

Green synthesis of silver nanoparticles (AgNPs) using *Alstonia scholaris* extract: Evaluation of their antioxidant, enzyme inhibitory, antimicrobial, and antimutagenic activities through in vitro and in silico studies

Shubhangi D. Shirsat (✉ Shubhangi.shirsat@gtit.edu.cn)

Guangdong Technion – Israel Institute of Technology

Manjusha Chakranarayan

MGM's Institute of Biosciences and Technology

Varenyam Achal

Guangdong Technion – Israel Institute of Technology

Mahendra Rai

SGB Amravati University

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Abstract

Alstonia scholaris is used as a traditional medicine for the treatment of various human and livestock diseases as it contains diverse types of biologically active compounds. In the current study silver nanoparticles (AgNPs) were synthesized by an eco-friendly method using *Alstonia scholaris* leaf extract (aqueous and methanolic). The synthesized nanoparticles (NPs) were characterized by UV-visible spectroscopy, Fourier transform infrared (FTIR), x-ray diffraction (XRD), and scanning electron microscopy (SEM). Green synthesized AgNPs were evaluated for their antioxidant, enzyme inhibition, antimicrobial, and antimutagenic potential. The AgNPs exhibited efficient antioxidant activities and considerable enzyme inhibition potential against α -amylase, and butyrylcholinesterase (BChE) enzymes. Meth-AgNPs showed strong antioxidant activity and high enzyme inhibitory effect as compared to Aq. AgNPs. Both AgNPs demonstrated significant antimicrobial activity. Synthesized AgNPs did not show any antimutagenic activity while Meth. AgNPs have demonstrated lower toxicities than previously reported AgNPs. Plant extracts are complex and contain several phytochemicals, which participate in the reduction and capping of AgNPs. Thus, despite the popularity of plant extract-mediated synthesis of AgNPs, the exact mechanism is still unclear. Here, we have used molecular simulation to reveal the interaction of phytochemicals and AgNP. Molecular dynamics simulation trajectory analysis showed that all phytochemicals- AgNP complexes reach an equilibrium within 100 ns. In addition, molecular docking experiments were performed to understand the mechanism of enzyme inhibition. The data from molecular simulation and dynamic support the capping of phytochemicals on the surface of AgNPs and is also responsible for the antioxidant, enzyme inhibition, and lower toxicity of AgNPs.

1 Introduction

The use of engineered nanoparticles (NPs) is exponentially increasing in fields such as biomedicine, materials science, agriculture, food, and consumer product industries. NPs are colloidal materials with sizes below 100 nm with physicochemical and biological properties distinct from those of their bulk counterparts (1). Despite the advantages of uniformity in the size of NPs synthesized by chemical methods, the green synthesis of NPs is emerging as an attractive alternative from an environmental standpoint (2–4). The absence of hazardous chemicals, environmental friendliness, uncomplicated procedures enormous diversity of raw materials, and cost-effectiveness make the plant extract-mediated green synthesis of NPs a superior method. Additionally, NPs synthesized using plant extracts are more biocompatible and biologically active than those synthesized via chemical methods (5–7). Plant extracts are very complex and contain several biomolecules belonging to various structural classes such as alkaloids, polysaccharides, vitamins, amino acids, proteins, enzymes, tannins, phenolic acids, saponins, terpenoids, etc. According to various studies, most of these molecules were predicted to participate in the green synthesis and capping of NPs (8, 9). The phytocompounds capped on NPs are responsible for their attractive and unique bioactive properties such as antimicrobial, anticancer, and antioxidant properties (10, 11). Different medicinal plants have different types of phytochemical profiles which are responsible for their unusual medicinal properties. Therefore, NPs synthesized from different medicinal plants have diverse bioactive molecules as reducing and capping agents which are responsible for their unique bioactivities (12, 13).

Alstonia scholaris belongs to the family Apocynaceae and is commonly known as *Devil's tree* or *Saptparni*. It is widely distributed in Asia, especially in India, China, and Malaysia (14). It has been used as a traditional medicine for the treatment of various human and livestock diseases and several biologically active compounds have been isolated from this plant (15,16). *Alstonia scholaris* is a very rich source of alkaloids, terpenoids flavonoids, etc. On the basis of traditional usage, the leaf extract was also developed to be a commercial *traditional Chinese medicine* in China. The rich diversity and high concentration of secondary metabolites in *Alstonia scholaris* are also responsible for its antimutagenic property (17–20). Noble metal NPs, such as silver nanoparticles, (AgNPs) have been studied extensively due to their multifunctional nature (21, 22). In ancient periods, the silver (Ag) was very popular known for its antimicrobial potential. The modern era exploits the nano form of silver i.e. AgNPs in a number of applications including viral inhibition assays, anti-cancer activities, wound dressing, food preservatives, and water treatment (23, 24). AgNPs have also been used extensively as antifungal, antibacterial, antioxidant, anti-inflammatory, and anti-angiogenic agents (25). However, the multiple toxicity and genotoxicity of AgNPs synthesized from different methods hinder their, environmental, and agricultural applications (26). Therefore, nontoxic AgNPs with all biological activities are needed.

The present work aimed to synthesize biologically stable AgNPs using leaf extract of *Alstonia scholaris* and to evaluate their antioxidant, enzyme inhibition, antimicrobial, and antimutagenic potentials. Although there are reports of the synthesis of AgNPs from *Alstonia scholaris* from bark extract, there is no clear scientific evidence for the antidiabetic, cholinesterase, and antimutagenic effects of AgNPs synthesized from *Alstonia scholaris*. Therefore, in the present study, AgNPs were synthesized by extract of *Alstonia scholaris*, characterized by UV-visible Spectrophotometry, FT-IR spectrophotometry, scanning electron microscopy (SEM), energy dispersive spectroscopy (EDAX), x-ray diffraction (XRD) and the antidiabetic, cholinesterase, and antimutagenic activities were evaluated by *in vitro* and *in silico* studies.

Many studies have suggested are responsible for the green synthesis of NPs (27). Molecular dynamics simulation is frequently used to obtain the interaction trajectories for a better understanding of biological processes. Atomic-scale theoretical methods can provide important insights into the solution-phase synthesis of AgNPs that involves the seeded growth and aggregation of silver clusters (28, 29). It also helps in understanding the mechanism of adsorption of stabilizing agents and solvent molecules onto the exposed facets of inorganic metal nanocrystals (30–32). Numerous MD simulation studies of metal NPs protected by organic ligand monolayers (33–36), polymers (37–41), peptides (42, 43), and proteins (44) have been conducted. In this work, we present a unified MD model that can be used to simulate AgNPs in an aqueous solution consisting of phytochemicals from *Alstonia scholaris*. The main goal of our study was to identify the interaction between these phytochemicals and the silver core. We also performed molecular docking of these phytochemicals with α -amylase and cholinesterase to understand the mechanism of enzyme inhibition by the AgNPs.

2 Materials and methods

2.1 Plant material

The leaves of *A. scholaris* (Apocynaceae) were collected after proper identification and authentication of the plant by Agharkar Research Institute, Pune, India. A voucher specimen (No. AHMA-23777) has been deposited for future reference. The average age of the plants from which samples were collected was more than 50 years. Fresh leaves of *A. scholaris* free from diseases were collected in July from rural areas of Aurangabad district, Maharashtra, India.

2.2 Extraction procedure

The leaves of *A. scholaris* were with distilled water and dried for 5–7 days in the dark at room temperature. After drying, the leaves were ground finely and placed in a porous bag (thimble) made of strong filter paper. The thimble was placed in the chamber of a Soxhlet apparatus. Two separate solvents (methanol and double distilled water) were used for extraction. The extraction assembly was set up as described by Handa et al., (45). Hot continuous extraction was carried out for 5–6 refluxing cycles. When no residue was found from the evaporated drop collected, the refluxing cycle was terminated. The solvent containing extract siphoned out into the distillation still was collected and allowed to cool. The liquid content was evaporated and condensed. This was stored in a sterile labelled container at 4°C until further use. Extracts were re-dissolved in sterile pyrogen-free water and sterilized using syringe filters prior to use (46).

2.3 Qualitative phytochemical analysis

The obtained extracts were subjected to qualitative phytochemical analysis of different bioactive compounds such as alkaloids, flavonoids, tannins, terpenoids, saponins, and steroids by using the following standard protocols (47, 48).

2.4 Green Synthesis of Silver Nanoparticles

For the green synthesis of AgNPs, 20 mL of 3 mM AgNO₃ (Sigma-Aldrich, Germany) was added to 50 mL volumetric flasks, and 2 mL of different extracts (aqueous and methanolic) was added and mixed for 5 min. Then, the reaction mixtures were stirred continuously on a benchtop stirrer at 100 rpm at room temperature in the dark. The colour changes from light yellow to dark brown were used as an indication of phyto reduction. The reaction mixtures were transferred to 50 mL Falcon tubes and centrifuged at 6000 rpm for 30 min to pelletize the AgNPs. The supernatants were removed and the pellets containing AgNPs were washed 5 times with distilled water. Finally, AgNPs were dried by lyophilization (EYELA, FDU-1200) (50).

2.5 Characterization of synthesized AgNPs.

The phytosynthesis of the AgNPs was confirmed with the help of ultraviolet–visible spectroscopy (Thermo Fisher, GENESYS-150). FT-IR spectral measurements were carried out for the identification of potential phytochemicals involved in the capping and stabilization of AgNPs using a spectrophotometer (BRUKER A220/D-01) with a frequency range of 4000–600 cm⁻¹. The morphology and size of the synthesized AgNPs were determined by scanning electron microscopy (Zeiss, Gemini 450), and energy dispersive X-ray (EDX) analysis was performed to analyze the elemental composition and chemical composition of the AgNPs. XRD analysis was conducted to examine the crystalline nature of samples (Rigaku, SmartLab (9KW)).

2.6 Antioxidant studies

The antioxidant activity of the synthesized AgNPs (20–100 µg/mL) was assessed in terms of the *in vitro* scavenging potential of samples for the free radical 2,2-diphenyl 1-picrylhydrazyl (DPPH). Briefly, a 0.1 mM solution of DPPH in methanol was prepared. Ascorbic acid was used as a standard. The reaction mixture was prepared by mixing 20 µL of each test sample with 180 µL of reagent solution. After a resting phase of an hour, each mixture was analysed at 517 nm using a spectrophotometer. The scavenging effect was calculated as follows:

$$\text{Scavenging effect (\%)} = [(A_0 - A_1) / A_0] \times 100$$

where A₀ is the absorbance of the control reaction and A₁ is the absorbance in the presence of the standard (30).

2.7 Enzyme inhibition assay

2.7.1 α-amylase Inhibition Assay.

The α-amylase inhibition activity of the phytosynthesized AgNPs was determined by The DNS method (51,52). The sample (500 µl) at different concentrations (10 to 100 µg/ml) was incubated with 500 µl of α-amylase solution. After preincubation, 1% starch in 0.02 M sodium phosphate buffer (500 µl) was added and incubated for 10 min. The reaction was stopped by adding 0.5 ml of dinitro salicylic acid and kept for 15 min in a boiling water bath. Then, 5 ml of distilled water was added in reaction mixture and the absorbance was measured at 540 nm. The blank was maintained without adding an enzyme. Acarbose served as standard compound to evaluate the inhibitory activities of AgNPs. The percent α-amylase inhibition was calculated as follows:

$$\% \alpha\text{-amylase inhibition} = (\text{Absorbance without inhibitor} - \text{absorbance with inhibitor}) / \text{absorbance without inhibitor} \times 100$$

The FT-IR spectra of pure amylase and Aq. AgNP + amylase, Meth. AgNPs + amylase and acarbose + amylase were read between 4500 and 400 cm⁻¹. The spectra of the samples were recorded thrice and averaged to ensure reproducibility.

2.7.2 Cholinesterase (AChE/BChE) inhibition assay

Modified Elman's method was used to investigate the inhibitory potency of synthesized AgNPs against acetyl-cholinesterase (AChE) and butyryl-cholinesterase (BChE) (53). Enzymes including acetylcholinesterase (AChE), source *Electrophorus electricus* (CAS number 9000-81-1), purchased from Sigma Aldrich, St. Louis, MO, United States, and butyrylcholinesterase (BChE), source equine serum (CAS number: 9001-08-5), Sigma Aldrich GmbH, Germany were used in the study. To perform the assay, the synthesized AgNPs were dispersed in PBS and other substrate solutions such as DTNB (5,5-dithiobisnitrobenzoic acid) (100 mM), butyrylcholine iodide (100 mM) and acetylcholine iodide (100 mM) were prepared in distilled water and kept at 8°C. The final concentrations of enzymes were 0.4 U/ mL for AChE and 0.4 U/mL for BChE. Galantamine hydrobromide was used as a positive control and prepared in methanol at a

concentration of 10 mg/mL, while the reaction mixture without a test sample was used as a negative control. Afterward, additional DTNB complexes exhibited yellow colour which was further used to record its absorbance at 412 nm using a UV-VIS spectrophotometer. The two biogenic AgNPs (Ag & Meth) and galantamine percent enzyme inhibition was calculated as:

$$\% \text{ Inhibition} = (\Delta\text{Abs control} - \Delta\text{Abs sample}) / [\Delta\text{Abs control}] \times 100$$

The FT-IR spectra of Pure AchE and Aq. AgNP + AchE, Meth. AgNPs + AchE and galantamine + AchE were read between 4500 and 400 cm^{-1} . Similarly, the FT-IR spectra of pure BchE and Aq. AgNP + BchE, Meth. AgNPs + BchE and galantamine + BchE were read between 4500 and 400 cm^{-1} . The spectra of the samples were recorded thrice and an average was taken for plotting the data.

2.8 Antimicrobial activity

The antibacterial activity of synthesized AgNPs was determined against four bacterial strains *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 9721), using agar well diffusion method (52). One-day-old inoculated microbial inoculums (OD 0.5) were spread on the agar surface and holes were punched (6 mm) with the help of a sterile hole borer. Approximately 20 μL of AgNPs of different concentrations (1.0 mg/ml,) were introduced. Subsequently, the plates were incubated at 37°C for the next 24 h.

2.8.1 Minimum Inhibitory Concentration Assay

The minimum inhibitory concentration (MIC) of phytosynthesized AgNP was measured using the broth microdilution method. In a 96-well microtiter plate, the bacteria were inoculated at a concentration of 10^6 CFU/ml. Then, the stock solution of AgNPs (1–10 mg/mL) was sonicated and inoculated with 100 μL of bacterial suspension with turbidity equivalent to 0.5 McFarland in the tubes. Column 1 inoculated with only medium served as a negative control. Then, a resazurin solution was mixed in all wells and incubated at 37°C for 24 h. If the color changes from blue or purple to pink or colorless, it refers to bacterial growth (53).

2.9 Antimutagenic Activity

2.9.1 Allium cepa root growth and chromosome aberration assay

The root primordia of the bulbs were removed before starting the treatment. Healthy onion bulbs (20–25 g) were grown in the dark in a cylindrical glass beaker at room temperature (28 ± 0.5)°C and given a renewed water supply every 24 h. When the roots reached 2 to 3 cm in length they were treated with AgNPs (25 ppm), copper sulfate (3ppm), and plant extract suspension for 24 h. Three replicates were made for each concentration. After treatment, the root tips were cut and fixed in a solution of ethanol (99%) and glacial acetic acid (3:1) for 24 h and washed with distilled water three times. Then roots were transferred to 70% alcohol and stored in a refrigerator until use (55).

2.9.2 Microscopic examination

The root tip squash technique was applied for the study of the mitotic index (MI) and chromosomal aberrations. The root tips were heated (60°C) in 1 M HCl for approximately 6 min followed by washing with distilled water three times and then staining with 40–45% Safranin. Staining was continued for about 5–6 min. The slides were analysed at $\times 1000$ magnification for cytological changes and chromosomal aberrations. The mitotic index was calculated as the number of dividing cells per 1000 observed cells (56). The number of aberrant cells was noted per total cells scored at each concentration (57).

2.9.3 Comet Assay (single-cell gel electrophoresis)

The nuclei of the root cells were isolated as described by Pfosser et al. (58). The roots were cut 5 mm from their ends and approximately 20 mg of root tips (from 15 to 20 seedlings) were immediately chopped with a razor blade in 1 mL of ice-cold 0.2 M Tris pH 7.5 containing 4 mM magnesium chloride and 0.5% w/v Triton X-100 (Tris– MgCl_2 buffer). The nuclei were filtered through a 52 μm nylon net, and precipitated by centrifugation at 200 g for 5 min (4°C) in a swinging bucket rotor. The pellet was then resuspended in

200 μL of Tris– MgCl_2 buffer. The degree of DNA damage was determined using the alkaline comet assay (59). The microscope slide was pre-coated with a layer of 1% normal melting point agarose and dried at room temperature overnight. Next, 100 μL of 0.8% low melting point agarose at 37°C was mixed with 20 μL of the nuclear suspension and dropped on top of the first layer. The slides were incubated at 4°C for 30 min and then immersed in ice-cold lysing solution (1 M NaCl; 30 mM NaOH, 0.5% w/v SDS, pH 12.3) for 1 h. After lysis, the slides were placed in a horizontal gel electrophoresis chamber and the DNA was allowed to unwind for 1 h in electrophoretic buffer (30 mM NaOH and 1.5 mM EDTA at pH 12.3) (59). The samples were electrophoresed for 20 min at 25 V (1 V cm^{-1}) in a chamber cooled on ice. Slides were then washed in distilled water and neutralized with 0.4 M Tris–HCl buffer pH 7.4. Following a 5 min wash in distilled water, slides were dried for 1 h at room temperature. The dried microscope slides were stained with DAPI, covered with a cover slip, and analysed using a fluorescence microscope (Olympus, Japan). The images of 50 randomly chosen nuclei were analysed visually. (60). The comet images were visualized and captured at 40X magnification. For each treatment 50 comets were scored from two slides. CometScore software was used to analyze and score the comets, out of a number of parameters available in the software, comets were analysed on the basis of the Olive tail moment (61).

2.10 Computational details

2.10.1 Molecular Dynamics (MD) Simulation

To modelled silver core coated by phytochemicals in aqueous environment we prepared a system containing crystalline, quasi-spherical AgNPs, phytochemicals, water, and neutralizing ions. For spherical single-crystalline AgNPs, a truncated octahedron has been predicted to be one of the

thermodynamic equilibrium shapes (62). Therefore, the silver core was modeled as a face-centered cubic (*fcc*) crystalline structure containing a fixed number of 1205 silver atoms. The topology, and parameters of AgNP are based on the interface force field. The Nanomaterial Modeler of CHARMM-GUI was used to build nanomaterial structures for molecular dynamics simulations (63).

The phytochemical constituents of different parts of *Alstonia scholaris* have been investigated intensively by various research groups (64–67). HPLC analysis of methanolic extract discovered that *A. scholaris* contains a variety of phenolic acids such as gallic acid, ellagic acid, catechin, kaempferol, Scholaricine, 19-epischolaricine, picrinine and vallesamine (67). For the interaction with AgNPs we selected four phytochemicals gallic acids, kaempferol, picrinine, and vallesamine for molecular simulation studies. Three-dimensional (3D) transformer of the phytochemical was downloaded from the PubChem database (<http://pubchem.ncbi.nlm.nih.gov>), and the PubChem CID for ligands gallic acid, kaempferol, picrinine and vallesamine are 370, 5280863, 46229104, and 101286269, respectively. The Ligand Reader & Modeler of CHARMM-GUI is used to build the topology and parameter files of the ligand using CHARMM36 (68). CHARMM-GUI's multicomponent assembly used to generate necessary files for molecular simulations (69). Molecular dynamics simulations were carried out using GROMACS 5.0.7 (70). The systems were minimized and equilibrated at 100ps employing the following method. First, energy minimization was carried out using the steepest descent algorithm for 50,000 steps. Then, the system was equilibrated using the NVT ensemble at 298 K, and in the NPT ensemble, the system pressure was equilibrated at 1 bar after 100 ps. Finally, the MD simulation was accomplished in 100 ns. After MD simulation, the trajectory was analysed using GROMACS. Molecular graphics and visualization were performed using UCSF Chimera.

2.10.2 Molecular docking

Molecular docking experiments were conducted to predict the theoretical binding affinities and possible mechanism of enzyme inhibition of phytosynthesized AgNPs. As the surface of AgNPs was coated with these phytochemicals so, we have used these selected phytochemicals for molecular docking with α -amylase and BChE. The crystallographic 3D structure of α -amylase and BChE (PDB ID: 6xsv, 5dyw) enzyme proteins was obtained from the RCSB PDB Protein Data Bank (<https://www.rcsb.org>). The protein receptors are pretreated by deleting unnecessary ligands and water molecules, hydrogenation, and completing incomplete amino acid residues. Only one chain from BChE was used for docking. UCSF Chimera software was used for protein preparation. Gasteiger charges were added to receptors. A grid box was established according to the interactions that corresponded to the predicted binding pocket and enzyme active sites. The chemical structures of all ligands (gallic acid, kaempferol, picrinine, vallesamine, acarbose (standard), and galantamine (standard)) were obtained from PubChem in a sdf format. Multiple ligand docking was performed by Autodock vina and openbabel. Next, the interactions between each ligand and the respective enzyme protein were analysed via Discovery Studio (DS, Dassault Systems BIOVIA, USA) to obtain binding parameters and binding energy (71).

2.11 Statistical analysis

Biological synthesis, phytochemical analysis, and biological activities of each sample were performed in triplicate and the results are presented as the mean or mean $\pm$ SD. Graphs were generated using the software Origin 8.5. One-way ANOVA with least significant difference test (LSD) was used to determine the statistical significance difference ($P < 0.05$) of the data with the aid of Windows XP/Microsoft Excel 2000 computer software.

3 Results and Discussion

3.1 Phytochemical screening

The composition and production of phytochemicals vary due to tissue-specific metabolism and seasonal variation. Plant samples were collected at the end of July, as in this period, the maximum number of phytochemicals is present. Leaves have the maximum concentration of phytochemicals, so leaves are chosen for AgNPs synthesis (72). The phytochemical characteristics of the leaf extract tested were summarized in Table 1. From the table, it is evident that methanolic extract contains a higher concentration of bioactive components than the aqueous extract. The *A. scholaris* extract displayed the presence of phytochemicals such as alkaloids, carbohydrates, phenol, protein, saponin, and terpenoids. Flavonoids are absent in aqueous extract and steroids are absent in both extracts.

Table 1
Phytochemical analysis of aqueous and methanolic extracts of *A. scholaris*.

Phytochemicals	Aqueous extract	Methanolic extract
Alkaloids	+ve	+ve
Flavonoids	-ve	+ve
Terpenoids	+ve	+ve
Tannins	+ve	+ve
Steroid	-ve	-ve
Saponins	+ve	+ve
Phenol	+ve	+ve
Proteins	+ve	+ve
Carbohydrate	+ve	+ve

3.2 Biosynthesis of AgNPs.

The AgNPs were synthesized from aqueous and methanolic extracts of *A. scholaris* by phytochemical chemical mediated reduction of AgNO₃ solution. The formation of AgNPs was indicated by a change in color of the reaction mixture from yellow to dark brown, and the same color change has been observed by many researchers worldwide. This color change is due to the vibration of the plasmon base, an optical property peculiar to noble metals (73).

3.3 Characterization of AgNPs.

3.3.1 UV-visible spectroscopy

UV-visible spectroscopy is a very important technique for the characterization of NPs.

The optical properties of AgNPs strongly depend on the characteristics of individual particles (their size and shape) and their environment, including the spatial ordering of particles. The visible spectrum of the bare AgNPs showed surface plasmon resonance (SPR) at 400 nm which is characteristic of bare AgNPs (74). The UV-vis spectra of the synthesized AgNPs from the aqueous and methanolic extract of *A. scholaris* showed the major peaks at 430 nm and 415 nm respectively (Fig. 1a). This shows that it has been coated by phytochemicals.

3.3.2 FT-IR spectroscopy.

The presence of phytochemicals in the plant extract and synthesized AgNPs was further confirmed by the FT-IR spectrum data (Fig. 1b) which was recognized as an important tool in the characterization and determination of functional groups. The aqueous extract of *A. scholaris* exhibited stretching vibrations at 3300 (-OH stretching) and 1640 cm⁻¹ (C = C stretching), while Aq. AgNPs exhibited stretching vibrations 1595 (C = O stretching of aromatic ring), 1384 (O-H bending) and 1250 (C-N stretching of amines.), 1042 (C-N (amines) stretch vibration of the proteins respectively.), 550 cm⁻¹ (C-Br stretching, which is characteristic of alkyl halides). The methanolic extract of *A. scholaris* exhibited peaks at 3300 (-OH stretching), 2945 and 2831 (C-H stretching of an aromatic compound), The band at 1631 cm⁻¹ in the spectra corresponds to C-N and C-C stretching indicating the presence of proteins (75), 1448 (N-H stretch vibration present in the amide linkages of the proteins.), 1117 (C-O stretching in primary alcohols.), 1010 (C-O stretching.), 610 cm⁻¹ (C-Br stretching, which is characteristic of alkyl halides) while Meth. AgNPs show peaks at 3375 (N-H stretching indicated the presence of primary amine groups of proteins.), 2924 (-CH stretching.), 2852 (C-H stretching of aromatic compound), 1733 (carbonyl stretching), 1688 (C = C stretching), 1457 (N-H stretch vibration present in the amide linkages of the proteins), 1376 (O-H bending), 1170 (C-N stretching of amines.), 1042 (C-O stretching), 997 (Characteristic absorption of polysaccharides), and 637 cm⁻¹ (C-Br stretching, which is characteristic of alkyl halides.). The overall results of the FTIR study of the synthesized NPs revealed that plant proteins and secondary metabolites were actively involved in the process of reduction, capping, and stabilization of AgNPs. Phytochemicals with hydroxyl (-OH), carboxyl (-C = O), and alkane (C-H) functional groups were mainly involved in the reduction and stabilization of AgNPs.

3.3.3 XRD analysis

XRD is a powerful tool for phase identification, determination of crystallinity and information on unit cell dimensions. XRD spectrum analysis was mainly used to check the crystalline nature of the NPs. Figure 1c clearly indicates that the green synthesized AgNPs were crystalline in nature. Peaks at approximately 37⁰, 44⁰, and 64⁰ correspond to three diffraction faces of silver. The XRD peak at around 37⁰ represented the Bragg reflection corresponding to the (111) plane. The presence of biological material in the free state might crystallize independently leading to Bragg reflections (75).

3.3.4 SEM and EDX analysis

SEM analysis was used to investigate the surface morphology and size of the biogenic AgNPs (76). Figure 2 shows that both AgNPs synthesized from *A. scholaris* are spherical in shape. The size of Aq. AgNPs (Fig. 2a) are in the range of 10–40 nm and EDX analysis (Fig. 2b) showed that they are mainly composed of silver atoms but other elements (C, O, Si, Cl) confirmed the involvement of phytochemicals from the aqueous extract of *A. scholaris*. The size of Meth. AgNPs (Fig. 2c) are in the range of 10–27 nm and EDX analysis (Fig. 2c) showed that they are mainly composed of silver atoms but other elements (C, N, O, Si, S, Cl) confirmed the involvement of phytochemicals from the methanolic extract of *A. scholaris*. The particle size of Aq. AgNPs are larger than the Meth. AgNPs, which is also confirmed from UV- Vis spectroscopy.

3.4 Antioxidant studies

A variety of factors including different diseases cause oxidative stress. Oxidative stress is caused by reactive oxygen species (ROS) which can be extremely dangerous for living organisms as they can result in cellular and DNA damage. Antioxidants are natural mitigating agents of ROS and hence an important weapon against oxidative stress. DPPH (purple) is a protonated radical that has a characteristic absorbance at 517 nm using a spectrophotometer, which decolorizes (yellow) upon activation with antioxidants. The DPPH scavenging activity is a fast and reliable method to assess the *in vitro* antioxidant activity of AgNPs solution. The *In vitro* antioxidant activity of synthesized AgNPs was studied using DPPH as a free radical scavenging assay. Figure 3 summarizes the results of antioxidant activity. The DPPH scavenging activity of AgNPs increased in a dose-dependent manner (Fig. 3). The DPPH scavenging ability of the Meth AgNPs (IC 50 – 8 µg /ml) is higher than that of Aq. AgNPs (87 µg /ml) and almost similar to that of standard ascorbic acid (5 µg/ml). Therefore, the Meth. AgNP solution exhibits a proton-donating ability and could serve as a free radical inhibitor per scavenger, possibly acting as a primary antioxidant. Previous studies have shown the antioxidant activities of bioinspired AgNPs synthesized from the root extract of *Nepeta leucophylla*, and the aerial extract of *Lippia nodiflora* (77, 78). The promising antioxidant potential of green synthesized NPs validates their importance as novel tools against a plethora of diseases.

3.5 α-amylase inhibition

An effective strategy to maintain adequate glucose levels in the blood is the inhibition of carbohydrate hydrolysing enzymes within an acceptable range (79). The enzyme α-amylase is responsible for the breakdown of carbohydrates into glucose, which increases the blood glucose levels. Effective inhibition of α-amylase may play a critical role in maintaining blood glucose levels. In this study, the antidiabetic potency of AgNPs was evaluated at a concentration of 1000

$\mu\text{g/ml}$ to 62.5 $\mu\text{g/ml}$ to assess α -amylase percent inhibition when subjected to AgNPs (Fig. 4A). Acarbose (std.) showed a maximum inhibition potential of (IC50-7 $\mu\text{g/ml}$), followed by Meth- AgNPs IC50-8 $\mu\text{g/ml}$, while Aq. AgNPs have less inhibitory action (IC50-32 $\mu\text{g/ml}$). The variation in α - amylase inhibition potential may be attributed to morphological and physicochemical features resulting from various capping and reducing agents in the extracts.

The binding of amylase with AgNPs was confirmed by FTIR analysis (4B). FTIR spectra revealed peak shifts in the test sample of amylase interacted with AgNPs. Binding of Meth- AgNPs to amylase cause narrowing of the peak at 1600 indicating the participation of amide group in attachment. The peak at 2837 and 2938 cm^{-1} can be interpreted as the vibration peak for C–H stretching from CH₂ or CH₃. Peaks at 1168 and 1033 cm^{-1} could be of the stretching and bending of aliphatic amines. Peaks at 1034 and 1013 cm^{-1} are assigned to C-H in plane bending. The FTIR bands found at 1631 and 1600 cm^{-1} are assigned to C-N stretching vibrations. Changes in the 900 cm^{-1} regions are due to the stretching mode of C-C bonds. This suggested that the binding of Meth. AgNPs cause more structural changes in α -amylase due to this there is more inhibition of α -amylase activity than Aq. AgNPs.

3.6 Anti-cholinesterase inhibition

In this study, various concentrations of AgNPs (1000 $\mu\text{g/ml}$ to 62.5 $\mu\text{g/ml}$) were used to inhibition of acetylcholinesterase (AChE) and butyryl-cholinesterase (BChE). Figure 5 (a, c) shows the inhibition inhibition of acetylcholinesterase (AChE) and butyryl cholinesterase (BChE) by AgNPs (Aq & Meth). The IC₅₀ value of Aq. and Meth. AgNPs were found to be 1537 $\mu\text{g/ml}$, and 1370 $\mu\text{g/ml}$ respectively, which is very much higher than the standard galantamine IC₅₀ value of 1 $\mu\text{g/ml}$ (Fig. 5a). The phytosynthesized AgNPs do not have much inhibitory action against AchE. While phytosynthesized AgNPs show higher inhibition of BchE, the IC 50 values for Meth. AgNPs is 15 $\mu\text{g/ml}$ and Aq. AgNPs were 1490 $\mu\text{g/ml}$. So, the IC 50 value of Meth. AgNPs are much lower than the standard galantamine (115 $\mu\text{g/ml}$) (Fig. 5c). From the given results, it is concluded that the inhibitory potential of Meth. AgNPs for BChE were higher than the AChE. Rajakumar et al. determined the cholinesterase activity of the AgNPs synthesized from the flower extract of *Milletia pinnata* and reported that NPs presented a remarkable increase in activity toward ChE compared with the plant extract (80). Until now, there is no report on the mechanism of inhibition of AchE and BchE by NPs.

3.7 Antimicrobial activity

Abuse treatment of microbial diseases led to the development of resistant strains (81, 82), so there is need to search for new drugs (83). Nanoscale materials have emerged as novel antimicrobial agents owing to their high surface area-to-volume ratio and their unique chemical and physical properties, which increase their contact with microbes and their ability to permeate cells. Furthermore, nanotechnology has amplified the effectiveness of AgNPs as antimicrobial agents (84). In this study, the AgNPs synthesized by using *A. scholaris* exhibited promising anti-bacterial activity against gram + ve bacteria: *Bacillus subtilis* (*B. subtilis*); *Staphylococcus aureus* (*S. aureus*), and gram – ve bacteria: *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Escherichia coli* (*E. coli*) (Fig. 6A). The minimum inhibitory concentration of Aq. AgNPs were found to be 1.33 ± 0.2 , 5.33 ± 0.2 , 0.66 ± 1.4 , 2.83 ± 1.4 , and for Meth. AgNPs were 0.66 ± 0.1 , 4.33 ± 0.2 , 0.43 ± 0.1 , and 2.75 ± 0.2 for *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa* respectively (Fig. 6B). Both Aq. and Meth. AgNPs show the highest antibacterial activity against *E. coli* and the lowest activity against *S. aureus*. Antimicrobial activity of Meth. AgNPs was slightly lower than the Aq. AgNPs in case of *B. subtilis*, *S. aureus*, *E. coli*.

3.8 Antimutagenic Activity

In the last couple of decades, natural products derived from plants, fruits, spices, herbs, etc., have been the main focus of research to ameliorate the threat posed by harmful chemicals, toxins, etc., from endogenous and exogenous sources. The hydroethanolic extract of *A. scholaris* has radioprotective efficacy and antimutagenic properties have been reported (17–20, 46). Most of the biomolecules present in the plant extract were predicted to participate in the synthesis and capping of AgNPs. These capped phytochemicals may also help in ameliorate the effect of toxic chemicals. In this study, we have investigated the antimutagenic potential of AgNPs synthesized from *A. scholaris*.

3.8.1 Allium cepa root growth and chromosome aberration assay

Allium cepa is an efficient test system routinely used to evaluate the genotoxic potential of chemicals in the environment; due to its sensitivity and good correlation with mammalian test systems. To identify the harmful effects of substances at different concentrations and exposure times, a variety of tests have been employed, such as chromosome aberration and comet assays (85). *A. cepa* root cells are sensitive to copper sulfate solution and show DNA damage induction and inhibition of growth in *A. cepa* root cells (86). The effect of copper sulfate, copper sulfate + plant extract (Aq & Meth), copper sulfate + AgNPs (Aq & Meth) and AgNPs (Aq & Meth) on the cell division and chromosome behaviour of *A. cepa* are presented in Tables 2 and 3. No chromosomal aberration was observed in the control and the mitotic index (MI) value was 60.6 ± 0.72^a %. Group B treated with copper sulfate showed chromosomal aberration such as sticky chromosome, chromatin bridge, and disturbed metaphase, and MI value (36.4 ± 0.17^b) was significantly lower than that of control group A. The same genotoxic effect of copper sulfate is already been mentioned by Yildiz et. al. (86). Representative aberrations are given in Fig. 7 (a)–(r). The MI value of group C was not significantly different from that of group B, suggesting that there is no antimutagenic effect of aqueous extract of *A. scholaris* but the MI value of group D (47.566 ± 1.9) is greater than that of group B and C but not equal to group A. This suggests that there could be certain compound(s) present in methanolic extracts that enhance DNA repair capacity. The radioprotective and chemoprotective effect of plant extract of *A. scholaris* was previously reported by the research group (46) and our results correlate with their findings. The MI value of group E was the lowest, ($23.8 \pm 0.2\%$) in the treatment groups, which might be due to both Aq. AgNPs and copper sulfate have a genotoxic effect and the DNA of cells is very much damaged so it cannot be replicated and due to this, the MI value was lowest. MI values of group F (37.5666 ± 2.7) and G (39.066 ± 1.05) did not differ significantly. Group H (51.9 ± 1.4) had the highest MI value in the treatment group after the control. Several groups reported the genotoxicity and cytotoxicity of AgNPs but in Meth. AgNPs synthesized from *A. scholaris* show little genotoxic effect as compared to previously reported AgNPs. Natural products derived from plants, fruits, spices, and herbs, contain several promising chemopreventive compounds like vitamins, minerals, carotenoids, and an array of other phytochemicals (87). From the results, we can

conclude that the methanolic extract has some compound(s) that get coated on AgNPs and help to ameliorate the adverse effect of AgNPs on root meristem cells of *A. cepa*.

3.8.2 Comet Assay

The comet assay is a versatile technique for assessing DNA damage and have been extensively used for human biomonitoring, molecular epidemiology, and ecogenotoxicology. It is highly sensitive, fast and economical and can detect even minor changes in the single and double-stranded DNA. During electrophoresis, the damaged DNA migrates away from the nucleus and produces a comet shape. Figure 8a and b show the DNA damage effects of copper sulfate, copper sulfate + plant extract (Aq & Meth), copper sulfate + AgNPs (Aq & Meth), and AgNPs (Aq & Meth) concentrations after 24 h treatments of *A. cepa* root meristem cells. The control group (A) does not show any DNA damage and no comet tail was observed. Group B which was treated with copper sulfate showed significant DNA damage and olive tail movement (OMT) with respect to the control group. This is because treatment of copper sulfate causes the formation of both free radicals and reactive oxygen species which is beyond the capacity of the antioxidant systems of cells, thus leading to oxidative DNA damage (86). In group B and C (copper sulfate + Aq. plant extract) there is no significant difference is seen. Group D (copper sulfate + Meth. plant extract) shows slightly lower OMT than group B. This might be to bioactive compounds from methanolic extract help in the amelioration of oxidative stress caused by copper sulfate. The DNA damage was significantly higher in Group E than in the control ($P < 0.05$). This might be due to both copper sulfate and AgNPs causing damage to the cells by oxidative stress and lipid peroxidation regulating the NP-induced DNA damage. Also, the DNA of group H (Aq. AgNPs) was significantly damaged. This suggests that Aq. AgNPs have a genotoxic effect on the cells. In group F (copper sulfate + Meth. AgNPs) the DNA damage is less as compared to group E. The minimum DNA damage was observed in group H, which was treated with Meth. AgNPs. Synthesized AgNPs does not show any antimutagenic property but Meth. AgNPs has lower toxicities than previously reported AgNPs. The results of the comet assay support the results of the microscopic data of root meristem cells of *A. Cepa*. So, therefore both *Allium cepa* root growth and chromosome aberration assay and comet assay confirm the lower toxicity of Meth. AgNPs.

Table 2

Chromosome aberrations obtained for the root meristem cells of *Allium cepa* treated with distilled water copper sulfate, copper sulfate + plant extract (Aq Meth), copper sulfate + synthesized AgNPs (Aq & Meth), and AgNPs (Aq & Meth.) alone

Treatment Groups	Sample	No. of counted cells	Normal metaphase	Normal anaphase	Sticky chromosome	Disturbed metaphase	Multipolar anaphase
Group A (control)	A1	1000	+	+	-	-	-
	A2	1000	+	+	-	-	-
	A3	1000	+	+	-	-	-
Group B (CuSO ₄)	B1	1000	+	+	+	+	+
	B2	1000	+	+	+	+	+
	B3	1000	+	+	+	+	+
Group C (CuSO ₄ + Aq extract)	C1	1000	+	+	+	+	+
	C2	1000	+	+	+	+	+
	C3	1000	+	+	+	+	+
Group D (CuSO ₄ + Meth extract)	D1	1000	+	+	+	+	+
	D2	1000	+	+	+	+	+
	D3	1000	+	+	+	+	+
Group E (CuSO ₄ + Aq AgNPs)	E1	1000	-	-	+	+	+
	E2	1000	+	-	+	+	+
	E3	1000	-	-	+	+	+
Group F (CuSO ₄ + Meth AgNPs)	F1	1000	-	-	+	+	+
	F2	1000	-	-	+	+	+
	F3	1000	-	-	+	+	+
Group G (Aq AgNPs)	G1	1000	-	-	+	+	+
	G2	1000	-	-	+	+	+
	G3	1000	-	-	+	+	+
Group H (Meth AgNPs)	H1	1000	+	+	-	+	-
	H2	1000	+	+	-	-	+
	H3	1000	+	+	+	-	-

Table 3
Effects of copper sulfate, plant extract and AgNP treatment on the mitotic index in the root meristem cells of *Allium cepa*

	Group A (Control)			Group B (CuSO ₄)			Group C (CuSO ₄ + Aq extract)			Group D (CuSO ₄ + Meth extract)			Group E (CuSO ₄ + Aq AgNPs)			Group F (CuSO ₄ + Meth AgNPs)			Group G
	A1	A2	A3	B1	B2	B3	C1	C2	C3	D1	D2	D3	E1	E2	E3	F1	F2	F3	G1
Dividing cell (total)	608	598	612	362	365	360	358	361	356	492	454	481	240	238	236	342	381	400	38
Prophase	583	568	592	358	359	358	351	360	354	462	429	471	239	236	234	341	378	398	38
Metaphase	12	19	13	2	2	2	3	1	0	14	12	4	1	1	0	1	1	1	3
Anaphase	10	7	4	1	3	0	3	0	1	11	10	5	0	1	1	0	2	0	2
Telophase	3	4	3	1	1	0	1	0	1	5	3	1	0	0	1	0	0	1	1
Mitotic index (%)	60.8	59.8	61.2	36.2	36.5	36	35.8	36.1	35.6	49.2	45.4	48.1	24	23.8	23.6	34.6	38.1	40	38
Mean	60.6 ± 0.72 ^a			36.4 ± 0.17 ^b			35.83333 ± 0.25 ^b			47.566 ± 1.9 ^d			23.8 ± 0.2 ^e			37.5666 ± 2.7 ^f			39

3.9 Molecular Dynamics (MD) Simulation

Plant-based bioconstituents such as steroids, sapogenins, tannins, terpenoids, polyols, alkaloids, polysaccharides, flavonoids, phenolics, proteins, amino acids, enzymes, and vitamins function as strong chelating, reducing and stabilizing agents, which provide stability, prevent agglomeration and help in tuning the shape and size of NPs (88, 89). FTIR analysis also showed the peaks of functional groups on synthesized AgNPs which clearly indicate the presence of biomolecules as stabilizing agents. Additionally, EDX analysis showed that synthesized AgNPs were mainly composed of silver atoms but also showed the presence of other elements (C, N, O, Si, S, Cl) which confirmed the involvement of phytochemicals from the plant extract of *A. scholaris*. Researchers have performed phytochemical analysis of the plant extract of *A. scholaris* and revealed that *A. scholaris* contains a variety of phenolic acids like gallic acid, ellagic acid, catechin, kaempferol, Scholasticine, 19-epischolasticine, picric acid and vallesamine etc (65–67). To investigate the interaction of these phytochemicals with silver metal we have performed molecular simulation studies. Molecular dynamics simulation is usually used to obtain the interaction trajectories for a better understanding of biological processes (90). For the interaction with AgNPs we randomly selected four phytochemical kaempferol, gallic acid, picric acid, and vallesamine for molecular simulation studies. The stability and degree of the interactions between AgNPs and the four small molecules were further elucidated using the GROMACS program. Of the different crystal surfaces— (Ag (110), Ag (100), and Ag (111) facets) which were employed to model the AgNP, we have considered the (111) plane of silver because our XRD analysis showed that synthesized AgNPs (111) plane. Figure 9a represents the initial configuration of bare AgNPs. The initial system represented in Fig. 9b silver core atoms are shown as a blue ball containing fixed 1205 silver atoms surrounded by kaempferol (red), gallic acid (yellow), picric acid (green), and vallesamine (purple) buffering ions Na⁺ and Cl⁻ as green and yellow balls and water (red lines). Figure 9 (c-d) MD snapshot small molecule distribution on the AgNP surface. The small molecules were adsorbed on the AgNP surface from the beginning of the simulation.

The capping of the four molecules with AgNPs was then subjected to analysis of the trajectory data obtained from the 100 ns MD simulations. Two main parameters affect the adsorption of these molecules on the AgNPs' surface: diminution of the hydrogen-binding interactions between these molecules and water, and binding to the AgNPs surface. The total number of hydrogen bonds between four molecules and water molecules was checked during the MD simulation (Fig. 10a). The number of hydrogen bonds between kaempferol, gallic acid vallesamine, and water was reduced from 140,130 and 45 at the beginning of the simulation process to 80,70 and 20 respectively after 10 ns, which demonstrated adsorption of kaempferol, gallic acid and vallesamine on the AgNPs' surface from the beginning of the simulation. However, the hydrogen bond between picric acid and water does not decrease throughout the simulation period.

Additionally, valuable information about these molecules aggregating on the AgNP surface could be obtained from the radius of gyration (Rg). The radius of gyration of the four systems was quantified and the relationship between the radius of gyration and the simulation time was plotted to check the adsorption as shown in Fig. 10b. The Rg values of kaempferol - AgNP, gallic acid -AgNP, vallesamine -AgNP, and picric acid-AgNP decrease rapidly during initial period of simulation and then become constant for kaempferol (1.88 ± 0.12) and gallic acid (1.89 ± 0.07) but for vallesamine (1.77 ± 0.19) they decrease and fluctuate. However, the Rg value of picric acid (2.09 ± 0.11) does not decrease significantly. The results show that the gyration radius of the vallesamine -AgNP complex is smaller than those of the other three systems, indicating that the structural tightness of the vallesamine-AgNP complex was better than that of other systems. These results represent the rapid aggregation of kaempferol, gallic acid, and vallesamine molecules around the AgNPs surface. It has been reported that van der Waals interactions had an important contribution to adsorption of these molecules on the AgNPs' surface. To evaluate the interaction between these molecules and AgNPs' surface, we measured the van der Waals forces throughout the simulation time to understand the system's stability. The negative value van der Waals forces change from - 153000 to -155500 kJ/mol initially and then remain constant showing the stable interactions between these molecules and AgNPs (Fig. 10c). From the above results we can conclude that kaempferol, gallic acid and vallesamine molecules capped the AgNPs. Previously, it has been reported that Kaempferol and gallic acid play an important role in the reduction and stabilization of Ag and Au NPs (91, 92). This also supported our simulation results and is the first report for the involvement of vallesamine in the synthesis of AgNPs. This is the first simulation proof for the interaction and coating of phytochemicals with AgNPs.

3.10 Molecular docking

Molecular dynamic simulation results showed that the surface of AgNPs were coated with kaempferol, gallic acid and vallesamine so, we used these molecules for molecular docking with α -amylase, and BChE. The interaction mechanism between α -amylase, BChE, and three small-molecule ligands along with standard acarbose and galantamine was studied by multiple ligand docking using AutoDoc vina. Table 4 shows the binding energy of these molecules with α -amylase and BChE. The lowest binding energy suggests the favourable binding of these molecules with the target proteins. Kaempferol, gallic acid, and vallesamine can all interact with α -amylase active sites by conventional hydrogen bonds, carbon – hydrogen bonds, Pi – Pi stacking, Pi-sigma, and Pi – alkyl interactions. Kampeferol (-8.553 kcal/mol) has the lowest binding energy when docked with α -amylase. It interacts with the Gln158, Tyr242, Tyr238 Lys213 residues of active site of α -amylase (Fig. 11). FTIR analysis of AgNPs along with α -amylase (Fig. 7b) shows the structural changes in α -amylase upon interaction with synthesized AgNPs (Aq and Meth). Maximum structural changes are seen when Meth. AgNPs bind with α -amylase as well as the activity of Meth. AgNPs are much higher than Aq. AgNPs. The α -amylase inhibition activity of kampeferol and gallic acid has recently been reported (93, 94)

Figure 12 shows the interaction of galantamine (std), kaempferol, gallic acid, and vallesamine with BchE. All interact with BchE active sites by conventional hydrogen bonds, carbon – hydrogen bonds, Pi – Pi stacking, and Pi – alkyl interactions. Vallesamine (-9.24 kcal/mol) had the lowest binding energy when docked with BchE, which was even less than galalntamine (std) (-6.042). Additionally, during enzyme inhibition activity we obtained higher activity than the standard (Fig. 5). Vallesamine interacts with the residues of active site of BchE (Fig. 12) such as Gly115 forming a carbon-hydrogen bond and, Tyr330, Ala326, Phe327, Phe396, and Trp229 with Pi-alkyal interactions. From these results, we can conclude that BchE inhibition activity is due to the presence of vallesamine on the surface of AgNPs.

Table 4
The binding energy of interactions between compounds and enzymes (α -amylase and cholinesterase (BchE))

Sr.no	Compound	α -Amylase	BchE
		Binding Energy (Kcal/mol)	Binding Energy (Kcal/mol)
1	Kaempferol (Kam)	-8.553	-8.33
2	Gallic acid (Gal)	-4.3	-6.2
3	Vallesiachotamine (Val)	-4.623	-9.24
4	Acarbose (Standard)	-8.017	–
5	Galantamine (Standard)	–	-6.042

4 Conclusion

Our study has demonstrated that Meth. AgNPs exhibit superior antioxidant capacity, enzyme inhibition, and reduced toxicity compared to Aq. AgNPs is because during extract preparation we used two solvents i.e. water and methanol which were used for the synthesis of AgNPs. The extraction solvent plays an important role in the yield, purity, and quality of the extract (95). Fig .3 shows that there is a significant difference in FT-IR peaks in aqueous and methanolic extract as well as in Aq. AgNPs and Meth AgNPs. The extraction of kaempferol is usually carried out by organic solvents such as methanol and ethanol because it gives a better yield (80%) of kaempferol, also most of the other phytochemicals are better extracted with methanol (96). As methanolic extract contains a high amount of different types of phytochemicals, AgNPs synthesized by methanolic extract show a different FT-IR profile than aqueous extract as well as higher enzyme inhibition and lower toxicity. Research has proven the antioxidant, anticarcinogenic, antidiabetic, and antimicrobial properties of the kampeferol (96). Gallic acid is also actively involved in nanoparticle synthesis as well as well-known for its antioxidant activity (93). Meth. AgNPs showed significant inhibitory activity against BchE but not against AchE. Similar results were reported for the methanolic extract of *A. scholaris* and here we confirm that the inhibition of BchE was due to vallesamine.

Declarations

Ethics approval and consent to participate

This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication

“Not applicable”

Availability of data and materials

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation, to any qualified researcher.

Competing interests

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Authors' contributions

Shubhangi Shirsat- Conceptualization, Methodology, Data curation, Writing- original draft preparation.

Manjusha Chakranarayan- Plant material collection, Analysis, Data curation.

Varenyam Achal- Supervision, Writing-reviewing and editing.

Mahendra Rai-Writing-reviewing and editing.

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Authors' information

Environmental Engineering Program, Guangdong Technion – Israel Institute of Technology, Shantou 515063, China.

Shubhangi D. Shirsat

MGM's Institute of Biosciences and Technology, Aurangabad, India

Manjusha Chakranarayan,

Environmental Engineering Program, Guangdong Technion – Israel Institute of Technology, Shantou 515063, China,

Technion – Israel Institute of Technology, Haifa 32000, Israel

Varenyam Achal

Department of Biotechnology, SGB Amravati University, Amravati-444602, Maharashtra, India

Department of Chemistry, Federal University of Piauí (UFPI), Teresina, Brazil

Mahendra Rai

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Figures

Fig 1

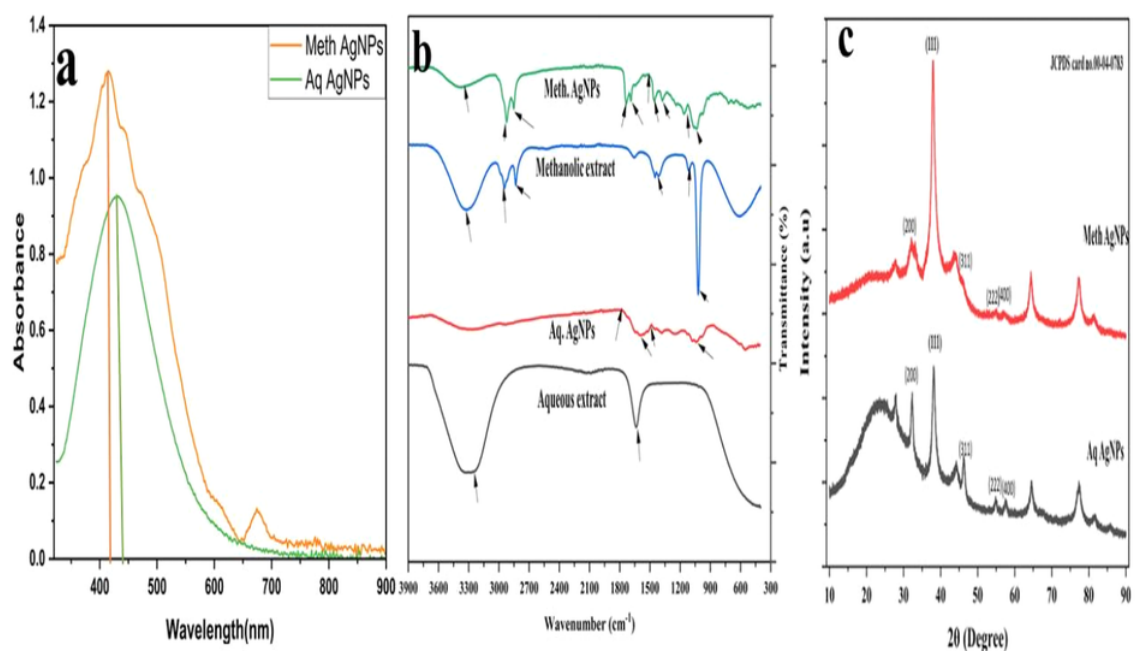


Figure 1

(a) Ultraviolet–visible spectrum of Aq. and Meth. AgNPs (b) FT-IR spectrum of aqueous, methanolic extract, Aq. AgNPs and Meth. AgNPs. (c) X-ray diffraction (XRD) of Aq. and Meth. AgNPs

Fig 2

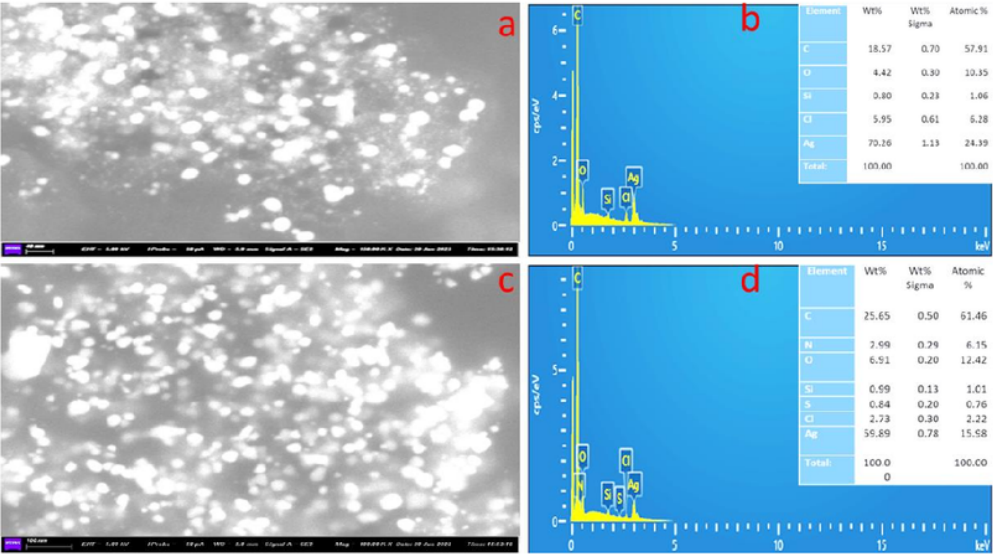


Figure 2
SEM and EDX analysis of Aq. AgNPs (a & b) Meth. AgNPs (d & c).

Fig 3

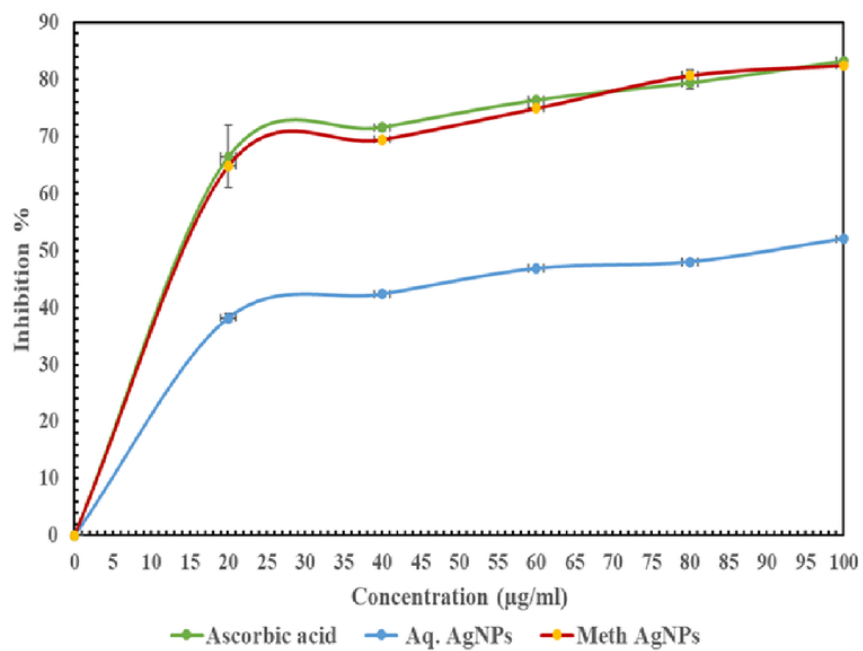


Figure 3

DPPH radical scavenging activity of synthesized AGNPs from extract of *A. scholaris*.

* Values expressed are means $\pm$ S.D. of three parallel measurements.

Fig 4

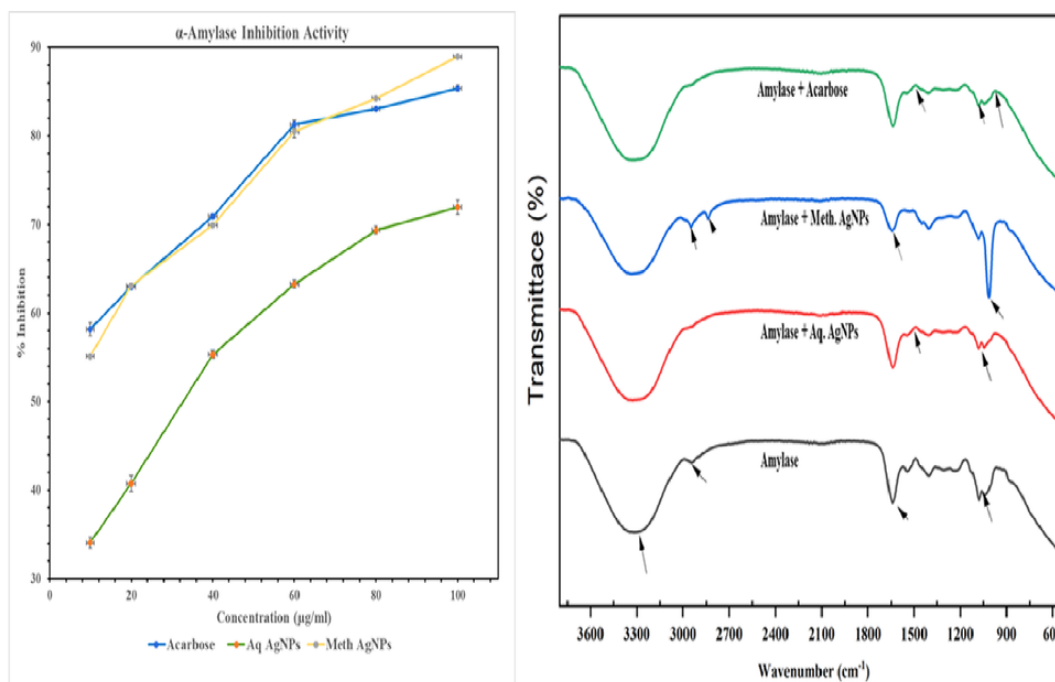


Figure 4

A α- Amylase inhibition activity of phytosynthesized AgNPs (Aq & Meth) and B-FTIR spectra of amylase (control) and AgNP-amylase (test).

Fig 5

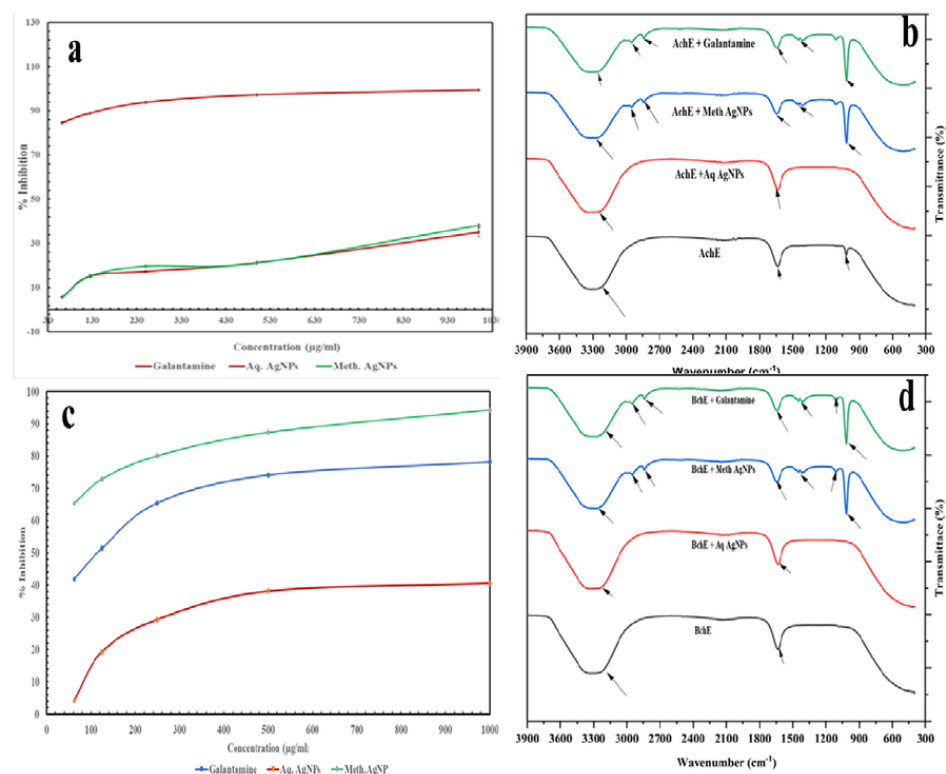


Figure 5
a- AchE inhibition activity of phytosynthesized AgNPs (Aq & Meth) and b-FTIR spectra of AchE (control) and AgNP-AchE (test), c- BchE inhibition activity of phytosynthesized AgNPs (Aq & Meth) and d-FTIR spectra of BchE (control) and AgNP-BchE (test).

Fig 6

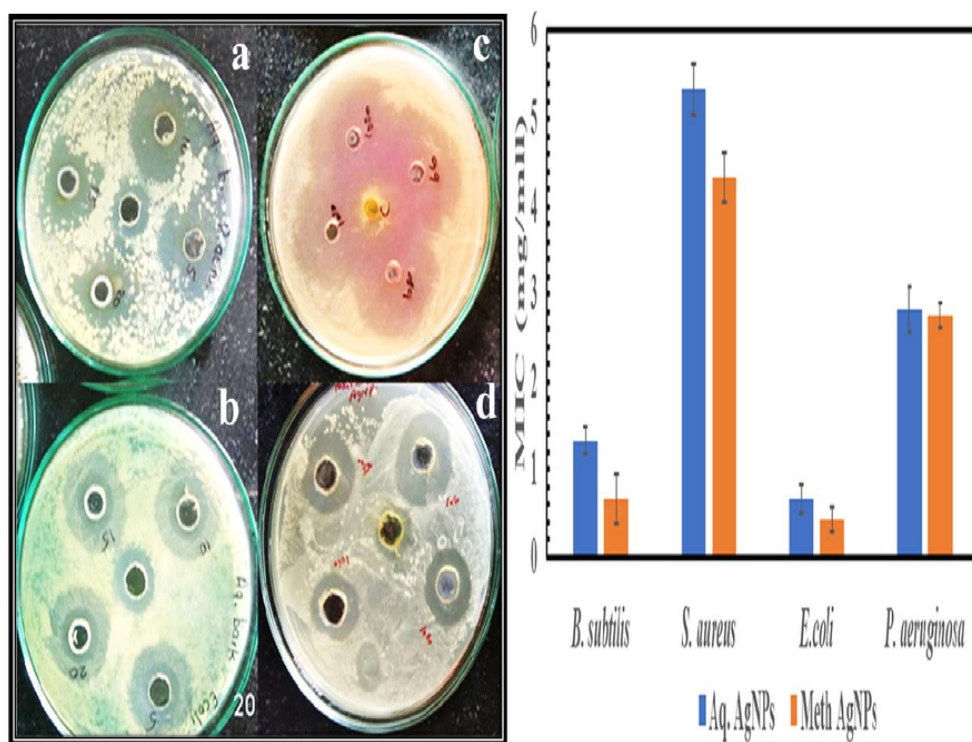


Figure 6
A-Antibacterial activity of AgNPs (Aq. and Meth.) (a) *B. subtilis*, (b) *S. aureus* (c) *E. coli* (d) *P. aeruginosa*. B- Minimum inhibitory concentration of AgNPs synthesized by *A. scholaris* against *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa*

Fig 7

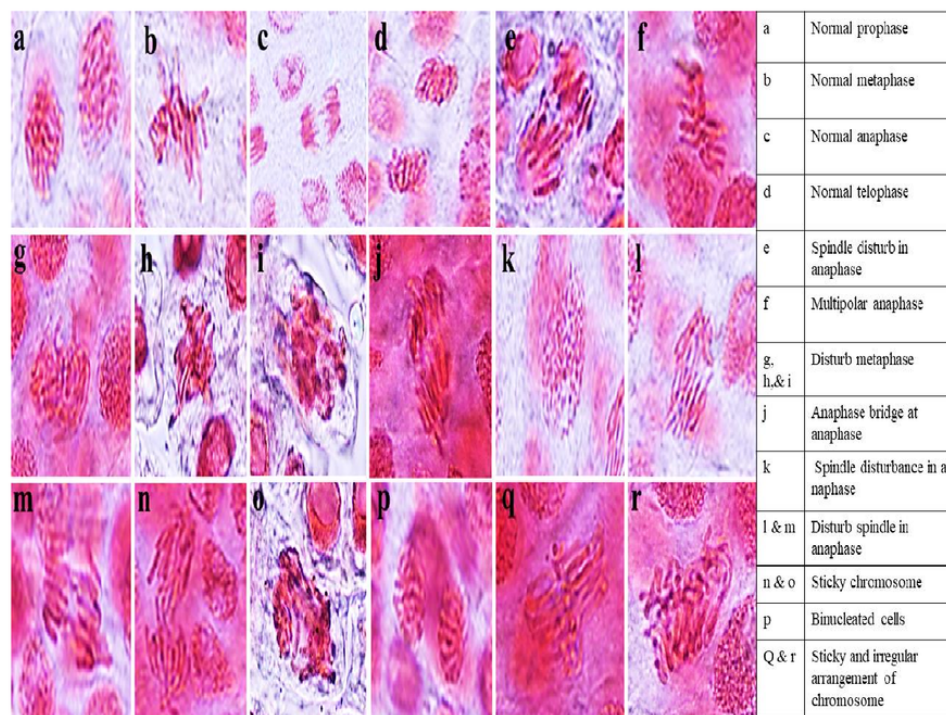


Figure 7

Copper sulfate and AgNPs induced chromosomal aberrations in *A. cepa*. root tip cells.

Fig 8

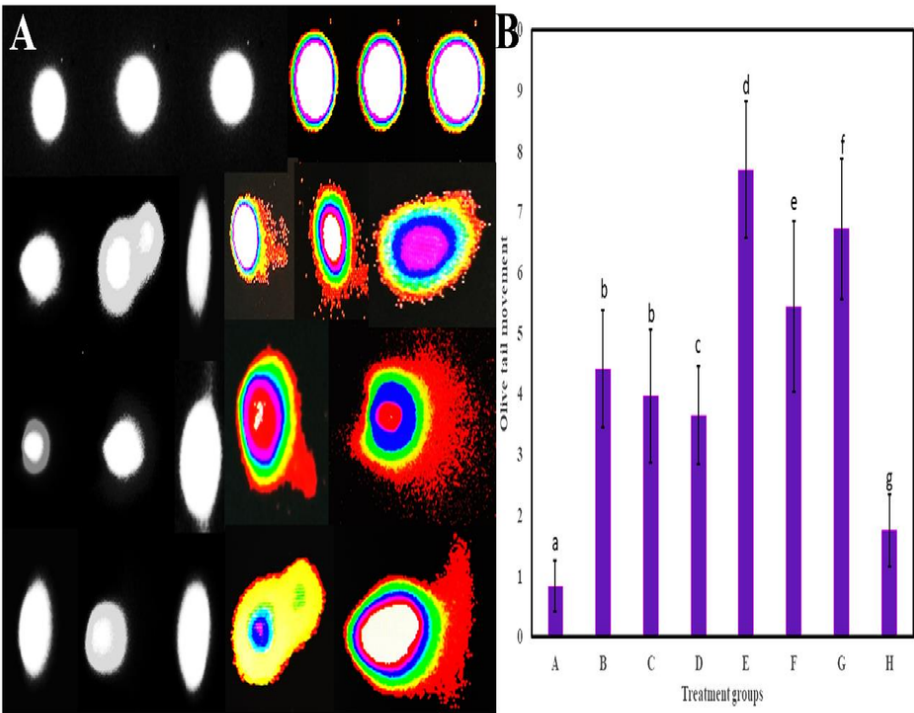


Figure 8
A Representative comet image B- Mean Olive tail movement of root meristem cells of *Allium cepa* treated with copper sulfate, plant extract, and AgNPs.

Fig 9

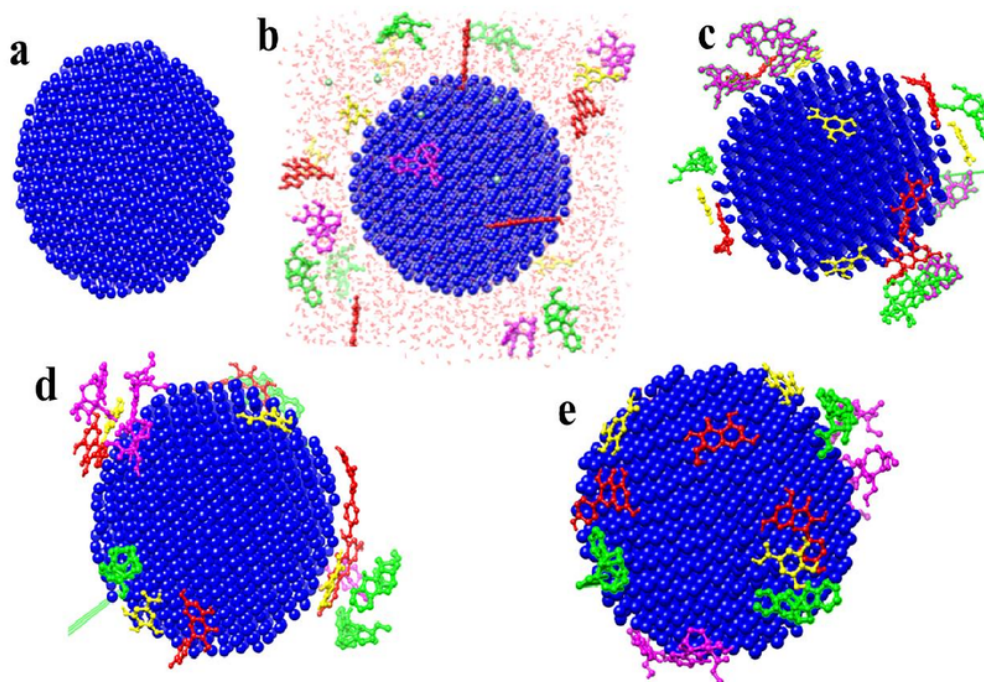


Figure 9

MD simulations of kaempferol, gallic acid, picric acid and vallesamine-AgNPs (a) The Ag (0) surface model (b) initial system containing AgNP (blue), kaempferol (red), gallic acid (yellow), picric acid (green) and vallesamine (purple) (c-e) Snapshots of MD simulations of interaction of these molecules with AgNPs in water solution from 0 ns to 100 ns.

Fig 10

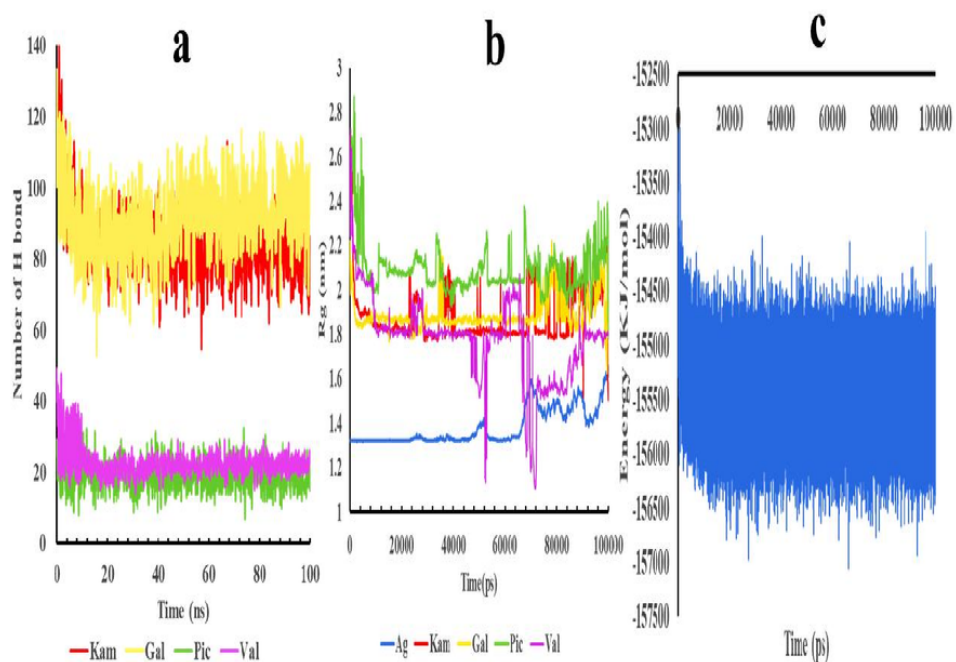


Figure 10

Molecular dynamics (100 ns) results of AgNP and kaempferol, gallic acid, picric acid and vallesamine (a) total number of hydrogen bonds between kaempferol, gallic acid, picric acid and vallesamine and water molecules as a function of simulation time (b) radius of gyration for kaempferol, gallic acid, picric acid and vallesamine-AgNP simulation and (c) the variations in van der Waals forces throughout the simulation time.

Fig 11

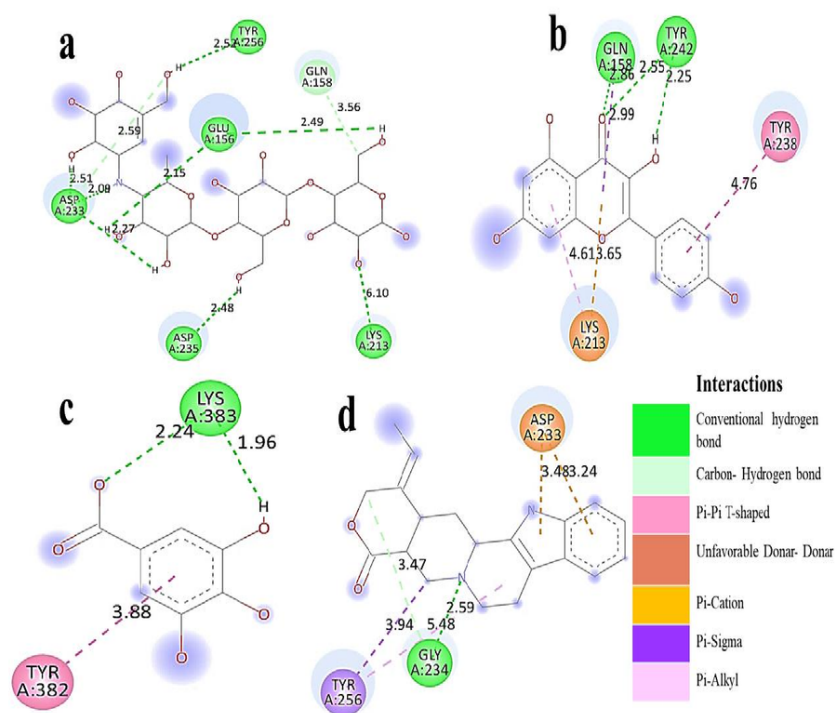


Figure 11

Interactions of α -amylase with (a) acarbose (Std) (b) kaempferol, (c) gallic acid, and (d) vallesamine.

Fig 12

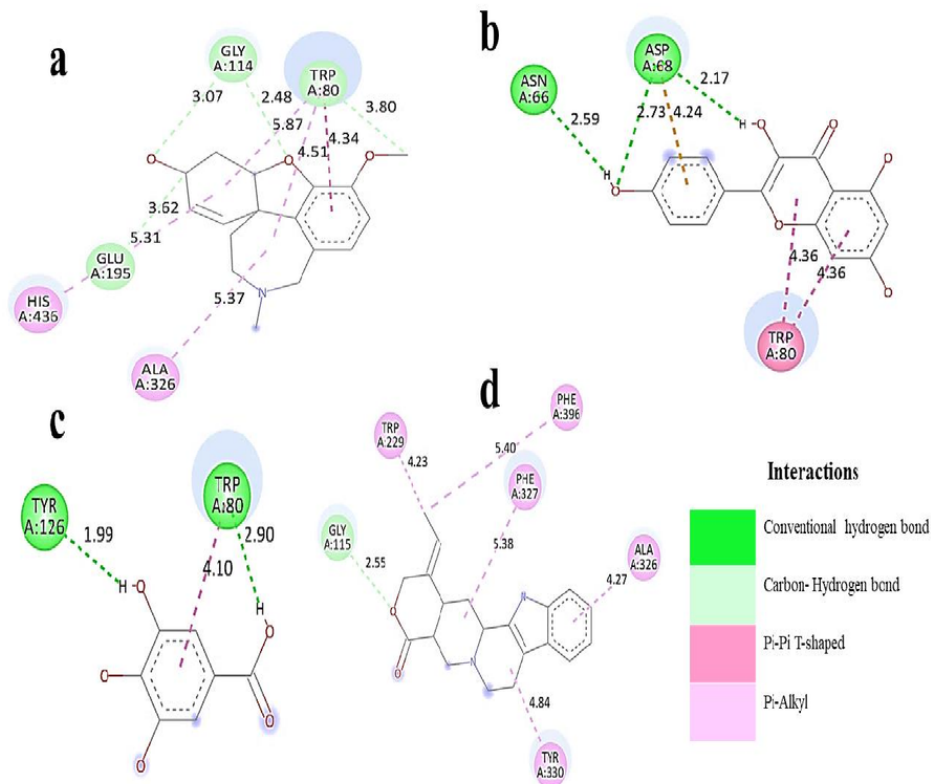


Figure 12

Interactions of BchE with (a) galantamine (Std) (b) kaempferol, (c) gallic acid, and (d) vallesamine.

Supplementary Files

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