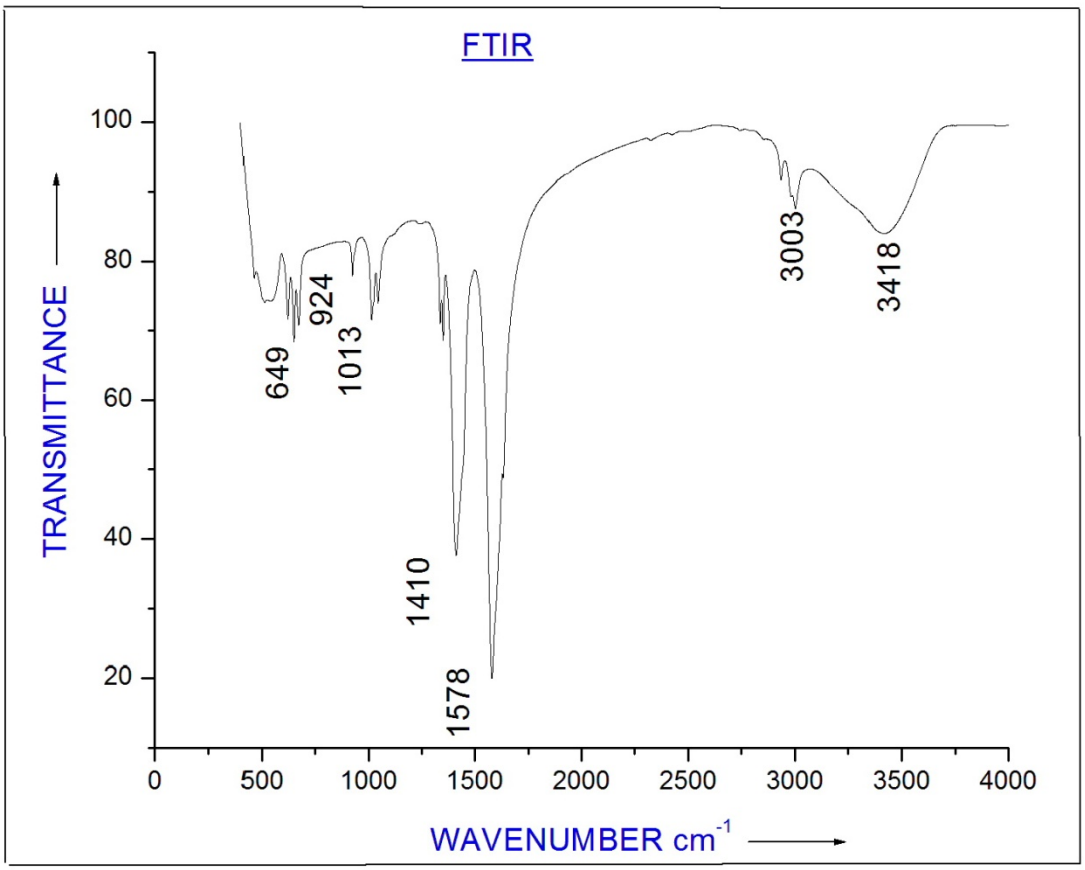


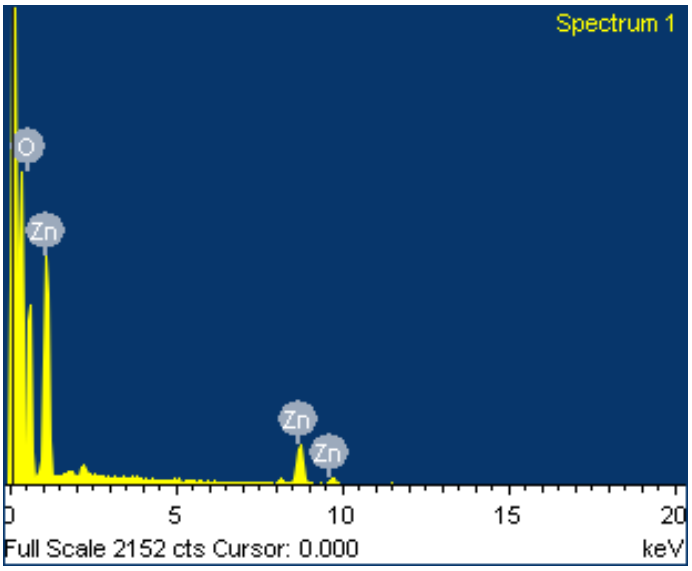
Table 6.1 shows Bonds present in the Zinc Oxide Nanoparticle

FREQUENCY RANGE (cm^{-1})	BOND	INTENSITY
3418.17	N-H Stretching	Strong
2988.27	CH_2 Symmetric Stretching	Strong
1013	SO_3 Symmetric Stretching	Strong
924	C-H out of Plane depending	Strong
649	C-S Stretching	Strong
1245	N=O Stretching	Strong
1578	NH_2 Bending	Strong

6.0 Energy Dispersive X-Ray Spectroscopy (EDAX)

Energy dispersive X-Ray Spectrometers take advantage of the photon nature of light. Latter on the ratification of the XRD result the sample was further analysis the EDX studies. The EDX analysis was confirmed the chemical composition of the ZnO NPs.

Figure 6.0: EDAX pattern for ZnO Nanoparticles



SUMMARY AND CONCLUSION

The biological production of metal nanoparticles is becoming a very important field in chemistry, biology, and material science. Metal nanoparticles had been produced chemically and physically for a long time; however, their biological production had only been investigated very recently. The biological reduction of metals by plant extracts had been known since the early 1900s however, the reduction products were not studied.

Moringa Oleifera plant virtually contains all the whole nutrients needed by man. They contain both the most essential and non essential nutrients. Increased awareness on the nutritional and medicinal potential of *Moringa* should form the priority of all health institutions especially those in the rural area where cases of malnutrition is prevalent. One way to achieve this is to encourage the cultivation of *Moringa Oleifera* in family compounds as such making it the family companion

The rapid biological synthesis of zinc oxide nanoparticles using flower extract of *Moringa Oleifera* provides an environmental friendly, simple and efficient route for synthesis of nanoparticles. The use of plant extracts avoids the usage of harmful and toxic reducing and stabilizing agents. The synthesized nano crystallites of ZnO nanoparticle are in the range of 30-35nm. Zinc nanoparticles exists as ions only in the presence of strong oxidizing substances. The environmental conditions will affect the stability of nanoparticle. The synthesis of ZnO nanoparticles is still in its infancy and more research needs to be focused on the mechanism of nanoparticle formation which may lead to fine tuning of the process ultimately leading to the synthesis of nanoparticles with a strict control over the size and shape parameters.

Synthesis of zinc oxide nanoparticles was achieved by using zinc acetate dehydrate by microwave irradiation method. Detailed structural characterization demonstrated that the synthesized products are spherical and crystalline in structure in various diameter. These structures were clearly evident from SEM and XRD, SEM results were in accordance with X- ray diffraction. Due to the large specific surface area and high energy, some nanoparticles aggregated. The aggregation occurred probably during the process of drying, XRD patterns of zinc oxide nanoparticles calcinated at $450C^{0,}$ the average particle size increased with the increase of calcination temperature X-ray diffraction (XRD) with Cu-Kα radiation was used for checking the size D was calculated by Debye-Sherrer formula.

Microorganisms used for antimicrobial activity such as Staphylococcus aureus, Escherichia coli and Candida albicans. The antibacterial activity performance of ZnO nanoparticles was done by disc diffusion method and antibiotic susceptibility had been tested by Kirby-Bauer method. The agar used is Meluller-Hinton agar that vigorously tested for composition and pH. Further the depth of the agar in the plate is a factor to be considered in the disc diffusion method. This method is well documented and standard zone of inhibition had been determined for susceptible and resistant values. Green synthesis of ZnO nanoparticle by moringa oleifera which is used in the field of food processing, production of antimicrobials, cosmetics, health care products. The green

synthesis of nanoparticles were reported to had advancement over chemical and physical methods. Future scope of this is study to synthesize another metal nanoparticles in *Moringa Oleifera* plant flower extract.

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