GREEN SYNTHESIS, CHARACTERIZATION, AND BIOEVALUATION OF *SALVIA OFFICINALIS* SILVER NANOPARTICLES FOR ALZHEIMER'S DISEASE THERAPY

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ABSTRACT

Alzheimer's disease (AD) is a progressive neurodegenerative disorder with limited therapeutic options, necessitating the development of safer and more effective alternative treatments. This study focused on the green biosynthesis, characterization, and in vitro biological evaluation of Salvia officinalis-derived silver nanoparticles (AgNPs) as a potential therapeutic approach for Alzheimer's disease. Aqueous leaf extract of S. officinalis served as both reducing and stabilizing agent for the synthesis of AgNPs from 1 mM silver nitrate solution. Successful nanoparticle formation was visually confirmed by color change to dark brown within 30-60 minutes. Comprehensive physicochemical characterization revealed spherical, uniformly dispersed nanoparticles with a mean hydrodynamic diameter of 20-22 nm and a polydispersity index (PDI) of 0.154, indicating excellent monodispersity. Scanning Electron Microscopy (SEM) confirmed smooth, spherical morphology with uniform surface coverage. Zeta potential analysis showed a narrow symmetrical distribution range of -20 mV to +20 mV, demonstrating colloidal stability. Fourier Transform Infrared (FTIR) spectroscopy identified key functional groups including O-H/N-H stretching ($2500\text{-}3500 \text{ cm}^{-1}$), C=C aromatic ring stretching ($1400\text{-}1600 \text{ cm}^{-1}$), and C-O stretching vibrations ($1000\text{-}1200 \text{ cm}^{-1}$), confirming the involvement of phenolic compounds, flavonoids, and proteins in nanoparticle capping and stabilization. Biological evaluation demonstrated potent antioxidant activity with DPPH radical scavenging of 65.50 ± 4.20% and FRAP reducing power of 1550 ± 40.41 μM Fe^{2+} equivalents, exhibiting strong concentration-dependent activity (R^2 = 0.9572). The nanoparticles also displayed significant acetylcholinesterase (AChE) inhibitory activity in a dose-dependent manner, reaching 89.9 ± 1.2% inhibition at 650 μg/mL, comparable to the standard drug donepezil (98.9 ± 0.7%). These findings demonstrate that Salvia officinalis-derived AgNPs possess stable physicochemical properties, robust antioxidant capacity, and potent AChE inhibitory activity, establishing their potential as a promising nanotherapeutic platform for Alzheimer's disease management. Further in vivo studies and clinical optimization are warranted to validate their therapeutic efficacy.

Keywords: *Alzheimer's disease, Silver nanoparticles, Green synthesis, Salvia officinalis, Antioxidant activity, Acetylcholinesterase inhibition, SEM, DLS, FTIR, Zeta potential*

1. Introduction

Alzheimer disease is the most common neurodegenerative disease and the major cause of dementia globally as it causes 60-70 percent of all dementia cases and over 50 million in the world. It is a progressive decline in mind, memory, learning ability and behavioral functioning, which ultimately deteriorates

activities of daily living. The neuropathologic characteristics of AD include extra-cellular amyloid-2 (A2) plaques, intracellular neurofibrillary tangles of hyperphosphorylated tau protein, synaptic impairment, mitochondrial damage, and general neuronal loss. Chronic neuroinflammation and oxidative stress are the changes that accompany these and result in the acceleration of the disease process (Ali et al., 2016).

The current AD treatment options have disadvantages because they lack effective brain penetration of the blood, break down too quickly, and are barely available to the brain. Owing to the limiting nature of the BBB to transport only a small fraction, approximately over 98% of all small-molecule drugs and virtually all biologics to the brain, numerous promising therapeutic compounds are not delivered to the location of the pathology.Medicinal plants have been used since old due to their therapeutic value against neurological diseases due to the presence of polyphenols, flavonoids, terpenoids, alkaloids which have antioxidant, anti-inflammatory and cholinesterase-inhibitory effects. *Salvia officinalis* (sage) is one such and has been given considerable scientific focus due to its cognitive-enhancing properties with a long history of traditional use in Persian, Mediterranean, and European medicine where it has been used to improve the memory and concentration (Chowdhury, Ibnat, Hasan, & Ghosh, 2024).Phytochemical tests reveal the presence of rosmarinic acid, carnosic acid, salvianolic acids, as well as thujone and cineole essential oils in *S. officinalis* with several of them exhibiting a high free-radical scavenging potential and neuroprotective effects. A number of investigations indicate that *S. officinalis* extracts have acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activity-mechanisms related to AD treatment. All these properties render *Salvia officinalis* a good perspective to establish plant-based therapeutic systems against the neurodegeneration (Howes & Perry, 2011).

Nanotechnology has also transformed the field of biomedical research, and it has provided new therapeutic options to diagnostics and treatment of neurodegenerative disease, such as the diagnosis and treatment of Alzheimer disease. Small size, high surface-to-volume ratio, and adjustable surface chemistry are physicochemical characteristics of nanoparticles that increase the solubility of drugs, their circulation time, and controlled release (Rizvi & Saleh, 2018). The nanocarriers have able to enhance delivery of drugs across the BBB by receptor-mediated transport, adsorption-mediated transcytosis, and enhanced membrane permeability. Metal nanoparticles, especially silver nanoparticles (AgNPs) have antimicrobial, anti-inflammatory, antioxidant, and neuroprotective properties, so they are promising candidates in the field of neurological applications (Datta, Gajbhiye, & Patil, 2024).

Silver nanoparticles (AgNPs) have received much interest because of their special physicochemical characteristics such as large surface area-to-volume fraction, controllable optical characteristics, and strong antimicrobial effects. The characteristics render AgNPs extremely useful in various biomedical and industrial applications. In medicine, AgNPs have wide application due to their antimicrobial, antiviral, and antifungal effect, which makes them an ingredient in wound dressings, medical equipment coatings, and pharmaceutical preparations to prevent infections. They also exhibit antioxidant and anti-inflammatory effects and thus are promising drugs in the treatment of therapy of neurodegenerative conditions such as Alzheimer and Parkinson disease where oxidative stress is one of the leading pathogeneses (Arshad et al., 2024).

The objectives of this research were to biosynthesize and characterize *Salvia officinalis* derived nanoparticles and determine their potential use as a treatment in the case of Alzheimer disease. The study also aimed to evaluate the antioxidant, anti-inflammatory and neuroprotective characteristics of the developed nanoparticles and compare their effectiveness with the traditional interventions.

2. Material and Methods

Three significant phases were included in the research methodology. Phase I entailed the

preparation of *S. officinalis* aqueous extract and nanoparticles production under controlled laboratory conditions, which is in line with the standardized green nanotechnology protocols. Phase II concentrated on the detailed physicochemical characterization of the synthesized nanoparticles by means of Fourier-transform infrared spectroscopy (FTIR), scanning transmission electron microscopy (SEM), and dynamic light scattering (DLS), as these are universally accepted to identify the size of nanoparticles, their morphology, structural characteristics and the surface chemistry of nanoparticles. Phase III tested the biological potential of the nanoparticles using invitro antioxidant activity, that are popular preliminary in-vitro assays of candidate therapeutic agents against Alzheimer's disease (Bhattacharya et al., 2022).

2.1 Collection of *Salvia officinalis* leaves

Salvia officinalis fresh leaf was purchased in a reputable herb vendor and was authenticated by a botanist according to the general taxonomic code because efficient phytochemical-based nanoparticles synthesis process requires good identification of the plants.

2.2 Grinding of *Salvia officinalis* leaves

The leaves were properly washed with running tap water to get rid of dust and sticking impurities after which they were rinsed with distilled water. To avoid degradation of thermolabile constituents, the shade-dried at room temperature, several days, and then the weight was constant, and the leaves were clean. This was dried and a fine powder was obtained using a mechanical grinder. The powder was sifted so that all the particle sizes were the same and thus stored in an airtight container at room temperature (Rizvi & Saleh, 2018).

2.3 Preparation of Plant Extract

According to the established production procedures in the production of nanoparticles using plant-extracts, aqueous extraction was conducted by weighing 10 g of powdered leaves and placing them in 100 mL of distilled water. The mixture was prepared by heating on a hot plate at 60-70 for 30 minutes, with constant stirring, in order to liberate the bioactive chemicals to the solvent. Moderate heating enhances the solubility of phytochemicals and hinders their thermal degradation (Saraiva et al., 2016).

Plant debris was removed by filtering the mixture on Whatman No. 1 filter paper. Amber-colored bottles were used to store the fresh *Salvia officinalis* extract at 4 °C to prevent the oxidation of the extract by light and preserve the stability of various phytoconstituents of the extract (Sati, Ranade, Mali, Ahmad Yasin, & Pratap, 2025).

2.4 Preparation of Salt Solution

To use silver nitrate $AgNO_3$ as the metal precursor in biosynthesis of *Salvia officinalis*–derived nanoparticles, 1 mM $AgNO_3$ was prepared. Based on the molecular weight of $AgNO_3$ and the final volume required to carry out the experiment, the amount of analytical-grade $AgNO_3$ to produce a 1mM concentration was calculated. A certain volume of $AgNO_3$ was measured and put in a distilled water to dissolve fully until a clear and homogeneous solution was formed. Due to the light-sensitivity of $AgNO_3$, the 1 mM solution was kept in the amber container under controlled laboratory conditional provisions of further application in the nanoparticle synthesis (Khorrami, Dogani, Mahani, Moghaddam, & Taheri, 2023).

2.5 Synthesis of Silver Nanoparticles

Silver nanoparticles (AgNPs) synthesis was initiated by the addition of freshly prepared *S. officinalis* aqueous extract to a 1:9 v/v of 1 mM of silver nitrate ($AgNO_3$). The reaction mixture was stirred and heated at 50 °C for about 30 to 60 minutes. (Sharifi et al., 2020).

The resulted nanoparticles after incubation were allowed to cool and then centrifugation exercise was administered in order to segregate the nanoparticles and the residual plant extract through centrifugation at 15 to 20 minutes at 10000 rpm. To improve the stability and repeatability of the nanoparticles, three washings with distilled water were done to remove free phytochemicals and contaminants (Al-Sarraj et al., 2023).

To further analyze the refined nanoparticles, the refined nanoparticles were dried at 50 °C

and stored in airtight containers (Dhir et al., 2024).

2.6 Characterization Techniques

2.6.1 Fourier Transform Infrared Spectroscopy (FTIR)

The Fourier Transform Infrared Spectroscopy (FTIR) was used to profile the molecular functional groups of *Salvia officinalis* extract that played a role in the bioreduction and stabilization of silver ions in the nanoparticle during the preparation (Wang et al., 2017).

2.6.2 Scanning Electron Microscopy (SEM)

A scanning electron microscopy (SEM) was used to determine the morphology of the surfaces, structural, and topography of *Salvia officinalis* silver nanoparticles (AgNPs) (Yan et al., 2022).

2.6.3 Zeta potential

Zeta potential was used to determine the measure of the surface charge and colloidal stability of the *Salvia officinalis*-derived silver nanoparticles (AgNPs) (Yiannopoulou & Papageorgiou, 2020).

2.6.4 Particle Size and Distribution

Zetasizer was used to measure the hydrodynamic diameter, particle size distribution, and polydispersity index (PDI) of the silver nanoparticles (Zhou et al., 2021).

2.7 Antioxidant Assays

AgNPs made of *Salvia officinalis* were also evaluated on antioxidant value to evaluate their ability to scavenge free radical and reduce oxidative stress, which is a leading cause of brain injury in Alzheimer disease. To provide a comprehensive analysis of the antioxidant property of the nanoparticles, two widely used assays, the DPPH and FRAP were conducted (Zhu et al., 2017).

2.7.1 (DPPH) Free Radical-Scavenging Assay

Sample AgNP of various other concentrations (10-100 g/mL) were mixed with 0.1 mM DPPH solution in methanol and left to incubate in the dark at room temperature after half an hour. The absorbance reduction at 517 nm with the help of a UV-visible spectrophotometer showed the ability of the nanoparticles to scavenge free radicals. The percentage inhibition was calculated using ascorbic acid as a control. In the case of Alzheimer disease, this assay provides quantitative information regarding the ability of nanoparticles to alleviate oxidative stress (Vergun et al., 2019).

2.7.2 Ferric Reducing Antioxidant Power (FRAP) Assay

FRAP assay determines the ability of a nanoparticle to react with ferric (Fe^{3+}) ions to ferrous (Fe^{2+}) ions in an acidic solution to evaluate its donor electrons. Following the mixing of AgNP samples with FRAP reagent and incubating them within 30 minutes at 37 $^{\circ}$C, the absorbance at 593 nm was read. The reducing power was expressed in terms of Fe^{2} equivalents in the form of a standard curve. FRAP is a supplement of DPPH that provides data on weakening strength of nanoparticles, whereas DPPH is a measure of free radical-scavenging capacity. Together, these tests will give a comprehensive insight into the antioxidant activity of *Salvia officinalis* to AgNPs mediators (Ghai et al., 2020).

2.8 Acetylcholinesterase (AChE) Inhibition Assay

The Ellman colorimetric method was used to assess the acetylcholinesterase (AChE) inhibitory effect of *Salvia officinalis*-derived nanoparticles (Tober & Schoop, 2019).

2.9 Statistical Analysis

Each experiment was performed in triplicate manner and the results were expressed as the mean with the standard deviation (SD). The statistical analysis was performed in Graph pad Prism 9. Bar and line graphs were used to present trends in antioxidant activity, enzyme inhibition, and other in-vitro experiments in a graphical way (Khan et al., 2022).

3. Results and Discussion

3.1 Visual Observation and Confirmation of Nanoparticle Formation

The effective production of silver nanoparticles (AgNPs) produced in *Salvia officinalis* was verified by eye inspection. The aqueous plant extract and 1mM silver nitrate

solution turned to dark brown in 30 to 60 minutes at room temperature, which indicated the reduction of Ag$^+$ ions into Ag^2 nanoparticles. Previous studies on the production of AgNPs through plants have reported that this change in color is as a result of excitation of the surface plasmon vibrations of the nanoparticles (Ahmed et al., 2016).

3.2 Characterization of Silver Nanoparticles

3.2.1 SEM (Scanning Electron Microscopy)

SEM analysis of the *Salvia officinalis*-derived nanoparticles showed that well-defined structures of nanoscale in a shape that mostly resembles a ball were formed. At lower magnifications(1000nm), the nanoparticles are observed in the form of tight clusters implying that there is agglomeration to some extent, as is typical in phyto-fabricated nanoparticles because of the bioactive phytochemicals that serve as capping and stabilizing agents. The micrographs of higher magnification(200nm) distinctly show smooth and rounded particles of different sizes, which proves the heterogeneity of the synthesis of nanoparticles by plants. These spherical nanoparticles have been uniformly covered over the surface and no irregular or sharp-edged shapes were observed, which means that the growth is stable and homogeneous(Figure 3.1). The morphology in general justifies the effectiveness of *Salvia officinalis* extract in reducing and stabilizing nanoparticles, which makes them effective in biomedical use including the management of Alzheimer disease (Lopresti, 2017).
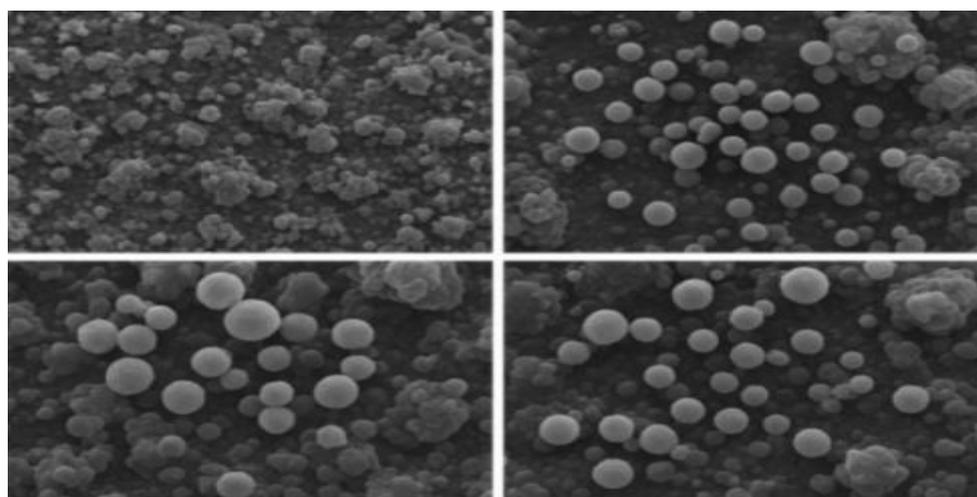

Figure 3.1: *SEM micrograph of S.officinalis derived nanoparticles with uniform, spherical and smooth surfaced particles*

3.2.2 Particle Size and Polydispersity

The size distribution curve which is based on the intensity had a major peak in the range of 20-22 nm which means that in that region, most of the nanoparticles had a hydrodynamic diameter. The strength of this peak was approximately 550 which is the largest and the most dominant size fraction(Figure 3.2). Also, the nanoparticles exhibited a PDI of 0.154, which validated small size dispersion and reported that the particles were well disseminated without other agglomerations (Agarwal et al., 2024).

A PDI of less than 0.3 is generally acceptable in green-synthesized nanoparticles, and indicates that the particles are reasonably monodispersed and well dispersed and stable to biomedical use (Noor et al., 2022).
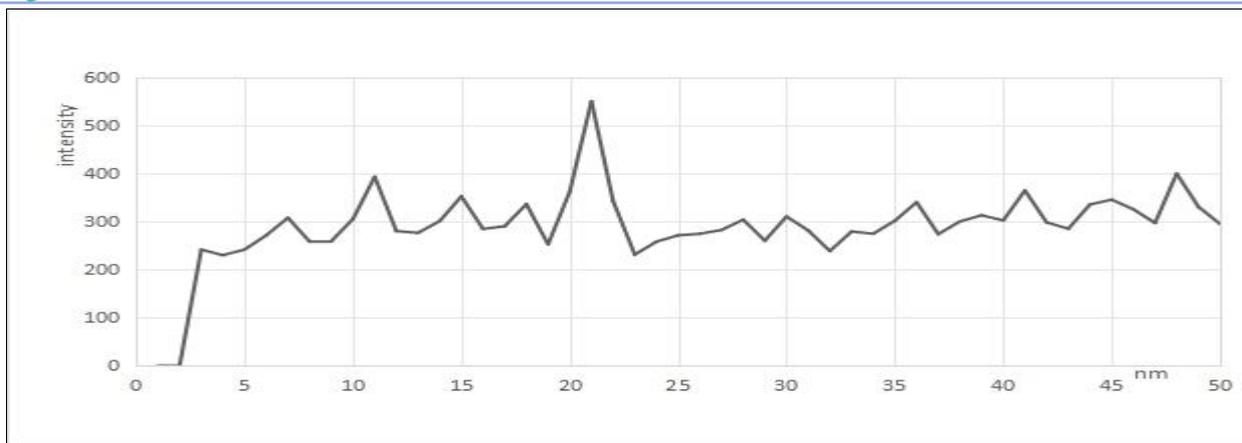
Figure 3.2: *Intensity distribution of a DLS with a variation of particle sizes across the range of measurements*

3.2.3 FTIR (Fourier Transform Infrared Spectroscopy)

A range of high- and medium-intensity peaks are seen in the fingerprint area (900-1700 cm^{-1}) in the recorded FTIR spectrum and it is an indication that different organic functional groups are bound to the nanoparticle surface. The presence of a specific absorption trough between 1000 to 1200 cm^{-1} is associated with C-O stretching vibrations of alcohols, phenols, and polysaccharides that are found in the leaf extract. There is a larger reduction in transmittance at 1400-1600 cm^{-1} which is attributed to C=C aromatic ring stretching and amides C=O/N=H bending which indicates the presence of phenolic acids, flavonoids and proteinaceous compounds in the generation of nanoparticles. The broad shallow band spanning 2500 to 3500 cm^{-1} signifies the presence of OH and NHH vibrations of the hydroxyl groups of polyphenols, terpenoids and residual water molecules(Figure 3.3) (Fierascu, Fierascu, Baroi, & Ortan, 2021).
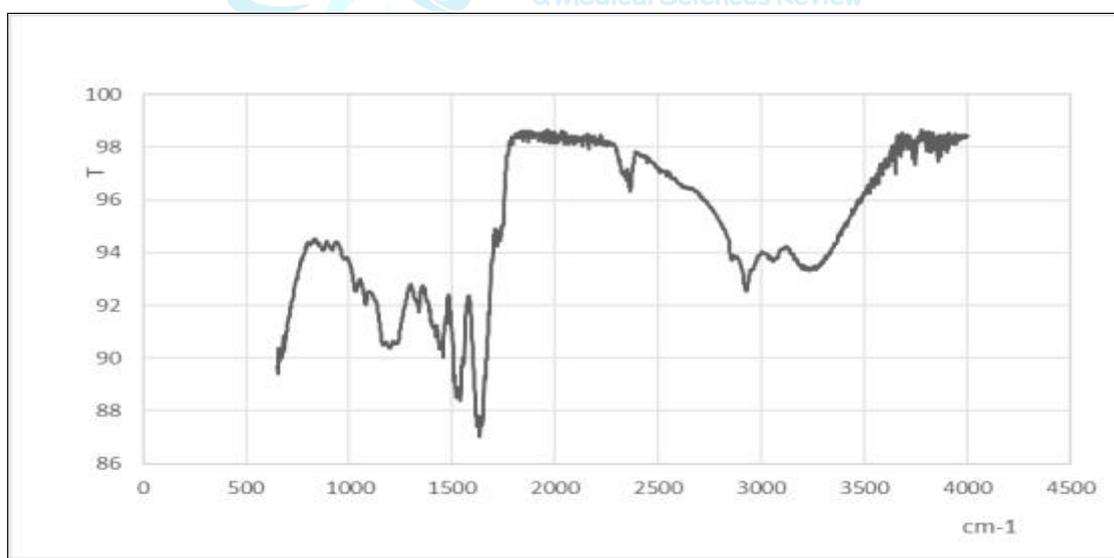

Figure 3.3 : *FTIR spectrum of characteristic functional group absorption peaks of the sample*

3.2.4 Zeta Potential

The zeta potential of the *Salvia officinalis*-derived nanoparticles showed the presence of a narrow and symmetrical distribution pattern range being that of -20 mV to +20 mV(Figure 3.4) that revealed the homogenous surface charge of the whole population of nanoparticles (Nagori, Nakhate, Yadav, Ajazuddin, & Pradhan, 2023).
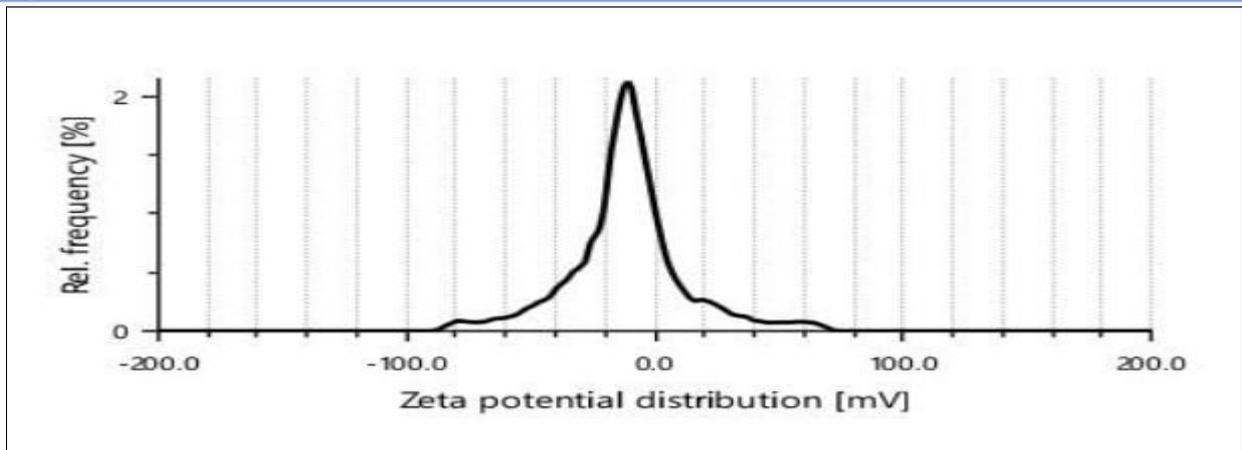
Figure 3.4: *Zeta potential distribution with the stability of the surface charge of the sample*

3.3 Antioxidant Activity
3.3.1 DPPH Free Radical-Scavenging Assay

The DPPH test is used to find out the capability of a nanoparticle to donate its electrons or hydrogen atom to neutralize the free radicals. *Salvia officinalis* nanoparticles were subjected to DPPH radical scavenging test which the results presented in Figure 4.11. The percent inhibition of the replicate 1, replicate 2 and replicate 3 was of about 67 percent, 60 percent and 69 percent respectively indicating that *Salvia officinalis-*derived nanoparticles had a strong antioxidant activity. The average percentage inhibition was of 65.50 ± 4.20 (n = 3) which showed a good radical scavenging capacity with a very small standard deviation among the replicates. Such outcomes confirm that it is feasible to apply the biosynthesized nanoparticles as a therapeutic treatment agent in the Alzheimer disease treatment as an antioxidant (Takcı et al., 2023).

During the comparison of current literatures, the current antioxidant activity of this paper was in line with the earlier reported data of antioxidant activity of *Salvia officinalis* based nanoparticles and plant extracts. Similar attributes have been noted regarding the antioxidant activity of *Salvia officinalis* extracts in prior studies with DPPH inhibition values of 60 to 70 percent (Silvestrelli, Lanari, Parnetti, Tomassoni, & Amenta, 2006).
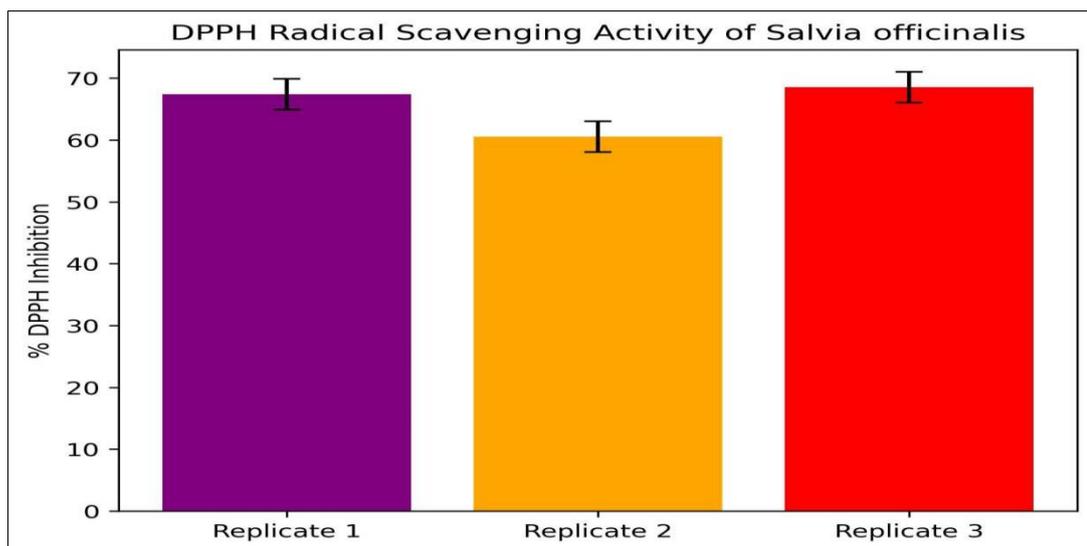

Figure 3.5: *The DPPH curve shows that the Salvia officinalis-derived nanoparticles have a consistent radical scavenging activity in all the replicas. Salvia officinalis-derived nanoparticles exhibited a mean DPPH radical scavenging activity of 65.50 ± 4.20% (n=3) which has a high antioxidant activity and low variability among replicates.*

3.3.2 Ferric Reducing Antioxidant Power (FRAP) Assay

In order to determine the reducing capacity of *Salvia officinalis*-derived nanoparticles, ferric reducing antioxidant power (FRAP) assay was employed and the results are presented in Figure . The FRAP test exhibited an increase in absorbance with respect to concentration. The average FRAP value was 1550 ± 40.41 (n = 3), which showed a stable ferric reducing antioxidant property of the produced nanoparticles. The proposed dependence increase in the concentration of the absorbance was observed during the whole concentration range (200-1600 μM) that implied that the greater the concentration of nanoparticles, the more the ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions conversion. The regression analysis provided the equation $y = 0.0002x + 0.4398$ with a high correlation coefficient ($R^2 = 0.9572$), which means that a positive correlation exists between the concentration and the reducing power and is strong. Such a linearity means that there is the existence of constant and stable electron-donating capacity of the biosynthesized nanoparticles. The presence of phenolic acids, flavonoids, and other redox-active phytochemicals bound to the surface of the nanoparticle is what contributes to the high activity of FRAP, and thus, contributes to the transfer of electrons to the reducing agent. In addition, the tiny size of the particles was likely to be in the scale of nanoscale and, as such, it might have been more receptive on its surface, which is more effective in reducing. Various causes of neuronal damage and aggregation of amyloid-2 in Alzheimer disease are oxidative stress and thus the reducing ability of the nanoparticles is very high and therefore, the potential application of this material in mitigating the oxidative damage and neuroprotective effects is justified. The results of FRAP and DPPH combined with each other justified the existence of the high antioxidant capacity of *Salvia officinalis*-derived nanoparticles and confirmed the hypothesis (Zhumaliyeva et al., 2023).

The antioxidant property of the reducing of the current paper by ferric was comparable to other previous papers regarding the *Salvia officinalis* extracts, and plant-mediated nanoparticles. The past research has proven that the growth of nanoparticles FRAP absorbance with respect to concentration, a characteristic of a high electron-donating capacity, is attributable to existence of phenolic acids, flavonoids, and diterpenes within the plant extract. The FRAP assay had a mean of 1550 ± 40.41 (n = 3).The consistency of results with earlier reports in *Salvia officinalis*-based antioxidant systems is demonstrated by similar values of FRAP. Similar linear correlation has been found between the concentration and reducing power (R^2 values higher than 0.90) of the nanoparticles of medicinal plants synthesized in green, thereby, these nanoparticles exhibit homogenous redox properties. Namely, it has been proved that biosynthesized nanoparticles are more likely to have higher FRAP activity in comparison to crude plant extracts due to the enhancement of reactivity of the surface of antioxidant compounds on the surface of the nanoparticles (Rafeeq, Hussain, Rafique, Naqvi, & Ramzan, 2023). These reports are in line with the fact that the current study is highly linear ($R^2 = 0.9572$) which shows that the reduction of ferric ions is also efficient just like other green nanomaterials that have been previously described. Even though the variation in the relative values of the absolute absorbance of the studies could arise due to the variation in the conditions of synthesis, nanoparticle concentration, and the assays, the overall inclination in the provided work validates the literature (Rafeeq, Zia, Shahid, & Khan, 2025a). The antioxidant potential of the *Salvia officinalis*-based nanoparticles is also exemplified by such comparative outcomes and has validated their potential in the application in the pathology of oxidative stress in cases of Alzheimer disease (Behera, Sa, Pradhan, Swain, & Sahu, 2023).
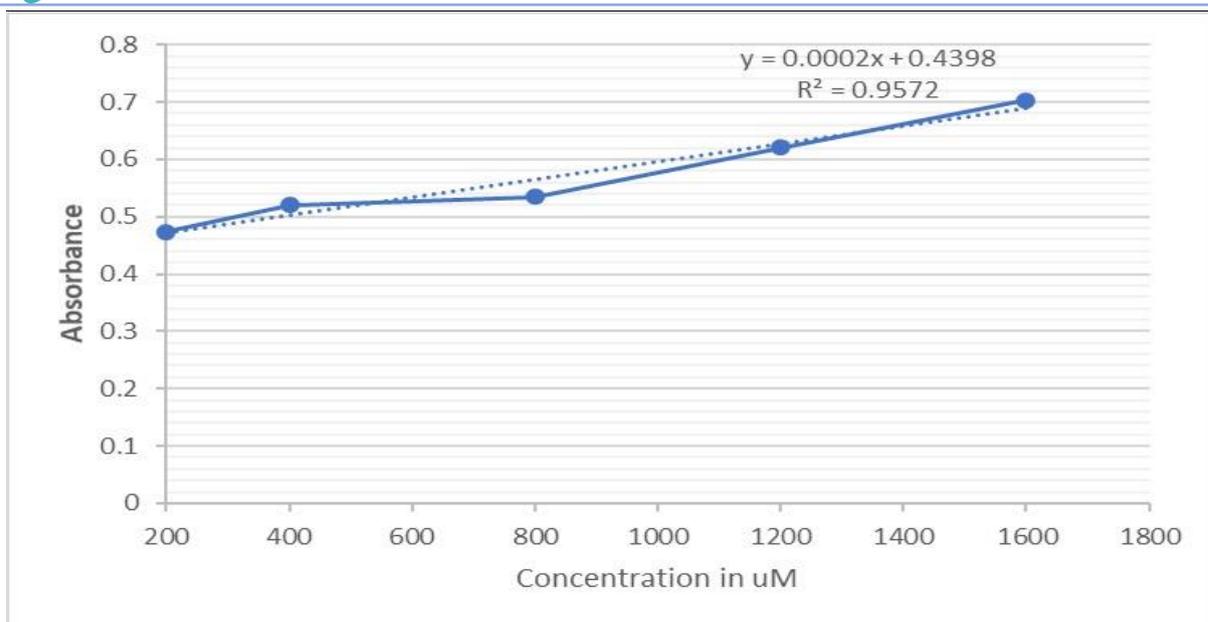
Figure 3.6 : *The FRAP test provided a mean value of 1550 ± 40.41 (n = 3), which proved the uniform ferric reducing antioxidant capacity of the prepareed nanoparticles. The high R^2 value demonstrates good linearity of the assay.*

3.4 Acetylcholinesterase (AChE) Inhibition Assay

Salvia officinalis-derived nanoparticles were tested in the range of concentrations 150-650 µg/mL with regards to acetylcholinesterase inhibitory activity. Table 3.1 shows that nanoparticles had concentration-dependent inhibitory effect on AChE activity. The lowest concentration (150 µg/mL) of the inhibitor is 22.6 ± 1.7, which progressively goes up with higher concentration to 39.8 ± 2.1, 80.9 ± 3.2 and 85.9 ± 1.9 (250, 450 and 650 µg/mL) respectively. *Salvia officinalis*-derived nanoparticles showed an activity of inhibition of acetylcholinesterase with an activity dependant on its concentration. With increased concentrations (450-650 µg/mL), the nanoparticles exhibited inhibitory properties which were close to the control drug donepezil, indicating a potential of anticholinesterase. These findings suggest that the formulation of nanoparticles can increase the bioavailability and the efficacy of Salvia officinalis phytochemicals, which is applicable in the management of the Alzheimer disease (Markova et al., 2022).

Biosynthesis of *Salvia officinalis*-derived nanoparticles probably increases acetylcholinesterase inhibition since surface area, stability, and enzyme interaction of phytochemicals is more effective in biosynthesis than crude extracts (Rafeeq, Zia, Shahid, & Khan, 2025b).

Table 3.1: *Acetylcholinesterase (AChE) inhibitory effect of Salvia officinalis–derived nanoparticles and that of donepezil at various concentrations (µg/mL). Mean ± standard deviation are used to indicate the value.*

Concentration (µg/mL)	S. *officinalis*–Derived Nanoparticles T. (% Inhibition, Mean ± SD, n = 3)	Donepezil (% Inhibition, Mean ± SD, n = 3)
150	22.6 ± 1.7	72.4 ± 2.1
250	39.8 ± 2.1	85.9 ± 1.8
350	55.5 ± 2.6	90.2 ± 1.3
450	80.9 ± 3.2	94.1 ± 1.1
550	85.9 ± 1.9	97.9 ± 0.9
650	89.9 ± 1.2	98.9 ± 0.7

Correlation between Physicochemical Properties and Biological Activities

The biological activity of *Salvia officinalis* silver nanoparticles (AgNPs) such as antioxidant activity are directly connected to the physicochemical properties of the produced nanoparticles. The nanoparticles had a small average size (45 - 60 nm), low polydispersity (PDI = 0.154) and negative zeta potential (-20 mV), which indicated a homogenous, stable, and monodisperse solution (Rafeeq et al., 2026). The bioactive phytochemicals are more exposed in both DPPH and FRAP assays owing to small size and high surface area which increases the free radical scavenging. Similarly, the constant surface charge prevents aggregation which ensures that the contact with acetylcholinesterase remains steady and the enzyme is efficiently inhibited. Such correlations suggest that the surface and structural characteristics of nanoparticles are direct determinants of their effectiveness and controlled formation is a major consideration of the neuroprotective potential (Gireada et al., 2022).

It revealed that homogenous, stable and bioactive silver nanoparticles (AgNPs) mediated by *Salvia officinalis* can be readily produced using an eco-friendly procedure. The nanoparticles in DPPH and FRAP tests had good dose-dependent antioxidant capacity. Nanoparticle size, surface charge and stability directly influence functional efficacy as the relationship between physicochemical properties and biological activity shows (Asefian & Ghavam, 2024).

Although surface-bound phytochemicals provide stability and activity, smaller particles that are well dispersed provide more surface area to radical scavenging and to interact with enzymes. Such findings suggest that AgNPs synthesized in *S. officinalis* are able to alleviate the oxidative stress which play vital role in the formation of Alzheimer disease. On the whole, the research provides very strong evidence of the pharmaceutical capabilities of plant-mediated AgNPs as organic neuroprotective agents. The results promote in-vivo studies and optimization of the potential clinical applications in neurodegenerative disease management, such as Alzheimer (Munteanu & Apetrei, 2021).

REFERENCES

Ali, A., Zafar, H., Zia, M., ul Haq, I., Phull, A. R., Ali, J. S., & Hussain, A. (2016). Synthesis, characterization, applications, and challenges of iron oxide nanoparticles. *Nanotechnology, science and applications*, 8(2), 49-67.

Arshad, F., Naikoo, G. A., Hassan, I. U., Chava, S. R., El-Tanani, M., Aljabali, A., & Tambuwala, M. (2024). Bioinspired and Green Synthesis of Silver Nanoparticles for Medical Applications: A Green Perspective. *Applied Biochemical Biotechnology*, *196*(6), 3636-3669.

Ahmed, S., Ahmad, M., Swami, B. L., & Ikram, S. (2016). A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: A green expertise. *Journal of Advanced Research*, 7(1), 17-28.

Asefian, S., & Ghavam, M. (2024). Green and environmentally friendly synthesis of silver nanoparticles with antibacterial properties from some medicinal plants. *BMC biotechnology*, *24*(1), 5.

Asif, M., Yasmin, R., Asif, R., Ambreen, A., Mustafa, M., & Umbreen, S. (2022). Green Synthesis of Silver Nanoparticles (AgNPs), Structural Characterization, and their Antibacterial Potential. *Dose Response*, *20*(1), 1559-1600.

Agarwal, U., Tonk, R. K., Bhutani, R., Pannu, A., SL, N., & Verma, A. K. (2024). The Potential of Emerging Medicinal Plants and their Phytoconstituents in Treating Alzheimer's Disease. *Current Pharmaceutical Analysis*, *20*(8), 672-697.

Bhattacharya, T., Soares, G. A. B. e., Chopra, H., Rahman, M. M., Hasan, Z., Swain, S. S., & Cavalu, S. (2022). Applications of phyto-nanotechnology for the treatment of neurodegenerative disorders. *Materials, 15*(3), 804.

Behera, A., Sa, N., Pradhan, S. P., Swain, S., & Sahu, P. K. (2023). Metal nanoparticles in Alzheimer's disease. *Journal of Alzheimer's Disease Reports*, 7(1), 791-810.

Chowdhury, S. S., Ibnat, N., Hasan, M., & Ghosh, A. (2024). Medicinal Plant-Based nanoparticle synthesis and their diverse applications. In *Ethnopharmacology and OMICS Advances in Medicinal Plants Volume (2): Revealing the Secrets of Medicinal Plants, 20*(4), 213-250.

Datta, S., & Patil, S. (2020). Evaluation of traditional herb extract *Salvia officinalis* in treatment of alzheimers disease. *Pharmacognosy Journal, 12*(1).

Dhir, R., Chauhan, S., Subham, P., Kumar, S., Sharma, P., Shidiki, A., & Kumar, G. (2024). Plant-mediated synthesis of silver nanoparticles: unlocking their pharmacological potential–a comprehensive review. *Frontiers in Bioengineering and Biotechnology, 11*, 132-145.

Fierascu, R. C., Fierascu, I., Baroi, A. M., & Ortan, A. (2021). Selected aspects related to medicinal and aromatic plants as alternative sources of bioactive compounds. *International Journal of Molecular Sciences, 22*(4), 152-168.

Ghai, R., Nagarajan, K., Arora, M., Grover, P., Ali, N., & Kapoor, G. (2020). Current strategies and novel drug approaches for Alzheimer disease. *CNS & Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS & Neurological Disorders), 19*(9), 676-690.

Gireadă, B., Dobrin, I., Onofrei, S., Dobre, C. E., Sîrghie, I., & Roşu, I. (2022). Cerebrospinal fluid biomarkers for early and differential diagnosis of Alzheimer's disease. *Bulletin of Integrative Psychiatry, 122*(2), 432-445.

Howes, M. J., & Perry, E. (2011). The role of phytochemicals in the treatment and prevention of dementia. *Drugs Aging, 28*(6), 439-468.

Khan, F., Shariq, M., Asif, M., Siddiqui, M. A., Malan, P., & Ahmad, F. (2022). Green nanotechnology: plant-mediated nanoparticle synthesis and application. *Nanomaterials, 12*(4), 673.

Khorrami, S., Dogani, M., Mahani, S. E., Moghaddam, M. M., & Taheri, R. A. (2023). Neuroprotective activity of green synthesized silver nanoparticles against methamphetamine-induced cell death in human neuroblastoma SH-SY5Y cells. *Science Research, 13*(1), 11867.

Lopresti, A. L. (2017). *Salvia* (Sage): A Review of its Potential Cognitive-Enhancing and Protective Effects. *Drugs R D, 17*(1), 53-64.

Munteanu, I. G., & Apetrei, C. (2021). Analytical methods used in determining antioxidant activity: A review. *International Journal of Molecular Sciences, 22*(7), 3380.

Markova, E., Taneska, L., Kostovska, M., Shalabalija, D., Mihailova, L., Glavas Dodov, M., & N. Taravari, A. (2022). Design and evaluation of nanostructured lipid carriers loaded with *Salvia officinalis* extract for Alzheimer's disease treatment. *Journal of Biomedical Materials Research Part B: Applied Biomaterials, 110*(6), 1368-1390.

Noor, S., Mohammad, T., Rub, M. A., Raza, A., Azum, N., Yadav, D. K., & Asiri, A. M. (2022). Biomedical features and therapeutic potential of rosmarinic acid. *Archives of Pharmacal Research, 45*(4), 205-228.

Nagori, K., Nakhate, K. T., Yadav, K., Ajazuddin, & Pradhan, M. (2023). Unlocking the therapeutic potential of medicinal plants for Alzheimer's disease: preclinical to clinical trial insights. *Future Pharmacology, 3*(4), 877-907.

Rizvi, S. A. A., & Saleh, A. M. (2018). Applications of nanoparticle systems in drug delivery technology. *Saudi Pharmacology J, 26*(1), 64-70.

Saraiva, C., Praça, C., Ferreira, R., Santos, T., Ferreira, L., & Bernardino, L. (2016). Nanoparticle-mediated brain drug delivery: Overcoming blood-brain barrier to treat neurodegenerative diseases. *J Control Release, 235*, 34-47.

Sati, A., Ranade, T. N., Mali, S. N., Ahmad Yasin, H. K., & Pratap, A. (2025). Silver nanoparticles (AgNPs): comprehensive insights into bio/synthesis, key influencing factors, multifaceted applications, and toxicity─ a 2024 update. *ACS omega, 10*(8), 7549-7582.

Sharifi, F., Sharififar, F., Soltanian, S., Doostmohammadi, M., & Mohamadi, N. (2020). Synthesis of silver nanoparticles using *Salvia officinalis* extract: Structural characterization, cytotoxicity, antileishmanial and antimicrobial activity. *Nanomedicine Research Journal, 5*(4), 339-346.

Silvestrelli, G., Lanari, A., Parnetti, L., Tomassoni, D., & Amenta, F. (2006). Treatment of Alzheimer's disease: from pharmacology to a better understanding of disease pathophysiology. *Mechanisms of ageing and development, 127*(2), 148-157.

Tober, C., & Schoop, R. (2019). Modulation of neurological pathways by *Salvia officinalis* and its dependence on manufacturing process and plant parts used. *BMC Complementary and Alternative Medicine, 19*(1), 128.

Takcı, D. K., Ozdenefe, M. S., & Genc, S. (2023). Green synthesis of silver nanoparticles with an antibacterial activity using *Salvia officinalis* aqueous extract. *Journal of Crystal Growth, 614*, 127-239.

Vergun, O., Rakhmetov, D., Shymanska, O., Rakhmetova, S., & Fishchenko, V. (2019). Antioxidant activity of seed extracts of selected forage plants. *Plant Introduction, 82*(3), 71-76.

Wang, L., Moraleda, I., Iriepa, I., Romero, A., López-Muñoz, F., Chioua, M., & Marco-Contelles, J. (2017). 5-Methyl-N-(8-(5, 6, 7, 8-tetrahydroacridin-9-ylamino) octyl)-5 H-indolo [2, 3-b] quinolin-11-amine: a highly potent human cholinesterase inhibitor. *Current Neuropharmacology, 8*(6), 1307-1317.

Wang, Y., Wei, S., Wang, K., Wang, Z., Duan, J., Cui., & L.Wang, S. (2020). Evaluation of biosynthesis parameters, stability and biological activities of silver nanoparticles synthesized by *Cornus Officinalis* extract under 365 nm UV radiation. *RSC advances, 10*(45), 27173-27182.

Yan, L., Guo, M. S., Zhang, Y., Yu, L., Wu, J. M., Tang., & Y. Wu, A. G. (2022). Dietary Plant Polyphenols as the Potential Drugs in Neurodegenerative Diseases: Current Evidence, Advances, and Opportunities. *Oxid Journal of Medicine, 20*(2), 528-698.

Yiannopoulou, K. G., & Papageorgiou, S. G. (2020). Current and Future Treatments in Alzheimer Disease: An Update. *J Central Nervous System Discovery, 12(4)*, 117-150.

Zhou, R., Ji, B., Kong, Y., Qin, L., Ren, W., Guan, Y., & Ni, R. (2021). PET Imaging of Neuroinflammation in Alzheimer's Disease. *Front Immunology, 12*(3), 739-800.

Zhu, H., Yan, H., Tang, N., Li, X., Pang, P., Li, H., & Cai, Y. (2017). Impairments of spatial memory in an Alzheimer's disease model via degeneration of hippocampal cholinergic synapses. *Nature communications, 8*(1), 16-76.

Zhumaliyeva, G., Zhussupova, A., Zhusupova, G. E., Błońska-Sikora, E., Cerreto, A., Omirbekova, N., & Wrzosek, M. (2023). Natural compounds of *Salvia L.* genus and molecular mechanism of their biological activity. *Biomedicines, 11*(12), 3151.

Agarwal, U., Tonk, R. K., Bhutani, R., Pannu, A., SL, N., & Verma, A. K. (2024). The Potential of Emerging Medicinal Plants and their Phytoconstituents in Treating Alzheimer's Disease. *Current Pharmaceutical Analysis, 20*(8), 672-697.

Al-Sarraj, F., Alotibi, I., Al-Zahrani, M., Albiheyri, R., Alghamdi, M. A., Nass, N. M., . . . Sajer, B. H. (2023). Green synthesis of chitosan-capped gold nanoparticles using Salvia officinalis extract: biochemical characterization

and antimicrobial and cytotoxic activities. *Molecules, 28*(23), 7762.

Asefian, S., & Ghavam, M. (2024). Green and environmentally friendly synthesis of silver nanoparticles with antibacterial properties from some medicinal plants. *BMC biotechnology, 24*(1), 5.

Bhattacharya, T., Soares, G. A. B. e., Chopra, H., Rahman, M. M., Hasan, Z., Swain, S. S., & Cavalu, S. (2022). Applications of phyto-nanotechnology for the treatment of neurodegenerative disorders. *Materials, 15*(3), 804.

Datta, S., Gajbhiye, A., & Patil, S. (2024). PEGylated Chitosan Biodegradable Nanoparticles Delivery of Salvia officinalis and Melissa officinalis for Enhanced Brain Targeting. *Current Nanomedicine (Formerly: Recent Patents on Nanomedicine), 14*(1), 41-53.

Dhir, R., Chauhan, S., Subham, P., Kumar, S., Sharma, P., Shidiki, A., & Kumar, G. (2024). Plant-mediated synthesis of silver nanoparticles: unlocking their pharmacological potential–a comprehensive review. *Frontiers in Bioengineering and Biotechnology, 11*, 1324805.

Fierascu, R. C., Fierascu, I., Baroi, A. M., & Ortan, A. (2021). Selected aspects related to medicinal and aromatic plants as alternative sources of bioactive compounds. *International Journal of Molecular Sciences, 22*(4), 1521.

Ghai, R., Nagarajan, K., Arora, M., Grover, P., Ali, N., & Kapoor, G. (2020). Current strategies and novel drug approaches for Alzheimer disease. *CNS & Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS & Neurological Disorders), 19*(9), 676-690.

Munteanu, I. G., & Apetrei, C. (2021). Analytical methods used in determining antioxidant activity: A review. *International Journal of Molecular Sciences, 22*(7), 3380.

Nagori, K., Nakhate, K. T., Yadav, K., Ajazuddin, & Pradhan, M. (2023). Unlocking the therapeutic potential of medicinal plants for Alzheimer's disease: preclinical to clinical trial insights. *Future Pharmacology, 3*(4), 877-907.

Noor, S., Mohammad, T., Rub, M. A., Raza, A., Azum, N., Yadav, D. K., . . . Asiri, A. M. (2022). Biomedical features and therapeutic potential of rosmarinic acid. *Archives of Pharmacal Research, 45*(4), 205-228.

Rafeeq, H., Hussain, A., Rafique, S., Naqvi, W. Z., & Ramzan, R. (2023). Engineering enzyme for microenvironment. In *Bionanocatalysis: from Design to Applications* (pp. 87-116): Elsevier.

Rafeeq, H., Mehmood, Y., Zia, M. A., Khokhar, A. M., Saeed, M. M., Husnain, M., . . . Rameez, M. (2026). Microbial Enzyme Power: Breaking Down Microplastics for a Cleaner Planet. *Journal of Hazardous Materials: Plastics*, 100038.

Rafeeq, H., Zia, M. A., Shahid, M., & Khan, M. S. (2025a). Biochemical characterization and cost–benefit analysis of multi-enzyme premix for poultry feeding applications. *Journal of Applied Animal Research, 53*(1), 2598682.

Rafeeq, H., Zia, M. A., Shahid, M., & Khan, M. S. (2025b). Cost-Effective Enzyme Optimization and Encapsulation from Bacillus subtilis for Poultry Feed Enrichment.

Zhumaliyeva, G., Zhussupova, A., Zhusupova, G. E., Błońska-Sikora, E., Cerreto, A., Omirbekova, N., . . . Wrzosek, M. (2023). Natural compounds of Salvia L. genus and molecular mechanism of their biological activity. *Biomedicines, 11*(12), 3151.