Green Synthesis of zinc oxide nanoparticles using *Ocimum basilicum* seed extract: characterization and evaluation of antioxidant and antidiabetic activities

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Abstract

The green synthesis of zinc nanoparticles using plant extract is utilized for its potential biological application and development of novel therapeutics. The biological method of nanoparticle synthesis has gained attention due to its therapeutic applications. This study aimed for the biosynthesis of zinc oxide nanoparticles using an ethanolic extract of Ocimum basilicum seeds and to characterize and to evaluate antioxidant and antidiabetic activity in in-vitro. The ZnO nanoparticles were characterized by UV-visible spectroscopy, X-ray diffraction, Infrared spectroscopy (FTIR) and SEM measurements. The synthesized ZnO NPs possess hexagonal and alkyl chloride compounds that exhibit cubically close-packed crystal structures.

Phytochemical analysis confirmed the presence of flavonoids, terpenoids, tannins, saponins, alkaloids and phenol. The percentage of inhibition for amylase and glucosidase was higher in O.basilicum ZnO NPs. It was found to be 76.10 ±0.83% in amylase and 70.68±0.83 % in the glucosidase inhibition assay. The present study results also showed that synthesized zinc nanoparticles possess antioxidant properties by scavenging the superoxide anion. It concluded that the ZnO nanoparticles synthesized using O.basilicum extract displayed remarkable antioxidant and antidiabetic activities and could be used for therapeutics.

Keywords: Antidiabetic activity, Antioxidant activity, Zinc oxide nanoparticles, *Ocimum basilicum*, α-amylase, α - glucosidase.

Introduction

Zinc oxide (ZnO) nanoparticles are one of the most important nanomaterials due to their unique electrical and optical properties. ZnO is the most studied nanomaterial and received much attention in biological applications such as drug delivery, nanomedicine, gene delivery and biological labeling which also exhibited antidiabetic, antibacterial, antioxidant and antifungal activities[22]. The nanoparticles are synthesized by several methods such as solvothermal, thermolysis and hydrothermal of precursor compounds which may have adverse effects on medicinal applications[12].

The synthesis of nanoparticles using plant extracts has increased awareness due to its compatibility, reliability, simplicity and eco-friendliness. Medicinal plants are a rich source of secondary metabolites which improve human health without any side effects[34]. There are different plants such as *Passiflora caerulea*, *Limonium pruinosum*, *Ocimum sanctum*, *Azadirachta indica*, *Mucuna pruriens*, *Moringa oleifera and Tamarindus indica* used to synthesize ZnO nanoparticles[16,18,20,27,28].

Ocimum basilicum (O. basilicum) also known as sweet basil, belongs to the family of the Lamiaceae. Phytochemical studies showed the presence of phenolic compounds, flavonoids, terpenoids and xanthones[20]. Several studies reported that *O. basilicum* possesses different pharmacological effects such as antimicrobial, anti-inflammatory, anticancer, antiviral, antimicrobial, antioxidant and anti-aging activities[23,30,35,36]. *O. basilicum* seeds are tiny and ellipsoid in shape. *O. basilicum* seed has been used as a traditional medicine for treating dyspepsia, colic ulcer, diarrhea, skin allergies and whoop cough[4,29].

Hence, it is hypothesized that the use of *O. basilicum* seeds to synthesize ZnO nanoparticles would benefit biological systems, especially for the treatment of diabetes. Thus, the present study aimed to biosynthesize ZnO nanoparticles using *O. basilicum* seed ethanol extract, to characterize them and to evaluate their antioxidant and antidiabetic properties.

Material and Methods

Chemical Reagents and Instruments: Glycogenase which is preferably referred as alpha amylase, DPPH (Diphenyl picryl hydrazyl), alpha-1,4-Glucosidase, a chromogenic substrate like PNP-α-D-Glucopyranose, 2-hydroxy-3,5-dinitrobenzoic acid, ascorbic acid, acarbose, soluble starch, phosphate buffer, sodium carbonate, gallic acid, tannic acid, 1,2-dihydroxybenzene, sodium biphosphate, a lipophilic compound like dibutyl hydroxyl toluene, sodium hydrogen carbonate, a phenolic reagent like Folin-Ciocalteau, nitrous acid, sodium hydrate, an inorganic compound known as aluminium trichloride, potassium ferric cyanide, iron trichloride, ammonium molybdate, Mohr's salt or commonly termed as ferrous alum, ammonium acetate, calcium disodium EDTA, sulfinyl bismethane, N-Methyl phenazonium methyl sulphate, triphospho pyridine nucleotide, sulfonamide, acetone, nitro blue tetrazolium chloride (NBT), dihydrogen dioxide, ethanoic acid, orthophosphoric acid, ferrozine disodium salt, nitroprusside

sodium, N-naphthyl-ethylene-diammonium chloride, hydrogen sulphate, tri chloro acetic acid (TCA) were procured from the market.

Preparation and extraction of *O. basilicum* seed: The seeds of *Ocimum basilicum* were washed in running water and air-dried for seven days. The dried seeds were powdered using a mechanical grinder. 100g of powdered sample was subjected to Soxhlet extraction with 800 ml of ethanol for 6 hr at 25^0C. The extract was filtered with sterile Whatmann no.1 filter paper and the ethanol in the extract was evaporated using a rotary evaporator at 40^0C. The extract was stored at 4^0C for further use.

Phytochemical analysis of *O. basilicum*: The preliminary qualitative analysis was carried out to find the availability of various primary metabolites in *Ocimum basilicum* sample. The evaluation of phytochemicals such as flavonoid, tannin, Triterpene glycoside, phenols, alkaloid and terpenoid was analyzed using the standard procedure.

Synthesis of zinc oxide nanoparticles: 20 mL of 0.05 M zinc acetate solution was prepared. After that 20 mL solution of *O. basilicum* seed extract was added drop-wise to the zinc acetate solution and the resulting solution was heated at 85ºC for 2 h. During the process of heating, the colour changes from black to brown which indicates the end of the reaction. The solution was kept at room temperature and centrifuged at 5000 rpm for 30 min. Then it was washed with ethanol 3 times and the product was dried in the oven for 3 h at 50 ºC.

Characterization of zinc oxide nanoparticles: The optical properties and chemical group of the sample are characterized using two common analyses which are UV-Vis spectroscopy and FT Infrared analysis. The size, shape, surface area, elemental composition and nature of the sample were analyzed by using SEM, XRD and EDAX.

Alpha amylase Inhibitory Method: The α-amylase method is explained as follows: 1000 µl of sample (20-100µg/ml) was taken in a test tube. 250µl of 0.02M buffer solution (sodium phosphate) and α-amylase was added. 0.02M of 1% starch solution is taken in buffer solution (phosphate) (pH-6.9). The reaction was terminated by the addition of 500µl of DNS (dinitro salicylic acid) reagent. These test tubes are placed in boiling water to 5 minutes and then cooled at room temperature.

Finally, the reaction is diluted with 5 ml of distilled water. By using a spectrophotometer, absorbance can be measured at 540nm[33]. Results are expressed as percentage of α-amylase inhibition activity:

Percentage inhibition = [Abs control –Abs sample/ Abs control] × 100

Alpha Glucosidase Inhibitory Method: The α-glucosidase inhibitory activity was carried out by the method of Suthindhiran et al[32]. The solution of p- NPG (nitrophenyl glucopyranoside) was prepared in buffer solution (phosphate-20mM) and pre-incubation was with 50µl of different concentrations. The α-glucosidase(100µg/ml) (pH-6.9) is added to the sample of different concentrations (20-100µg/ml). The incubation of the mixture was placed at 37ºC for 10 minutes. This process is stopped by adding 2ml of sodium carbonate (0.1M). This glucosidase activity is measured by yellow-colored p-NPG at 450nm[32]. Results are expressed as percentage of α- glucosidase inhibition activity:

Percentage inhibition = [(Abs control - Abs sample) / Abs control] x 100

DPPH Assay Method: The antioxidant activity of the sample was examined by stable DPPH free radical activity [3]. Ethanolic solution of DPPH (0.05 mM) (500µl) was added to 1000µl of synthesized nanoparticles with different concentrations (20-100µl). The freshly prepared DPPH solution was kept in the dark at 4˚C. Then 96% (2.7 ml) of ethanol was added to the mixture and shaken vigorously. The mixture was kept to stand for 5 minutes and the absorbance was measured at 540nm. Absorbance was set to zero by using ethanol. A blank sample containing the same amount of ethanol and DPPH was prepared. Experiment was performed in triplicate. The radical activity of the tested samples expressed as a percentage of inhibition was calculated:

Percent (%) inhibition of DPPH activity = [(Control-Test)/control] × 100.

Scavenging of ABTS radical cation: To 1 ml of various concentrations of the extract or standard (Ascorbic acid), 1.0 ml of distilled DMSO and 3 ml of ABTS solution were added and incubated for 20 min. Absorbance of these solutions was measured spectrophotometrically at 734nm [25].

Percentage inhibition = [(Control- Test)/control]×100

Scavenging of hydrogen peroxide: A solution of hydrogen peroxide (20mM) was prepared in phosphate buffer saline (PBS, pH 7.4). Various concentrations of the extract or standard ascorbic acid (20-100 µg/ml) in ethanol (1 ml) were added to 2ml of hydrogen peroxide solution in PBS. After 10 min, the absorbance was measured at 230 nm[13]:

Percentage inhibition = [(Control- Test)/control]×100

Superoxide anion radical Scavenging assay: To various concentrations of the extracts (20-100 µg/ml), 1.0 ml of phosphate buffer (0.1 M, pH 7.2), 1.0 ml of NADH (2 mM), 1.0 ml of NBT (0.5 mM) and 0.1 ml of PMS (0.03 mM) were added. After 5 minutes incubation at room temperature, the absorbance was read at 562 nm against a reagent blank to determine the quantity of formazan generated. Gallic acid was used as the standard[8]:

% inhibition = [(Control- Test)/control]×100

Metal Chelating ability on ferrous ions: The reaction mixture contained 1.0 ml of various concentrations of the extracts (20-100 µg/ml) and 0.05 ml of 2 mM $FeCl_3$. The reaction was initiated by the addition of 0.2 ml of 5 mM ferrozine. The reaction mixture was shaken vigorously and left standing at room temperature for 10 min and the absorbance of the reaction mixture was measured at 562 nm against a reagent blank. A lower absorbance of the reaction mixture indicated a higher ferrous ion chelating ability. The control contained all the reagents except sample. Ascorbic acid was used as standard for comparison[15].

% Inhibition = [(Control- Test)/control] ×100

Statistical Analysis: All assays were conducted in triplicate. Results were expressed as mean±SEM.

Results

Comparative analysis of bioactive compounds from ethanolic extract of seed of *Ocimum basilicum*: The ethanolic extract of *Ocimum basilicum* shows the presence of tannic acid, phlorotannin, flavonoid, steroids, phenol, saponin, glycosides and cardiac glycosides, leuco anthocyanin, terpenoids, vacuolar pigments like anthocyanins, anthranoids, xanthoproteic acid, coumaric acid lactone, emodol, proteins, alkaloids and carbohydrates (Table 1). It suggested that the presence of high concentrations of phenolic compounds and flavonoids could help to protect against various diseases. Previous studies also reported the presence of flavonoids, saponins, alkaloids, phenols and tannic acids in both aqueous and ethanolic extracts of *O. basilicum* leaves[11,19].

Quantitative analysis of seed of *Ocimum basilicum:* The quantitative analysis of *Ocimum basilicum* seed extract showed the presence of different phytochemical constituents. The present study report shows that higher amounts of flavonoids, terpenoids and tannins are present in the extract of *Ocimum basilicum* seed compared to saponin, alkaloid and phenol (Table 2).

Table 1
Qualitative screenings of ethanolic extract of seed of *Ocimum basilicum*

Test No	Phytochemical compounds	Observation	*Ocimum basilicum* Seed result
1	Tannin	Brownish green	+++
2	Phlobatannin	Red precipitate	+
3	Saponins	Blue colour	+++
4	Flavonoids	Yellow colour	+++
5	Steroids	Bluish colour	+++
6	Terpenoids	Reddish brown	+++
7	Cardiac glycosides	Brown ring	+++
8	Leucoanthocyanin	Absence of Reddish layer	_
9	Anthocyanin	Bluish violet	++
10	Anthraquinone	Red	+++
11	Coumarin	Yellow colour	+++
12	Protein	White precipitate	+++
13	Glycosides	Green colour	+++
14	Phenol	Blue black	+++
15	Xanthoprotein	Reddish orange precipitate	+++
16	Alkaloids	Yellow colour	+++
17	Emodin	Red colour	+++
18	Carbohydrates	Reddish violet colour	+++

(+) - Trace (++) - Moderate (+++) - Strong (-) - Absence

Table 2
Quantitative analysis of *Ocimum basilicum* seed extract

S.N.	Phytochemical compounds	*Ocimum basilicum*(mg/100g)
1	Saponin	1.4
2	Tannins	2.1
3	Alkaloids	1
4	Phenol	0.8
5	Terpenoids	2.8
6	Flavonoids	3.1

The quantitative analysis of *Ocimum basilicum* leaves exhibits the presence of phytochemicals such as tryptophan, P-coumaric acid, limonene and alpha terpene[31]. Another study revealed that ethanolic extract of *Ocimum basilicum* leaves and fruits contains the predominant amount of flavonoid and total alkaloid. In aqueous extracts of *Ocimum basilicum*, the total flavonoid content was observed. In contrast, n-hexane extracts showed a poor availability of total flavonoid content than the ethanolic extract of *Ocimum basilicum*[10].

UV- Spectral Analysis: Ultra-violet visual analysis is commonly established to determine the emergence of ZnO NPs and is reported at the range of 277.40 nm. This directly projects the existence of ZnONPs in the sample (Fig. 1). Further, UV-spectral analysis results depicted the appearance of a light green to pale yellow color which represents the existence of ZnO NPs. The previous study reported that the obtained UV spectrum showed the maximum absorption for the synthesized ZnO nanoparticle at 305.01 nm from the *Ocimum basilicum* leaves extract[6].

FT Infrared analysis: In FTIR analysis, the following peaks were obtained in ZnONP seeds extract, the higher peak acquired at 3437.63 cm^{-1} correlated with stretching vibrations of OH. 1632.99 cm^{-1} and 1384.4097 cm^{-1} ranges were in assistance with aromatic rings that hold C=C stretchings, C=O in phenolic acids and C-N in amide region of the protein. Weaker ranges acquired at 1015.60 cm^{-1} and 760 cm^{-1} stated the availability of amino acid stretching, C-H and C-N stretching. A peak between 952.76 cm^{-1} and 670.05 cm^{-1} shows the hexagonal and alkyl chloride compounds. FTIR analysis firmly assessed the existence of terpenoids and flavonoids, which play a major role in the formation of ZnO NPs by oxidizing zinc ions (Fig. 2).

Similarly, the FT-IR spectrum of silver nanoparticles predicts the molecular configuration of different functional groups present in the extract and the peaks are more characteristics of linalool, eugenol, flavonoids and methylchavicol[2].

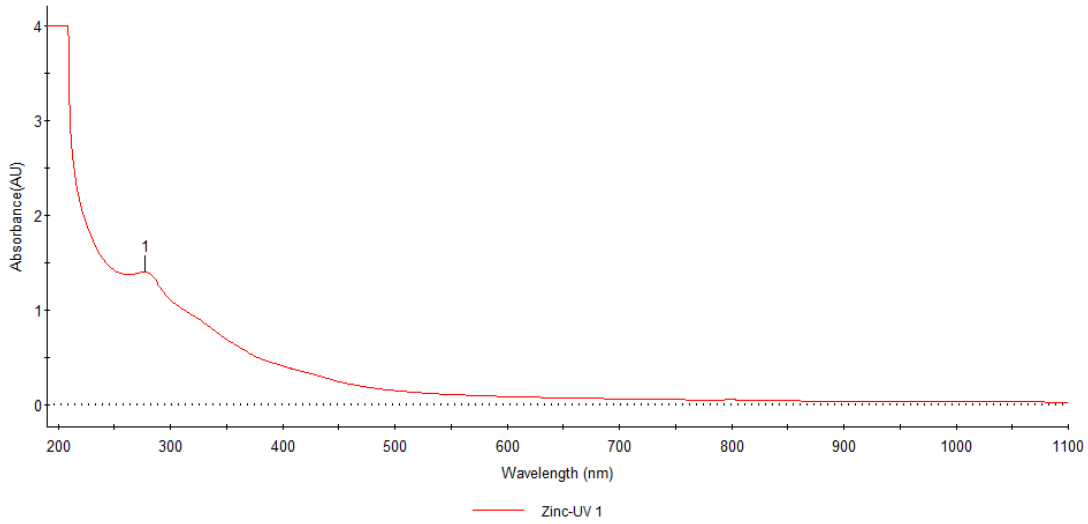

Figure 1: UV Spectral analysis of synthesized ZnONPs using *Ocimum basilicum* seeds

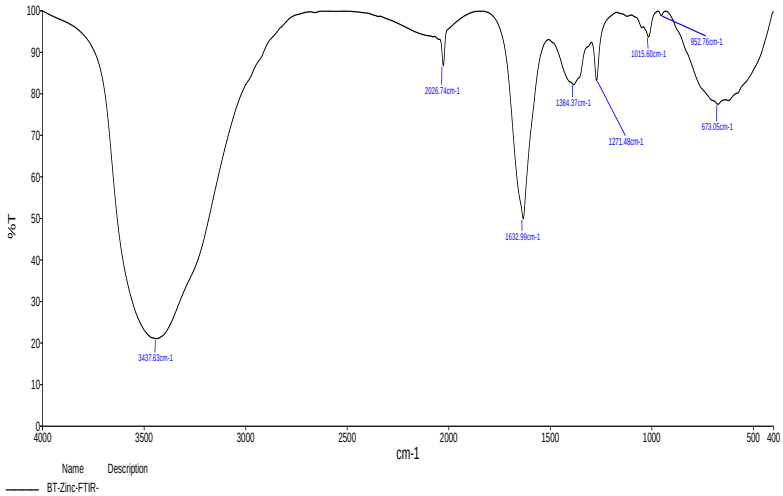

Figure 2: FTIR spectral analysis of synthesized ZnONPs using *Ocimum basilicum* seeds.

X-Ray Power Diffraction (XRD) analysis: In X-ray crystallography, the crystalline nature of ZnO nanoparticles was confirmed using *Ocimum basilicum* seeds (Table 3). The synthesized ZnO nanoparticles pattern performed by XRD is projected in figure 3. The acquired crests are found to be 31.69°, 35.33°, 39.34°, 46.35°, 47.73°, 60.80°, 66.65° and 74.84° correlates to the frame plane of (100), (002), (101), (102), (110), (112), (201) suggesting the cubically close-packed crystal structure of the synthesized ZnONPs. These peaks are in accordance with those data cards (JCPDS 36- 1451).

SEM analysis of synthesized ZnONPs using *Ocimum basilicum* seeds extract: SEM analysis was carried out to anticipate the shape, size of ZnONPs. From the SEM image, it was confirmed that the synthesized ZnO nanoparticles using seeds of *Ocimum basilicum* were consistent in shape and size. SEM images of the ZnO nanoparticles lie between 93.57 -160.3 nm region of ZnO NPs. ~ 200 nm is considered as the general size range of ZnO NPs and they are considered as globular shaped NPs (Figure 4). Another study revealed that the size of synthesized ZnO nanoparticle ranged from 37.77 to 64.13 nm by SEM micrograph[17]. Dogaroglu et al[7] reported that the shape of synthesised ZnO-NPs using leaf extract of *Mentha spicata* and *Ocimum basilicum* was triangular.

EDAX analysis: The energy in the depiction is established by X-axis in KeVEDAX spectra for synthesized zinc oxide nanoparticles from seeds of *Ocimum basilicum* was shown in figure 5 with supplementary peak of O and Zn. It represents the biomolecules attached along with the ZnO nanoparticles surfaces while the total counts of X-rays were denoted by Y- axis. The weight of the zinc oxide in ZnO NPs reduced by the seeds of *Ocimum basilicum* was found as 63.25% which was summarized in the table 4. The purity of the prepared ZnO NPs was finalized by EDAX analysis which revealed the presence of zinc and oxygen[24].

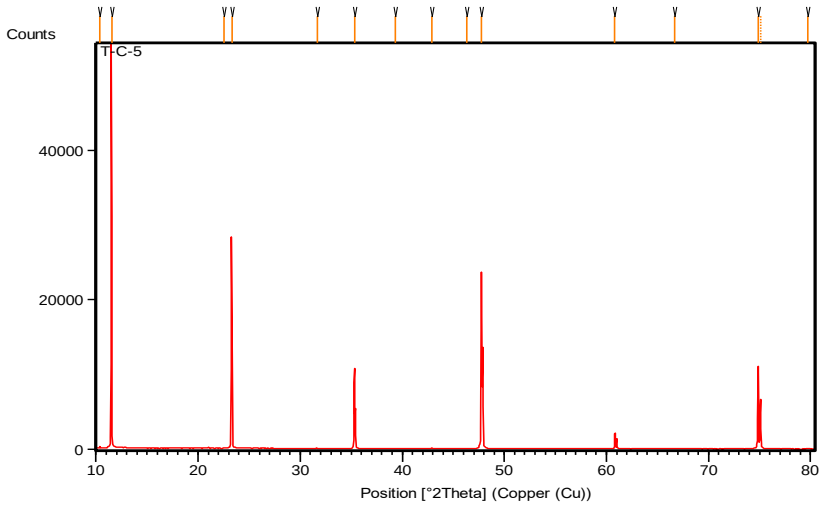

Figure 3: X-ray power diffraction analysis of synthesized ZnO NPs using *Ocimum basilicum* seeds

Table 3
Synthesized ZnO nanoparticles using *Ocimum basilicum* were analysed by XRD patterns

Pos. [°2Th.]	Height [cts]	FWHM Left [°2Th.]	d-spacing [Å]	Rel. Int. [%]
10.3699	109.10	0.2362	8.53084	0.20
11.5269	55820.44	0.1181	7.67701	100.00
22.5739	64.64	0.2362	3.93892	0.12
23.3016	20265.64	0.1181	3.81754	36.31
31.6979	25.04	0.4723	2.82289	0.04
35.3353	9082.66	0.1574	2.54020	16.27
39.3478	25.20	0.2362	2.28991	0.05
42.8907	112.55	0.1181	2.10861	0.20
46.3591	41.20	0.4723	1.95861	0.07
47.7393	27201.72	0.1574	1.90516	48.73
60.8099	2217.44	0.1181	1.52326	3.97
66.6524	42.12	0.3149	1.40322	0.08
74.8497	11830.70	0.0960	1.26752	21.19
75.0829	6505.79	0.0960	1.26730	11.65
79.7298	24.82	0.2880	1.20175	0.04

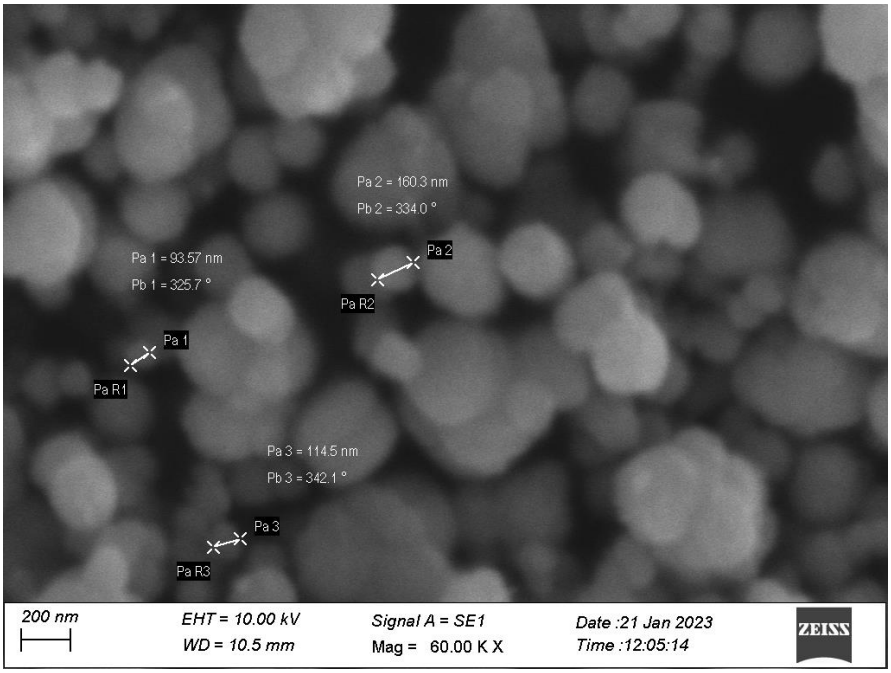

Fig. 4: SEM analysis of synthesized ZnONPs

Table 4
Elemental composition of *Ocimum basilicum* mediated ZnO NP

Element Line	Weight %	Weight % Error	Atom %
O K	25.17	± 0.71	23.18
Zn K	63.25	± 2.63	44.20
Zn L	---	---	---
In L	11.58	± 1.42	32.62
Total	100.00		100.00

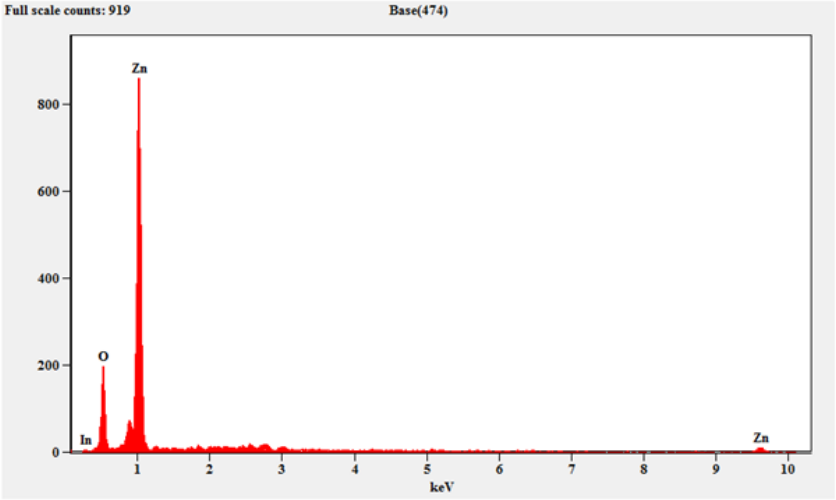

Fig. 5: EDAX spectra of synthesized ZnONPs

Antidiabetic activity ZnO NPs of *Ocimum basilicum*

***In vitro* α-Amylase Inhibitory Assay:** The ability of the zinc nanoparticles of *Ocimum basilicum* seed extract to inhibit α-amylase enzyme activity was determined at different concentrations. The synthesized zinc nanoparticles of *Ocimum basilicum* seed extract showed higher % of the inhibitory activity against the enzyme (Table 5). The synthesized zinc nanoparticles of *Ocimum basilicum*(100µg/ml) possess 76% α-amylase inhibitory activity whereas *Ocimum basilicum*(20µg/ml) showed 43% of inhibition. Acarbose was used as standard compound for the enzyme inhibitor and at the concentration of 20-100

µg/ml, it expressed the activity from 54% to 84%. The prepared ZnO NPs using *Ocimum basilicum* showed the higher alpha-amylase inhibition activity whereas chemical approach of ZnO NPs production expresses the lower antidiabetic activity.

Another *in vitro* study revealed that the presence of phytochemical compounds in methanolic extract of *Ocimum basilicum* leaves could be responsible for the antidiabetic properties[14].

***In vitro* α-Glucosidase inhibition activity:** α- glucosidase enzyme inhibitory assay in synthesized zinc nanoparticle of *Ocimum basilicum* seed extract revealed the remarkable action of inhibition towards the enzyme. The synthesized zinc nanoparticles of *Ocimum basilicum* seed extract of various concentrations (20-100µg/ml) exhibit a dose proportional enzyme activity. The percentage of α-glucosidase inhibition ZnO NPs was found in the range from 40.51 ± 0.831 to 70.68 ± 0.830 (20-100 µg/ml) (Table 6). The maximum of 70% inhibition was observed by the highest concentration (100 µg/ml) which proves that the synthesised ZnO NPs of *Ocimum basilicum* postponed the digestion of carbohydrates. This process mainly biological produced ZnO NPs than chemically fabricated NPs [26].

Free Radical scavenging activity of synthesized zinc nanoparticles by DPPH assay method: The synthesized zinc nanoparticles showed 22% concentration for 20µg/ml and 100µg/ml exhibiting 48% of scavenging activity. Ascorbic acid was used as a standard which shows 59% of scavenging effect for 100µg/ml. The hydroxyl free radical is one of the most reactive oxygen species which causes biological damage that leads to severe pathologic condition[15]. The DPPH assay suggests that NPs of *Ocimum basilicum* exhibiting the scavenging of free radicals. Although DPPH assay is considered as the most popular method to detect the antioxidant property, it is necessary to perform additional assays to confirm its antioxidant potential.

Scavenging of ABTS radical cation: The antioxidant effect of the synthesised ZnO NPs was determined from the decolourization of ABTS radical cation. Interaction with the synthesised ZnO NPs or ascorbic acid suppressed the absorbance of the radical cation which is expressed as % inhibition of absorbance.

Table 5
α-amylase inhibitory activity of ZnO NPs of *Ocimum basilicum* at different concentration.

S.N.	Concentration	Alpha Amylase (%)	
		Sample	Acarbose
1.	20 (µg//ml)	43.36±0.834	54.86±0.825
2.	40 (µg//ml)	47.78±0.826	61.94±0.824
3.	60 (µg//ml)	57.52±0.824	70.79±0.832
4.	80 (µg//ml)	65.48±0.824	76.99±0.832
5.	100 (µg//ml)	76.10±0.832	84.07±0.826

Each value was obtained by calculating the average of three experiments and data are presented as mean±SEM.

Table 6
α-glucosidase inhibitory activity of ZnO NPs of *Ocimum basilicum* at different concentrations.

S.N.	Concentrations	α -Glucosidase (%)	
		Sample	Acarbose
1.	20 (µg//ml)	40.51±0.831	59.48±0.810
2.	40 (µg//ml)	47.41±0.823	65.51±0.814
3.	60 (µg//ml)	50.86±0.832	72.41±0.831
4.	80 (µg//ml)	62.93±0.824	75±0.824
5.	100 (µg//ml)	70.68±0.830	82.75±0.824

Each value was obtained by calculating the average of three experiments and data are presented as mean±SEM.

Table 7
Free radical scavenging activity of ZnO NPs by DPPH assay

S.N.	Concentration (µg/ml)	Percentage of scavenging effect	
		ZnO NPs	Ascorbic Acid
1	20	22±0.823	38±0.813
2	40	27±0.838	42±0.812
3	60	30±0.816	48±0.838
4	80	33±0.813	54±0.823
5	100	48±0.812	59±0.816

Each value was obtained by calculating the average of three experiments and data are presented as mean±SEM.

The result showed that different concentrations of synthesized zinc nanoparticles of *Ocimum basilicum* seed extract exhibit varying degree of scavenging effect for radicals in concentration dependent manner. The synthesized zinc nanoparticles of *Ocimum basilicum* seed extract showed maximum inhibition of 78.24 ± 0.824 at 100 µg/mL among different concentrations of 20, 40, 60, 80 and 100 µg/mL (Table 8). Studies on *Ocimum basilicum* leaves showed significant ABTS radicals scavenging activity than DPPH radical scavenging activity[9,21].

Hydrogen peroxide scavenging assay: The hydrogen peroxide scavenging assay revealed that the ZnONPs synthesized by ethanolic seed extract of *O. basilicum* possess radicals scavenging activity in a dose-dependent manner. The hydrogen peroxide scavenging percentage was found as 59.48 to 72.34% for the concentration of 20 to 100 µg/ml respectively. Similarly, the percentages of hydrogen peroxide scavenging activities were close to the standard ascorbic acid which shows 70.38 to 82.49% at the concentration of 20 to 100 µg/ml respectively (Table 9). The present finding concludes that the ZnONPs of *O. basilicum* had higher radical scavenging activity which attributed to the presence of secondary metabolite such as phenolics, tannins and flavonoids. The antioxidant activity of leaves of *O. basilicum* was also reported by Nadeem et al[19].

Scavenging assay of Superoxide anion: Table 10 depicts the superoxide radical scavenging effect of synthesized ZnO NPs from seed extract of *O. basilicum* and ascorbic acid in a dose-dependent manner. The results showed that the synthesized ZnO NPs from seed extract of *O. basilicum* exhibited 56.28 % at 20 µg/ml whereas 100 µg/ml showed 80.32 %. The percentage inhibition was found significantly higher in the case of ascorbic acid showing 69.82 % to 84.32 % at 20-100 µg/ml. Superoxide anion is the byproduct produced during the catalysis of xanthine oxidase. Superoxide anion produces singlet oxygen and hydroxyl radical that induced oxidative stress and contributed to damage of various biomolecules[5].

The superoxide scavenging activity of ZnO NPs of *O. basilicum* seed extract significantly scavenged the superoxide anion radicals. These observations confirm the presence of antioxidant in the synthesised ZnO nanoparticles using *O. basilicum* seed extract.

Table 8
ABTS radical cation scavenging activity of the ZnO NPs

S.N.	Concentration	ABTS radical cation (%)	
		Sample	Standard Ascorbic acid
1	20 (µg/ml)	61.35±0.824	70.27±0.816
2	40 (µg/ml)	64.27±0.816	73.87±0.812
3	60 (µg/ml)	57.23±0.838	75.67±0.842
4	80 (µg/ml)	70.58±0.832	81.98±0.838
5	100 (µg/ml)	78.24±0.824	83.78±0.824

Each value was obtained by calculating the average of three experiments and data are presented as mean±SEM.

Table 9
Hydrogen peroxide radicals scavenging activities of ZnONPs.

S.N.	Concentration	Hydrogen peroxide (%)	
		Sample	Standard Ascorbic acid
1	20 (µg/ml)	59.48±0.813	70.38±0.824
2	40 (µg/ml)	61.25±0.847	74.76±0.823
3	60 (µg/ml)	63.42±0.854	75.26±0.812
4	80 (µg/ml)	68.09±0.847	80.73±0.847
5	100 (µg/ml)	72.34±0.812	82.49±0.854

Each value was obtained by calculating the average of three experiments and data are presented as mean±SEM.

Table 10
Superoxide radical scavenging activity of synthesized ZnO nanoparticles

S.N.	Concentration	Superoxide anion radical (%)	
		Sample	Standard Ascorbic acid
1	20 (µg/m	56.28±0.843	69.82±0.832
2	40 (µg/m	64.89±0.824	71.24±0.824
3	60 (µg/m	72.13±0.813	77.65±0.812
4	80 (µg/m	76.78±0.812	83.46±0.843
5	100 (µg/m	80.32±0.812	84.32±0.813

Each value was obtained by calculating the average of three experiments and data are presented as mean±SEM.

Table 11
Scavenging of Metal Chelating of Synthesized Zinc Oxide Nanoparticles

S.N.	Concentration	Metal Chelating (%)	
		Sample	Ascorbic acid
1	20 (µg/ml)	58.94±0.823	64.67±0.813
2	40 (µg/ml)	60.32±0.813	75.14±0.824
3	60 (µg/ml)	63.56±0.83	79.48±0.832
4	80 (µg/ml)	67.92±0.824	84.83±0.812
5	100 (µg/ml)	74.37±0.008	89.12±0.824

Each value was obtained by calculating the average of three experiments and data are presented as mean±SEM.

Metal chelating ability on ferrous ions: The synthesized ZnO NPs from seed extract of *O. basilicum* showed the highest metal-chelating ability from 58.94 to 74.37 % for the concentration 20- 100 µg/ml respectively. A standard drug ascorbic acid exhibits the activity from 64.67 to 89.12 % (20-100 µg/ml) (Table 11).

In this assay, Fe (III) salt gets reduced through an antioxidant by an electron transfer mechanism. The present study results showed that ZnO NPs of *O. basilicum* seed extract seemed to be more potent in chelating iron. The reducing power ofZnO NPs of *O.basilicum* is directly proportional to its anti oxidativeproperty[1].

Conclusion

ZnO NPs were synthesized using *Ocimum basilicum* seed extract in an eco-friendlier and effective approach. The size, morphology and structure of synthesised NPs were analysed by UV–vis spectroscopy, FTIR, XRD, SEM and EDAX characterization. The phytochemical screening of ZnO NPs of *Ocimum basilicum* seed extract showed the presence of higher amount of flavonoids, terpenoids, tannins as compared to saponin, alkaloids and phenol. Moreover, the present study suggested that *Ocimum basilicum* seed extract acted as promising component to synthesize ZnO NPs due to the presence of phytochemicals.

In this study, *Ocimum basilicum* ZnO NPs exhibited significant antidiabetic activity and possessed antioxidant activity by scavenging free radicals. This study showed that the green synthesis of *O. Basilicum* ZnO NPs could be produced in large scale with high quality which is stable at high temperature and can be used for biomedical applications. Therefore, the green synthesized *O. Basilicum* ZnO NPs can be used as a safer alternative in the treatment for diabetes and various diseases.

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