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Original Article

Green synthesis of gold nanaoparticles using *Delphinium Chitralense* tuber extracts, their characterization and enzyme inhibitory potential

Síntese verde de nanaopartículas de ouro utilizando extratos de tubérculos *Delphinium chitralense*, sua caracterização e potencial inibitório enzimático

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Abstract

Green synthesis has been introduced as an alternative to chemical synthesis due to the serious consequences. Metal nanoparticles synthesized through green approach have different pharmaceutical, medical and agricultural applications. The present study followed a green and simple route for the preparation of potentially bioactive gold nanoparticles (Au NPs). Au NPs were prepared via green synthesis approach using crude basic alkaloidal portion of the tuber of *Delphinium chitralense.* The green synthesized Au NPs were characterized by transmission electron microscopy (TEM), scanning electron microscopy (SEM), X-ray diffraction (XRD) fourier transform infrared (FTIR), and UV-Visible spectrophotometer. Morphological analysis shows that Au NPs have cubic geometry with different sizes. UV-Vis spectroscopic analysis confirmed the synthesis of Au NPs while XRD proved their pure crystalline phase. The Au NPs showed promising dose dependent inhibition of both AChE and BChE as compared to the crude as well as standard drug.

Keywords: gold, nanoparticles, *Delphinium chitralense*, green synthesis, enzyme inhibition activity.

Resumo

A síntese verde foi introduzida como uma alternativa à síntese química devido às graves consequências. As nanopartículas metálicas sintetizadas através da abordagem verde têm diferentes aplicações farmacêuticas, médicas e agrícolas. O presente estudo seguiu uma rota verde e simples para a preparação de nanopartículas de ouro potencialmente bioativas (Au NPs). As NPs de Au foram preparadas via abordagem de síntese verde usando a porção alcaloide básica bruta do tubérculo de *Delphinium chitralense*. As NPs de Au sintetizadas verdes foram caracterizadas por microscopia eletrônica de transmissão (TEM), microscopia eletrônica de varredura (MEV), difração de raios X (DRX), infravermelho com transformada de Fourier (FTIR) e espectrofotômetro UV-Visível. A análise morfológica mostra que as NPs de Au possuem geometria cúbica com tamanhos diferentes. A análise espectroscópica UV-Vis confirmou a síntese de Au NPs enquanto a XRD provou sua fase cristalina pura. O Au NPs mostrou inibição dependente da dose promissora de AChE e BChE em comparação com a droga bruta e padrão.

Palavras-chave: ouro, nanopartículas, *Delphinium chitralense*, síntese verde, atividade de inibição enzimática.

1. Introduction

Nanoparticles are materials having at least one dimension less than 100 nm (Khan et al., 2019). Metal NPs are of great interest due to their distinctive features such as magnetic, catalytic, optical and electrical properties (Dubey et al., 2010). Among metal NPs noble metal NPs have received increased attention due to their promising contribution in the fields of plasmonics, renewable energy, catalysis, photocatalysis and in biomedical applications (Bogireddy et al., 2018; Chaicherd et al., 2019). Noble metals NPs such as gold exhibit excellent chemical physical and

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biological properties due to their nanosize, produced in different shapes and sizes and can be easily functionalized with polymers, antibodies, drugs, diagnostic probes, genetic material, etc (Ramírez Castro et al., 2018). Gold nanoparticles have been studied extensively as they exhibit completely improved and new properties when compared to its bulk form (Ganesh Kumar et al., 2011; Suchomel et al., 2018). Gold nanoparticles (Au NPs) are a promising class of nanomaterials having varieties of applications and widely employed in catalysis, medicine, cancer hyperthermia treatment, biotechnology, infrared radiation absorbing optics, surface-enhanced Raman spectroscopy, magnetic resonance imaging (MRI) optoelectronics, photoacoustic imaging, photothermal therapy and targeted drug delivery (Polte et al., 2010; Aljabali et al., 2018; Chen et al., 2018; Slepička et al., 2019; Fan et al., 2020). Au NPs are widely used for biomedical applications because of their compatibility of synthesis and functionalization, facility of detection and less toxicity (Herizchi et al., 2016). Au NPs bring advances in the early detection and treatment of cancer and can be targeted to tumor site more effectively due to its small size (Yee et al., 2015). Au NPs are potentially utilized in carrier systems due to their enhanced stability, biocompatibility and oxidation resistance (Sengani et al., 2017). Au NPs can easily penetrate cells to interact with molecules without causing any damage making it suitable candidate for cancer therapy, drug delivery and other applications (Badeggi et al., 2020). In photothermal therapy gold nanorods behave as thermal scalpels to destroy infected cancer cells (Zada et al., 2018). The applications of Au NPs in medicine are due to the easy loading of antibodies, drugs and also other molecules to their surfaces (Ahmad et al., 2020). In biomedical applications Au NPs are employed as a potential candidates in different fields such as in bio-sensing (Baek et al., 2020; Panariello et al., 2020; Chen et al., 2020), antibiotic agents (Rotimi et al., 2019; Katas et al., 2019; Wang et al., 2020; Shikha et al., 2020), drug delivery (Fuller et al., 2020), cancer treatment (Rajeshkumar, 2016; Attaalmanan et al., 2020), enzyme inhibitory agent (Badeggi et al., 2020; Hameed et al., 2020; Zainab et al., 2021) etc.

Various physical (Mafuné et al., 2002) and chemical methods (Oliveira et al., 2020; Hussain et al., 2020) are reported for the synthesis of Au NPs. The physical methods has a high production cost as it requires high energy and pressure during the synthesis procedure (Wongyai et al., 2020). In chemical methods, toxic compounds as a reducing agent are used to synthesize NPs which are not appropriate for long-term environmental sustainability, toxic to human health and environment and produce toxic hazardous byproducts in large quantity (Folorunso et al., 2019; Dzimitrowicz et al., 2019; Islam et al., 2019), and greatly limits their applications in the fields of biomedical, especially in clinical purposes (Kunoh et al., 2018). Thus the green synthesis of NPs is developed as an alternative to conventional chemical and physical methods and can overcome some of its destructive effects (Goyal et al., 2019; Salem and Fouda, 2021). The uses of plant extracts for the NPs synthesis are the most eco-friendly and green approach and attracted much attention due to easy availability and wide distribution of plants (Jadoun et al., 2021). The use of plant extracts in NPs synthesis has an advantage over other biomaterials as it is easier, ecologically friendly method, less expensive, short production time, much safer for human use and allows NPs synthesis in one-pot process (Lee et al., 2016; Keijok et al., 2019; Rodríguez-León et al., 2019). Plant extracts may work as reducing as well as stabilizing agents in the NPs synthesis (Sadeghi et al., 2015).

In the present work, Au NPs were successfully prepared through green approach using crude basic alkaloidal portion of the tuber of *Delphinium chitralense* and then the potential of as-synthesized Au NPs were utilized for enzyme inhibitory activity. *Delphinium chitralense* is 40 cm long annual herb, found at an altitude of 1520 to 1830 m. *Delphinium* genus is considered as a rich source of bioactive alkaloids representing cardio tonic, antipyretic, analgesic and sedative activities (Ahmad et al., 2016a) Recently many diterpenoids and norditerpenoids having anticholienesterase potential have been reported from various aconitum and delphinium species (Nisar et al., 2009; Ahmad et al., 2016a, 2017b, a, 2018b, a). According to the available literature, *Delphinium chitralense* is used for the first time for the green synthesis of Au NPs and the enzyme inhibitory activity of Au NPs are also still unreported.

2. Experimental Work

2.1. Materials

Methanol commercial grade was used during extraction. Gold chloride and Dimethyl sulfoxide were obtained from Sigma Aldrich.

2.2. Collections of samples and extraction

Delphinium chitralense (aerial parts) were collected from Kumrat valley, KPK, Pakistan in August 2016 (3 Kg dry weight) after identification by Assistant Professor, Dr. Zahid Ullah, Institute of plant Sciences, University of Swat, KPK, Pakistan. A voucher specimen (accession number= CD.US-015) was deposited in the herbarium of this institute. The plant material was chopped into small pieces, followed by grinding into fine powder using electrical blender. This fine powder was soaked in methanol (80%) for three days followed by concentration on a rotary evaporator (40 °C) to yield a gummy crude. The total alkaloids were obtained using traditional acid-base method and successive pooling in chloroform at various pH levels. The alkaloidal part obtained at pH 10 (basified with 5% NaOH) was selected for the synthesis of Au NPs.

2.2.1. Synthesis of Au NPs

To prepare the alkaloidal solution, the basic fraction was dissolved in deionized water (mg/mL), filtered and centrifuged at 3000 rpm to collect any suspensions. The process was repeated thrice so to get a clear solution. The resulted supernatant was dissolved in deionized water to make 0.3% of basic alkaloidal solution 1. The solution 1 was then mixed with Gold chloride solution (0.001 M) in 2:8 ratio in a 250 mL conical flask. The mixture was

stirred at 80 °C for 1 hr on magnetic hotplate followed by cooling and monitoring the color change. The solution was then centrifuged at 13000 rpm on 10 °C for 10 minutes, supernatant was removed while the resulted pallet (Au NPs) was re-dissolved in small amount of deionized water and subjected to drying in oven at 100 °C.

2.3. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibition assays

Elman method with slight modifications according to Rocha et al. (1993) was used to determine the AChE and BChE inhibition activities of Au NPs (Rocha et al., 1993). Accordingly, the samples solutions in various concentrations (10-50 µg/mL in DMSO) were separately mixed with 50 µL of 5,5′-dithiobis-2-nitrobenzoic acid (0.2 mM, pH 8, prepared in sodium phosphate buffer (300 µL, 62 mM). This mixture was allowed to stand for some time. Additionally, 10 µL each of AChE and BChE were separately added into the previous solutions and incubated for 15 min at 25 °C. To start the reaction, 40 µL (15 mM) each of acetylthiocholine and butyrylthiocholine were added to the previous solutions to produce yellow-colored anion (5-thio-2-nitrobenzoate). The absorbance was spectroscopically monitored at 412 nm with BMS spectrophotometer (USA) for 15 min. Similar samples were prepared for the standard drug, Allanzanthane. Percentage inhibition was calculated from the comparisons of samples, standard and blank (Tris buffer only) by using the following Formula 1.

$$\text{Percentage inhibition} = \frac{(E-S)}{E} \times 100 \quad (1)$$

where *E*= activity of the enzyme without test sample; *S*= activity of test sample.

3. Results and Discussion

3.1. Morphological study

Morphological studies of materials are very important for analyzing the shape, size and surface. Figure 1 represents the SEM images of Au NPs at various magnifications. Images show that Au NPs have cubic morphology and their size in the range of 100-300 nm. The images represent that the cubes have different sizes and are porous in nature. The cubes are mostly is dispersed form however small portion also gets agglomerated. Some tiny size Au NPs are also found in deposited form over the cubic structures. The green synthesized Au NPs are more deeply examined via TEM analysis as shown in the Figure 2, which support the results of SEM analysis.

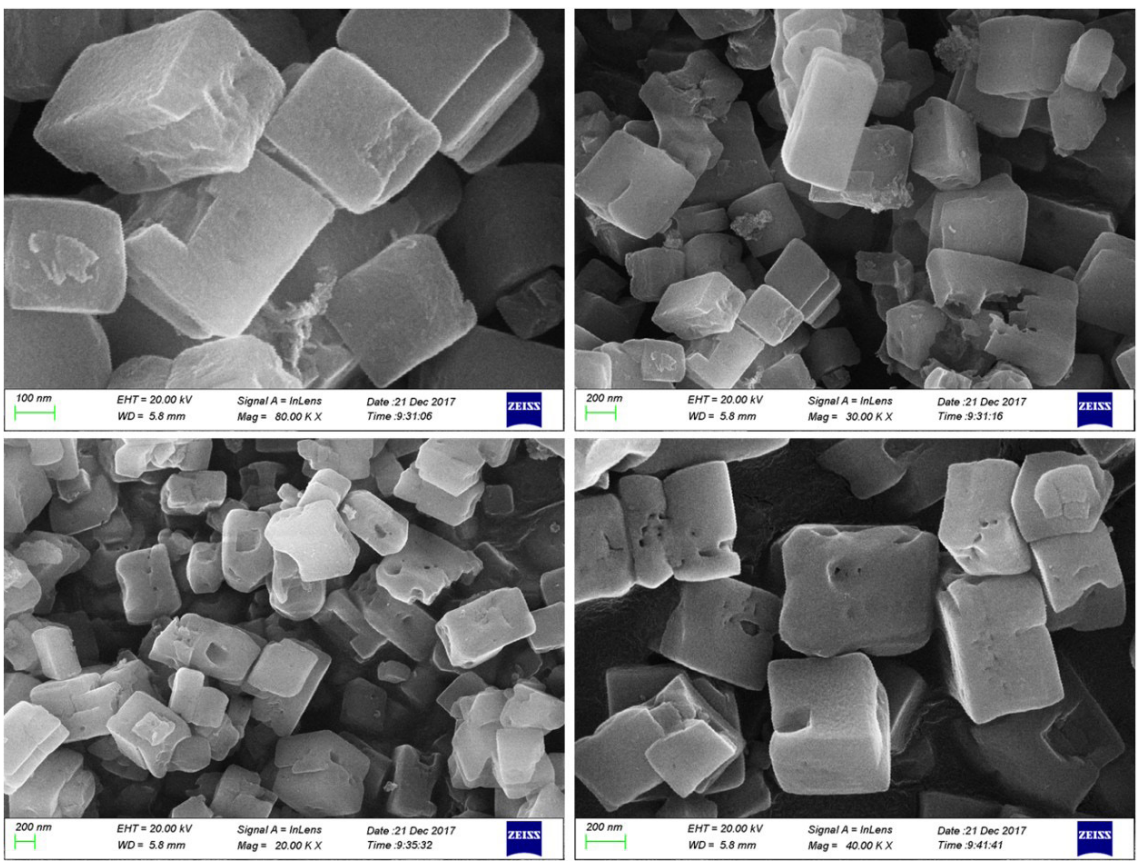

Figure 1. SEM images of green synthesized of Au NPs at different magnifications.

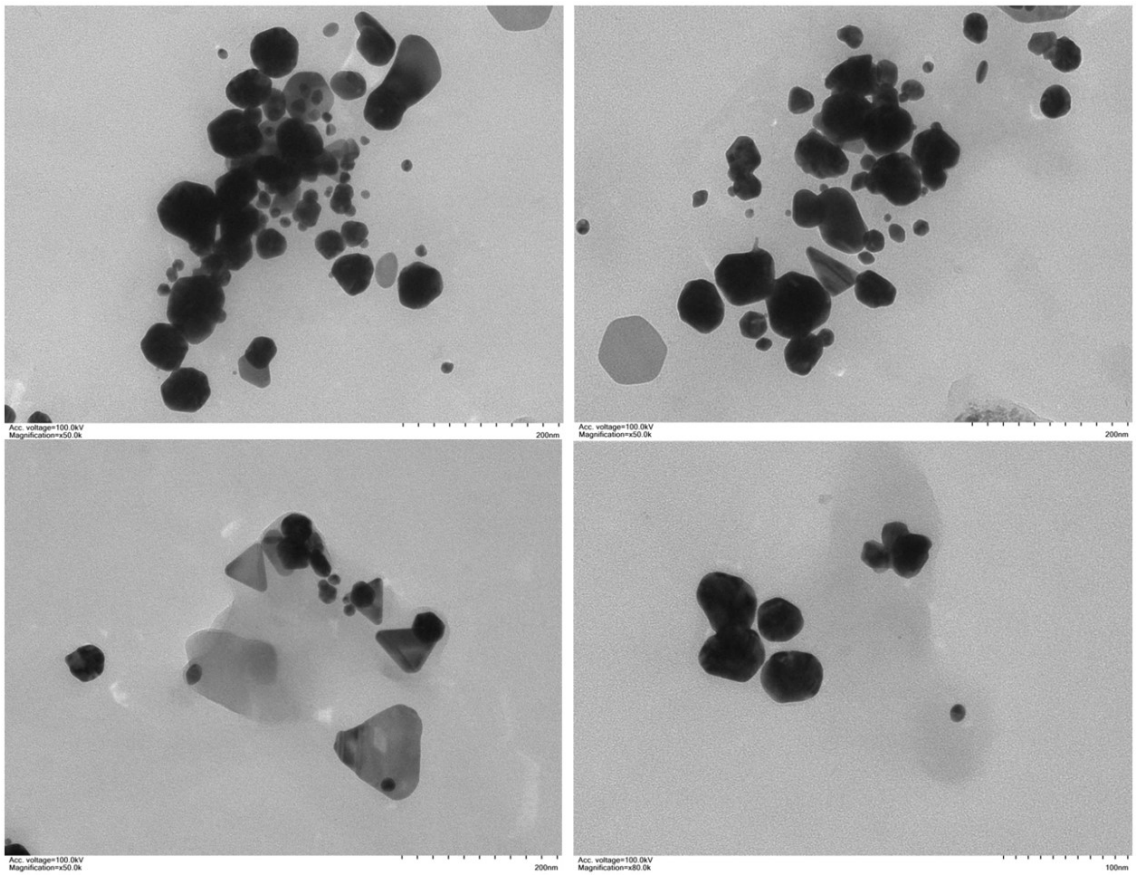

Figure 2. TEM images of green synthesized Au NPs at different magnifications.

3.2. FTIR, XRD and UV-Visible analysis

FTIR analysis of green synthesized Au NPs was carried out for detecting various functional groups present of tuber extracts. Figure 3 represents the FTIR spectrum of green synthesized Au NPs. The long peaks observed at 3439 cm^{-1} is assigned to the O–H bonds which indicates the presence of alkaloid compounds as the basic fraction of *Delphinium chitralense* contain alkaloid portion as reported in our previous study (Ahmad et al., 2016a). Alkaloids were also confirmed through 'Dragendorff's test, which is a common test for the detection of alkaloids (Abraham et al., 2020). The sharp peak at 1626 cm^{-1} is attributed to C=C aromatic group. The peaks observed at 2851cm^{-1} and 2921 cm^{-1} are due to the stretching vibration of C–H bond (Ahmad et al., 2016b; Yulizar et al., 2017). The C–OH shows vibration at 1054 cm^{-1} but here it shows a strong peak at 1029 cm^{-1} which is due to its binding to the Au NPs (Ismail et al., 2018). Figure 4 represents the XRD pattern of the as-synthesized Au NPs. The XRD spectrum gives an intense peaks at 2θ = 38.5°, 44.8°, 66.5°, 78° and 82° which correspond to the (111), (200), (220), (311) and (222) plane proving the face center cubic (fcc) structure of green synthesized Au NPs (Xin Lee et al., 2016; Bogireddy et al., 2018). The synthesis of Au NPs is also confirmed through UV-Visible spectroscopy. Figure 5 represents the UV-Visible absorbance spectra of the Au NPs which gives maximum absorption at around 550-560 nm. These results of UV-Visible absorbance spectra of Au NPs are in good agreement with available literature (Wongyai et al., 2020). Peak broadening and red shift were observed and noted in alkaline medium (Islam et al., 2019).

3.3. Enzyme inhibition activity

The Au NPs showed promising dose dependent inhibition of both AChE and BChE as compared to the crude as well as standard drug (Figure 6 and 7). The most potent dose of Au NPs obtained through assay was 50 µg mL^{-1} which inhibited AChE 88 ± 0.56% while at the same dose, the crude inhibited 70 ± 0.8%, while the percentage inhibition obtained for Allanzanthane was 98 ± 0.25. The IC_{50} values calculated include Au NPs = 15±0.42; crude= 29±0.8; Allanzanthane = 10±0.12 µg mL^{-1} (Table 1).

The same pattern of inhibition was obtained for all the samples against BChE however, much potent effect was observed against BChE as compared to AChE. The Au NPs inhibited 92 ± 0.37% of BChE at 50 µg/mL concentration which is almost very close to the inhibition obtained for standard at this concentration (98 ± 0.33%) (Table 1). The IC_{50} values calculated for Au NPs (12±0.42 µg mL^{-1}) is

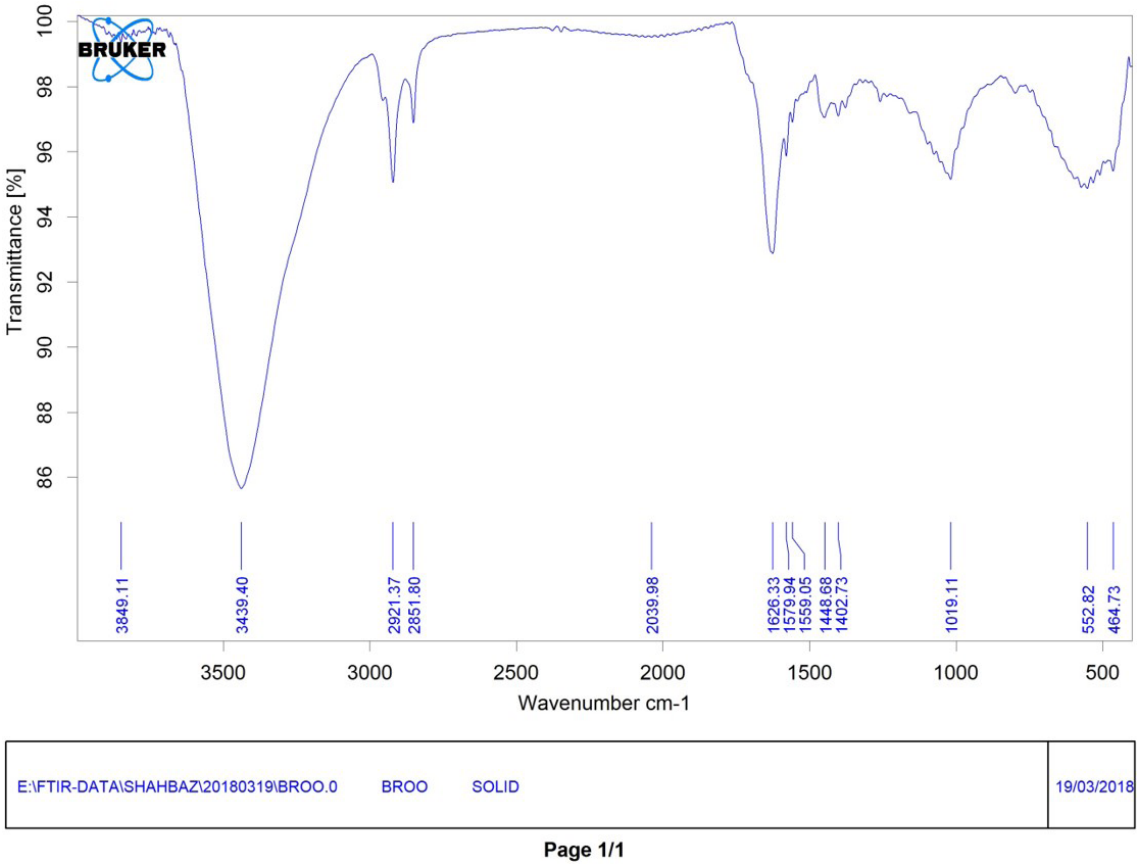

Figure 3. FTIR spectrum of green synthesized gold nanoparticles.

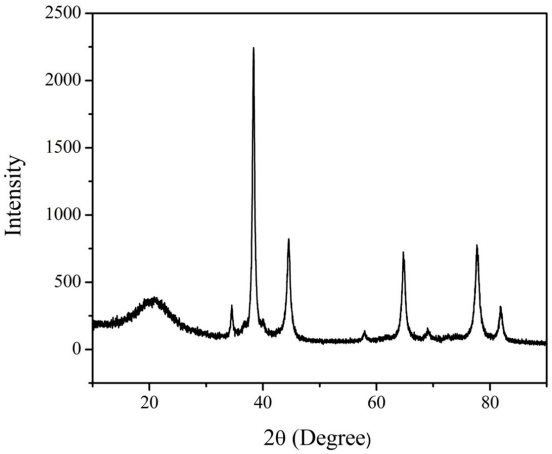

Figure 4. XRD patterns of green synthesized gold nanoparticles.

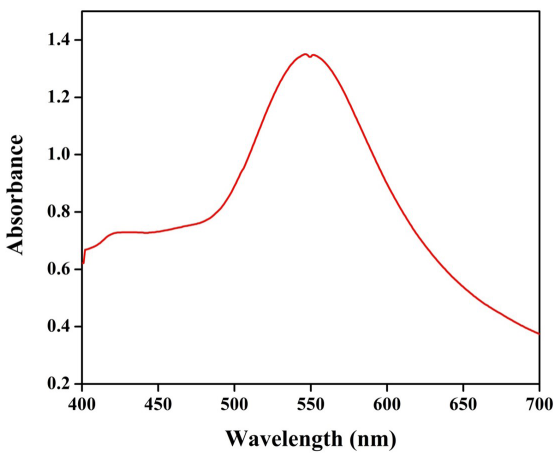

Figure 5. UV-Visible spectra of green synthesized gold nanoparticles.

very close to that of standard against BChE (10±0.16 µg mL^{-1}), thus showing the potency of Au NPs against BChE. In either case; Au NPs showed higher inhibition of both AChE and BChE as compared to crude extract.

Cerebral dementia or other brain memory related diseases are usually correlated with the excess of reactive oxygen species as well as deficiency of the neuro transmitter, acetylcholine which consequently result in Alzheimer's disease (AD). These conditions are generally overcome by the natural antioxidants in our foods that eliminate any reactive oxygen species (ROS), a cause towards oxidative damage of neuronic cells and resulting in dementia. The molecular basis of dementia is also related to the function of acetylcholine, which is

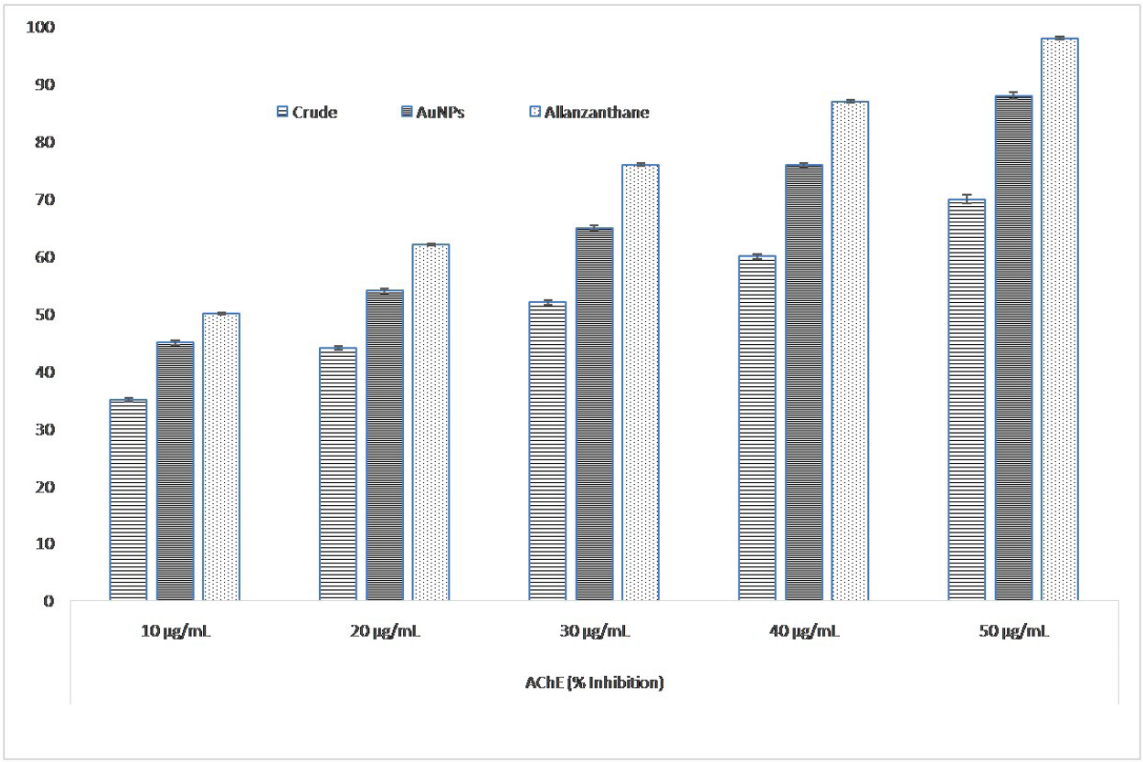

Figure 6. Comparative %age inhibition of AChE by Au NPs, crud extract and allanzanthane.

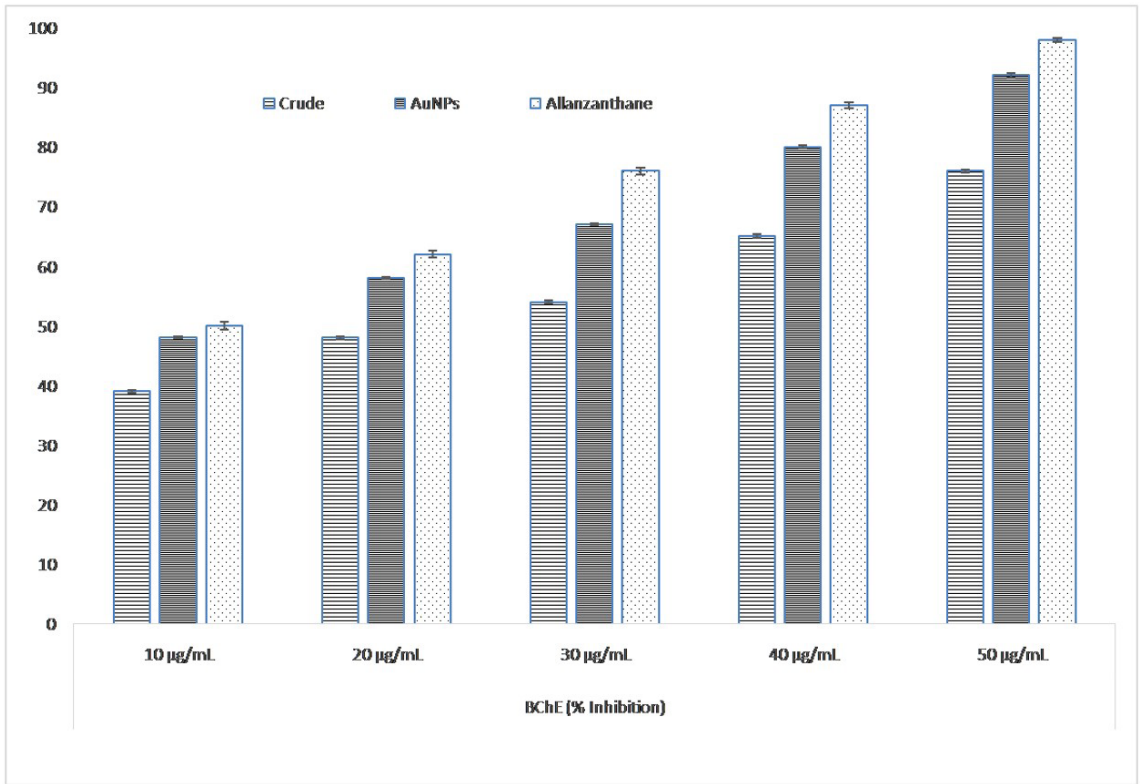

Figure 7. Comparative %age inhibition of BChE by Au NPs, crude extract and Allanzanthane.

Table 1. Cholinesterase inhibitory effect of crude and Au NPs from *Delphinium chitralense* tuber extract.

Sample	AChE (% Inhibition)					
Concentration	**10 μg mL^{-1}**	**20 μg mL^{-1}**	**30 μg mL^{-1}**	**40 μg mL^{-1}**	**50 μg mL^{-1}**	**IC_{50} μg mL^{-1}**
Crude	35 ± 0.34	44 ± 0.32	52 ± 0.39	60 ± 0.45	70 ± 0.8	29±0.8 μg mL^{-1}
AuNPs	45 ± 0.44	54 ± 0.43	65 ± 0.41	76 ± 0.33	88 ± 0.56	15±0.42 μg mL^{-1}l
Allanzanthane	50 ± 0.15	62 ± 0.17	76 ± 0.19	87± 0.23	98 ± 0.25	10±0.12 μg mL^{-1}
	BChE (% Inhibition)					
Crude	39 ± 0.21	48 ± 0.23	54 ± 0.26	65 ± 0.34	76 ± 0.25	29±0.6 μg mL^{-1}
AuNPs	48 ± 0.15	58 ± 0.19	67 ± 0.21	80 ± 0.21	92 ± 0.37	12±0.42 μg mL^{-1}
Allanzanthane	50 ± 0.71	62 ± 0.56	76 ± 0.58	87± 0.43	98 ± 0.33	10±0.16 μg mL^{-1}

often blocked by excessive release of AChE and BChE. Diets containing several vitamins (A, C and D) and β-carotene have been reported to be useful in tackling AD especially in aged people (Wong et al., 2017; Mecocci et al., 2018) however, some medicinal plant mediated formulations also act as free radical scavengers (Ali-Shtayeh et al., 2008). Specifically, drugs like donepezil or galantamine have been used as cholinesterase inhibitors however, due to their low efficacy, medicinal plant mediated drugs might be an alternative towards AD therapy (Gauthier et al., 2003; Mehta et al., 2012). Our findings could lead towards Au NPs based medication from *Delphinium chitralense* tubers against AD related diseases in particular.

4. Conclusion

Green synthesis is a simple, economic and safe way for the synthesis of Au NPs. The Au NPs formed cubic structure with different sizes. Morphological analysis shows that Au NPs are in cubic with porous structure. FTIR analysis indicates the presence of various organic functional groups and their possible involvements in the reduction process. UV-Visible analysis confirmed the synthesis of Au NPs while XRD indicates its pure crystalline phase. Au NPs showed potent anticholinesterase activities in dose dependent manner with promising IC_{50} values of 15±0.42 against AChE and 12±0.42 μg mL^{-1} against BChE respectively.

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