RJC

RASĀYAN *J. Chem.*
Vol. 16 | No. 1 |214-222| January - March | 2023
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM *Scoparia dulcis* L. PLANT EXTRACT AND ITS *In-vitro* ACETYLCHOLINESTERASE, ANTIOXIDANT ACTIVITY

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ABSTRACT

Silver oxide nanoparticles exhibit distinctive material and biotic properties that contain passionate research impact as vital applications in the pharmaceutical, biomedical, paper and textile industries. The goal of the current task is to synthesize AgNPs through a facile, low-cost, environmentally friendly route. In this method, we use the aqueous leaf extract of *Scoparia dulcis* Linn, found in the Western Ghats region, because the use of an aqueous medium is critical for reducing time and minimizing the possibility of side effects. The synthesized Ag nanoparticles were evaluated using UV-Visible, FTIR technique, scanning electron microscopy (SEM) with EADX, transmission electron microscopy (TEM), X-ray powder diffraction (XRD). Results confirmed that UV-visible spectroscopic analysis showed the absorbance peak at 460 nm, which implies the formation of silver nanoparticles. The FTIR spectrum aimed to such that biomolecules involved in wrapping and binding AgNPs synthesized by the plant extract. SEM and TEM are used to visualize the size and shape of nanoparticles. The XRD analysis revealed spherical structures with an average grain size of 21nm and a diameter range of 2 to 20nm. The EDAX study also exhibits the presence of 86.34 % Silver and 13.6 % Oxygen. Alzheimer's disease symptoms were controlled with a drug known as an acetylcholinesterase inhibitor. The *in vitro* acetylcholinesterase inhibitory activity of the AgNPs from *Scoparia dulcis* plant extract was evaluated using an Ellman's test with an IC_{50} value of 149µg/ml. The generated nanoparticles significantly inhibit AChE. The SD-AgNPs were examined for antioxidant activity via the DPPH and ABTS assays having IC_{50} values of 73.29µg/ml and 55.29µg/ml, respectively.

Keywords: Green Synthesis, *Scoparia dulcis*, Alzheimer's Disease, Ellman's Assay, Acetylcholinesterase (AChE), Antioxidant Activity.

RASĀYAN *J. Chem.*, Vol. 16, No.1, 2023

INTRODUCTION

Nanotechnology is regarded as a well-known cutting-edge technology with numerous applications in industries such as chemical, pharmaceutical, mechanical, food processing, power generation, optics, drug delivery and environmental sciences.[1] The importance of "green chemistry" which tries to decrease or remove potentially harmful compounds to human health and the environment when designing, developing, and using chemical processes and products has increased.[2,3] Researchers are increasingly embracing greener synthesis techniques due to the using less dangerous chemicals their eco-friendly nature and the one-step synthesis of nanoparticles.[4] Being environmentally benign and biocompatible, green nanoparticle formation with plants is advantageous since it avoids using toxic chemicals, high pressure, energy, or temperatures.[5] The use of plants for the creation of nanoparticles is a fast, inexpensive, environmentally friendly, one-step process that could be straightaway used for drug delivery and other similar applications without any coating techniques.[6] Because of their important medical potential, silver nanoparticles exhibit unique biological and physical characteristics that have generated great investigation research.[7] *Scoparia dulcis* Linn is a perennial herb with several bioactive ingredients in traditional medicine belonging to the Scrophulariaceae family. Its therapeutic characteristics were cancer diagnosis, diarrhea, diabetes, stomachaches, hepatitis, bronchitis, fever, hypertension and ulcers[8] which also exhibited antibacterial, anti-diabetic, anti-fungal, anti-inflammatory, antiviral, and hypercholesterolemic properties. The phytoconstituents for *Scoparia dulcis* L., which can all mediate the production of AgNPs, were in prominent abundance and included diterpenoid, triterpenoid, alkaloids, flavonoids, phenols, and steroids.[9] Phenolic components have a greater ability to reduce metals for their

http://doi.org/10.31788/RJC.2023.1618075

anti-oxidative properties.[10] Acetylcholinesterase (AChE) a neurotransmitter hydrolase is a factor in the stimulus transmission's termination for the number of cholinergic routes in the CNS and peripheral nervous systems.[11] By blocking AChE and lowering acetylcholine cleavage, Acetylcholinesterase inhibitors enhance cholinergic neurotransmission. Based on this inhibitory mechanism, First-line drugs for Hypertension, early-onset dementia, dizziness, congenital myasthenia syndrome, and Psoriatic arthritis are used with AChE inhibitors.[12] Although donepezil, Rivastigmine, and tacrine are frequently used to control neurological impairment their side effects such as digestive upset due to this reason nowadays researchers look for more natural cures.[13] Acetylcholine esterase inhibitors arise from plant-derived bioactive compounds due to their positive effects on cognitive impairments; they are attractive candidates for the therapeutics of Alzheimer's disease. Acetylcholinesterase (AChE) study was analyzed using Ellman's method.[14] The present study is a non-toxic and environmentally friendly generation of AgNPs using *Scoparia dulcis*. Several characterization methods were applied to investigate the shape and morphology of silver nanoparticles, including UV-Vis, IR, SEM, TEM, XRD, and EDAX. Ellman's approach was used to examine the inhibition of enzymes associated with Alzheimer's disease Acetylcholinesterase (AChE) studies on green generated AgNPs of *Scoparia dulcis* extract. Using the DPPH and ABTS assays, the antioxidant potentials of generated AgNPs of *Scoparia dulcis* extract were studied *in vitro* method.

EXPERIMENTAL

Collection of Plant

Fresh leaves of *Scoparia dulcis* were found in the Western Ghats region of Pollachi, Tamilnadu, South India (10035' 12.96" N, 77014' 37.37" E) between July 2021 and August 2022. The PG Department of Botany, NGM College, Pollachi recognized and verified the plant material. In order to be used as a reference in the future, the specimen NGMPCY47 was preserved in the PG Department of Chemistry at the NGM Campus.

Preparation of Plant Extract

About 100 g of fresh *Scoparia dulcis* leaves shown in Fig.-1 were sliced and immersed in a 500 mL flask with 100 mL Milli-Q water after over-cleaned with water. For 8 minutes, the solution was boiled at 70ºC. The leaf extract was filtered through Whatman No. 1 filter paper after a time to cool the extract was stored for use in further experiments.[15]

Fig.-1: *Scoparia dulcis* (Linn.)

Silver Nanoparticles Synthesis

The biosynthesis of silver nanoparticles was performed utilizing a 1 mM silver nitrate solution ($AgNO_3$) prepared in deionized water. *Scoparia dulcis* plant extracts of 10 ml and 90 ml of 1 mM silver nitrate were taken in a 250 ml beaker. This content was then heated to 90 °C for an hour. As evidenced by the color change from colorless to dark brown that was seen over time AgNPs have formed.[16] The silver colloidal solution (1:6 ratio of extract to salt solution) is centrifuged at 4500 rpm for 30 minutes to get rid of superfluous Ag+ ions. Using a cooling centrifuge, collected particles are centrifuged at 10,000 rpm for 15 minutes after being rinsed among a phosphate buffer (pH 7.0). The prepared silver nanoparticles were lyophilized at -20 °C to obtain dry SD-AgNPs powder.[17]

Characterizations of Biosynthesized Ag NPs

UV-Vis Analysis

The synthesized AgNPs from aqueous extract of *Scoparia dulcis* leaves are identified using a UV–Visible spectrophotometer (SHIMADZU, UV-1800, PG Department of Botany, Nallamuthu Gounder Mahalingam College, Pollachi), between 350 to 750 nm; with 10-mm quartz cuvettes have a resolution of 1 to 21nm.

FT-IR Spectroscopy Analysis

FTIR analysis was used to investigate functional groups and the interaction between proteins and AgNPs, in contrast to the transmittance mode. FT-IR instrument (BR-CNR RAO Research Centre, Avinashilingam Institute of HSHE for Women, Coimbatore) was operated via 400–4000 cm^{-1} wave number range. KBr pellets are purchased from Merck Chemicals and separately pulverized with SD AgNPs. Utilizing the hydraulic pellet press to create thin sample pellets, an FT-IR study was performed.[18]

X-ray Diffraction Analysis, TEM, and SEM with EDAX

The elemental compositions of *Scoparia dulcis* AgNPs were identified by energy-dispersive X-ray analysis (EDXA, model H-7593, SRM Institute of Science and Technology, Kancheepuram). The solution of biosynthesized AgNPs was centrifuged at 15000 rpm for 10 minutes before being washed with deionized water to separate the silver NPs. It was dried in an oven overnight at around 80–90 °C. XRD analysis was performed on these dried nanoparticles. After being drop coated the powdered AgNPs are examined using XRD using Cu Ka 1.5418Ao, 30 kV and 20mA with a scan speed of 2 nm across 2 hrs. Temperature range for 20°C to 80°C maintained. A TEM (JEM-1400, SRM) and SEM with EDAX were used to observe their morphology and dimension (FE-SEM S4800, Karunya Institute of Technology and Sciences, Coimbatore, India). The synthesized SD-AgNPs performed EDAX analysis after being dissolved in pure ethanol and ultrasonically processed. A small amount of these suspensions was placed on a Cu grid, dried in the air, and then evaluated.[19]

Acetylcholinesterase Inhibition Activity

Scoparia dulcis-produced AgNPs were investigated for their potential to prevent acetylcholineesterase (AChE) using Ellman's method.[14]

Antioxidant DPPH Radical Scavenging Activity

Utilizing the DPPH approach, it was possible to measure SD-AgNPs' capacity to capture free radicals.[20]

ABTS•+ Decolorization Assay

Scoparia dulcis silver nanoparticles' ability in order to defend antioxidants against the ABTS•+ radical cation was examined using this ABTS technique.[21]

RESULTS AND DISCUSSION

Visual Observation

The green synthesis of AgNPs using *Scoparia dulcis* fresh leaves aqueous extract is illustrated in Fig.-2a. The formation of SD-AgNPs in Fig.-2b confirmed that the pale green color had turned dark brown.

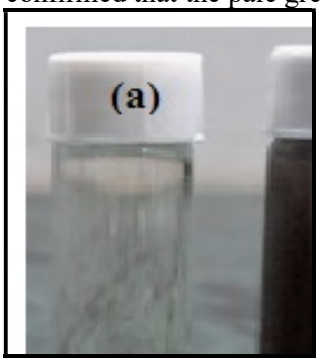

Fig.-2(a): *S. dulcis* Plant Extract, Fig.-2(b): Synthesized AgNPs using *S.dulcis*

UV-visible Analysis

UV-visible spectroscopy was used to confirm the formation of AgNPs. As a result of SPR effect activation[18] for AgNPs a free electron has existed, which is combined vibration of the metal nanoparticles electrons in resonance with the light wave is produced in the SPR absorption band.[19] The gradual reduction of Ag ions to AgNPs is indicated along a peak at 460 nm.[17,22,23] The UV-visible spectra of SD-AgNPs as displayed in Fig.-3.

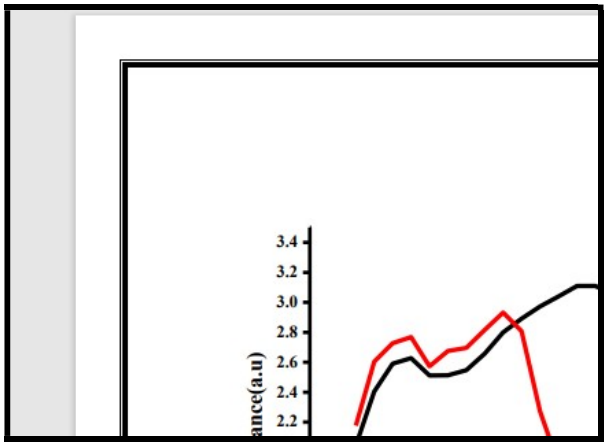

Fig.-3: UV-Visible Spectrum of AgNPs Synthesized by *Scoparia dulcis*

FT-IR Analysis

The FT-IR spectroscopy for greenly synthesized Ag-NPs was adapted to investigate the functional groups in control of Ag+ ion reduction and biosynthesized AgNPs stability. Fig.-4(a) and Fig.-4(b) shows that FTIR spectra of synthetic AgNPs and a control plant extract, respectively. Plant extract had strong bands at 3379, 2970, 1720, 1450, 1373, 1286, 1126, 1080, 879, 740, and 594 cm^{-1}, while manufactured silver nanoparticles had peaks at 2916, 1728, 1604, 1512, 1365, 1288, 1118, 1072, and 1041 cm^{-1}, carboxylate ion peak on 1365 cm^{-1} (–COO–) is in charge of maintaining silver nanoparticle stability (Fig.-4b). The intense and wide band observed by 3379 cm^{-1} (*S. dulcis* extract) corresponds to the alcoholic or phenolic O–H stretching band.[24] The amine N–H bending group[25] of leaf extract has a peak at1720 cm^{-1}, while the C–H stretching vibrations of $–CH_3$, $–CH_2$ has a peak at 2970 cm^{-1} (Fig.-4a). These peaks are missing from AgNPs - FTIR spectra, as shown in Fig.-4(b), showing that the O–H groups are a part of the synthesis with AgNPs. In addition, the IR spectra of AgNPs showed an absorption band at 1365 cm^{-1}, which corresponded to the NO_3 stretching caused by the silver nitrate residue, while the bands at 1373 cm^{-1}, 1286 cm^{-1} signified the aromatic tannins present in *Scoparia dulcis* extract.[26] The C–O group was responsible for the peak at 1080 cm^{-1}, which changed to 1041 cm^{-1} after silver reduction. The N–H bond in 1°, 2° amines found in peptides correspond to the 879 cm^{-1}. The methyl (CH_3) rocking vibrational band is represented by a prominent band at 740 cm^{-1}. Biological components are interacting with metal salts and affect reduction processes via these functional groups.[27] Functional groups such as aldehydes, amines, carboxylic acids, and alcohols were found in the FTIR spectrum of AgNPs and the aqueous extract from *S.dulcis* proving that they effectively reduce and cap the silver ions.[28] According to FTIR analysis, the *Scoparia dulcis* leaf extract (–C=O), (–OH), (N–H) groups remain predominantly responsible for the reduction of Ag^+ to Ag^0 nanoparticles and the synthesis of nanoparticles stabilized by carboxylic functionality.[29]

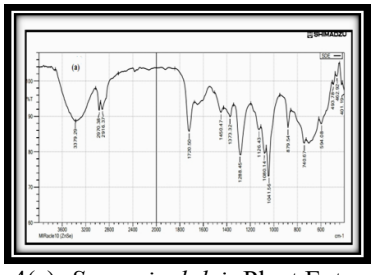

Fig.-4(a): *Scoparia dulcis* Plant Extracts

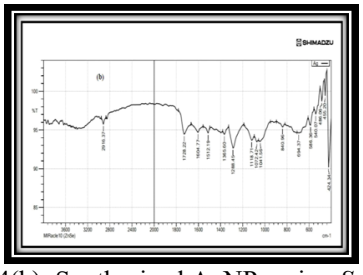

Fig.-4(b): Synthesized AgNPs using *S. dulcis*

SEM and EDAX

SEM analysis was analyzed to imagine the shape & size of Ag nanoparticles. The appearance of SD-AgNPs in addition to morphological characteristics by the SEM, regular size from 1µm to 10µm was obtained. It can be shown that the *Scoparia dulcis* extract is applied as a capping and reducing agent to produce AgNPs with a spherical shape and average diameter of 2µm. The SD-AgNPs SEM pictures are given in Fig.-5

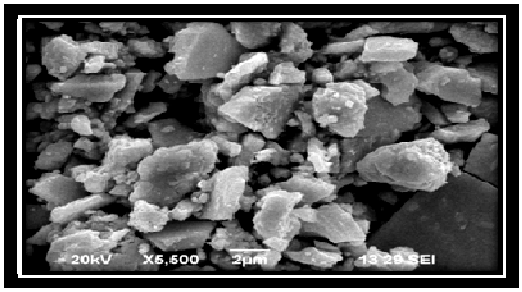

Fig.-5: SEM Image of AgNPs in Magnification Range for 2µm

EDAX analysis was performed to verify the existence of a single peak of oxygen at 0.5 KeV and a peak of Ag at 3.5 KeV. From EDAX spectra, it is evident that AgNPs reduced by *Scoparia dulcis* (Linn.) have silver's weight percent as 86.30 % and 13.6 % oxygen. EDAX spectrum for the SD-AgNPs was seen in Fig.-6.

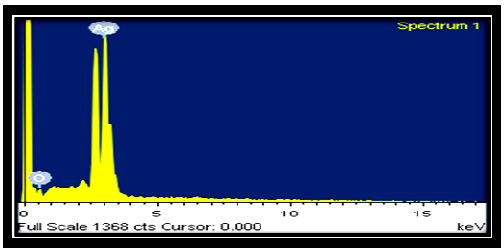

Fig.-6: EDAX Spectrum of AgNPs

TEM

The TEM image in Fig.-7 reveals that it is spherical and very small. The average particle size for SD-AgNPs ranged from 2 to 20 nm. According to the free AgNPs, Ag ions can be reduced by extract, and inhibit aggregation since the biomolecules serve as protective agents. In contrast, bio compounds perform as reducing agents at higher extract concentrations.

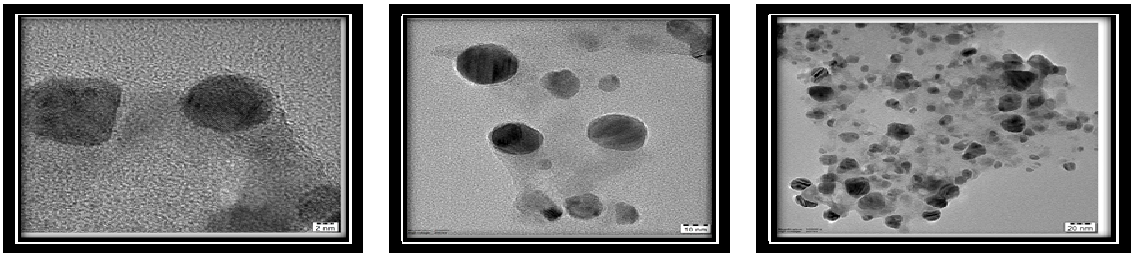

Fig.-7: TEM Image of AgNPs

XRD Analysis

The crystal identity of biosynthesized silver nanostructure from *Scoparia dulcis* leaves extract was exhibited and sustained by the distinct slopes detected in the XRD. It displayed significant diffraction peaks and the XRD pattern revealed Braggs reflections at 2θ= 27.9°, 32.3°, 38.2°, 44.4°, 46.3°, 54.9°, 57.4°, 64.5°, and 77.5° which are referred to as (JCPDS)[17] Fig.-8.

AChE Activity of SDE-AgNPs

AChE inhibitors are therapeutically utilized to treat Alzheimer's syndrome, because they make more acetylcholine accessible in cholinergic synapses, they increase cholinergic activity. For the acetylcholine esterase inhibition trials in this investigation, *Scoparia dulcis* was utilized at doses of 20, 40, 60, 80, and 100µg/mL that also varied with dosage shown in Fig.-9. When compared to the normal Rivastigmine, the

synthesized SDE -Ag NPs had an IC_{50} value of 149 g/mL. For the first time, significant confirmation of the SDE–Ag NPs' AChE activity has been reported Table-1. Hence, *Scoparia dulcis* alternative Acetylcholinesterase inhibitor medications or acts as a starting point for the construction of AChE inhibitors.

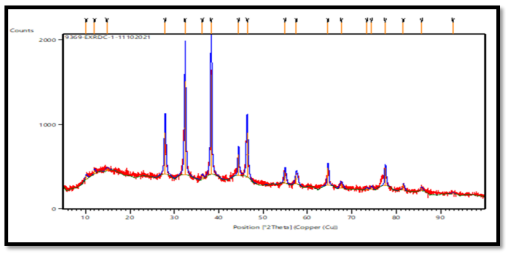

Fig.-8: XRD Spectrum of Synthesized AgNPs

Table-1: Acetylcholinesterase Activity of Synthesized AgNPs *Scoparia dulcis*

S.No	Concentration (µg/ml)	% of Zone of Inhibition	
		Ag NP's	Rivastigmine
1.	20	5.7	75.1
2.	40	13.1	78.5
3.	60	19.8	84.8
4.	80	27.3	90.4
5.	100	34.5	95.3
6.	120	38.6	-
7.	IC_{50} value	149.71	73.29

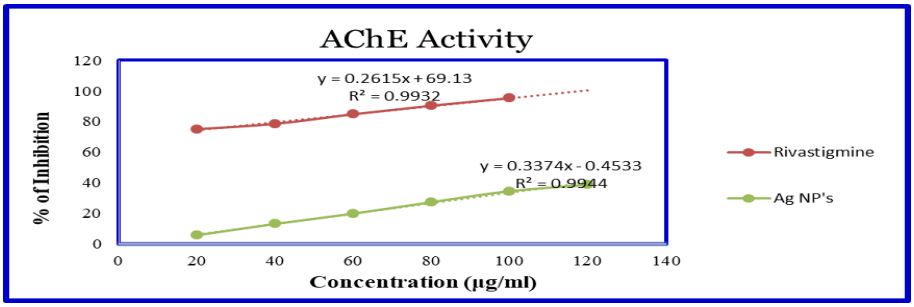

Fig.-9: Acetylcholinesterase Inhibition of Synthesized AgNPs

Antioxidant Assays of Synthesized SD-AgNPs

Fig.-10 and Fig.-11 represents the outcomes of the DPPH and ABTs tests performed to assess the antioxidant potentials of SD-AgNPs. The antioxidant ability of SDE-AgNPs was correlated with that of standard ascorbic acid. SDE-AgNPs showed the greatest radical scavenging performance against the DPPH radical, with displayed IC_{50} values of 69.79 µg/mL and 111.84 µg/mL for ascorbic acid. The displayed IC_{50} values for SD-AgNPs were 55.29 µg/mL for SD-AgNPs and 33.44 µg/mL for standard. SDE-AgNPs' antioxidant action against ABTS was reported for the first time. SDE AgNPs could thus eventually be used as a source of improved antioxidant inhibitors. The antioxidant activity of SDE-AgNPs was determined using DPPH, and ABTS assays with ascorbic acid at 20, 40, 60, 80, and 100 µg/ml concentrations are shown in Tables-2 and 3.

Table-2: Antioxidant Activity of Synthesized SDE–AgNPs by DPPH Assay

Concentration (µg/ml)	% of Inhibition	
	Ascorbic acid	AgNPs
20	78.1	12.3
40	84.3	28.7
60	87.4	41.5
80	93.2	59.6
100	95.6	71.5
IC_{50} value	111.84	69.79

Table-3: Antioxidant Activity of Synthesized AgNPs Using ABTS Assay

Concentration (µg/ml)	% of Inhibition	
	Ascorbic acid	AgNPs
20	32.3	18.9
40	58.5	35.2
60	78.3	61.2
80	89.4	74.1
100	94.2	79.4
IC_{50} value	33.44	55.29

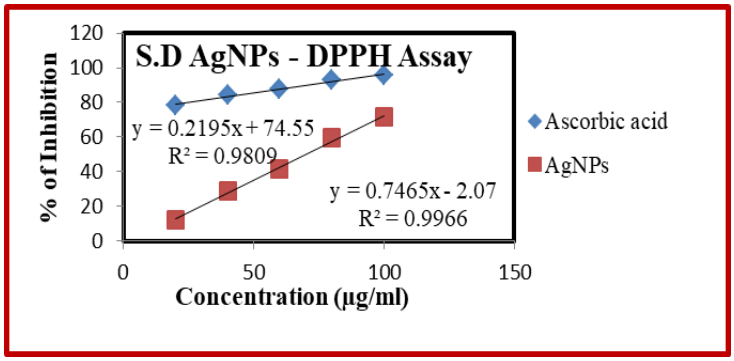

Fig.-10: SDE–AgNPs Antioxidant Activity by DPPH Assay

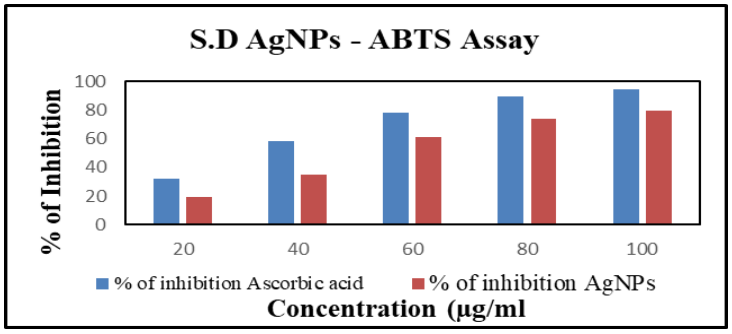

Fig.-11: SDE–AgNPs Antioxidant Activity by ABTS Assay

CONCLUSION

This study used Ag NPs, which were synthesized by employing a green technique from *Scoparia dulcis* (L.) leaf aqueous extract at room temperature. Silver nitrate has been converted into silver oxide nanoparticles using *S. dulcis* leaf extract act as a reducing agent. Green synthesized silver oxide nanoparticles are confirmed by a color change, which was observed by UV-Visible at 460 nm. The spherical AgNPs are further characterized by SEM and TEM investigation, which reveals that their particle sizes range between 2 to 20 nm. From the XRD data, the SD-AgNPs had a typical size of 21 nm. FTIR spectrum revealed the structure with the appropriate bands of the synthesized nanoparticles. SD-Ag NPs demonstrated specific antioxidant and acetylcholinesterase activities. *Scoparia dulcis* could be used to make alternative AChE inhibitor medicines or as a starting point for AChE inhibitor syntheses. According to the findings of this study, nanoformulation of manufactured AgNPs using *S. dulcis* aqueous plant extract is perhaps a better way to improve the potency of plant extracts in the future.

ACKNOWLEDGMENTS

The management of Nallamuthu Gounder Mahalingam College (Autonomous) in Pollachi is deserving of my deepest gratitude for providing the initial financing for our project. I thank the Post Graduate Botany Department of NGM College for capturing UV-Visible Spectra.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/ editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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[RJC-8075/2023]