International Journal of Scientific Research in Engineering and Management (IJSREM)
Volume: 07 Issue: 08 | August - 2023 SJIF Rating: 8.176 ISSN: 2582-3930

Green Synthesis of Zno Nano Particles using Hibiscus Sabdariffa and its Anti Diabetic and Anti Inflammatory Applications

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Abstract

ZnO nanoparticles is green synthesized using Hibiscus sabdariffa plant which has very high medicinal applications. The synthesized ZnO nanoparticles were characterized by using UV-visible spectrophotometer, Scanning electron microscope(SEM) . Their biological activities like anti diabetic and anti inflammatory activity of ZnO nanoparticles were studied.

Keywords: ZnO nanoparticles, Hibiscus Sabdariffa, anti diabetic and anti inflammatory activity.

Introduction

Nanotechnology is associated with nano-meter sized objects [1]. Living organisms are made up of cells. These cell parts, however, are nano sized. Nanotechnology basically deals with design, production and characterization on nano sized particles. Nano sized particles are basically small objects that act as a whole unit in accordance with their transport and properties. Fine particles have the range of 100-2500 nm and ultrafine particles have the size of 1- 100nm. They can also be designed to improve the pharmacological and therapeutic effects of the drugs[2]. They also have a very high surface area and they permit many functional groups to be adhered to them which in turn, can bind to tumor cells. They have proven to be an excellent replacement for radiation and chemotherapy as they can easily assemble in the micro environment of the tumor.

Recent studies have developed a number of nano-sized particles such as metals, semiconductors and polymeric particles utilized in molecular imaging and particulate delivery vehicles. Polyethleneimine liposomes, silica nanoparticles, micelles and chitosans play an important role in drug delivery with minimized side effects[3].

Objective of using ZnO nanoparticles

Zinc oxide nanoparticles have drawn considerable attention from researchers and scientists in the past 4–5 years due to its wide applications field of the biomedical field as well as in optics and electronics. ZnO nanoparticles are of great interest due to inexpensive to synthesize, safe, and easy method of synthesis. These nanoparticles possess high exciton binding energy of 60 meV and a large bandgap of 3.37 eV, and due to this, these show various semiconducting properties such as high catalytic activity, wound healing, anti inflammatory, ultraviolet filtering properties and extensively used in various cosmetics such as sunscreen[4]. These nanoparticles revealed various biomedical applications too such as antifungal, antibacterial, drug delivery, antidiabetic, anticancer. Up to now, numerous works have been reported for ZnO synthesis and utilization by plants, microorganisms, and others. Plant parts like flower, root, seed, leaves, etc., are used for the synthesis of ZnO nanoparticles.

In recent years ZnO NPs have drawn attention of many researchers for their unique optical and chemical behaviors which can be easily tuned by changing the morphology. Within the large family of metal oxide NPs ZnO NP has been used in various cutting edge applications like electronics, communication, sensor, cosmetics, environmental protection, biology and medicinal industry [5]. Moreover, ZnO NP has a tremendous potential in biological applications like biological sensing, biological labeling, gene delivery, drug delivery and nano-medicine [6] along with its antibacterial, antifungal, acaricidal, pediculocidal, larvicidal and anti-diabetic activities. Recently, synthesis of NPs via eco-friendly routes have become popular among researchers due to its low cost, synthesis in ambient atmosphere, non-toxicity, environmental compatibility etc. and ease of applications as the resulting particles are highly soluble in water, biocompatible, and devoid of toxic stabilizers. Plant extracts are very promising tool for facile synthesis of NPs via green routes[7].

Green synthesis of ZnO nanoparticle using Hibiscus sabdariffa

The origin of *H. sabdariffa* is not fully known, but it is to believe to be native from India to Malaysia, where it is commonly cultivated, and must have been carried at an early date to Africa. It has been widely distributed in the Tropics and Subtropics of both hemispheres, and in many areas of the West Indies and Central America has become naturalized. *H. sabdariffa* is used in many folk medicines. It is claimed as a Thai traditional medicine for kidney stones and urinary bladder stones. *H. sabdariffa* also is said to have diuretic effects, used effectively in folk medicines for treatment of inflammatory diseases and cancer . The positive effect of *H. sabdariffa* extract consumption to decrease blood pressure has been proved in study on both man and rats . More recently, the antihypertensive action of *H. sabdariffa* has been confirmed with experimental hypertension. In addition, studies on humans also proved the anti-inflammatory effect of *H. sabdariffa* consumption .*H. sabdariffa* extract is also reported used as an antibacterial, antifungal, diuretic, uricosuric, and mild laxative substance . In addition, the components of *H. sabdariffa* extract exhibit anti-tumor characteristics, immune-modulating and anti-leukemic effects. Oil extracted from seeds of H. sabdariffa has been shown to have an in vitro inhibitory effect on bacillus anthracis and staphylococcus albus[8].

Fig.1.Hibiscus sabdariffa Plant

PREPARATION OF EXTRACT

5 grams of *H. sabdariffa* leaves were washed thoroughly with plenty of distilled water and both surface of leaves were sterilized using alcohol by gently rubbing. These leaves were heated for 30 min in 100 ml of distilled water at 50 °C. Then the extract was filtrated with Whatman filter paper no 1 and further filtered using vacuum filter. The final filtrate was stored in cool dry place for further use[9].

Green synthesis of zinc oxide nanoparticles:

20 ml of the plant extract was heated at 50 °C for 10 min and 50ml of 91 mM of zinc acetate solution (1 gm of zinc acetate was dissolved in 50 ml of distilled water) was added drop wise to it under stirring. The reaction mixture became yellowish and cream coloured precipitate of zinc hydroxide was formed. -The reaction mixture was left for 30 min for complete reduction to zinc hydroxide. Then the precipitate was collected by centrifugation at 16000 rpm for 10 min at 4 °C. The precipitate was vacuum dried at 30 °C and the sample was stored for further studies.

EXPERIMENTAL METHODS

The experimental methods used for the characterization of ZnO nano particles are

- FT-IR Spectroscopy
- UV-Visible Spectroscopy
- SEM (Scanning electron microscopy)
- Energy dispersive analysis of x-rays (EDAX)

Biological studies carried over are as follows

- Ani-diabetic
- Anti-inflammatory

RESULT AND DISCUSSION

This chapter covers all the aspects that are related to current research which is based on green synthesis of ZnO nanoparticles of Hibiscus sabdarifa leaves extract and biological characterization as follows

UV-VISIBLE SPECTROMETER ANALYSIS

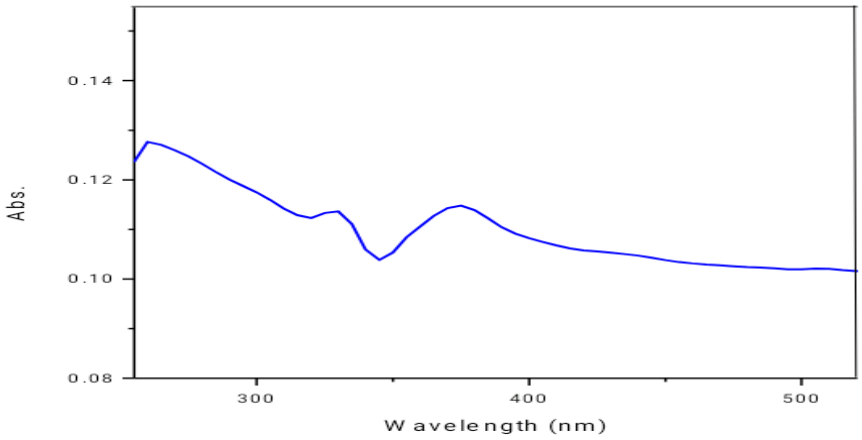


Fig.2.UV-Visible Absorption spectra of Hibiscus sabdariffa

UV -Visible spectroscopy of ZnO nanoparticles is obtained at the wavelength range of 380-390nm.

SCANNING ELECTRON MICROSCOPY

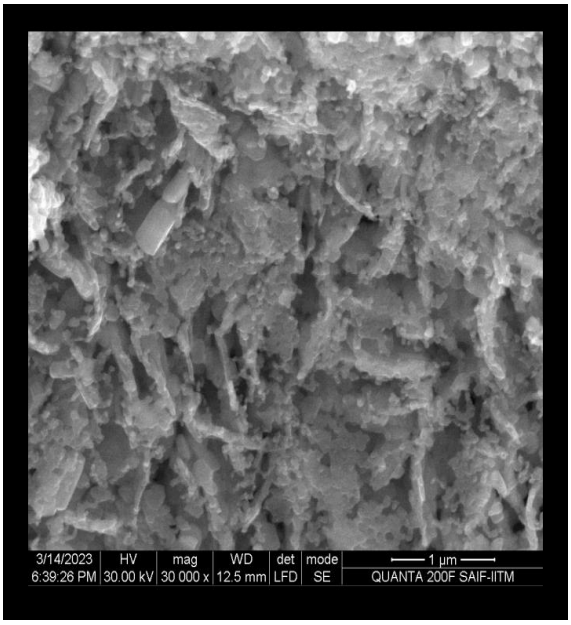


Fig.3 SEM images of ZnONPs by the aqueous extract of Hibiscus sabdariffa leaves at 1µm

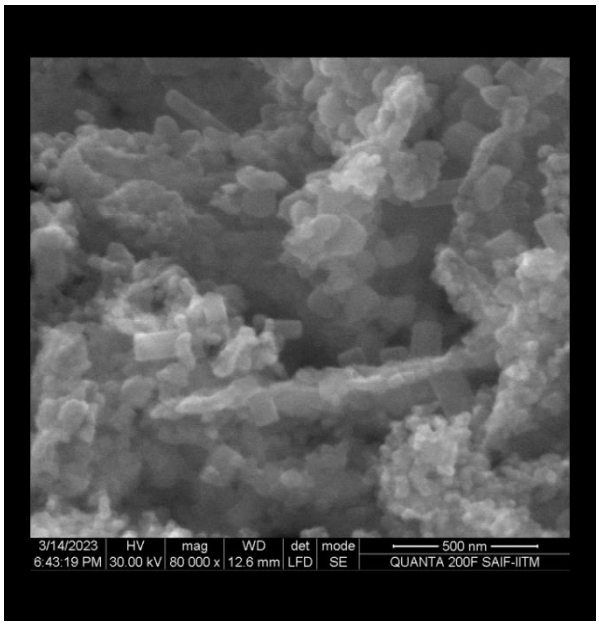


Fig. 4. SEM images of ZnO NPs by aqueous extract of hibiscus sabdariffa leaves at 500nm.

Surface morphology of NPs were thoroughly studied by FESEM micrographs. From the micrographs , it was observed that PZN30 showed irregular surface morphology was amorphous in nature, previously confirmed by XRD . Spherical structure was found in PZN60 which on higher magnification showed aggregation of group of smaller spherical particle ranging from 16-60 nm (showed in arrow) together forming cauliflower like structure around 300-400 nm in diameter. PZN100 was more crystalline in nature and were forming a dumb bell shaped structure (showed in arrow) with a length of 200-230 nm, 30-50 nm diameter in head and 70-80 nm broad at base region . The transformation of shape in PZN100 was induced by crystal growth and loss of bioactive compounds (stabilizers) of PZN60 due to higher temperature. The smaller particles in PZN60 became aligned and the crystals grew in such a way that outer particles formed the head and central parts of the aggregates formed the body of dumbbell structure in PZN100[10].

FTIR SPECTROSCOPY

FTIR spectra showed presence of characteristic bands for several functional groups in aqueous extract of *H. sabdariffa*, PZN60 and PZN100 respectively. IR peaks for –OH stretching of water was observed at around 3441, 3478 and 3450 cm−1 . Aromatic compounds were present and confirmed by C=C stretching of aromatic amine, aromatic C-H, asymmetric stretch of C=C-C, symmetric stretch of -C-C=C and C=C were observed at around 1381, 2889, 1474,1576 and 1780 cm-1 , Bending vibration of the alcoholic -C-OH, -NH2 stretching vibration of` secondary amine and -C=O groups from the aromatic ring having conjugation were reflected from the presence of peak at 1102, 1629 and 1422 cm-1. Presence of ZnO was confirmed by a peak at 482 cm-1 in both PZN60 and PZN100 . Peaks for glycosidic linkage of C-O-C and secondary alcoholic group observed at 1565 and 1225 cm-1 respectively. Current findings are supported by some previous reports on

phytochemical constituents of *H. sabdariffa* . Aqueous extract of plant contains phenolic compounds, flavonoids, saponins, tannins and alkaloids, amines. IR bands of these compounds justified their presence.A significant difference in the FTIR spectra of PZN60 and PZN100 were observed. FTIR spectrum of PZN60 confirmed the presence of intense stretching and vibrational bands for several compounds. FTIR spectrum for PZN60 showed characteristic vibrational bands of aromatic compounds around 1381, 2889, 1474, 1576 and 1780 cm-1 which correspond to C=C stretching of aromatic amine, aromatic C-H, asymmetric stretching of C=C-C, symmetric stretch of -C-C=C and C=C respectively. Bending vibration of the alcoholic -C-OH, -C=O groups from the aromatic ring having conjugation and secondary alcoholic group were reflected at 1102, 1422 cm 1 and 1225 cm-1 . This result supported that, bioactive compounds were absorbed on the surface of ZnO particles in PZN60. On the other hand FTIR spectrum of PZN100 showed that those phyto-active compounds were either absent or remained absorbed on ZnO nanoparticles in small amount. These differences were due to rise in temperature as the bioactive compounds were lost in PZN100 as they were calcinated at higher temperature[11].

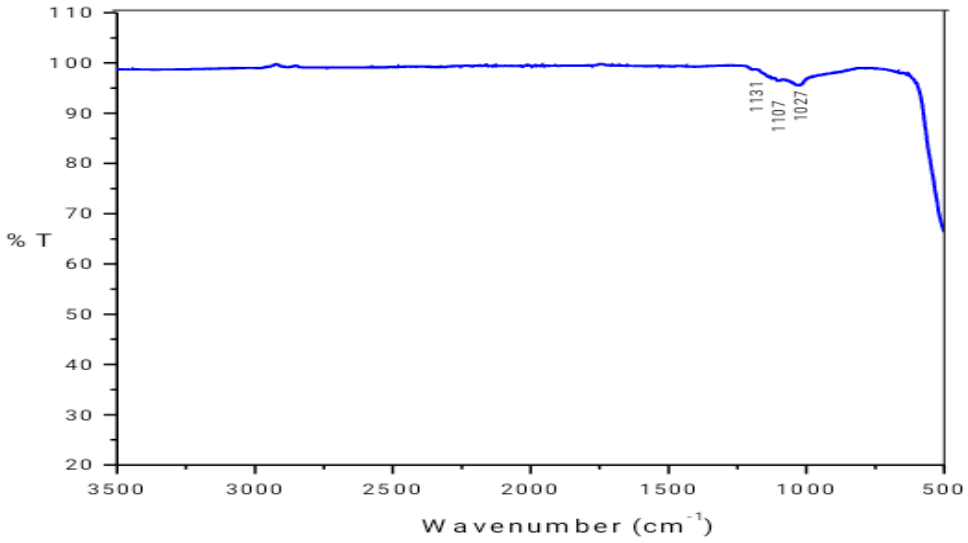
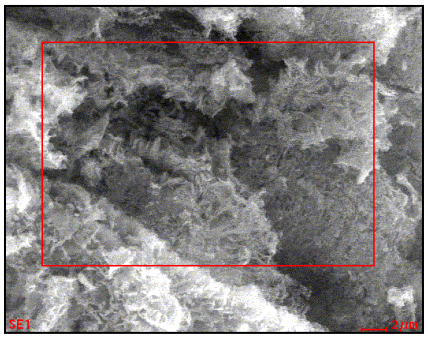


Fig.5 FT-IR Spectrum of Hibiscus sabdariffa

Energy Dispersive X-ray (EDX)

EDX of the samples indicated the presence of zinc and oxygen at stoichiometric ratio. Carbon, nitrogen and some other element in the EDX spectra showed the presence of stabilizing agents which were originated from plantextract. Presence of carbon and nitrogen in indicating bioactive compounds were adsorbed on PZN60which were absent in PZN100 due to temperature rise[12].



Element	*Wt%*	*At%*
CK	27.75	55.01
OK	16.60	24.71
ZnK	55.65	20.27
Matrix	Correction	ZAF

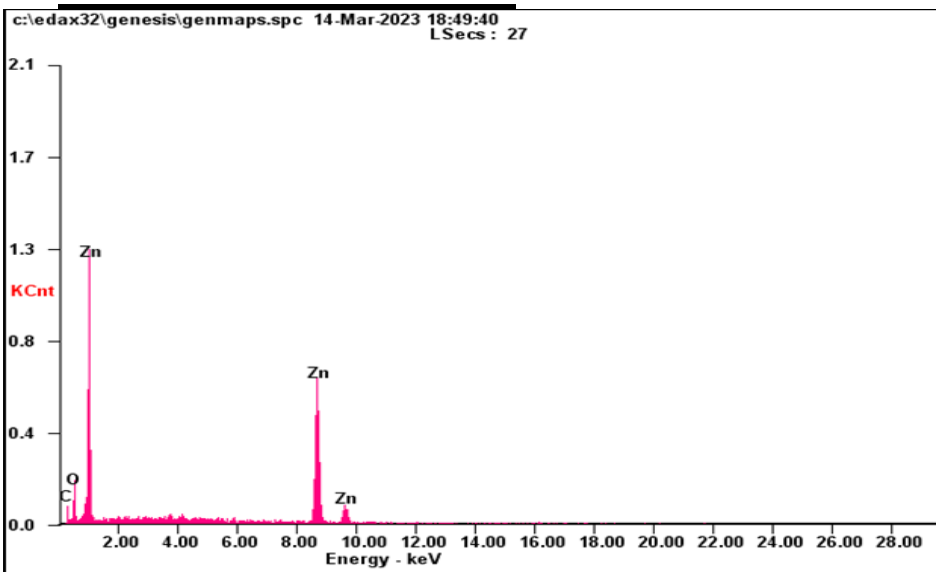


Fig.6 .EDAX Spectrum of ZnO NPs by aqueous extract of Hibiscus sabdariffa leaves at 2µm.

ANTI DIABETIC STUDIES

α-amylase inhibition technique

The antidiabetic activity of the samples was performed using α-amylase inhibition method. Briefly, Amylase (0.2%) was incubated with and without samples (in 1.5 mL) and standard for 10 min at 25^0C. This experiment was performed in 0.2M phosphate buffer (pH 6.9). After pre incubation, the 1% starch solution (0.5mL) was added and the reaction mixture was incubated for 30 min at 25^0C. In order to stop the enzymatic reaction, DNSA reagent (0.5 mL) was added as the color reagent and then incubated in a boiling water bath for 90 min. After cooling down to the room temperature, 0.5 mL of samples were diluted to 2.5mL of distilled water and the absorbance measured at 540 nm using a UV-Visible spectrophotometer. The measured absorbance was compared with that of the control experiment. The percentage inhibition was calculated from the given formula.

% of Inhibition = 100 x[Ac-At /Ac]

At: Absorbance of test

Ac: Absorbance of control

CONCENTRATION (µmL)	PERCENTAGE INHIBITION	
	SAMPLE	STANDARD
20	38.7832	30.5449
40	65.7794	53.4854
80	81.2420	72.3700
200	95.0570	90.1140
400	99.1128	97.8453

TABLE.1 Comparison between standard and sample

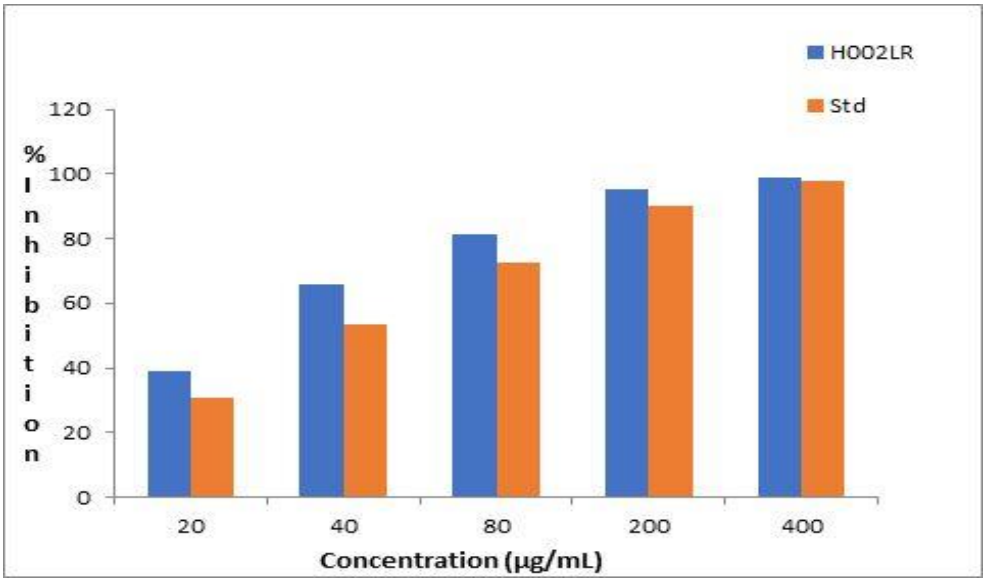


Fig.7. Anti-Diabetic activity

Antidiabetic activity of ZnO nanoparticles are comparatively higher than the standard at all concentration which is shown in the table given above. This show very good antibiotic activity of green synthesized nanoparticle compared to standard chemicals.

ANTI-INFLAMATORY STUDIES

BSA denaturation technique

The synthesized compound and standard diclofenac sodium were screened for anti-inflammatory activity by using the inhibition of albumin denaturation technique with minor modification. The standard drug and compound were dissolved in minimum quantity of Dimethyl formamide (DMF) and diluted with phosphate buffer (0.2 M, PH 7.4). The final concentration of DMF in all solution was less than 2.5%. Test Solution (2.5 mL) containing different concentrations of the drug was mixed with 1 mL of 1 mM Bovine serum albumin solution in phosphate buffer and incubated at 37 °C in an incubator for 10 min. Denaturation was induced by keeping the reaction mixture at 70°C in a water bath for 10 min. After cooling, the turbidity was measured at 660 nm. Percentage of Inhibition of denaturation was calculated from control where no drug was added. The percentage inhibition of denaturation was calculated by using the following formula.

% of Inhibition = 100 x[Ac-At /Ac]

CONCENTRATION	PERCENTAGE INHIBITION %	
	SAMPLE	STANDARD
20	19.0621	20.1834
40	27.7268	29.9694
80	38.8379	41.1824
200	54.5361	58.0020
400	72.2731	75.7390

TABLE 2 Comparison between standard and sample

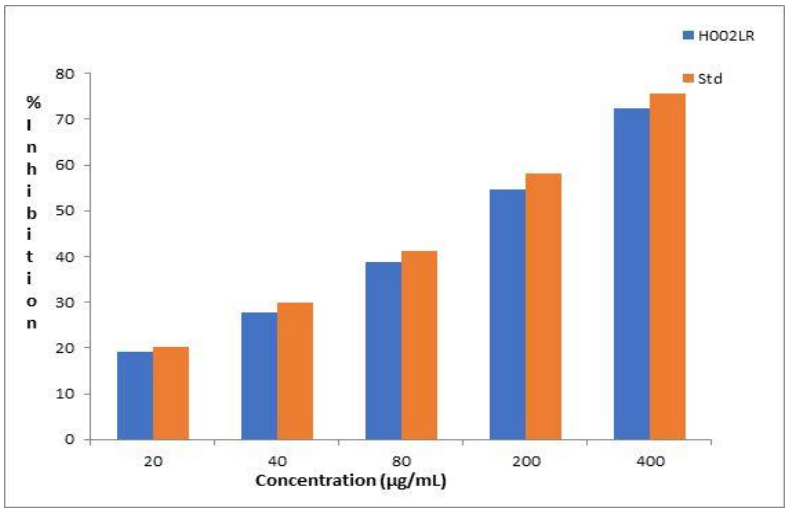


Fig.8 Anti- Inflammatory Activity

The anti-inflammatory activity of ZnO nano particles are observed slightly less than standard one which shows anti-inflammatory activity of ZnO nano particle is comparatively as good as the standard.

CONCLUSION

The ZnO nanoparticle is synthesized using Hibiscus sabdariffa shows very good antidiabetic activity and moderate anti-inflammatory activity. The UV-Visible absorbance obtained at 380-390nm and the size of the nanoparticle using FE-SEM is shown by 500nm. The ZnO nanoparticles have other important biological applications like antibacterial, antifungal, antioxidant activities etc. which can be used for further research work in future.

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