Research Article

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Green synthesis of *Kickxia elatine*-induced silver nanoparticles and their role as anti-acetylcholinesterase in the treatment of Alzheimer’s disease

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Abstract: The synthesis of silver nanoparticles (AgNPs) by the green method is favored as compared to chemical synthesis due to their appreciable properties of less toxicity and simple synthesis. The current study designed the biosynthesis of AgNPs in one step by using the plant *Kickxia elatine* (KE) extract and then investigated its inhibiting activity against rat’s brain acetylcholinesterase (AChE) ex vivo. Ultraviolet spectrum at 416 nm confirmed the formation of AgNPs. X-ray diffractometer calculated size was reported to be 42.47 nm. The SEM analysis confirmed spherical-shaped AgNPs. FT-IR suggested that the phytochemicals groups present in the KE extract and their nanoparticles (NPs) are responsible for the biosynthesized sizes of NPs. EDX analysis presented that Ag was the chief element with 61.67%. Both KE extract and AgNPs exhibited non-competitive type inhibition against AChE, i.e. $V_{\text{max}}$ decreased (34.17–68.64% and 22.29–62.10%), while $K_m$ values remained constant. It is concluded that KE and AgNPs can be considered an inhibitor of rats’ brain AChE. Furthermore, the synthesis of AgNP-based drugs can be used as a cheaper and alternative option against diseases such as Alzheimer’s disease.

Keywords: *Kickxia elatine*, AgNPs, brain homogenate, acetylcholinesterase, kinetics

1 Introduction

Nanotechnology is the generation of solid nanometer-sized nanoparticles (NPs) with restricted shapes and sizes and gaining enormous attention all over the world in the last many years [1]. The importance of Nanoscience is concerned with the outstanding and unique optical, physical, and chemical properties of NPs and their use in the fields of the medicines, agriculture, cosmetics, garment, and food industries [2,3]. Recently, metallic NPs are synthesized using gold (Au), zinc (Zn), copper (Cu), iron (Fe), silver (Ag), magnesium (Mg), etc. [4]. Among
NPs, AgNPs are gaining significant attention due to their small size in the range of 1–100 nm and related to the size of human body proteins [5,6]. The nanostructured AgNPs have a vital role as antimicrobial agents [7], water purifiers, surgical, and wound care products, antioxidants [8], acetylcholinesterase (AChE) inhibitors [9], and anti-diabetic agents [10,11]. Nobel AgNPs have attracted enormous attention due to their broad-spectrum application in growing demand goods such as cosmetics, textiles, electronics, biosensors, catalysts, and medicine products. The essential characteristics of biosynthesized NPs such as shape and size are particularly attributed to the type and biochemical’s constituents of selected natural sources. It is worth mentioning that no study has been reported yet examining the effects of collective biomaterials on NP fabrication and their biological activities. Thus, this research focuses on the combinative effect of abundant and low cost of marine-derived materials of biopolymer chitosan and the brown marine algae extract as an extracellular green platform on bio-fabrication of AgNPs [1].

AgNPs have attracted a great deal of interest due to their excellent antimicrobial, antioxidants, anti-Alzheimer’s disease, anticancer, and catalytic properties. The main drawback with the chemical and physical methods of AgNPs formation is that they are extremely costly and also involve the use of toxic, hazardous chemicals and they contain potential environmental and biological stakes. However, the use of harmful substances and the high-energy consumption required for the preparation of AgNPs represent disadvantages that limit their large-scale production. Currently, researchers are using green methods for the biosynthesis of AgNPs where the bio-extracts are used as mediators for the fabrication of AgNPs. These metabolites (e.g., vitamins, enzymes, proteins, polysaccharides, amino acids, and organic acids) involve directly in the reduction of Ag ions to produce NPs. The secondary metabolites in the reduction of metal ions into NPs and in supporting their subsequent stability have also been postulated [12]. Recently reported plant extracts that were used for the biosynthesis of AgNPs included Urtica dioica [13], Tropaeolum majus [14], Skimmia laureola [15], Azadirachta indica [16], and Aracuraria angastifolia [17].

Alzheimer’s disease (AD) is associated with memory and thinking impairment, behavioral problems, and disturbance in daily living activities [6]. AD is common in old people due to irreversible neuronal loss. The deficiency of acetylcholine (ACh) in synapses of the cerebral cortex is one of the important sufferers of AD [18] and can be treated by the inhibition of AChE that hydrolyzes ACh into choline and acetate [18]. Synthetic AChE inhibitors (tacrine, donepezil, and rivastigmine) cause significant side effects [19]. Therefore, researchers have been focused on herbal inhibitors of AChE (tacrine, donepezil, and galantamine), which can be used in the treatment of AD with very less or no side effects [20].

Kickxia elatina (KE) is an annual plant used in conventional medicine as a wound-healing agent, sedative, and general tonic, and also in case of bleeding and laceration. Worldwide only a few species of Kickxia have been analyzed for its phytochemical constituents, which result in the isolation of flavonoids and iridoid glycosides and conferred good antiglycation activity (inhibition of α-glycosidase). [21] extracted four types of flavonoids (flavone, glycosides, pectolinarin, and acetylpectolinarin) from KE, which suggests its strong antioxidant activity and prevents the development of heart ailment, cancer, diabetes, and some other disorders like Alzheimer’s disease and dementia [22]. Linarioside extracted from Kickxia spuria, Kickxia elatina (L.) Dumort, and Kickxia commutata expressed strong anti-diabetic activities [23]. KE is one of the least explored members of Plantaginaceae. In the present project, AgNPs’ synthesis from KE was undertaken that has not been evaluated previously. The present work is based on the hypothesis that KE extract and synthesized K. elatina silver nanoparticles (AgNPs) will bear effective anti-Alzheimer’s disease activity due to the presence of biologically active ingredients in KE.

2 Materials and methods

2.1 Collection and processing of KE

The KE plant was obtained from the Ghoriwala area of district Bannu, KP Pakistan, in June 2021. The plant was recognized by Dr. Tahir Iqbal, an expert taxonomist, and a voucher specimen was deposited (NH II) at the Botany Department, University of Science and Technology, Bannu. The plant was dried and powdered. About 100 g of KE was extracted with 500 mL of ethanol and concentrated with a rotary evaporator to harvest crude extract (KE). The concentrated KE was air-dried at 37°C and then stored at 4°C for further investigation.

2.2 KE-mediated AgNPs

AgNPs were synthesized using a standard procedure [24]. About 1.5 g KE crude extract was dissolved in 100 mL of deionized water. The supernatant was stored for activity.
About 1,000 mL of 1 mM AgNO₃ solution was prepared and adjusted the pH at 9. Furthermore, 100 mL of supernatant (1 g·100 ddH₂O⁻¹) and 1,000 mL of AgNO₃ (1 mM) solution were mixed in a 1:10 ratio and incubate the mixture at 40°C. The color change to reddish-brown after 1 h is an indication of chemical reduction, i.e., the formation of AgNPs. The solution was further incubated for 24 h at 40°C. AgNPs were definite by ultraviolet-visible (UV–Vis) spectrometry between 200 and 800 nm. AgNPs were centrifuged at 14,000 rpm. The obtained pellet of NPs was dried in an incubator at 50°C and used for further characterization.

2.3 Size and shape optimization of AgNPs

AgNPs’ synthesis was optimized by using different intrinsic factors such as KE volume, temperature, reaction time, Ag salt molarity, pH, and stability time. To assess the effect of KE concentration on the fabrication of AgNPs, its concentration varied as 0.25, 0.5, 1, 1.5, and 2 mL. AgNPs were prepared at different temperatures 20°C, 40°C, 60°C, 80°C, and 100°C and at varied time intervals (1, 2, 3, and 24 h) to estimate the effect of temperature and time. To analyze the effect of Ag salt concentration, NPs were prepared at different dilution (0.5, 1, 1.5, and 2 mM) of Ag salt. To assess the effect of pH, AgNPs were prepared at varied pH from 5 to 12, respectively, because NPs’ synthesis is supported by both acidic and basic conditions. The NPs’ stability was checked after 24 h, 30 days, and 3 months.

2.4 Characterization of AgNP

AgNPs’ synthesis was confirmed by Shimadzu UV Spectrophotometer (UV-1800). KE and NPs were inquired for functional groups by using Nicolet iS50 FT-IR. The crystalline structure of AgNPs was examined by using the JEOL X-ray diffractometer (XRD) model (JDX-3532, Japan). SEM analysis was used to examine the morphology of AgNPs. EDX was done to know the elemental constituents of AgNPs.

2.5 Experimental animals

The study was ethically approved by the Biotechnology Ethical Committee, USTB, Bannu, Pakistan, on March 17, 2020, under reference number USTB-534/2020. The experiment was performed on male Sprague Dawley rats that were obtained from a Veterinary Research Institute, Peshawar, Pakistan. The average age of the animals was 3–3.5 months, and their weight was 310–350 g. The rats were retained in a controlled temperature well-aired room in steel cages with a 12 h dark–light period. The rats had free entree to food. Rats were not subjected to any specific treatment before slaughtering. The animals were sacrificed by cervical dislocation. The brain was excised, weighed, and washed with 50 mM phosphate buffer.

2.6 Preparation of brain homogenate (BH)

About 1 g of brain was minced and homogenized in 10 mL of 50 mM phosphate buffer and followed by centrifugation at 4°C for 10 min at 10,000 rpm in a high-speed cooling centrifuge.

2.7 Protein estimation

The protocol was used to estimate the proteins in BH. Bovine serum albumin was used as standard [25].

2.8 Anti-acetyl cholinesterase activity

AChE inhibition strength was evaluated by the methodology of Ahmed et al. [26]. The 1 mL mixture assay contained DTNB 10 mM, 50 mM phosphate buffer of pH 7.4, 100 µL of BH as an enzyme, 67 µL water, and different volumes of KE and AgNPs (75, 125, and 175 µL). The mixture was subjected to incubation for 5 min at 37°C. With the addition of substrate acetyl thiocholine (0.05–1 mM), the reaction was started. The ACh hydrolysis rate was measured every 15 s for the 90 s by the development of thiolate di-anion that reacts with DTNB. The activity of the enzyme was scrutinized by the extent of the yellow color formation. The activity was repeated in triplicate to eliminate the errors and % inhibition is calculated by the following equation:

\[
\% \text{ Inhibition} = \frac{\text{Control} - \text{Sample/Control}}{\text{Control}} \times 100 \quad (1)
\]

2.9 Kinetic determinations

For assessing the type of inhibition, kinetics studies were done. The interaction of KE, AgNPs, and enzyme BH was assessed by the double reciprocal plot [27]. 1/V was assessed at ACh (0.05–1 mM) with and without KE and
its NPs. Michaelis constants \((K_m)\) were deliberated by V vs V/S [28] and 1/V vs 1/S plots [27]. The inhibition constant \((K_i)\) was calculated by Cornish-Bowden plots [34]. IC\(_{50}\) was calculated by inhibition/activity vs concentration of samples.

2.10 Statistical analysis

For data analysis, a two-way analysis of variance was performed. Data were shown as mean ± standard. The graphs were drawn in Origin Pro 8.5 and Slide Write.

3 Results

3.1 UV–Vis spectroscopy of AgNPs

The aqueous extract of KE was green which changed to reddish-brown after the addition of AgNO\(_3\) solution and incubation at 40°C for 24 h. The color change was observed due to the bio-reduction of Ag\(^+\).

After chromatic observation, the AgNPs were subjected to UV–Vis spectral analysis in the range of 200–800 nm and displayed a peak at 416 nm with the absorption of 1.98 at optimal conditions as 24 h incubation time, 1.5 mL of aqueous extract concentration, 1 mM silver nitrate solution concentration, and 40°C temperature at pH 9, while the plant extract did not exhibited absorption spectrum in 200–800 nm (Figure 1).

3.2 Factors affecting biosynthesis of AgNPs

The AgNPs’ stability was checked after 24 h, 30 days, and 3 months. The sharp peaks at 416 and 409 nm were reported after 24 h and 30 days and confirmed the presence of AgNPs, but after 3 months, this peak becomes broader with the low absorbance of 0.50 (Figure 2a).

To standardize the NPs’ formation route, different volumes of KE extract varied from 0.25, 0.5, 1, 1.5, and 2 mL were tested in this study, which exhibited a spectrum of absorption in the range of 200–800 nm with the increase in the intensity of peaks from 0.76 to 2.22, respectively (Figure 2b).

The UV–Vis spectra outcomes publicized a rise in the absorbance intensity of the AgNPs with time (Figure 2c). An absorption peak (390 nm) of very low intensity (0.79) appeared after 1 h of reaction, which transformed into a sharp and visible peak of 416 nm after 24 h and represented the formation of stable and spherical AgNPs.

Figure 2d shows the temperature stability of AgNPs (20–100°C) and suggests that SPR peaks became sharper by increasing the temperature from 20°C to 40°C. Further increase in temperature (up to 100°C) results in the broadening of peaks.

Assessment of the influence of AgNO\(_3\) concentration (0.5–2 mM) on AgNPs’ synthesis showed absorbance at 407, 416, 403, and 411 nm, respectively (Figure 2e).

The pH effect (5–12) on the biogenic synthesis of NPs was also evaluated (Figure 2f). At pH 5 and 6, no absorption peaks appeared. But sharp bands were detected as the pH was increased from 6 to 9. At higher pH beyond 9, broader peaks formed.

3.3 FT-IR spectroscopy of KE and synthesized AgNPs

The FT-IR spectrum of KE and AgNPs is presented in Figure 3. Different peaks were demonstrated that correspond to the different functional groups (Table 1). The various peaks reported by KE and AgNP at 3,272.72 and 3,300.39 cm\(^{-1}\) indicate O–H stretch, 2,916.20 and 2,922.52 cm\(^{-1}\) signify the C–H stretch, 2,854.45 and 2,847.93 cm\(^{-1}\) correspond to the OH group of carbonyl compounds of the protein, 2,174.70 and 2,181.18 cm\(^{-1}\) for C≡C stretch, 2,078.26 and 2,023.71 cm\(^{-1}\) for N=C=S stretch, 1,975.49 and 1,906.71 cm\(^{-1}\) indicate C=C=C stretch of allenes, 1,728.06 and 1,735.17 cm\(^{-1}\) resemble the C=O vibrations of conjugated aldehydes, 1,185.77 and 1,233.99 cm\(^{-1}\) resemble C–O stretching of
Figure 2: UV spectra of AgNPs at different stability periods (a), extract volume (b), time intervals (c), temperature (d), silver nitrate solution concentration (e), and pH (f).
alcohol, ester, ether, and carboxylic acid, 1,062.45 and 1,024.22 cm$^{-1}$ represent C–N aliphatic amines, and 643.47 and 630.03 cm$^{-1}$ attribute to C–Br stretch.

3.4 SEM of AgNPs

The nanostructure and surface morphology studies of size and shape were interpreted by SEM (Figure 4a). The outcomes showed that AgNPs are mono-dispersive with the constant arrangement in the matrix. The particle size distribution graph was deliberated by Nano Measurer software (Figure 4b), which demonstrated that 90% of AgNPs are in an average size of 50 nm.

3.5 XRD analysis of AgNPs

XRD exploration of AgNPs presented four strong Bragg reflections 38°, 44°, 64°, and 77° at 2θ values, which attributes to the plane of (111), (200), (220), and (311) reflections and conferring face-centered cubic crystal

Table 1: FT-IR interpretation of KE extract (whole plant) and its silver nanoparticles

<table>
<thead>
<tr>
<th>S. no.</th>
<th>KE extract peak position (cm$^{-1}$)</th>
<th>AgNPs peak position (cm$^{-1}$)</th>
<th>Corresponding frequency range (cm$^{-1}$)</th>
<th>Chemical bond</th>
<th>Functional group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3,272.72</td>
<td>3,300.39</td>
<td>3,500–3,200 (s)</td>
<td>O–H (stretch)</td>
<td>Alcohol and phenol</td>
</tr>
<tr>
<td>2</td>
<td>2,916.20</td>
<td>2,922.52</td>
<td>3,000–2,850 (m)</td>
<td>C–H (stretch)</td>
<td>Alkanes</td>
</tr>
<tr>
<td>3</td>
<td>2,854.45</td>
<td>2,847.93</td>
<td>3,300–2,500 (m)</td>
<td>O–H (stretch)</td>
<td>Carboxylic acid</td>
</tr>
<tr>
<td>4</td>
<td>2,174.70</td>
<td>2,181.18</td>
<td>22,60–2,100 (w)</td>
<td>C=C (stretch)</td>
<td>Alkynes</td>
</tr>
<tr>
<td>5</td>
<td>2,078.26</td>
<td>2,023.71</td>
<td>2,140–1,990 (s)</td>
<td>N≡C≡S (stretch)</td>
<td>Metal carbonyl</td>
</tr>
<tr>
<td>6</td>
<td>1,975.49</td>
<td>1,906.71</td>
<td>2,000–1,900 (m)</td>
<td>C≡C≡C (stretch)</td>
<td>Allene</td>
</tr>
<tr>
<td>7</td>
<td>1,728.06</td>
<td>1,735.17</td>
<td>1,740–1,720 (s)</td>
<td>C=O (stretch)</td>
<td>Conjugated Aldehyde</td>
</tr>
<tr>
<td>8</td>
<td>1,632.41</td>
<td>1,604.74</td>
<td>1,650–1,600 (m)</td>
<td>C=C (stretch)</td>
<td>Conjugated ketone and alkene</td>
</tr>
<tr>
<td>9</td>
<td>1,336.75</td>
<td>1,371.54</td>
<td>1,390–1,310 (m)</td>
<td>O–H (bend)</td>
<td>Phenols</td>
</tr>
<tr>
<td>10</td>
<td>1,185.77</td>
<td>1,233.99</td>
<td>1,320–1,000 (s)</td>
<td>C=O (stretch)</td>
<td>Alcohol, ester, ether, carboxylic acid</td>
</tr>
<tr>
<td>11</td>
<td>1,062.45</td>
<td>1,024.22</td>
<td>1,250–1,020 (m)</td>
<td>C–N (stretch)</td>
<td>Aliphatic amines</td>
</tr>
<tr>
<td>12</td>
<td>877.47</td>
<td>863.24</td>
<td>890–850</td>
<td>C–Br (stretch)</td>
<td>Aromatic compounds</td>
</tr>
<tr>
<td>13</td>
<td>643.47</td>
<td>630.03</td>
<td>690–515 (s)</td>
<td></td>
<td>Halo compounds</td>
</tr>
</tbody>
</table>
FCC structure Figure 5. The size (42.47 nm) was calculated by the Debye–Scherrer equation (Eq. 2) and presented in Table 2.

$$D = \frac{K \lambda}{\beta \cos \theta}$$

(2)

3.6 EDX analysis of AgNPs

EDX was conducted for identifying the elemental constituents involved in the biosynthesis of AgNPs (Figure 6a). The EDX spectrometry indicated that AgNPs contain 61.67% of silver, 15.82% carbon, 15.65% oxygen, 4.93% sodium, 0.83% calcium, 0.82% chlorine, and 0.28 silicon by percent weight (Figure 6b). Therefore, the EDX analysis reveals the elemental composition of AgNPs.

3.7 Anti-Alzheimer’s disease activity

At ACh concentration (0.5 mM), BH acetylcholinesterase (BH-AChE) inhibition ability of KE, AgNPs, and AgNO₃ was evaluated. Figure 7a represents that KE, AgNPs, and AgNO₃ salt exhibited the highest 65.02%, 75.25%, and 21.68% inhibition against AChE at 175 µg mL⁻¹ concentration, respectively.

3.8 Calculation of IC₅₀

The IC₅₀ values are displayed in Figure 7b and showed IC₅₀ values of KE and AgNPs that are 135.16 ± 1.64 and 117.76 ± 5.21 µg mL⁻¹, respectively, while AgNO₃ salt did
not cause 50% inhibition at any concentration due to a lack of phytochemicals from the plant extract.

### 3.9 Effects of plant and AgNPs on $K_m$ and $V_{\text{max}}$

Kinetics studies revealed that both AgNPs and KE caused a non-competitive inhibition of AChE (Figure 8a and b), respectively. In such type of inhibition, $V_{\text{max}}$ decreased (34.17–68.64% and 22.29–62.10%) in the concentration-dependent mode for KE and AgNPs, respectively, while $K_m$ values remain constant (Table 3).

### 3.10 Influence of AgNPs and KE on $K_{\text{iapp}}$ and $V_{\text{maxiapp}}$

The effect of AgNPs and KE on $K_{\text{iapp}}$ and $V_{\text{maxiapp}}$ was studied (Figure 9a and b) and showed that with the increase in substrate concentration (0.05–1 mM), $K_{\text{iapp}}$ remains constant while $V_{\text{maxiapp}}$ values decreased from 48.43% to 150.12% and 7.66% to 49.52%, respectively, for AgNPs and KE (Table 4).

### 3.11 Determination of $K_i$, $K_i$, and $K_m$ of AChE

The dissociation constant ($K_i$) was calculated for AgNPs and KE as 46.69 and 30.95 µg respectively (Figure 9a and b). While the inhibitory constant ($K_i$) was calculated to be 32 (Figure 10a) and 19 µg (Figure 10b) for AgNPs and KE, respectively. $K_m$ (Michaelis–Menten constant) was 0.018 and 0.037 mM for KE and AgNPs, respectively (Table 5).

### 4 Discussion

Engineered nano-medicine is a fast-developing and important field that is related to the fabrication of metallic NPs by using metals like silver, gold, and platinum [30] and exhibits in the field of biomedicines, applied sciences, and material sciences. The rat’s BH-AChE inhibition by KE extract and AgNPs was done in this study. AgNPs’

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**Table 2: The grain size, interplaner spacing, and lattice constant of AgNPs**

<table>
<thead>
<tr>
<th>S. no.</th>
<th>$\theta$ Value</th>
<th>Element</th>
<th>hkl</th>
<th>FMHM $(\beta)$ of intense peak (radians)</th>
<th>Particle size $(D)$ [nm]</th>
<th>d spacing [Å]</th>
<th>Lattice constant $(a)$ [Å]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>38.07</td>
<td>Ag</td>
<td>111</td>
<td>0.0035</td>
<td>40.76</td>
<td>2.361</td>
<td>4.089</td>
</tr>
<tr>
<td>2</td>
<td>44.32</td>
<td>Ag</td>
<td>200</td>
<td>0.0033</td>
<td>44.71</td>
<td>2.041</td>
<td>4.083</td>
</tr>
<tr>
<td>3</td>
<td>64.39</td>
<td>Ag</td>
<td>220</td>
<td>0.0037</td>
<td>37.56</td>
<td>1.445</td>
<td>4.088</td>
</tr>
<tr>
<td>4</td>
<td>77.40</td>
<td>Ag</td>
<td>311</td>
<td>0.0030</td>
<td>46.84</td>
<td>1.231</td>
<td>4.084</td>
</tr>
</tbody>
</table>

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**Figure 6:** EDX spectra (a) and elemental profile (b) of biosynthesized AgNPs.

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characterization is an important tool for their stability, collection, bio-dispersity, and biological efficacy in the cells. Metal-based NPs have free electrons and excitation of electrons with the light of a specific wavelength [31,32]. AgNPs have brown color due to a change in the SPR band detected by the UV–Vis spectrophotometry and appeared at 416 nm with higher absorption of 1.98 showing the reduction and stabilization of Ag+ ions in the aqueous solution. A similar absorbance band at 420 nm was reported by ref. [33] in AgNPs synthesized from M. pulegium leaves.

The size, shape, and fabrication of the AgNPs are affected by extract volume, temperature, time, AgNO3 concentration, and pH [34]. Therefore, in the current study, these parameters were studied to optimize and control the size and shape of the AgNPs and revealed the optimum conditions that produced stable and small-size AgNPs were 1.5 mL (extract concentration), 1 mM (silver nitrate salt concentration), 40°C (temperature), 24 h (reaction time), and pH 9. The current investigation is also supported by the report of [15] on Hippeastrum hybridum-induced silver NPs.

**Figure 7:** (a) In vitro ache inhibitory capability of silver nanoparticles (AgNPs), KE extract, and AgNO3. (b) IC_{50} values of AgNPs and KE cause 50% inhibition of BH ache.

**Figure 8:** (a and b) Lineweaver–Burk (reciprocal of enzyme velocity vs reciprocal of ACh) plot showing the non-competitive type of inhibition (K_{m} constant and V_{max} decrease) caused by AgNPs and KE, respectively.
The biological activities of the silver NPs are greatly influenced by their stability. The results are supported by Jabariyan and Zanjanchi \[35\] who used grape juice for the biosynthesis of AgNPs and found no significant changes in the shape, position, and symmetry of the SPR absorption peak for 30 days. But after 3 months the SPR band became broader with low absorbance of 0.50 and showed that AgNPs lost their stability.

Plant extract consists of various phytochemicals such as tannins, phenols, alkaloids, flavonoids, anthocyanins, phenols, polysaccharides, and polyphenols that attribute to the reduction of Ag\(^+\) and the formation of stable and isotropic AgNPs \[36\]. At an optimal concentration of 1.5 mL, the sharp band (416 nm) attributes to the fabrication of small and isotropic particles. These findings are in agreement with earlier reports \[37,38\] that have proposed

<table>
<thead>
<tr>
<th>S. no.</th>
<th>AgNPs</th>
<th>KE extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration (µg)</td>
<td>(V_{\text{max}}) (µmol·min(^{-1})·mg(^{-1}) protein)</td>
</tr>
<tr>
<td>0</td>
<td>0.3137</td>
<td>0</td>
</tr>
<tr>
<td>75</td>
<td>0.2065</td>
<td>34.17</td>
</tr>
<tr>
<td>125</td>
<td>0.1657</td>
<td>47.17</td>
</tr>
<tr>
<td>175</td>
<td>0.0984</td>
<td>68.64</td>
</tr>
</tbody>
</table>

Table 3: Influence of KE and AgNPs on \(K_m\) and \(V_{\text{max}}\) of Sprague Dawley rats brain homogenate (AChE)

<table>
<thead>
<tr>
<th>S. no.</th>
<th>AgNPs</th>
<th>KE extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ACh concentration (mM)</td>
<td>(V_{\text{max,app}}) (µmol·min(^{-1})·mg(^{-1}) protein)</td>
</tr>
<tr>
<td>0.05</td>
<td>0.2563</td>
<td>0</td>
</tr>
<tr>
<td>0.1</td>
<td>0.3804</td>
<td>48.43</td>
</tr>
<tr>
<td>0.25</td>
<td>0.4420</td>
<td>72.47</td>
</tr>
<tr>
<td>0.5</td>
<td>0.4980</td>
<td>94.33</td>
</tr>
<tr>
<td>1</td>
<td>0.6410</td>
<td>150.12</td>
</tr>
</tbody>
</table>

Table 4: Effect of KE and AgNPs on \(K_{\text{app}}\) and \(V_{\text{max,app}}\) of Sprague Dawley rats’ brain homogenate (AChE); the \(V_{\text{max,app}}\) and \(K_{\text{app}}\) were calculated by Dixon plot of Figure 9a and b

Figure 9: (a and b) Determination of \(K_{\text{app}}\) and \(V_{\text{max,app}}\) for AgNPs and KE using Dixon plot for BH-AChE.

The biological activities of the silver NPs are greatly influenced by their stability. The results are supported by Jabariyan and Zanjanchi \[35\] who used grape juice for the biosynthesis of AgNPs and found no significant changes in the shape, position, and symmetry of the SPR absorption peak for 30 days. But after 3 months the SPR band became broader with low absorbance of 0.50 and showed that AgNPs lost their stability.

Plant extract consists of various phytochemicals such as tannins, phenols, alkaloids, flavonoids, anthocyanins, phenols, polysaccharides, and polyphenols that attribute to the reduction of Ag\(^+\) and the formation of stable and isotropic AgNPs \[36\]. At an optimal concentration of 1.5 mL, the sharp band (416 nm) attributes to the fabrication of small and isotropic particles. These findings are in agreement with earlier reports \[37,38\] that have proposed
that the rise in *M. pulegium* leaves extract concentration is linked positively with the stability of AgNPs. Moreover, the time influence on nanofabrication and size of AgNPs was also tested at different radiation times of 1, 2, 3, and 24 h. After 1 h of reaction, an absorption peak of 390 nm appeared at very low intensity, which transformed into a sharp and visible peak of 416 nm after 24 h suggesting the enhancement of synthesized NPs and complete reduction of Ag ions into stable and spherical AgNPs. Thus, to get maximum- and small-sized NPs, the optimum time was suggested to be 24 h. Awwad and Salem [39] reported 60 min for stable mulberry leaves extract-based AgNPs.

Generally, the NPs are prepared at room temperature, but the reaction is very slow and a long time is required for a complete reaction at room temperature. Therefore, the rate of reaction can be accelerated by elevating the temperature. The SPR peaks became sharper by raising the temperature from 20°C to 40°C suggesting that an increase in temperature up to 40°C led to a complete and rapid reduction of Ag⁺ and ultimately uniform nucleation of Ag nuclei that result in the formation of small-sized NPs [40]. Furthermore, an increase in temperature up to 100°C resulted in the broadening of peaks and revealed the biosynthesis of large-sized NPs. The AgNPs tend to be poly-dispersed with the increase in temperature beyond 40°C [41].

In the current study, the various concentrations of AgNO₃ were tested to get the optimum size of AgNPs (Figure 2e) and revealed that at a concentration of 0.5 mM silver nitrate was deficient for the fabrication of NPs. Interestingly, a 1 mM concentration of AgNO₃ supported the rapid formation of minute-sized silver NPs. Thus, a very little amount of AgNO₃ salt is required for the biosynthesis of potential and ideal-size AgNPs. With further increase in reactant concentration (1.5–2 mM), the rate of reduction of Ag ion will decrease and the accumulation of AgNO₃ makes the mixture solution foggy that subsequently resulting in broad peaks. The broad peak indicates large-size NPs [42]. The broad peak indicates large-size NPs. Kaya et al. [43] reported that small nanometer-sized NPs are biologically more active.

One of the important parameters is pH, which affects the rate of biosynthesis of NPs by changing the electrical load of phytochemicals and alternatively affects the capping and stabilizing ability of Ag ions [44]. Khan et al. [45] reviewed that the size of NPs is probably higher in the acidic medium as compared to the basic media. In the present report, the sharp SPR bands were detected as the pH was increased from 6 to 9 and indicated the biosynthesis of isotropic and stable AgNPs in the basic medium. The alkaline condition assisted in the reduction of biomolecules present in extracts. At higher pH beyond 9,
formations of broader peaks indicate large-size NPs. Thus, a pH study showed that the fabrication of AgNPs is enhanced by basic conditions while repressed by acid conditions [46].

FT-IR analysis identifies the existence of phyto-molecules, which account for Ag metal reduction and their collaboration with the stabilization and capping of AgNPs. FT-IR analysis confirmed various bond stretches at their respective peaks of KE and AgNPs, which conferred the presence of polyhydroxy, carboxyl, phenol, lipids, proteins, alkynes, amide, aliphatic amines, halo compounds, alkene, etc. The phenolic and alcoholic compounds are potentially stabilizing mediators involved in the reduction of AgNPs [47]. The carbonyl compounds of proteins attribute to the stabilization of AgNPs by capping them [48]. In the present study, FT-IR confirmed the interaction between biomolecules and different functional groups of KE extract in the reduction and biosynthesis of AgNPs. Thus, from the IR spectrum, it may be presumed that these biomolecules are involved in the stabilization and capping of AgNPs [49].

The morphological studies of size and shape were interpreted by SEM and showed that AgNPs are monodispersive with uniform alignment in the matrix. The grain sizes of the NPs valued from the SEM (50 nm) are comparable to XRD data (42.47 nm). The results are consistent with an earlier report in which AgNPs synthesized using Morus alba leaf are spherical with sizes ranging below 50 nm [50].

XRD investigation of AgNPs presented the four strong Bragg reflections that correspond to the pure silver metal with FCC symmetry. These results are in line with earlier reports by [51] where AgNPs synthesized from Euphorbia serpens were FCC crystals of 50 nm size and similar diffraction planes were observed. Hublikar et al. [52] reported that Carissa carandas L. leaf-synthesized AgNPs are crystalline with crystallite sizes 35 and 30 nm at 25°C and 60°C, respectively.

EDX analysis gives information concerning the participation of elements in the reduction of Ag⁺ both qualitatively and quantitatively [53]. The quantitative profile of elements indicated that AgNPs contain an appreciable and high percentage of silver, followed by carbon, oxygen, sodium, calcium, chlorine, and silicon. The silver showed a high-intensity peak at 3 keV due to SPR and confirmed the complete reduction of Ag⁺ to AgNPs [53]. The presence of carbon and oxygen corresponds to an aromatic compound that gives stability to reduced Ag [54,55].

Neurodegenerative disorders such as Alzheimer’s disease occur due to a low level of the neurotransmitter ACh or a high level of AChE [56]. The enzyme AChE catalyzes the hydrolysis of ACh after its liberation at the cholinergic synapses to return to its resting state after activation [57] and results in the reduction of contact time between the postsynaptic membrane and neurotransmitter ACh. Thus, delaying the transfer of information that leads to memory loss. Therefore, the main therapeutic approach in the prevention of Alzheimer’s disease involved the increasing level of ACh and controlling the activity of AChE by using reversible inhibitors of AChE that will balance the cholinergic system and allow more contact time for information transformation through neurons [56,58]. The effects of silver ions and AgNPs against AChE were also studied in vivo by [46] in which they used three different doses of the synthesized AgNPs and revealed decreased AChE activity. Currently, we confirmed the inhibition of Sprague rat’s BH-AChE by KE extract, AgNPs, and AgNO₃ (Figure 7a) and found that at a fixed 0.5 mM concentration of substrate (ACh), KE, KE-NPs, and AgNO₃ exhibited 65.02%, 75.25%, and 21.68% inhibition at 175 μg·mL⁻¹, respectively. The IC₅₀ values of AgNPs (117.76 ± 5.21 μg·mL⁻¹) are significantly higher as compared to KE (117.76 ± 5.21 μg·mL⁻¹) and suggest that reduced AChE activity by AgNPs might be due to quercetin in the extract that is responsible for capping off the nanofabricated NPs. Abdelhafiez et al. [59] reported a decrease in AChE activity in rats treated with biosynthesized AgNPs and are in best agreement with our present study.

According to kinetic studies, both KE and AgNPs inhibit AChE in a non-competitive mode ($K_m$ remains constant while $V_{max}$ decreased). In such a type of inhibition, the KE and AgNPs bind to an allosteric site and alternatively decline the efficacy of the enzyme [60]. The release of silver ions by AgNPs due to surface oxidation might be the possible mode of action as it increases interaction with AChE and subsequently inhibits its activity [61,62]. AgNPs are more effective compared to plant extract due to their morphology [63–65].

5 Conclusion

KE, an indigenous plant found in abundance in Pakistan, has been successfully utilized for the biosynthesis of simple, quick, colloidal, and stable AgNPs. The NPs were characterized by UV–Vis spectroscopy, SEM, FT-IR, EDX, and XRD analysis. AgNPs were also scrutinized for anti-AChE activities in BH and showed good inhibitory enzymatic properties as compared to their respective KE extract and AgNO₃. Thus, it may be concluded that green-synthesized AgNPs can be used as a cheaper and alternative option against AD disease.
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References


