



RESEARCH ARTICLE

Bio synthesis, characterization of ZnO nanoparticles from *Scoparia dulcis* L. plant extract and its *in-vitro* antioxidant, acetylcholinesterase activity

R. Mini, V. Prabhu*, K. Poonkodi, K. Vimaladevi, M. Anusuya & M. Vasuki

PG Department of Chemistry, Nallamuthu Gounder Mahalingam College, Pollachi 642 001, Tamil Nadu, India

*Email: prabhunmr@gmail.com



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Abstract

The current investigation is focused on the use of green synthesis methods for zinc oxide nanoparticles (ZnO NPs) from *Scoparia dulcis* L. extract (SDE). SDE-mediated ZnO NPs (SDE-ZnO-NPs) were made using a simple and eco-friendly method that required little reaction time and calcination temperature. UV-Vis, FT-IR, X-ray powder diffraction, SEM, TEM, and EDAX were used for the characterization of biosynthesized ZnO nano material. The UV-Visible spectroscopy absorption peak for SDE-ZnO-NPs was found to be at 380 nm, confirming the formation of ZnO NPs. The FTIR spectrum also revealed bio-active functional groups as well as metal-oxygen groups. Synthesized ZnO NPs had a rod shape in 200 nm, according to TEM examination. The Zn and O in the produced ZnO NPs were approved by the EDAX analysis. The XRD data revealed that the SDE-ZnO NPs have a crystal structure. AChE activity of the synthesized nanoparticles showed potential inhibitory activity with IC₅₀ values of 75.34 µg/mL. The antioxidant activity of the biosynthesized ZnO-NPs was investigated using the DPPH and ABTS assays.

Keywords

Scoparia dulcis, SDE-ZnO NPs, SEM, TEM, XRD, EDAX, Acetylcholinesterase (AChE) Activity, DPPH, ABTS assay.

Introduction

Scoparia dulcis L. belongs to Scrophulariaceae family found in Brazil, Nigeria, southern China, India and other countries. This herb has been used in traditional medicine for its stomachic, diuretic, antitussive, heat-clearing and toxin-absorbing properties. Plant decoctions were used to treat malaria, psoriasis, throat irritation, enterocolitis, flu, snuffle with heat, sore throats and colds. *Scoparia dulcis* was applied in ethnomedicine to treat lung problems, Edema, liver infections, gastrointestinal issue (1). Flavonoids, diterpenoids, triterpenoids, steroids, phenolics, fatty acids and hydrocarbons were found in this herb's chemical analysis, which can be used to prove its clear pharmacological effects. The therapy of metabolic syndrome (2) is related to the pharmacological activities of *S. dulcis* extract, which include anti-diabetic, anti-hyperlipidemic, anti-inflammatory, anti-atherosclerotic, anti-arthritis, hepatoprotective, anti-oxidative and anti-urolithiasis effects (3). *S. dulcis* extracts have hepatoprotective effects by delaying the degradation of the antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GRd), and glutathione-S-transferase. These effects include reducing fasting blood glucose levels, elevating

plasma insulin levels, stimulating insulin secretion to treat diabetes and reducing fasting blood sugar levels (GST) (4). Recent studies on the chemical components of *Scoparia dulcis* aerial parts from China were isolated and their structures were predicted (5). Wistar albino rats were utilised to investigate the phytochemical components of S.D. leaves for their ability to reduce inflammation through the induction of paw edoema by carrageenan in Nigeria (6). S.D. aerial parts are extracted using 3 distinct solvents (methanol, butanol, and ethyl acetate) and studied for Alzheimer's disease from Thailand(7).

Novel applications of nanotechnology can be found in the fields of computation, energy production, ophthalmology, drug discovery and environmental sciences. Numerous nanoscale devices have been developed since the advent of nanotechnology using a range of techniques, including physical, chemical and ecologically friendly ones (8). The biosynthesis of metal nanoparticles using *Scoparia dulcis* extracts has been widely researched and so described below. Cytotoxicity and antibacterial activities are evaluated for silver nano particles synthesised from S.D in West Bengal (9), and then antifungal activity against *Aspergillus niger*, *Aspergillus flavus* and *Fusarium oxysporum* demonstrated their fungicide potentials in Vietnam.(10) as well as the assessment of their antimicrobial property on Andhra Pradesh (11). S.D. AgNPs are obtained by 5-45 nm of a smaller dimension from Vietnam and are well-separated in a variety of shapes such as spherical, triangular, hexagonal and quadrilateral (12). A series of porous copper oxide nanostructures (CSD) were made utilizing the Solution Combustion Synthesis (SCS) process in a very short amount of time. Two model dyes (Methylene blue and Methyl orange) are converted to colourless degradation products by the produced nanoparticles improved photo catalytic research after 180 min with an efficiency of over 90%. All CSD NPs exhibit significant in vitro anti-inflammatory action, protecting the HRBC by more than 82%. The CSD NPs demonstrated a substantial cytotoxic effect on human alveolar basal epithelial cells that were adenocarcinomic, with an IC₅₀ value greater than 95 µg/mL. The CSD NPs also are believed to have numerous applications in the biological and environmental sectors in Karnataka (13). The encapsulation technique has been able to produce AuNPs. The form of the gold nanoparticles was deduced from spectroscopic and microscopic analyses to be spherical and hexagonal. A triterpenoid Methyl commate C, has been discovered and has been identified by spectoscopic analysis and used in the biomedical field in Tamilnadu.(14). *In-vitro* propagation of *S. dulcis* shows that callus induction, Sprout regrowth and root generation capacity with the effect of casein protein has stabilized the AgNPs, AuNPs and CuONPs in Kerala (15). Biosynthesized SD-Fe₂O₃ nanoparticles used as bioelectrodes of a sensor for the detection of paracetamol and ISD NPs were tested for antioxidant and antidiabetic activity (16). Synthesized SD ZnO NPs were investigated for the antibacterial, antioxidant and antifungal activity from Kerala (17).

Our focus has been green synthesis of nanoparticles from plant sources because of their usage in the biological field (18). Compared to pharmaceutical medications, nanomaterials made from plant extracts are apparently safer for the body (19). For this reason, we selected green synthesized ZnO NPs from *Scoparia dulcis* exhibits specific biological and medicinal properties. It serves as a constituent of nano-fertilizers for agriculture, a bio-imaging agent, a selective drug and gene delivery system (20).

Prior literatures has revealed that ZnO NPs from the leaves of *Scoparia dulcis* reduce oxidative stress, inflammation and bacterial diseases (21). Then, we proposed that ZnO NPs may provide protection against neurotoxicity linked to hypogonadism. Due to its non-toxic nature and smaller size, it may facilitate their entry into any part of the tissues. ZnO NPs derived from *S. dulcis* leaves may be used as a nanotherapeutic for treating neurological disorders in patients (22).

The current study was to produce a green, non-toxic approach of ZNO NPs from *Scoparia dulcis*. Several characterization methods, are UV-Vis, FTIR, SEM, TEM, XRD and EDAX, were predicted the structure and morphology of the ZNO-NPs. DPPH and ABTS assays were used to examine SD - ZnO NPs for *in-vitro* antioxidant properties. For the first time, we studied the Acetylcholine esterase (AChE) activity of SD- ZnO NPs using Ellman's method. AChE enzyme connected to Alzheimer's disease (23).

Materials and Methods

Plant Materials

Plant collection

Fresh *Scoparia dulcis* leaves were harvested between June and July 2021 in the region of Pollachi, Tamil Nadu (Pollachi, India latitude: 10035'12.96" N, longitude: 77014'37.37" E). Botanical Survey of India, Coimbatore identified the plant material, and the voucher specimen with number NGMPCY47 has been preserved by the PG Department of Chemistry for future references.

Plant extract Conservation

50g of fresh *Scoparia dulcis* leaves (Fig. 1) was chopped and immersed in vacuum flask with 100 mL Milli-Q water after being cleaned by using distilled water. For 8 min, the solution was boiled at 70 °C. The whatman number-1 used to filter the leaf extract and the filtrate (fresh extract) was used for the synthesis. (24).

Synthesis

Synthesis of Zinc oxide Nanoparticles

50 mL of 1mM Zinc acetate dihydrate solution mixed with 25 ml of *Scoparia dulcis* plant extract, 20 ml of NaOH solution was added to the mixture and it was stirred for 3 hrs for the appearance of a yellow color confirming the formation of ZnO NPs. By centrifuging the solution at 8000 rpm for 15 mins. The resulting pellet washed with distilled water then dried in a hot air oven at 80 degrees for 2 hrs .(25).



Fig. 1. *Scoparia dulcis* habit.

Characterizations

Characterization of Greensynthesized ZnO NPs from *Scoparia dulcis*

S. dulcis extract and SDE- ZnO NPs are diluted then recorded in a UV-Visible spectrophotometer. (Shimadzu UV-1800). Background upon UV-Vis measurement was adjusted using deionized water. The 300 to 500 nm wavelength range was chosen. Both the lyophilized *S. dulcis* extract and the *S. dulcis* ZnO NPs were combined with KBr and subsequently formed into pellets for FTIR spectroscopy. The FTIR analysis was compared to the transmittance mode in the wave number range of 400-4000 cm^{-1} . Energy Dispersive X-ray Analysis was used to determine the constituent compositions of *S. dulcis* ZnO NPs (EDXA, model H-7593). A TEM (JEM-1400) and SEM with EDAX were used to investigate their morphology and dimensions (FE-SEM S4800). Using Cu $\text{K}\alpha$ radiation and a 2θ range of 20° to 80° , XRD was performed to characterise and determine the phase structure of crystalline ZnO NPs (25).

Acetylcholinesterase Inhibition Analysis

Acetylcholinesterase (AChE) inhibition activity was evaluated for the synthesized *S. dulcis* ZnO NPs based on Ellman's method (23).

Antioxidant DPPH Radical Scavenging activity

The green synthesized *S. dulcis* ZnO NPs is tested for *in vitro* DPPH antioxidant activity as previously proposed method (26, 27).

ABTS+ Decolorization Assay

The *in vitro* ABTS radical scavenging activity of biosynthesized *S. dulcis* ZnO NPs was carried out using the earlier published approach (28).

Results and Discussion

Photographic observation

The nanoparticle formation initially observes a visual colour change. Biosynthesis of ZnO-NPs using *S. dulcis* L. fresh leaves extract displayed in fig. 2, it is observed by a colour change from pale yellow to half-white (29).

UV-Vis Study

Zinc oxide nanoparticles confirmed by using UV-visible Spectroscopy. Due to the SPR effect, conducting electrons



Fig. 2. ZnO NPs utilising plant extract from *Scoparia dulcis*.

begin to oscillate at a specific wavelength range. The strong absorption peak obtained at 380 nm clearly demonstrates the presence of ZnO NPs in the reaction mixture (30, 31). The UV-visible spectra of SD-ZnO NPs are shown in fig. 3.

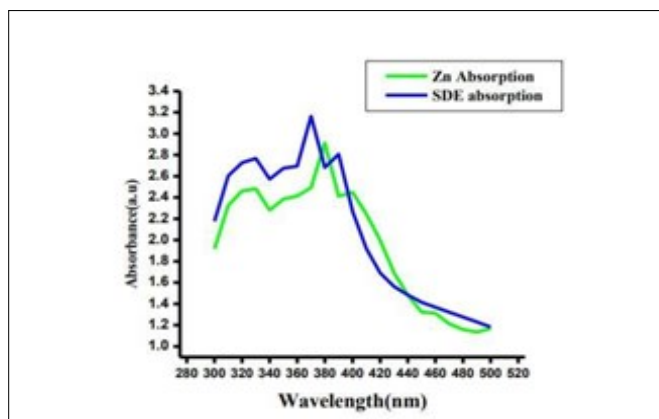


Fig. 3. UV-Vis spectrum of ZnO NPs synthesized by *Scoparia dulcis*.

FT-IR analysis

The FTIR spectrum of biosynthesized SD-ZnO nanoparticles showed a sharp and intense band at 540 cm^{-1} , indicating the presence of Zn-O vibrations (32). Existence of halogen compounds are insisted broad peak produced at $1018, 864 \text{ cm}^{-1}$ which matched toward C-O stretching and C-H bending vibrations. The strong peaks at 1550 cm^{-1} N-H bending, C=C and C-H bending and 540 and 493 cm^{-1} respectively (Fig. 4a). The plant extract itself had displayed a broad range at 3379 cm^{-1} corresponding to O-H stretch in the alcohol group. Sharp peaks were found at 1720 and 1450 cm^{-1} , which corresponded via C=O stretches the carbonyl group, -C-H bending alkane group respectively was shown in fig. 4b (33). This result implies the role of alkaloids, phenols, flavonoids and other phytochemical compounds present in the *S. dulcis* extract (34).

SEM and EDAX

Scanning electron microscopy analysis of the synthetic *S. dulcis*-capped ZnO NPs low resolution image (Fig. 5a) indicates agglomeration for the ZnO NPs which is a

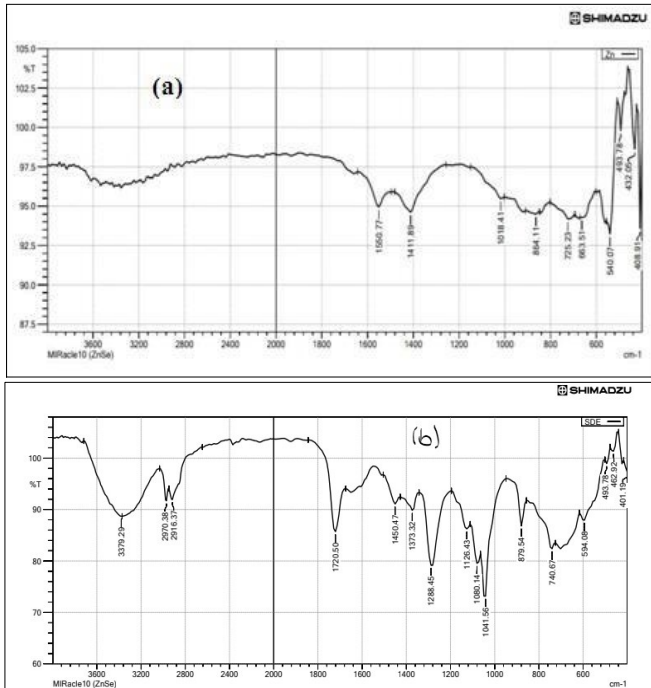


Fig. 4. FT-IR Spectrum of (a) ZnO NPs (b) *Scoparia dulcis* plant extract.

characteristic feature of the plant extract-mediated synthesis. On the other hand, the high resolution in the image (Fig. 5b) clearly represents that ZnO NPs possess rod like morphology ranged from 2 μm to 0.5 μm (35). The reason for agglomeration can be due to the existence of phytochemical moieties on the surface of the particles or due to the experimental conditions in the synthesis like pH of the medium, temperature etc. (36).

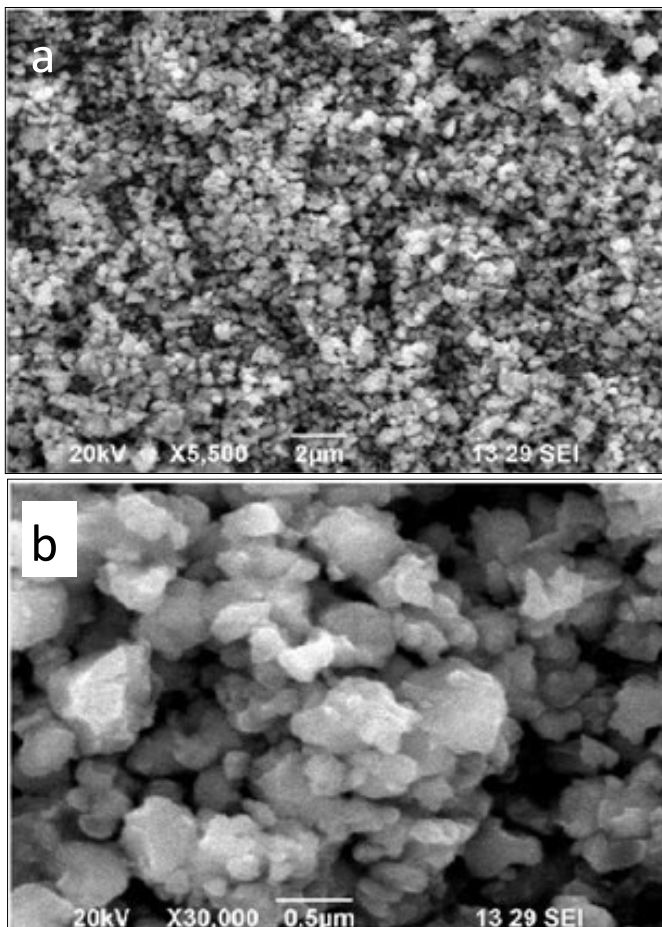


Fig. 5. ZNO NPs SEM images at various magnification ranges (a) 2 μm (b) 0.5 μm .

EDAX analysis confirmed the presence of a single peak of Oxygen at 0.5 KeV and 3 peaks of Zinc arise at 1, 8.6 and 9.6 KeV. The chemical composition of ZnO NPs was inferred from the EDAX spectra by the weight percentages of the components zinc and oxygen, which was 69.2% and 30.7% respectively was shown in fig. 6.

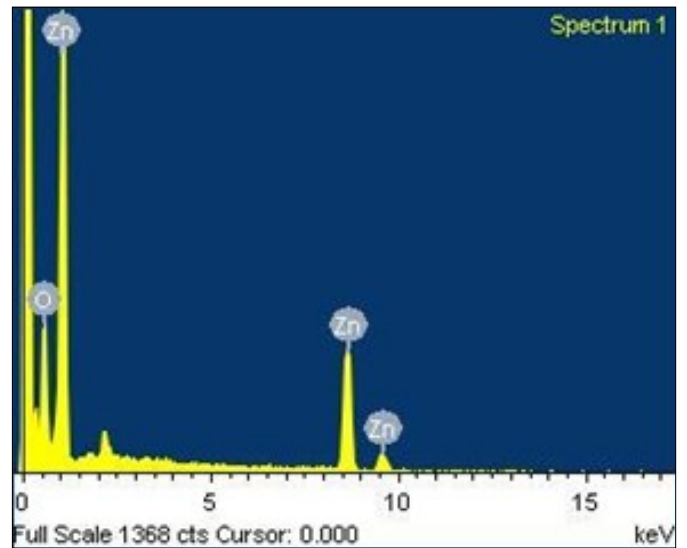


Fig. 6. EDAX spectra of ZNO Nps.

TEM analysis

The conformation of rod shape with a size range was 200 nm for SDE-ZnO NPs came through TEM analysis (Fig. 7). The free ZnO NPs showed that the plant extract can lower ZnO ions and prevent them from aggregating as a result of the bio molecules acting as protective agents (35).

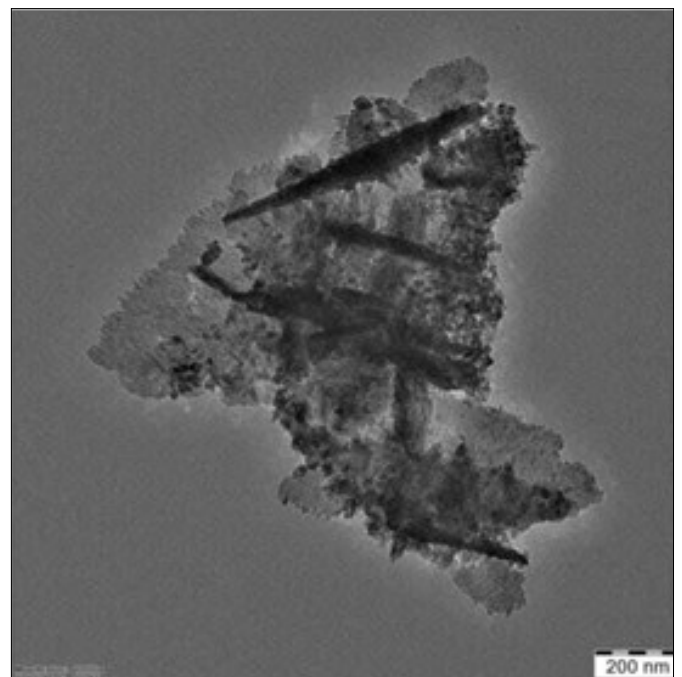


Fig. 7. TEM images for SDE - ZnO NPs magnification range 200 nm.

XRD Analysis

The SD-ZnO NPs crystallinity can be derived from its XRD spectrum. The Debye - Scherrer equation was used to determine the nanoparticles size (25). Synthesized ZnO NPs have good crystallinity and purity, which are evident from

the sharp peaks. The diffraction peaks were found to arise from the miller planes of (100), (002), (101), (102), (110), (103), (112), (202) which corresponds to the angle $2\theta = 31.8^\circ, 34.4^\circ, 36.4^\circ, 47.5^\circ, 56.6^\circ, 62.8^\circ, 68.0^\circ, 77.5^\circ$ respectively. This confirms that the ZnO NPs possess a hexagonal phase consistent with the Wurtzite structure [JCPDS No: 89-7102] (37). The XRD data indicates that plant extracts significantly impacted the development of ZnO NPs with good crystalline nature, as evident from the peak intensities (38). XRD Spectra of *S. dulcis* ZnO NPs (Fig. 8).

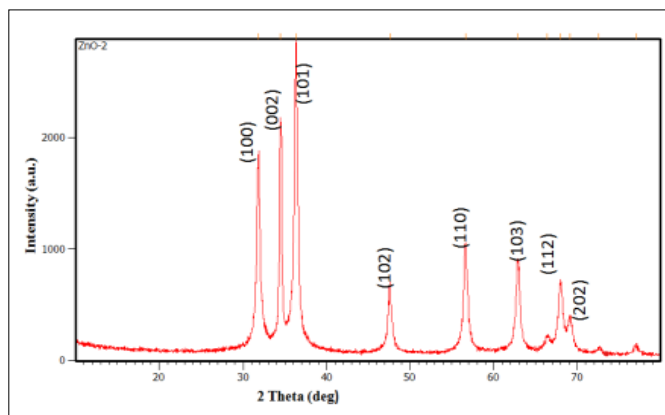


Fig. 8. XRD image of SDE - ZnO NPs.

In Vitro Acetylcholine Esterase (AChE) Inhibitory Activity of Synthesized SD -ZnO NPs

AChE inhibitors are therapeutically utilised to treat Alzheimer dementia. They increase the acetylcholine's availability in cholinergic synapses, which boosts cholinergic activity. For the acetylcholine esterase inhibition trials in this investigation, the previously reported AChE activity of *S. dulcis* aerial parts extract using 3 different solvents (methanol, butanol, and ethyl acetate) in Alzheimer's disease from Thailand. The butanol extract was the most potent AChE inhibitor, with an IC_{50} value of 93 $\mu\text{g}/\text{mL}$. Galantamine was used as a positive control (IC_{50} of 13 $\mu\text{g}/\text{mL}$) *S. dulcis* could be used as a therapeutic agent for those suffering from AD or other diseases characterized by a cholinergic deficit (7).

The substantial Acetyl cholinesterase inhibitory action for produced ZnO nano particles from the *Camellia sinensis* leaf extract was reported in Nilgiris (39). ZnO NPs made from *Moringa oleifera* leaves were tested for Acetyl cholinesterase inhibitory action from Nigeria (40) and the results revealed significant activity. In our current investigation, considerable confirmation of the AChE activity of the *S. dulcis* and SDE - ZnO NPs has been revealed for the first time (Table 1). It revealed that the methanol extract of *S. dulcis* leaves displayed percentage inhibitions for 3.20%, 8.90%, 13.20%, 19.60%, 24.60% and 27.20% and that the IC_{50} value was 90 $\mu\text{g}/\text{mL}$. Inhibitory percentages for synthesized SD-ZnO NPs ranged from 16.70% to 29.40% to 39.70% to 58.70% to 67.30% to 70.20%, having an IC_{50} value of 75.34 $\mu\text{g}/\text{mL}$. Both the drug and the common medication Rivastigmine were compared. The drug was dose-dependent at 20, 40, 60, 80 and 100 $\mu\text{g}/\text{mL}$ respectively, according to the results (Fig. 9). Hence, SDE-ZnO could serve as a starting point for the development of an alternative Acetyl cholinesterase inhibitor medications.

Table 1. AChE activity of Synthesized SDE - ZnO NPs

S.No	Concentration ($\mu\text{g}/\text{ml}$)	% of Zone of Inhibition		
		SD - ZnO NP's	<i>Scoparia dulcis</i> Methanol Extract	Rivastigmine
1.	20	16.70	3.20	75.10
2.	40	29.40	8.90	78.50
3.	60	39.70	13.20	84.80
4.	80	58.70	19.60	90.40
5.	100	67.30	24.60	95.30
6.	120	70.20	27.20	-
7.	IC_{50} value	75.34	90.00	73.29

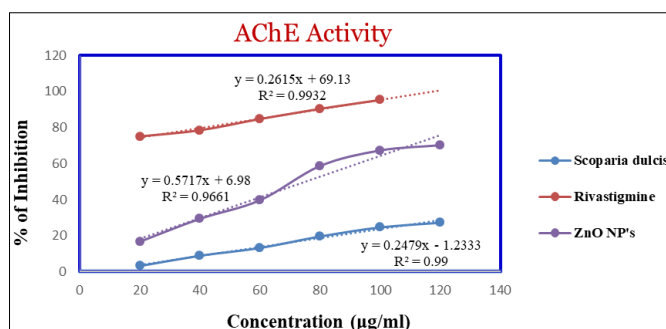


Fig. 9. Acetylcholine Esterase Inhibition Analysis of biosynthesized SDE - ZnO NPs.

Antioxidant Activity of Synthesized SD-ZnO NPs by DPPH assay

The *in-vitro* antioxidant activity of synthesized SDE - ZnO NPs was examined at 20, 40, 60, 80 and 100 $\mu\text{g}/\text{mL}$ concentrations with standard ascorbic acid listed in table 2.

Table 2. Antioxidant Activity of Synthesized SDE - ZnO NPs by DPPH assay

Concentration ($\mu\text{g}/\text{ml}$)	% of Inhibition	
	Ascorbic acid	ZnO NPs
20	78.10	14.20
40	84.30	30.70
60	87.40	54.80
80	93.20	68.70
100	95.60	88.90
IC_{50} value	111.84	58.44

The SDE - ZnO NPs exhibited inhibitory % between 14.20%, 30.70%, 54.80%, 68.70% and 88.90% which also varied on dose dependent (Fig.10) through IC_{50} of synthesized SDE - ZnO NPs is 58.44 $\mu\text{g}/\text{mL}$. SDE - ZnO NPs

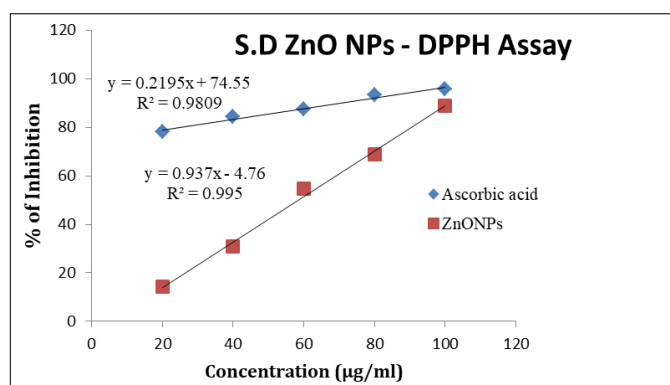


Fig. 10. Antioxidant Activity of ZnO NPs using DPPH Assay.

therefore may provide an alternate source of superior antioxidant inhibitor medications. The previously reported antioxidant activity of the synthesized ZnO NPs was evaluated using the DPPH assay from Kerala, which indicated an excellent IC₅₀ value of 1.7 µg/mL that shows high antioxidant activity. All these results proved that the *S. dulcis* plant extract-mediated synthesis method is a simple, low-cost, eco-friendly procedure for preparing efficient ZnO NPs for biomedical applications (17).

Antioxidant Activity of Synthesized SD-ZnO NPs by ABTS assay

Antioxidant activity of green synthesized SDE – ZnO NPs was exhibited % of inhibition between 21.90%, 45.40%, 71.70%, 89.80% and 95.60% (Fig. 11) through the IC₅₀ value is 59.79 µg/mL was shown in table 3 this is the first report on Antioxidant activity using ABTS assay. Alternative powerful antioxidant inhibitor medicines may come from the *S. dulcis*.

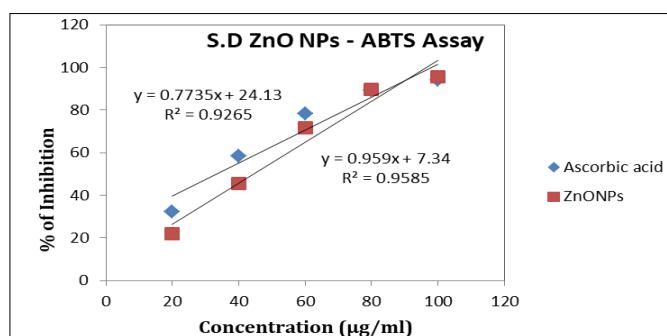


Fig. 11. Antioxidant Activity of Synthesized ZnO NPs using ABTS Assay

Table 3. Antioxidant Activity of SDE – ZnO NPs by ABTS assay

Concentration (µg/ml)	% of Inhibition	
	Ascorbic acid	ZnO NPs
20	32.30	21.90
40	58.50	45.40
60	78.30	71.70
80	89.40	89.80
100	94.20	95.60
IC50 value	33.44	59.79

Conclusion

By using *S. dulcis* aqueous extracts as a reducing agent in this study, we successfully synthesised ZnO nanoparticles in a green and environmentally safe manner. The creation of ZnO NPs is confirmed by the UV-Visible spectrum. Metal oxygen groups and bioactive functional groups were visible in the FTIR spectrum. A rod structure among mean particle size of 200 nm for ZnO NPs synthesized through SDE extract was visible by TEM examination. According to XRD results, a *S. dulcis* extract produced ZnO NPs with comparable structural shapes. Ellman's assay was used to measure the SDE-ZnO NPs AChE activity, while DPPH and ABTS assays were used to measure their antioxidant activity. *S. dulcis* may be used as a starting point for the synthesis of AChE inhibitors or as a source of alternative Acetylcholinesterase inhibitor medications. Alzheimer's disease symptoms have been treated with Acetylcholinesterase (AChE) inhibitors as a medication (AD).

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Authors contributions

RM completed the report as well as the characterization study for ZnO nanoparticles. VP wrote the paper used the data's from the UV-Visible, FT-IR, XRD, SEM with EDAX and TEM to characterise ZnO nanoparticles. KP was involved in acetylcholinesterase activity. KV carried out the DPPH assay. The ABTS test was carried out by MA. MV synthesised the ZnO nanoparticles from *Scoparia dulcis* L. The final manuscript was read and approved by all writers.

Compliance with ethical standards

Conflict of interest: There is no conflict of interest.

Ethical issues: None.

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