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ASSESSMENT OF SILVER NANOPARTICLES DERIVED FROM *AVERRHOA CARAMBOLA* LEAF EXTRACT AS A NOOTROPIC -INTERVENTION FOR ALZHEIMER'S DISEASE

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ABSTRACT: Alzheimer's disease (AD), a progressive neurodegenerative disorder, is characterized by cognitive decline and memory impairment, often associated with oxidative stress, amyloid plaque accumulation, and neuroinflammation. In the quest for alternative therapeutic strategies, nanotechnology combined with phytochemistry offers a promising avenue. This study investigates the synthesis, characterization, and neuroprotective potential of silver nanoparticles (AgNPs) synthesized using the aqueous leaf extract of *Averrhoa carambola* - a plant known for its antioxidant and medicinal properties. The green synthesis approach yielded stable, bio-functionalized AgNPs, as confirmed through UV–Vis spectroscopy, FTIR, SEM, and TEM analyses. *In-vitro* antioxidant assays demonstrated significant free radical scavenging activity of the synthesized nanoparticles. Furthermore, *in-vivo* experiments using an Alzheimer's-induced murine model revealed that treatment with *A. carambola*-derived AgNPs improved cognitive performance, reduced oxidative biomarkers, and attenuated acetyl cholinesterase activity in the brain. Histopathological assessments also indicated a reduction in amyloid deposition and neuronal degeneration. These findings suggest that bioengineered AgNPs from *Averrhoa carambola* may serve as a novel, biocompatible nootropic agent with therapeutic potential in mitigating the pathophysiology of Alzheimer's disease. Further investigation into pharmacokinetics, toxicity, and long-term effects is warranted to support clinical translation.

INTRODUCTION: Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive decline, memory loss, and behavioral disturbances. Despite decades of research, effective treatments that can halt or reverse disease progression remain elusive. Current pharmacological therapies provide only modest symptomatic relief and are often associated with significant side effects.

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This has led to growing interest in exploring alternative therapeutic strategies, including the use of natural compounds and nanotechnology-based interventions. Nanoparticles, particularly silver nanoparticles (AgNPs), have emerged as promising agents in biomedical research due to their unique physicochemical properties, including high surface area, bioavailability, and ability to cross the blood-brain barrier.

AgNPs have demonstrated anti-inflammatory, antioxidant, and neuroprotective effects, which are relevant to the pathophysiology of Alzheimer's disease. *Averrhoa carambola*, commonly known as star fruit, is a tropical plant rich in phytochemicals such as flavonoids, polyphenols, and alkaloids, which possess potent antioxidant and neuro-

protective properties. Utilizing plant-based extracts for the green synthesis of nanoparticles not only provides a sustainable and eco-friendly approach but also enhances the therapeutic potential of the nanoparticles through synergistic effects of the bioactive compounds.

This study aims to assess the potential of silver nanoparticles synthesized using *Averrhoa carambola* leaf extract as a nootropic intervention for Alzheimer's disease. By integrating phytomedicine with nanotechnology, the research seeks to explore a novel therapeutic avenue that may offer enhanced efficacy, reduced toxicity, and improved patient outcomes in the management of neurodegenerative disorders [4].

FIG. 1: *AVERRHOA CARAMBOLA* PLANT

MATERIAL AND METHODS:

Collection of *Averrhoa carambola* Leaves: The fresh leaves of *Averrhoa carambola* was utilised for the blending of *Averrhoa carambola* leaf extract. The fresh leaves were procured from *Averrhoa carambola* plant in the surroundings and this specimen samples was recognized and authenticated by Botany Department, Kerala University, Trivandrum.

Extraction of *Averrhoa carambola* Leaves: Fresh leaves were cleansed exhaustively with distilled water, weighed 60g of leaves, added 240ml of distilled water, admixed, heated at 80°C for 3 hours with progressive stirring and result ant extract was then strained thoroughly utilizing Whatman filter paper [15].

Preliminary Screening of Phytochemicals: Phytochemicals Preliminarly screening of alkaloids, tannins, saponins, phenols, flavonoids, glycosides were sustained out in *Averrhoa carambola* leaf extract by the following tests [14, 18].

Synthesis of Green Ag Nano-particle: 30mL aqueous leafy extraction of *Averrhoa carambola* was ad mixed to 270 ml of 1mM aqueous silver nitrate solution, followed by heating at 80°C for 3 hrs with regular stirring. The formation of AgNPs was preliminary detection by change in colour from yellowish green to darkish brown. Obtained AgNPs were separated by centrifugation at 10,000 rpm for 25 minutes [8].

Spectral Characterization: The explored Silver Nanoparticles have been characterized by FTIR, UV, Scanning electron microscopy and X-ray diffraction [10].

UV-visible Spectra: To perceive the full bioreduction of AgNO3 to silver nanoparticles the spectra, the sample or medicament were checked using a Shimadzu U.V. probe1800 in the scanning series 400 to 800 nm within are solution of 1 nm [13].

IR Spectra: IR spectra of the integrated silver nanoparticles were taken down within the range of 4000-300cm^{-1} on Shimadzu FTIR spectro-photometer using KBr pellet method. FTIR involves in the verification of bioactive agents covalently grafted onto silver, copper, and zinc together with any other NPs [14].

Scanning Electron Microscopy (SEM): For the analysis of the nanostructures of the samples, thin films are prepared in glass slides and then observed in Scanning Electron Microscopic (SEM, Hitachi, S-3000N). Finest films within samples are prepared by using spin coater (Delta spin) and the films are dried by placing it receiving the IR lamp (Philips) for 5 min.

RESULTS:

Extraction of *Averrhoa carambola* Leaves: *Averrhoa carambola* leaf extraction obtained after 3 hours continuous heating at 80°C.

FIG. 2: *AVERRHOA CARAMBOLA* LEAF EXTRACT

Preliminary Phytochemical Screening: Phytochemical exploration of *Averrhoa carambola* leaf extract were carried out. Phytochemical constituents such as glycosides, tannins, flavonoids, alkaloids, saponins were identified [11, 12].

TABLE 1: PHYTOCHEMICAL SCREENING OF *AVERRHOA CARAMBOLA* LEAF EXTRACT

Qualitative tests	Result
Test for alkaloids	
Dragendroff's test	+
Mayer's test	+
Hager's test	+
Wager' stest	+
Test for Tannins	
Ferricchloride test	-
Lead acetate test	+
Potassium dichromate test	+
Test for saponins	
Foam test	+
Liebermann Burchard's test	+
Test for phenols	
Lead acetate test	+
Potassium dichromate test	+
Test for flavonoids	
Shinoda test	-
Lead acetate test	+
Sodium hydroxide test	+
Pew's test	+
Test for glycosides	
Ferric chloride test	-
Bromine water test	+
Keller Killiani's test	+

Green Synthesis of Silver Nanoparticle: As the aqueous leaf extraction of *Averrhoa carambola* ad mixed to silver nitrate solution, the colour change of the solutions changing from a light brown to blackish brown subsequent to 3 hours continuous stirring at 80°C, and this indication relates synthesis of biomolecules of *Averrhoa carambola* AgNPs [20].

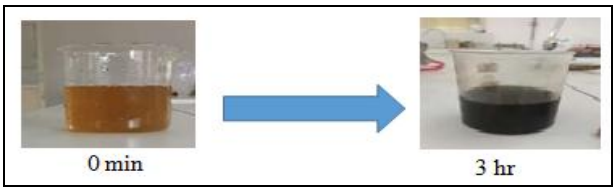


FIG. 3: COLOR CHANGES OF REACTING SOLUTION WITH TIME

Characterization of Silver Nanoparticles:

UV-Visible Spectroscopic Analysis: UV-visible spectroscopy as one most broadly used technique for structural characterization of nano-particles. The absorbance was taken at 300-800 nm. A peak was obtained at 420nm corresponding with the standard value between400-450 nm.

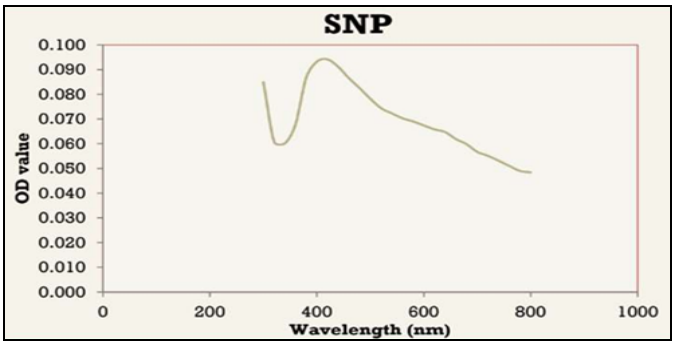


FIG. 4: UV SPECTRAL ANALYSIS

FTIR Spectroscopic Analysis: The synthesized AgNPs were explored using FTIR spectral analyte. The observed intensity bands comparison within standard values for identify the functional groups. FTIR spectrum shows absorption bands at 3431, 2917, 2849, 1650, 1141, 1327, 1033, 775 cm^{-1}. The bands at 3431 cm^{-1} in the spectra nearest to O-H stretching vibration indication the existance of alcohol along with phenol. Band at 2917 cm^{-1}exploring presence of methyl C-H stretching. Thus band 2849 cm^{-1} indicate the presence of alkyl C-H stretching. The peak area at 1650 cm^{-1} corresponding to double bond (C=C, C=O) and aromatic stretching. The band at 1441 cm^{-1} relates to the heteroaromatic C-C ring stretching. The band at 1327 cm^{-1} corresponding to O-H bending. The b and at 1033 cm^{-1} corresponds to O-H stretching of phenol. The band 775 cm^{-1} corresponds to aromatic C-H bending. The bands at 668 cm^{-1} indicates the strong binding affinity with the metal suggesting the encapsulation of metal nanoparticles [17, 20].

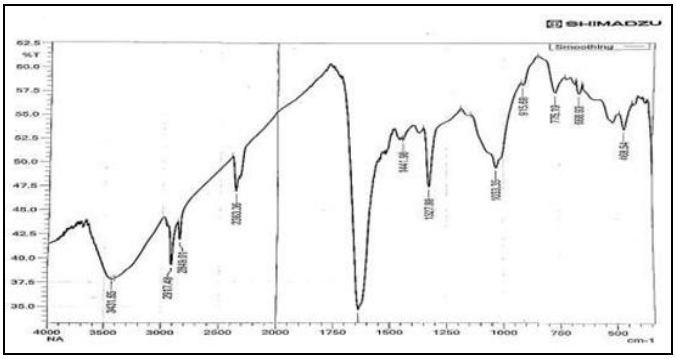


FIG. 5: FTIR SPECTRUM OF AgNP

X-ray Diffraction: X-Ray diffraction peaks of synthesized AgNPs were obtained diffraction angles at 38^o, 44^o, 69^o and 77^o which corresponds to the silver nanoparticles [17, 20].

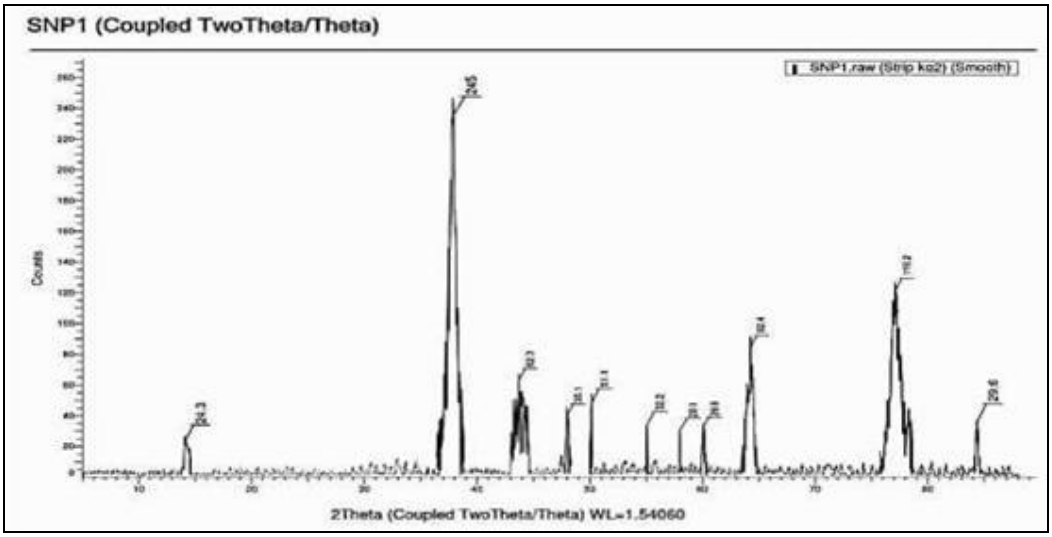


FIG. 6: XRAY DIFFRACTION OF AgNP

Scanning Electron Microscopy (SEM): AgNP was exposed to Scanning electron microscopy (SEM), the image obtained is given in **Fig. 7.**

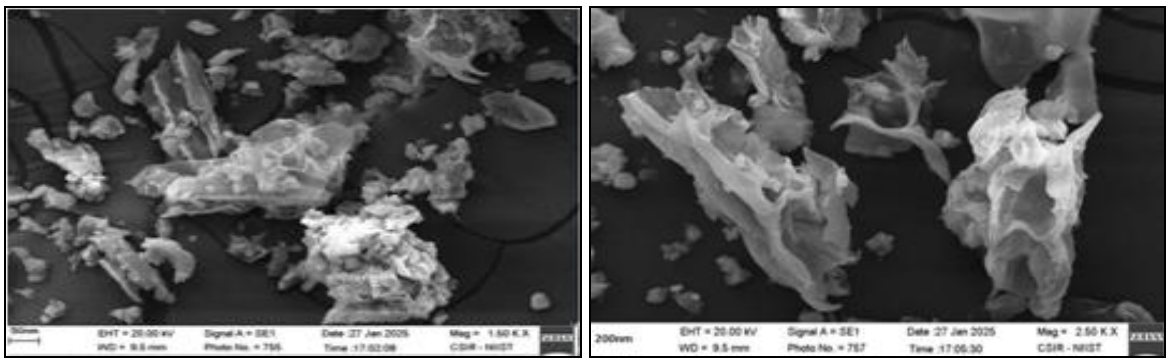


FIG. 7: SEM OF AgNP

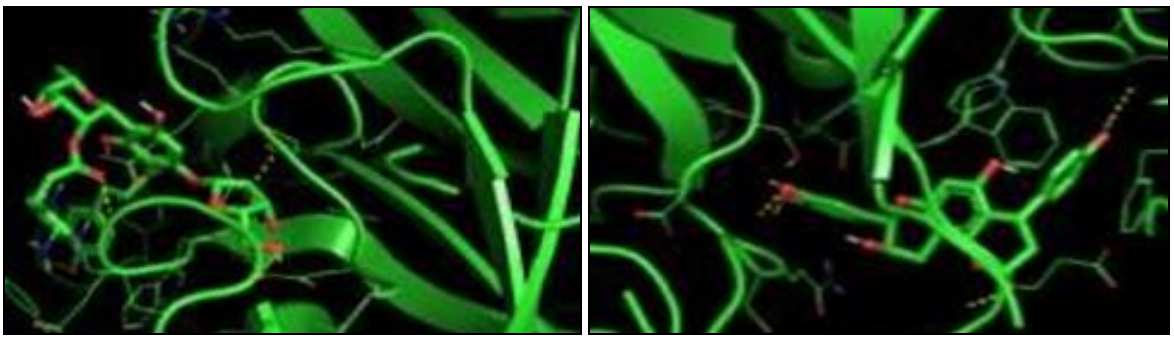
The SEM image indicate the size range of Silver nanoparticle from 50-200nm.

Molecular Docking:

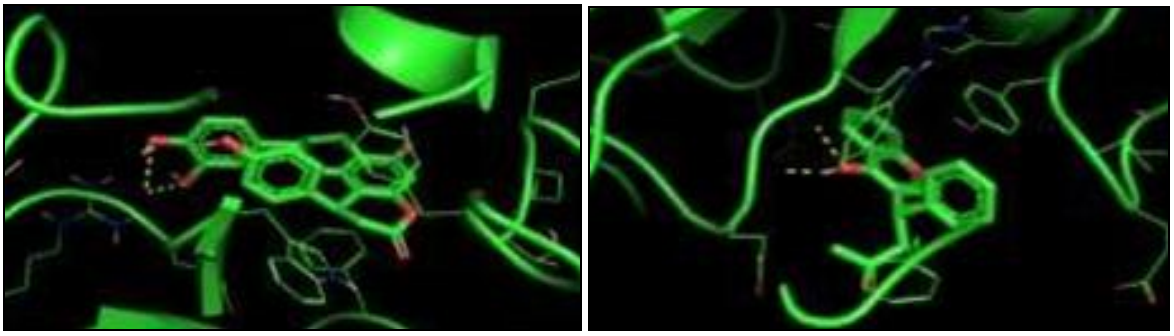
Molecular Docking of Antioxidant Activity: Carambolaside R1, Epicatechin, Quercetin derivatives and Ascorbic acid as drug standard have docked using Human peroxiredoxin (1HD2) for antioxidant activity. Here, the Carambola side has highest docking score for antioxidant activity than Quercetin and Epicatechin derivatives.

TABLE 3: DOCKING SCORE OF ANTIOXIDANT ACTIVITY

Compound Name	Binding Affinity
Carambolin	-9.3
Quercetin	-9.0
Epicatechin	-7.8
Standard Drug : Ascorbic acid	-7.6



CARAMBOLIN **EPICATECHIN**



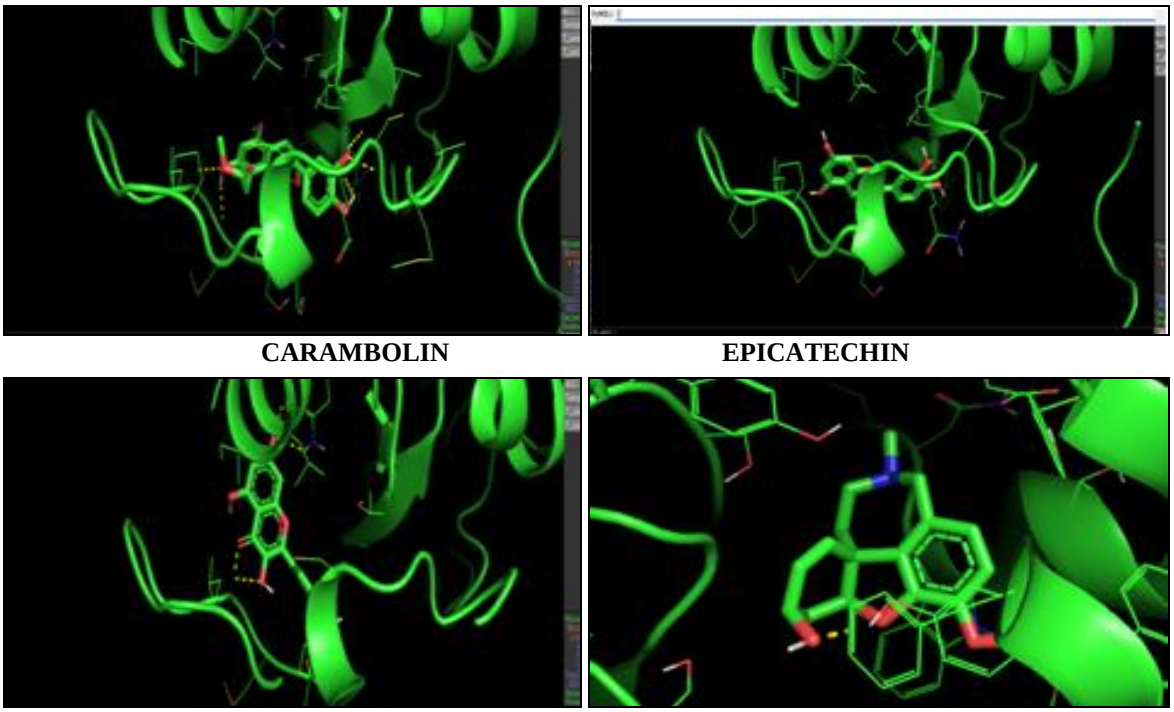
QUERCETIN **ASCORBIC ACID**

FIG. 8: MOLECULAR DOCKING IMAGES OF ANTIOXIDANT ACTIVITY

Molecular Docking of Anticholinesterase Activities: Carambolin, Epicatechin, Quercetin, derivatives along with Ascorbic acid as drug standard have docked using Recombinant Acetyl Cholinesterase (3LII) for anticholinesterase activity. Here, the Carambolaside has highest docking score for antioxidant activity than Epicatechin derivatives [17].

TABLE 4: DOCKING SCORE OF ANTI-CHOLINESTERASE ACTIVITY

Compound Name	Binding Affinity
Carambolin	-10.2
Quercetin	-9.0
Epicatechin	-9.5
Standard Drug: Galantamine	-9.1



CARAMBOLIN **EPICATECHIN**

QUERCETIN **GALANTAMINE**

FIG. 9: MOLECULAR DOCKING IMAGES OF ANTICHOLINESTERASE ACTIVITIES

***In-vitro* Studies:**

Determination of Antioxidant Activity: Various concentration of AgNPs sample ranging from 25µg/ml to 800µg/ml were tested for the free radical scavenging activity by DPPH method. Percentage inhibition of free radical concentration of AgNPs sample was estimation, the result were compared with same concentration of Ascorbic acid as its standard. Percentage inhibition of AgNPs sample showed IC_{50} 484.861 when encountered to Ascorbic acid with IC_{50} 24.589. The results are shown in **Table 5**. Antioxidant activity was empowered by DPPH radical scavenging assay method by using Ascorbic acid as standard. Comparison utilizing Ascorbic acid, the obtained AgNPs has antioxidant activity. Thereby percentage inhibition of AgNPs sample shows IC_{50} 484.861 while Ascorbic acid with IC_{50} 24.589 [18].

TABLE 5: ANTIOXIDANT ACTIVITY OF ASCORBIC ACID AND AgNPs

Concentration (µg/ml)	Percentage inhibition	
	Ascorbic acid	AgNPs
25	39.04±2.900	22.47±0.5554
50	52.75±1.6947	28.65±1.3559
100	60.82±1.356	30.71±1.4119
200	68.86±2.3636	38.98±0.8751
400	83.69±1.5106	47.66±1.1895
600	98.74±2.8096	55.60±1.4956
800	116.90±2.4275	68.04±1.0686

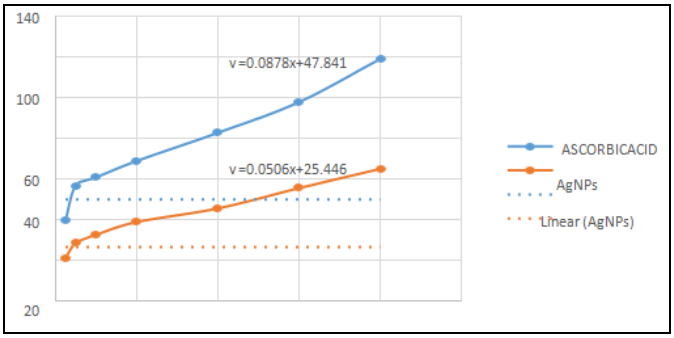


FIG. 10: ANTIOXIDANT ACTIVITY OF ASCORBIC ACID ANDAGNPS

Determination of Anticholinesterase Activity: Alzheimer's activity was evaluated using Ellmanns method by using Galantamine as standard. When compared with Galantamine the obtained AgNPs has Alzheimer's activity. The percentage inhibition of AgNPs sample shows IC_{50} 63.33 while Galantamine with IC_{50} 37.33.

TABLE 10: ANTICHOLINESTERASE ACTIVITY OF ASCORBIC ACID AND AgNP

Concentration (µg/ml)	Percentage Inhibition	
	AChE	
	AgNP	Galantamine
25	38.24±1.26	27.69±1.283
50	67.07±0.971	43.92±1.732
100	87.11±2.753	66.71±1.328

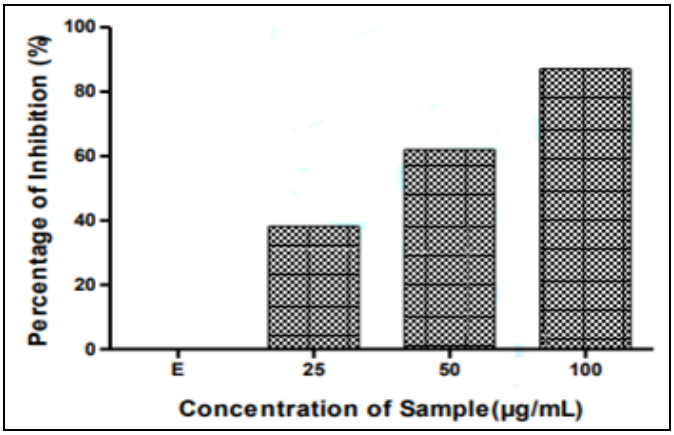


FIG. 11: ANTICHOLINESTERASE ACTIVITY OFASCORBIC ACID AND AgNP OF DIFFERENT CONCENTRATION

CONCLUSION: The research work explored the silver nanoparticles by green synthesis from *Averrhoa carambola* leaf extract. Spectral characterization of AgNPs were done with FTIR, UV-visible, X-ray diffraction and SEM. Docking studies of phytoconstituents along with evaluation of Alzheimer's activity and antioxidant activities carried out. AgNPs showed reliable Alzheimer's (Nootropic) and Antioxidant activities utilising DPPH radical scavenging assay for Ascorbic acid as standard and Ellmanns method for Alzheimer's activity. Thus, the results concluded that the synthesized AgNPs has comparable Alzheimer's activity and Antioxidant activities. The *in-vitro* studiesindicatetherelevanceoftheworkandthisanalog uecanbesubjectedto further future studies.

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CONFLICTS OF INTEREST: Nil

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