Journal of Advanced Zoology

ISSN: 0253-7214

*Volume **44** Issue **S-5 Year 2023** Page **1101:1115***

Green Synthesis of Silver *Atropa Acuminata* Nanoparticles: Characterization and Anti-Diabetic Potential

Priyanka Thakur[1]*, Vinay Pandit[2]

*[1]*Department of Pharmacology, Shiva Institute of Pharmacy, Chandpur, Bilaspur, HP, India- 174004*
2Department of Pharmaceutics, Laureate Institute of Pharmacy, Kathog, Kangra, HP, India- 176029

**Corresponding author's: Priyanka Thakur*

Article History

Received: 06 June 2023
Revised: 05 Sept 2023
Accepted: 17 Oct 2023

Abstract

Background: *Atropa acuminata plant, which is also known as the maitbrand or Indian belladonna, belongs to the family Solanaceae and is closely related to the deadly nightshade of Europe and North Africa used in the treatment of various diseases.* ***Objectives:*** *The main objective is to investigate the antidiabetic potential of silver nanoparticles of Atropa acuminata.* ***Methods:*** *In this study green synthesis of silver nanoparticles was carried out. The current study engross the green synthesis of A. acuminata roots by reducing silver ions, where ultraviolet (UV) spectral analysis and transmission electron microscopy confirmed nanoarchitecture. The optimized silver nanoparticles were characterized for shape, size and morphological features by various techniques viz., SEM, TEM EDXA and XRD. The optimized formulation was further subjected for lipid peroxidation assay and in vitro antidiabetic assay in order to understand the antidiabetic potential of formulated silver nanoparticles.* ***Results:*** *The Silver nanoparticles of Atropa acuminata roots was successfully prepared by optimizing different concentration of plant extract at different temperatures and stirring speed. The Resultant optimized nanoparticles showed a particle size around 20 nm and -28 mv zeta potential. Further the characterization of optimized AgNPs was carried out. SEM provides the shape, size, and morphological features, whereas EDXA confirms the compositions and distribution of nanoparticles through spectrum and elemental mapping. XRD diffraction analysis revealed the crystalline structure of nanoparticles. Further, Nanoparticles showed a maximum scavenging potential of 82.633±0.116 for superoxide anion free radicals at 100 µg/mL concentration. Among two anti- diabetic assays, the αamylase assay shows a better result of percent inhibition 63 ± 1.32 at 75 µg/ml concentrations. Lastly, in vitro, drug release study revealed a 101.50% cumulative release from Ag- NPs formulation up to 1 hour, which was better than the standard one.* ***Conclusion:*** *This study explores the novel technology of green synthesis for various biomedical applications.*

Keywords: *Ag-NP, Atropa acuminata, Antidiabetic, Biocompatible; Bioassay*

1. Introduction

Atropa acuminata plant, which is also known as the mait-brand or Indian belladonna, belongs to the family Solanaceae and is closely related to the deadly nightshade of Europe and North Africa used in the treatment of various diseases [1]. The four species of this plant are *A. acuminata Royle, Atropa belladonna L., Atropa baetica Wilk, and Atropa pallidiflora* [2]. Plant species' dried roots, leaves, and stems carry numerous phytochemicals like atropine, scopolamine, hyoscyamine, and other tropane alkaloids [3]. The plant has been medicinally described as an antidote, anodyne, analgesic,

hallucinogenic, Parkinsonism, encephalitis, carcinoma, spastic dysmenorrhoea, mydriatic, narcotic, and sedative properties, according to literature review

[4] [5] [6]. The field of research known as nanotechnology studies molecular and nanoscale processes. There are many instances of nanoscale objects in nature, such as DNA, water molecules, viruses, red blood cells (RBCs), etc [7] [8]. Researchers now have the chance to alter several treatments due to recent advances in nanotechnology. With the use of this technology, it is now possible to modify the biological and physicochemical characteristics of nanomaterials to enable more effective medication-targeted delivery to produce therapeutic effects [9] [10].

As per the literature survey, Singh et al. [11] have described that the *A. acuminata* plant is used to manage CNS and memory-related disorders. Majid et al. [12] have found anti-inflammatory potential due to the active constituents in this plant. Jayakanthi et al. [13] have reported the hepatoprotective role of the A. acuminata plant in acetaminophen-induced oxidative stress and hepatotoxicity in rat liver. Rahman et al. [14] analyzed the anti-cancer potential of the plant by inhibiting protein kinase enzymes. Khan et al. [15] reported that plant tissues are rich in antioxidant molecules. Literature also revealed that the plant has numerous medicinal and aromatic properties but is declining in wildlife [16]. The major threat to this species is overexploitation due to sociocultural patterns [17]. I propose that this plant species should be preserved by seed propagation; otherwise, it will be totally abolished if no action is taken [18] [19]. Mohammad et al. [20] reported that the propagation of *A. acuminata* plant should be optimized through numerous plant growth regulator sources as this plant will be critically endangered [21]. So, to achieve this rationality, research is focused on highlighting this plant's worth as it is highly medicated.

Pharmaceutical and other medical corporations avail themselves of nanotechnology applications worldwide in numerous biotechnology, bioengineering, and biomechanics disciplines, which swap the trendy scenario of researchers looking after this field as an alternative drug delivery system [22]. So, nanotechnology has become one of today's most exciting research zone. Nanomedicine and nanodelivery systems are emerging sciences in which Nanoscale materials are used as diagnostic tools to deliver therapeutic agents to specific targets [23]. Because the quantum energy of these particles can be easily ascertained due to their smaller size, this field makes it possible to design the biomaterials with the desired Nanoscale size, shape, and morphology [24].

Nanotechnology is creating nanoparticles of copious metal oxides like gold, silver, zinc, magnesium, titanium, etc., with sizes ranging from 1 to 100 nm [25]. Among all these oxides, the free radical scavenging and antioxidant potential of silver oxides grabbed the mind of scientists to think deeply. Further, the operating procedure of silver nanoparticles inside the body implies releasing silver ions, generating reactive oxygen species, and penetrating the targeted cell membrane, followed by the blockage of deoxyribonucleic acid [26]. Furthermore, these particles have outstanding optical properties due to their small size, large surface area, and distinct colloidal, optical, and electrical properties [27]. In addition, plants have an abundance of phytochemicals that serve as reducing agents in the emergence of Ag-NP, increasing the probability of synergistic effects within the biological systems [28]. Agricultural nanoparticles are becoming popular because of their outstanding features and practical versatility. AgNPs are primarily used in antimicrobial and anti-cancer remedies but are also used as a vaccine adjuvant, an anti-diabetic agent, and a biosensor [29].

In the evident nanotechnology, top-up and bottom-down approaches were used where top-up approaches included Physical vapor deposition, Chemical vapor deposition techniques, etc., and bottom-down approaches involved Sol-gel synthesis, Colloidal precipitation techniques, etc. to produce the nanomaterial [30]. Different physical, chemical, and biological methods (Pulse Laser Ablation, sol-gel method, microorganism method) were used to synthesize the nanoparticles [31]. Among all these methodologies, physical methods are expensive, cause radiation exposure, require high energy, temperature, and pressure, and generate a large amount of waste, whereas, in chemical methodology, the chemicals used in the procedures are costly and toxic, and making them hazardous to the environment as well as for human health [32]. However, biological methodology does not involve hazardous chemicals for reduction and stabilization. Moreover, biological methods are eco-friendly, biocompatible, synthesized in big batches, economical, safe, and target specific effects due to their

smaller size and very few side effects [33]. Therefore, this current research preferred the biological green synthesis method for nanoparticle formulation.

In this current investigation, the research aims to scrutinize this critically endangered plant *A. acuminata* roots for their green synthesis of Ag-NP and confirm the formulation by UV spectral analysis and transmission electron microscopy.

2. Materials And Methods

Chemicals

Fuming nitric acid, methanolic potassium hydroxide, acetone, solution of nitrated residue, 1mM aqueous solution of silver nitrate, platinum, DMSO, NBT, 5 mM NaOH, Tris-HCl buffer, 78 μM of β-nicotinamide adenine dinucleotide, 50 M of nitro blue tetrazolium, stock solution, 0.1mM methanolic solution of superoxide radical, 0.02 M sodium phosphate buffer (pH 6.9), 1U of αamylase, 30-100 g/mL of ANE, 1% starch solution, dinitrosalicylic acid reagent, , 2.4 U/mL αglucosidase, 2.5 mM pNPG in the buffer, and 100 mM phosphate buffer (pH 6.8), sodiumcarbonate solution, 4-nitrophenol, phosphate buffer (pH 8), ethanol, water, PMS-NADH, epigallocatechin gallate, acarbose.

All the chemicals were of analytical grade and procured from Sigma Aldrich. Some ingredients were purchased from Franco-Indian Pharmaceuticals Pvt. Ltd. The plant material was collected from local region of Mandi and Kullu district of Himachal Pradesh and authenticated by Dr. Madhava Chetty,

Assistant Professor in the Department of Botany at Sri Venkateswara University in Tirupati, Andhra Pradesh, India (SVU/SC/76/321/20-21).

Plant roots collection

The Himachal Pradesh districts of Mandi and Kullu are the regions from where the plant material was gathered. The plant roots were identified and verified by Dr. Madhava Chetty, Assistant Professor in the Department of Botany at Sri Venkateswara University in Tirupati, Andhra Pradesh.

Preparation of*A. acuminata* root extract

After the collection, plant roots were first swept with plain water to get rid of unnecessary waste. After being shade-dried for up to 2 weeks at room temperature, crush it into a powder using an electric mixer grinder (Superflame GX 11), and then store it in an airtight container. Crushed powder of plant roots was strained through sieve number 40. The soxhlet apparatus is suffused with 1000 gm of powdered medication, and ethanol: water solvent in a 50:50 ratio and subjected to an extraction process (Figure 01). The residual extract solvent is then incubated with continuous stirring, filtered through Whatman Paper No. 1 and evaporated using the phenomena of decreasing pressure distillation by rota evaporator (Rota vapour, R-210/215, Buchi, Switzerland). After concentrating the filtrate, the extract was stored at a low temperature (5˚C) for further experiments.

Figure 01: Soxhlet extract apparatus assembly for extraction of phytochemicals from Plant Root Extract

Preliminary Screening for Qualitative Phytochemicals of Extract

Atropa acuminata plant root extract lug numerous important phytochemical components that were qualitatively assessed in accordance with the literature review [35]. Alkaloids, phenolic chemicals,

flavonoids and tannins were thus found by screening evidence, but no inference evidence for glycosides, terpenoids, proteins, or carbohydrates was found [36] [37]. According to accepted methodologies, the findings showed that the alkaloids make up the majority of this plant species [38]. For the confirmation of type of alkaloids Vitali Morin Test was performed. Sample solution was reacted with fuming nitric acid and then allowed to evaporate until it converted into dried residue. Afterwards, dried residue were treated with methanolic potassium hydroxide, acetone and solution of nitrated residue which produce violet colour indicate the presence of tropane alkaloids [39].

Thin Layer Chromatography

Extract sample was applied on TLC plates where different solvents ratio were selected on the basis of their ascending polarity (Toluene: Ethylacetate= 1:9/ 9:1, Ethylacetate: Butanol: Distilled water= 2:4:4, Toluene: Ethylacetate: Formic acid= 1:1:1, Ethyl acetate: chloroform:formic acid:water = 7:1.5:1.5:1 etc.) to optimize the solvent system for the elution of phytochemicals effectively on TLC plate. The yellowish green spots were produced in silica plate which indicates the presence of flavonoids, alkaloids and phenols in the sample (Figure 02). Chloroform: Methanol: Acetone: 25% Ammonia (75:15:10:1.8 v/v) elutes the phytochemicals most effectively which will be further used for qualitative and quantitative analysis.

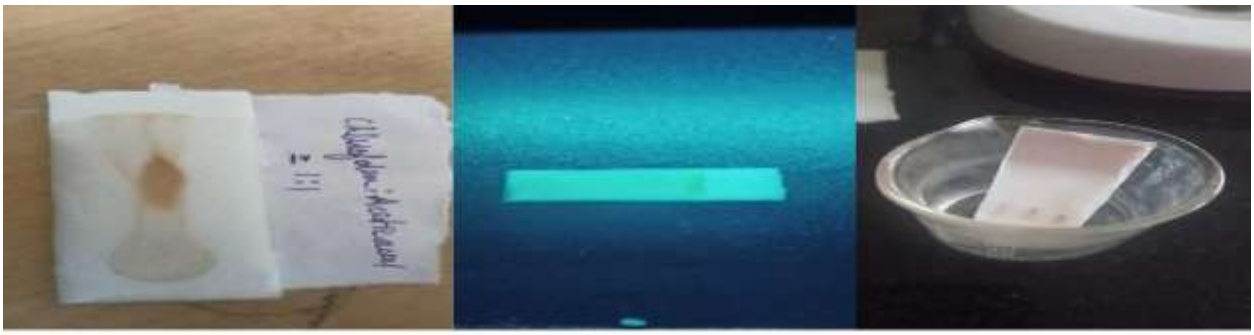

Figure 02: Band Appearance and visualization under UV Chamber in TLC Plate.

Synthesis of *Atropa acuminata* AgNPs

For the production of AgNPs of root extract, a 1mM aqueous solution of silver nitrate (AgNO3) was prepared in a 250ml volumetric flask. In a nutshell, according to established procedures, 10 ml of *A. acuminata* root extract was dissolved in 90 ml of an aqueous solution containing 1 mM silver nitrate [40] [41]. The transformation of color from colorless to dark brown demonstrated that AgNO3 had completely reduced to Ag+ ions. After that, the colloidal mixture was securely packed and kept for later use. Using UV-Visible spectroscopy (Varian Inc., USA) in range between 300 to 600 nm, the production of AgNPs of root extract was examined. The solution was diluted 20 times for UV spectroscopic monitoring. A high peak at 430–450 nm confirms the synthesis of root extract Ag–NPs, which was confirmed by the use of millipore water as a blank to reset the baseline. All the reactions were repeated for three times.

Characterizations Surface Plasma Resonance (SPR)

Green synthesized *A. acuminata* root extract AgNP formulation was inspected for the reduction of silver ions into nanoparticles using UV-visible spectroscopy Tecan Multimode Microplate Reader (InfiniteM200). The baseline was calibrated using millipore water, and spectra were monitored between 300 and 700 nm which indicate the bio-reduction of silver ions to produce AgNPs.

Analysis of Particle Size and Zeta Potential

The dilution approach was used to examine the particle size distribution, which is one of the most important element in determining the quality, efficacy, and safety of a nanoparticle formulation [42]. The mixture was assessed using Malvern Zeta Sizer ZS technology after being diluted up to ten times with distilled water. In a disposable cuvette, a 1 ml diluted sample is obtained and evaluated between a 250 and 900 angle. Helium was employed as the light source, and Particle diffusion by Brownian motion

phenomenon was used to determine particle size. Zeta potential is checked using the sample in the capillary tube [43].

Transmission Electron Microscopy (TEM)

Sample was blended with 10 ml of distilled water after being centrifuged at 20,000 rpm for 30 mins, and it was subsequently stored at 15-20°C for at least one full day. The entire mixture in suspension is subsequently kept within the lyophilizer (Jenco CP135-M4-2). By putting a few drops of the processed sample inside the instrument's copper grid, the processed sample is combined with double-distilled water. The process of dry heat sterilization is employed to eliminate any remaining water from the treated sample for a maximum of two hours at 55 ˚C [44].

XRD Analysis

The attributes of crystalline structure, phase nature, lattice parameters, and crystal size is frequently accomplished by XRD analytical instrument X'Pert MRD (PAN analytical, Almelo, Netherlands). When electrons impact nanoparticles, they scatter X-rays with a wavelength of 2 A˚ [45]. A glass slide was coated with solid AgNPs to serve as the sample container. The XRD machine, which is connected to an integrated program, houses the holder. Using CuK radiation (=1.54056) in the range of 20° to 80° at 40keV, the XRD pattern of AgNPs was captured by a Bruker D8 diffractometer. The Powder X computer program ascertains the lattice parameters.

SEM and EDXA analysis

Even for extremely small particles smaller than 10 nm, scanning electron microscopy is a high resolution imaging technique. Particle size, shape, and texture are utilized to assess surface fractures, defects, impurities, and corrosions [46]. Initially, a sample container containing carbon tape and AgNP was coated with platinum and viewed under S-4200 for 120 secs (Hitachi, Tokyo, Japan) [47]. Afterwards, synthesized AgNPs were examined using a SEM device connected to an EDXA Inc., Mahwah, NJ, USA).

Antioxidant Assay of *Atropa acuminata* Ag-NP Formulation *In vitro* Free Radical Scavenging Activity Synthesized Ag-NP *in vitro* antioxidant activity was determined using standard techniques which entail the suppression of free radicals. After samples were added up to a system that gives rise to free radicals, the suppression of free radical activity is perceived by the following formula [48].

$$\% \text{ inhibition } = \frac{[\text{Absorbance of Control} - \text{ Absorbance of Test}] \times 100}{[\text{Absorbance of Control}]}$$

The IC50 values calculated represent the sample concentrations required to scavenge 50% of free radicals.

Superoxide Radical Assay

To evaluate the superoxide anion scavenging activity, a reaction mixture including 0.2 ml of each AgNP, substance, and standard in DMSO, 0.2 ml of NBT (1 mg ml-1 solution in DMSO), and 1 ml of alkaline DMSO (5 mM NaOH in 1 ml water) was prepared. In this assay, superoxide radicals emerged in 3.0 ml of Tris-HCl buffer (16 mM, pH 8.0), which also included 1 mg of test sample (Ag-NP) dissolved in 50% ethanol, which was added at various concentrations (31.25-1000 µg/ml), 78 µM of βnicotinamide adenine dinucleotide (reduced form NADH), 50 M of nitro blue tetrazolium (Ransod kit, Randox Laboratories Ltd, Crumlin, UK) [49]. Numerous concentrations of solutions have been prepared of concentrations ranging from10-100 µg/ml from stock solution and then were mixed with 0.1mM methanolic solution of superoxide radical. This solution was incubated for 30 min at room temperature and was recorded at 560 nm.

Antidiabetic potential of A. acuminata AgNPs α-Amylase Inhibition Assay

The α-amylase assay combination includes 0.5 mL of 0.02 M sodium phosphate buffer (pH 6.9), 1U of α- amylase, and 30-100 g/mL of ANE-AgNPs that had been pre incubated for 250 min at 37 °C. About 20 mins at 37 °C of incubation, 200 L of a 1% starch solution in the previously mentioned buffer was introduced to the tubes. By administering 0.5 mL of dinitrosalicylic acid reagent and heating in a boiling

water bath for 15 mins, the reaction was ceased. After cooling the tubes, absorbance at 540 nm was measured [50]. α-

glucosidase enzyme inhibitory activity

The microplate-based approach was slightly modified to assess the α-glucosidase inhibitory activitiesof the Ag-NP [51]. 75 L of the reaction mixture for the sample group included 15 L of each of thefollowing: experimental drug, 2.4 U/mL α-glucosidase, 2.5 mM pNPG in the buffer, and 100 mMphosphate buffer (pH 6.8). The reaction was stopped by adding 75 L of a sodium carbonate solutioncontaining 0.2 mol/L to each well after the 96-well plates had been incubated at 37°C for 25 min. At405 nm, the absorption of 4- nitrophenol was observed. The reaction mixture's composition in thecontrol group was identical to that in the sample group, with the exception that solvent was employedin place of the test substance. The Ag-NP sample group's composition was identical to that of thecontrol blank group's, with the exception that α-glucosidase

was employed in place of the buffer.

Using the following formula, the inhibitory activity was determined:

The following formula was used to evaluate the % inhibition of α-glucosidase enzyme activity:

% Inhibition= Abs control- Abs Test/Abs control X 100

Drug Release Studies

In order to establish the effectiveness, therapeutic activity, formulation development and stability, it is very important to evaluate the *in vitro* drug release study. Keshary-Chien (K-C) diffusion cell and dialysis membrane 60 LA390-1MT (Thames chemical, Ludhiana) of molecular weight 13000 Da, pore size 2.4 nm and surface area 3.14 cm2 was used which is ideal for diffusion and osmosis work. The temperature of outer jacket of diffusion cell was maintained using water at temperature 37 ± 2˚C whereas 10 ml of freshly prepared receptor medium was used as phosphate buffer (pH 8) stirred at 100 rpm. 1 gm of *A. acuminata* plant extract Ag-NP was loaded inside the diffusion cell and during the whole experiment sink condition, 37± 2 C temperature was maintained. 5ml of sample was withdrawn at predetermined time intervals. Samples collected in test tubes were then analyzed using UV visible spectroscopy at 420 nm [52] against the similarly treated blank.

3. Results and Discussion

Analysis of Phytochemicals and Percentage yield

Harvested shade dried powdered material was extracted using ethanol and water as the solvents and overall percentage yield 19.25% from the 1000 gm weight of powdered material was determined utilizing extract. Weight of crude extract was considered to be 192.5 gm (Table 01). Preliminary phytochemical screening test was accompained to reveal the presence of alkaloids, phenolic compounds, and flavonoids while glycosides, terpenoids, proteins, and carbohydrates were lacking from the roots extract. Specific confirmatory testing of alkaloids (Tropane alkaloids) was also escorted by Vitali Morin Test as atropine is the prime phytochemical accountable for antioxidant and antidiabetic potential According to literature survey [53].

Table 01: Calculation of Percentage Yield of Crude Hydroethanolic Extract of *A. acuminata* roots

Sr. No.	Name of Plant	Extract Type	Weight of Powd Plant Material (g)	Weight Extract (g) c	r%age Yie Extract
1.	Atropa acum plant roots	i Hydroalcoholic	1000g	192.5	19.25

***A. acuminata* root extract Silver Nanoparticles:** The Ag-NP from the roots of the *Atropa acuminata* plant was initially established by visual inspection of reaction mixture (root extract and AgNO3) from pale yellow to dark reddish brown color, specifying clearly the reduction of cationic silver to its metallic counterpart and synthesis of Ag-NPs of different root extract concentrations in an experimental laboratory (Fig. 02). Reaction mixture of concentration 10 mg/ml has been augmented

by using two state variable firstly by keeping temperature constant (40 ˚C) and stirring variable, subsequently stirring constant (500 rpm) and variable temperature (Table 02). Reaction mixture (10mg/ml) was considered to be as stable colloidal nanoparticles with no lumps or precipitate formation.

Table 02: Green synthesis of plant extracts Ag-NP of numerous Concentrations and formulation optimization using two Sate Variables.

Sr. No.	Plant Extract Conc.(mg/ml)	Temperature (0C)	Stirring (rpm)Speed
1.	2	25	200
2.	4	30	300
3.	6	35	400
4.	8	40	500
5.	**10**	**50**	**600**
6.	12	60	700
7.	14	70	800
8.	16	80	900
9.	18	90	1000
10.	20	100	1100

Surface Plasma Resonance (SPR)

Surface Plasma Resonance (SPR) of green synthesized silver nanoparticles of *A. acuminata* root extract was analyzed by employing UV spectroscopy at 420nm. Spectroscopic SPR revealed the arithmetic sequence meaning at low concentration (Below 6 mg/ml) peaks are less sharp and at very higher concentration (above 14 mg/ml) peaks were immense (Fig. 03). Spectroscopic plot recommended that at low concentration of plant root extract peak was obtuse that means concentration is insufficient to reduce silver ions into Ag-NP while at very higher concentration also become insufficient for synthesis consequently; an optimum level is needed for its synthesis.

It was noticed from graphical analysis that below the 6 mg/ml concentration the values of λmax were almost alike that may be due to identical size and shape of nanoparticles in the formulation. All the samples remained as a quiet stable suspension for up to 1 month with no precipitate or lumps formation. Fig 03 shows how the blending of optimum concentration of plant root extract and AgNO3 with heating, stirring and time leads to synthesis of Ag-NP within 2-5 min and it takes maximum up to 25 min for its complete reduction and synthesis.

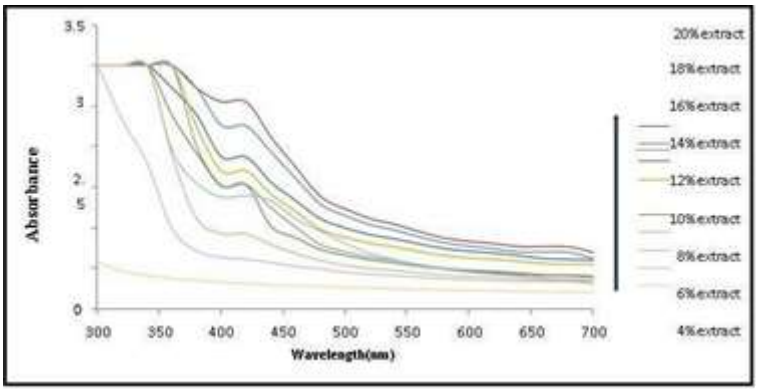

Figure 03: UV-visible absorption spectra of Ag-NPs synthesized by different concentrations of *A. acuminata*

plant extract Conc. (2, 4, 6, 8, 10, 12, 14, 16, 18 and 20%) against 1mM AgNO3 by Green synthesis Method.

Particle Size and Zeta Potential Analysis

The formulation was processed to determine the particle size and zeta potential using Nano Zeta Sizer ZS (Malvern,UK) equipment which act in accordance with the laser Doppler electrophoresis and dynamic light scattering phenomenon. The particle size and zeta potential of optimized formulation (10 mg/ml) was found to be 20 nm and -28 Mv respectively (figure 04). It was observed from report that the formulation was a stable colloidal suspension with no precipitations.

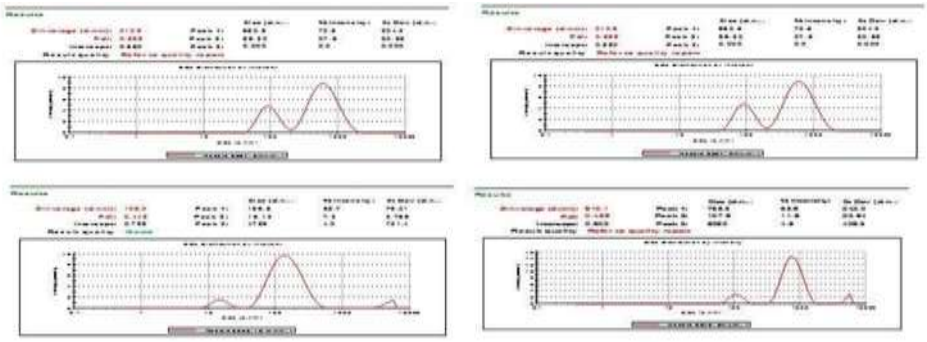

Figure 04: Particle size, PDI and Zeta Potential distribution Report produced by Nano Zeta Sizer ZS(Malvern, UK) equipment based on intensity (8 mg/ml, 10 mg/ml, 12 mg/ml, and 14 mg/ml of different *A. acuminata* Root Extract Concentration Ag-NPs.

Transmission Electron Microscopy

To ascertain the particle size, shape and morphological attributes of green synthesized Ag-NP of *A. acuminata* root extract, UHRTEM instrument was used which analyze spherical, oval shaped nanoparticles of average size ±20nm (Figure 05). The surface morphological features were recorded at atomic level by transmitting wave through the set of lattice plane of the crystals in formulation. Bright and dark stripped images of lattice revealed the fringes of silver metal in nanoparticles (Figure 05).

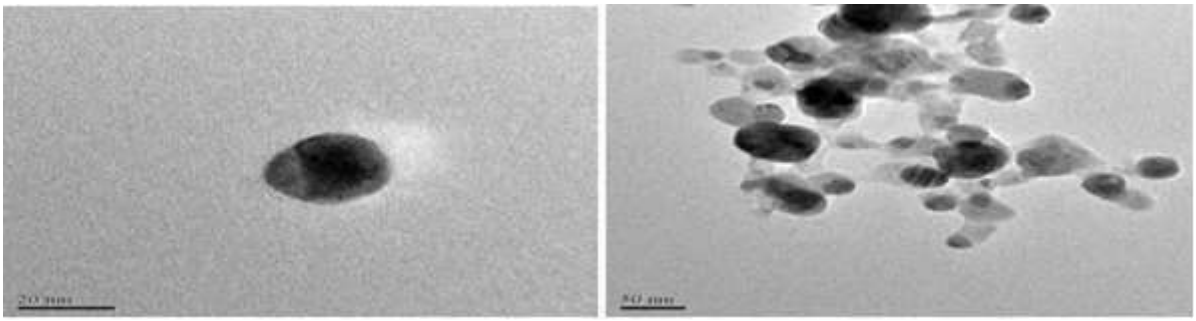

Figure 05: UHRTEM of Green Synthesized *A. acuminata* Ag-NPs emerged at optimum concentration (10 mg/ml)

Dispersive XRD Analysis

XRD analysis of green synthesized Ag-NP of Plant *A. acuminata* ethanolic root extract revealed the face centered cubic crystalline arrangement of nanoparticles in the formulation which is reflected by the peaks in the spectrum with distinct planes (Figure 06, 7a). Size of the nanoparticles are at all times the determining factor for XRD peak pattern [54]. XRD data displayed in figure 06 9manifest the five Bragg reflections characteristic 2θpeaksat 32.18°, 38.04°, 46.13°, 54.63°, and 77.08° of Ag-NPs with respect to the (120),

(206), (115), (207) and (317) planes respectively (Figure 06).

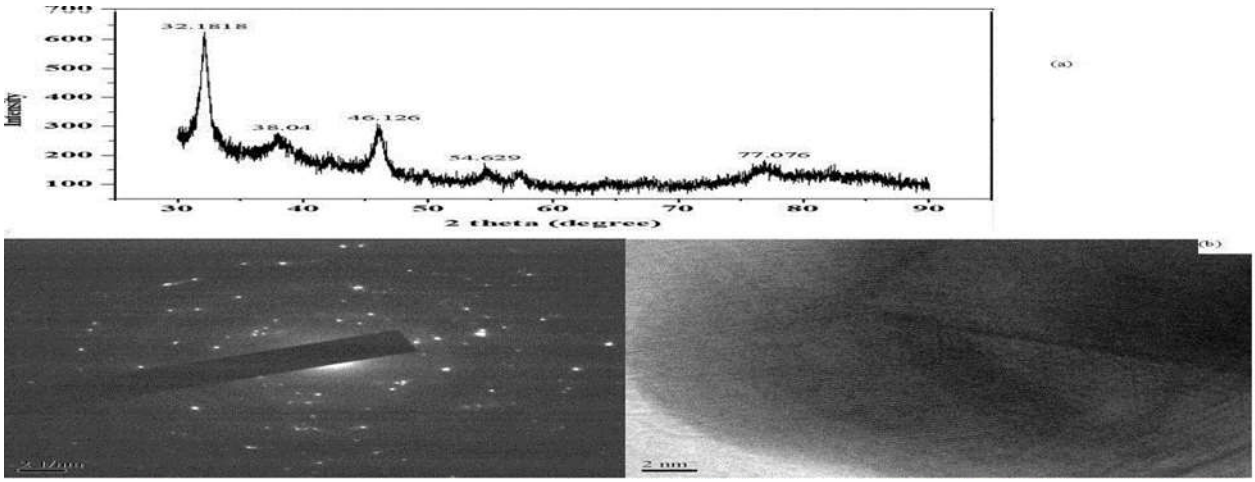

Figure 06: (a) XRD Pattern of the Green synthesized Ag-NP using *A. acuminata* Root Ethanolic extract (b) Crystalline confirmation by Electron Diffraction Pattern.

SEM and EDXA Analysis

Atomic force Microscopic and scanning electron microscopic analysis technique was used to ascertain the shape and morphological dimensions of silver nanoparticles [55]. Micrograph (Figure 07a) clearly indicates the approximate 20 nm sized spherical, tubular and some cuboidal shaped Ag-NPs which may be due to the availability of different reducing agents in extract phytochemicals. Micrograph analysis in Figure 07b, c and Table 03 revealed 72.43% weight percentage of Ag in formulation.

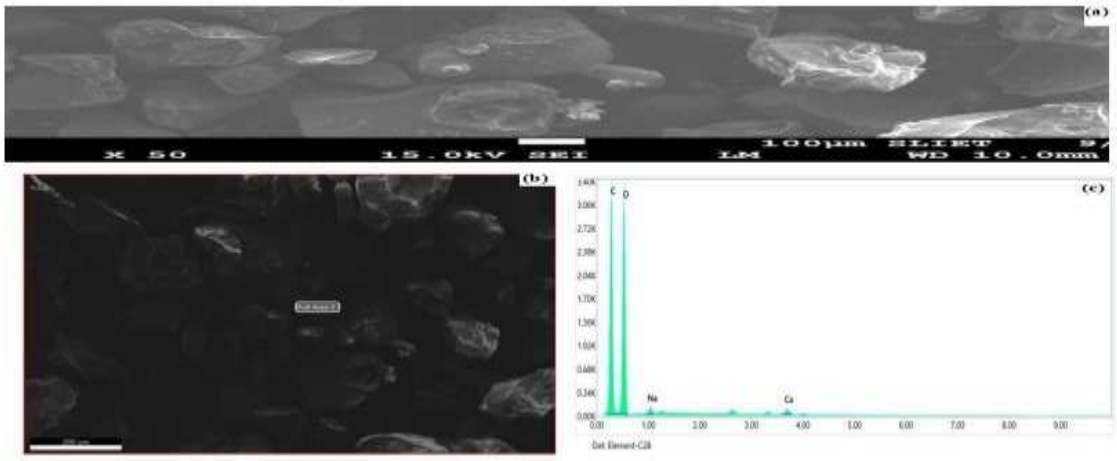

Figure No. 07: (a) Scanning electron microscopy Micrograph of Green synthesized Ag-NPs of *A. acuminata* Ethanolic Root Extract **(b)** EDXA micrograph Analysis of silver nanoparticles of *Atropa acuminata* plant Root Hydroethanolic Extract **(c)** and Corresponding EDX spectra.

Table 03: Weight and Atomic Percentage of Different Elements in Ag-NP of *A. acuminata* Ethanolic Root Extract

Element	Weight %	Atomic %
C, K	3.16	2.53
O , K	27.65	38.9
Na, K	7.6	3
Ca, K	14.7	6.3
Ag	72.43	28.45

Superoxide radical Antioxidant Assay

The antioxidant potential of Ag-NPs was measured on the basis of ability of Ag ions to quench the superoxide radical in a reaction mixture of formulation and free radicals. It was observed that superoxide radicals showed maximum inhibition 82.633±0.116 at 100 µg/mL while minimum 22.124± 0.203 at 10

µg/mL concentration when analyzed at 560 nm (Figure 08 and Table 04). The ability to reduce NBT by PMS- NADH coupling can determine the superoxide radicals emerged from dissolved oxygen [56].

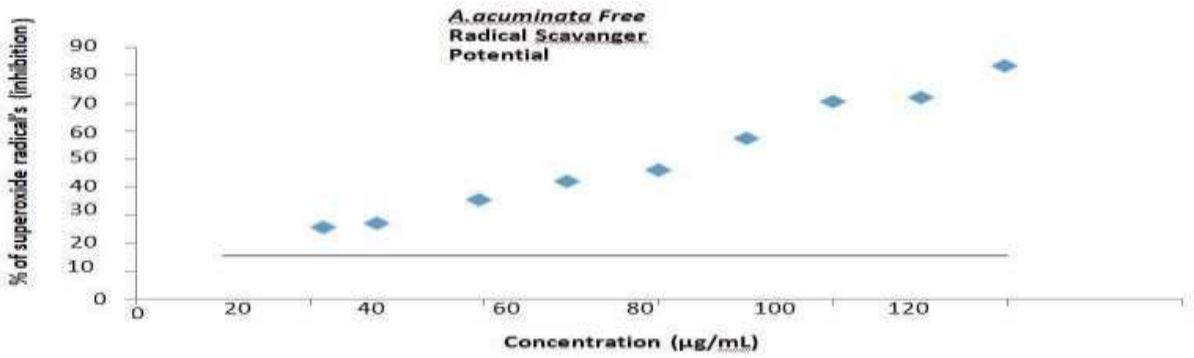

Figure 08: Antioxidant (Superoxide anion scavenging) activity of *A. acuminata* Root Extract Ag-NPs as percentage of superoxide anion radical's inhibition

Anti-diabetic Potential of AgNPs Nanoparticles Formulation

α-Glucosidase and α-Amylase Enzyme inhibitory activity

The α-glucosidase enzyme was significantly inhibited by the *A. acuminata* hydroethanolic extract AgNPs. The varied concentrations of Epigallocatechin gallate, i.e., 0.3, 0.5, and 0.7 µg, shown 51 ± 5.32%, 53 ± 4.43%, and 63 ± 6.76% of α- glucosidase enzyme inhibition, and the IC50 value was observed 48.4 µg/ml. In contrast, Ag-NPs, at concentrations of 25, 65, and 75 µg/ml, demonstrated 49 ± 7.65, 50± 6.54, 59± 7.65% inhibitions respectively at 405 nm (Figure 09, Table 04). While in αAmylase assay, on alike Acarbose concentrations percent inhibition was 56 ± 4.34, 61 ± 2.34, 68 ± 4.53 µg/ml and IC50 value was 52 .3 µg/ml. And for similar concentrations of Ag-NPs percent inhibition of α-Amylase was51 ± 3.76, 57 ± 5.43, 63 ± 1.32 µg/ml and IC50 value was 49.3 µg/mlat 540 nm respectively (Figure 09, Table 04).

Table 04:*In vitro* α-glucosidase and α-amylase enzyme inhibition by comparing %inhibition and I value of two samples of Standard &*A. acuminata* Root Hyroethanolic Extract at different concentrat (µg/ml)

Sample (Reference/Test)	Concentration (µg/ml)	Concentration (µg/ml) inhibition α-	IC50/(µg/ml)	Concentration (µg/ml) inhibition α-	IC50/(µg/m
α-glycosidase - Epigallocatechin gallate **α-amylase** -Acarbose	0.3 µg	51 ± 5.32	48.4	56 ± 4.34	52.3
	0.5 µg	53± 4.43		61 ± 2.34	
	0.7 µg	63± 6.76		68 ± 4.53	
Ag-NPs of Plant Root Hydroethanolic Extract	25 (µg/ml)	49 ± 7.65	39.3	51 ± 3.76	49.3
	65 (µg/ml)	50± 6.54		57 ± 5.43	
	75 (µg/ml)	59± 7.65		63± 1.32	

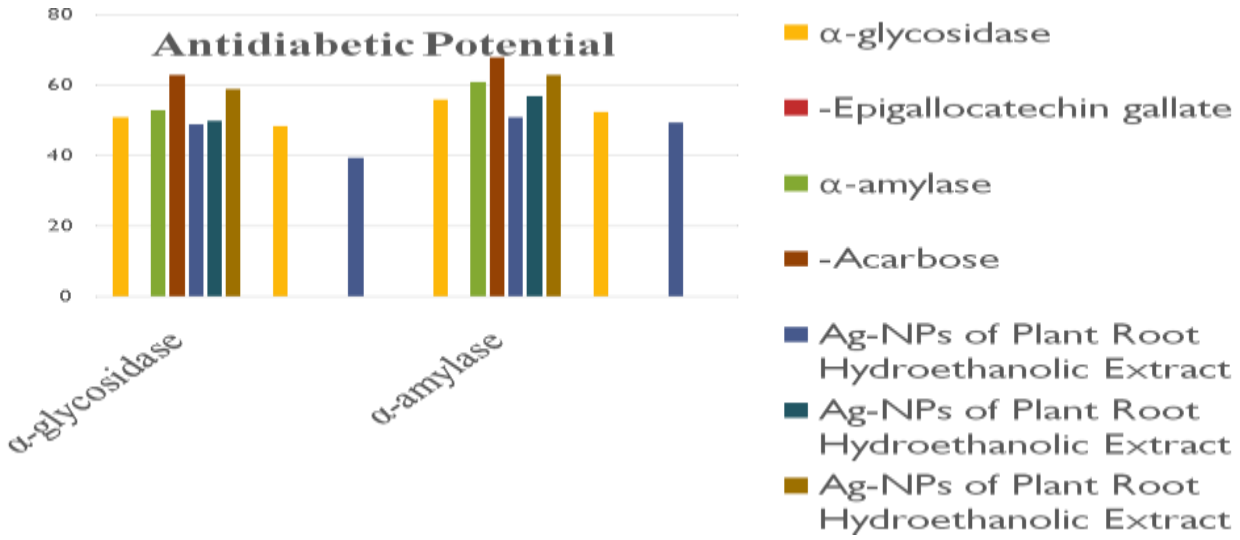

Figure 09: The comparison of percent inhibition (µg/ml), and IC50 value shown in the above figure for both Standard & Ag-NPs.

Drug Release Study

In drug release study, percent cumulative drug release for the both standard (atropine) and test sample (Ag- NPs) of various concentrations was calculated at different time interval by analyzing the absorbance under UV visible spectroscopy at 420 nm [57] (Table 05 and Figure 10). It was observed from the result that with the passage of time *in vitro* drug release for Reference was 99.60% and for Ag-NPs was 101.50% respectively (Table 05). All the data is expressed as the mean value plus the standard deviation minus one. Using SPSS software and the 5% threshold of significance, analysis was performed using the ANOVA test and the Duncan's multiple range tests.

Table 05: Percent cumulative release of Reference and Test sample at different Time Intervals

Sr. No.	Time (Min)	PercentCumulativeReference (%) Release	Percent Cumulative Release for NPs (%)
1.	5	39.80	18.50
2.	10	80.50	41.00
3.	15	97.40	60.60
4.	20	97.80	76.90
5.	25	97.70	90.00
6.	30	98.00	98.10
7.	45	99.60	101.50

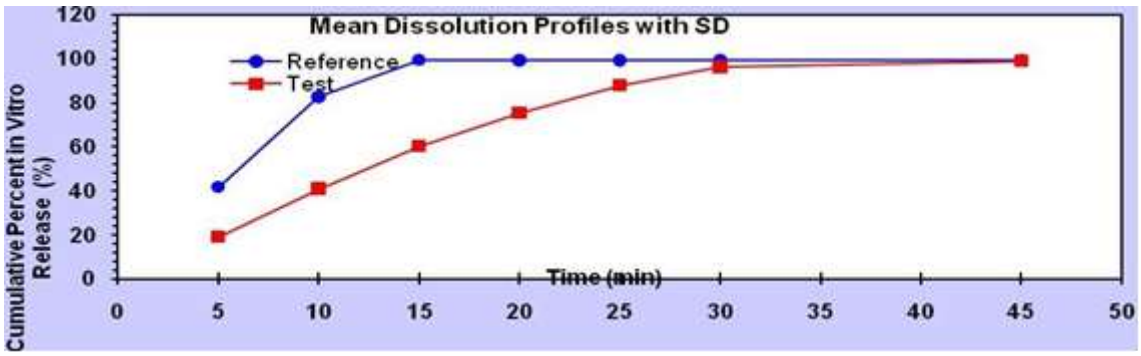

Figure 10: Cumulative percent in vitro release of Green Synthesized Silver Nanoparticles of Standard Atropine and Ag-NPs of *Atropa acuminata* Hydroethanolic Extract

4. Conclusion

In this research, green synthesis of Silver nanoparticles of *A. acuminata* hydroethanolic root extract was carried out to check its antioxidant and anti-diabetic potential. UV spectral analysis, TEM, SEM, EDXA confirm the spherical, cuboidal shaped 20 nm nanosized particles. XRD revealed the crystalline lattice of nanoparticles.Synthesis of silver nanoparticles wassupported by the phytochemicals available in plant extract which work as reducing agent for the reduction of silver ions to nanoparticles. This method of Nano architecture was very simple, easy, economic, ecofriendly, biocompatible and much superior to chemical synthesis method. Nanoparticles shows maximum scavenging potential 82.633±0.116 for superoxide anion free radicals at 100 µg/mL concentration. Among two anti-diabetic assay, α-amylase assay shows better result of percent inhibition 63 ± 1.32 at 75 µg/ml concentration. Lastly *in vitro*, drug release study revealed the 101.50% cumulative release from Ag-NPs formulation up to 1 hour which was found to be better than the standard one. It indicated that the plant have excellent antioxidant and anti-diabetic potential. So, there should be more and more germination of this critically endangered species for medicinal worth prospectives.

Acknowledgement

Authors like to acknowledge IIT, Delhi for TEM Central Instrumental Facility, LPU for Nano Sizer, SLIET Central University for XRD, SEM, EDXA and Shiva Institute, HPTU for providing facility of synthesizing formulation and to carry out Drug Release Study.

Funding

This research did not receive any specific grant from funding agencies in the public and commercial sectors.

Conflict of interest

The authors declare no conflict of interest.

References:

1. Lu, B., Mi, H., Zhu, Y., & Piggott, J. (2017). A sustainable long-term health care system for aging China: A case study of regional practice. *Health Systems & Reform*, *3*(3), 182-190.
2. Kong, F. Y., Zhang, J. W., Li, R. F., Wang, Z. X., Wang, W. J., & Wang, W. (2017). Unique roles of gold nanoparticles in drug delivery, targeting and imaging applications. *Molecules*, *22*(9), 1445.
3. Ren, B., Wan, Y., Wang, G., Liu, Z., Huang, Y., & Wang, H. (2018). Morphologically modified surface with hierarchical micro-/nano-structures for enhanced bioactivity of titanium implants. *Journal of Materials Science*, *53*(18), 12679-12691.
4. Marchioni, M., Jouneau, P. H., Chevallet, M., Michaud-Soret, I., & Deniaud, A. (2018). Silver nanoparticle fate in mammals: Bridging in vitro and in vivo studies. *Coordination Chemistry Reviews*, *364*, 118-136.
5. Khan, M., Al-Marri, A. H., Khan, M., Shaik, M. R., Mohri, N., Adil, S. F., ... & Siddiqui, M. R. H. (2015). Green approach for the effective reduction of graphene oxide using Salvadora persica L. root (Miswak) extract. *Nanoscale research letters*, *10*, 1-9.
6. Li, H., Qiao, R., Davis, T. P., & Tang, S. Y.(2020). Biomedical applications of liquid metal nanoparticles: a critical review. *Biosensors*, *10*(12), 196.
7. Roknuzzaman, M., Zhang, C., Ostrikov, K., Du, A., Wang, H., Wang, L., & Tesfamichael, T. (2019). Electronic and optical properties of lead-free hybrid double perovskites for photovoltaic and optoelectronic applications. *Scientific reports*, *9*(1), 718.
8. Zhang, X. F., Liu, Z. G., Shen, W., & Gurunathan, S. (2016). Silver nanoparticles: synthesis, characterization, properties, applications, and therapeutic approaches. *International journal of molecular sciences*, *17*(9), 1534.
9. Fonseca-Santos, B., Gremião, M. P. D., & Chorilli, M. (2015). Nanotechnology-based drug delivery systems for the treatment of Alzheimer's disease. *International Journal of nanomedicine*, 4981-5003.
10. Kiseleva, R. Y., Glassman, P. M., Greineder, C. F., Hood, E. D., Shuvaev, V. V., & Muzykantov, V. R. (2018). Targeting therapeutics to endothelium: are we there yet?. *Drug delivery and translational research*, *8*, 883-902.

11. Tahwia, A. M., El-Far, O., & Amin, M. (2022). Characteristics of sustainable high strength concrete incorporating eco-friendly materials. *Innovative Infrastructure Solutions*, *7*, 1-13.
12. Uddin, A. I., Phan, D. T., & Chung, G. S. (2015). Low temperature acetylene gas sensor based on Ag nanoparticles-loaded ZnO-reduced graphene oxide hybrid. *Sensors and Actuators B: Chemical*, *207*, 362- 369.
13. Yang, Y., He, J., Li, Q., Gao, L., Hu, J., Zeng, R., ... & Wang, Q. (2019). Self-healing of electrical damage in polymers using superparamagnetic nanoparticles. *Nature nanotechnology*, *14*(2), 151-155.
14. Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D. G., & Lightfoot, D. A. (2017). Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants*, *6*(4), 42.
15. Singh, R., Shedbalkar, U. U., Wadhwani, S. A., & Chopade, B. A. (2015). Bacteriagenic silver nanoparticles: synthesis, mechanism, and applications. *Applied microbiology and biotechnology*, *99*, 4579-4593.
16. Carnegie, A. J., Kathuria, A., Pegg, G. S., Entwistle, P., Nagel, M., & Giblin, F. R. (2016). Impact of the invasive rust Puccinia psidii (myrtle rust) on native Myrtaceae in natural ecosystems in Australia. *Biological Invasions*, *18*, 127-144.
17. Hedayati, A., Hemmaty, S., Nourozi, E., & Amirsadeghi, A. (2021). Effect of yeast extract on h6h gene expression and tropane alkaloids production in Atropa belladonna L. hairy roots. *Russian Journal of Plant Physiology*, *68*, 102-109.
18. Wangchuk, P., & Tobgay, T. (2015). Contributions of medicinal plants to the Gross National Happiness and Biodiscovery in Bhutan. *Journal of ethnobiology and ethnomedicine*, *11*, 1-13.
19. González-Gómez, L., Morante-Zarcero, S., Pérez-Quintanilla, D., & Sierra, I. (2022). Occurrence and chemistry of tropane alkaloids in foods, with a focus on sample analysis methods: A review on recent trends and technological advances. *Foods*, *11*(3), 407.
20. Shi, Z., Zou, W., Zhu, Z., Xiong, Z., Li, S., Dong, P., & Zhu, Z. (2022). Tropane alkaloids (hyoscyamine, scopolamine and atropine) from genus Datura: Extractions, contents, syntheses and effects. *Industrial Crops and Products*, *186*, 115283.
21. Smith, F. (2022). Therapeutics within a Naturopathic Approach. In *Naturopathic Medicine: A Comprehensive Guide* (pp. 129-202). Cham: Springer International Publishing.
22. Tariq, A., Mussarat, S., Adnan, M., Abd_Allah, E. F., Hashem, A., Alqarawi, A. A., & Ullah, R. (2015). Ethnomedicinal evaluation of medicinal plants used against gastrointestinal complaints. *BioMed research international*, *2015*.
23. Das, P., Ghosal, K., Jana, N. K., Mukherjee, A., & Basak, P. (2019). Green synthesis and characterization of silver nanoparticles using belladonna mother tincture and its efficacy as a potential antibacterial and anti-inflammatory agent. *Materials Chemistry and Physics*, *228*, 310-317.
24. Kantati, Y. T., Kodjo, K. M., Dogbeavou, K. S., Vaudry, D., Leprince, J., & Gbeassor, M. (2016). Ethnopharmacological survey of plant species used in folk medicine against central nervous system disorders in Togo. *Journal of ethnopharmacology*, *181*, 214-220.
25. Tuia, D., Kellenberger, B., Beery, S., Costelloe, B. R., Zuffi, S., Risse, B., ... & Berger-Wolf, T. (2022). Perspectives in machine learning for wildlife conservation. *Nature communications*, *13*(1), 792.
26. Sher, H., & Barkworth, M. E. (2015). Economic development through medicinal and aromatic plants (MAPs) cultivation in Hindu Kush Himalaya mountains of District Swat, Pakistan. *Journal of Mountain Science*, *12*, 1292-1301.
27. McCauley, D. J., Hardesty-Moore, M., Halpern, B. S., & Young, H. S. (2017). A mammoth undertaking: harnessing insight from functional ecology to shape de-extinction priority setting. *Functional Ecology*, *31*(5), 1003-1011.
28. Travlos, I., Gazoulis, I., Kanatas, P., Tsekoura, A., Zannopoulos, S., & Papastylianou, P. (2020). Key factors affecting weed seeds' germination, weed emergence, and their possible role for the efficacy of false seedbed technique as weed management practice. *Frontiers in Agronomy*, *2*, 1.
29. Dar, S. A., Nawchoo, I. A., Tyub, S., & Kamili, A. N. (2022). In vitro culture and biochemical and antioxidant potential of the critically endangered medicinal plant Atropa acuminata Royle ex Lindl of Kashmir Himalaya. *In Vitro Cellular & Developmental Biology-Plant*, *58*(4), 540-550.
30. Mahmood, N. (2016). A review of α-amylase inhibitors on weight loss and glycemic control in pathological state such as obesity and diabetes. *Comparative Clinical Pathology*, *25*(6), 1253-1264.
31. Vancamelbeke, M., & Vermeire, S. (2017). The intestinal barrier: a fundamental role in health and disease. *Expert review of gastroenterology & hepatology*, *11*(9), 821-834.
32. Assefa, S. T., Yang, E. Y., Chae, S. Y., Song, M., Lee, J., Cho, M. C., & Jang, S. (2019). Alpha glucosidase inhibitory activities of plants with focus on common vegetables. *Plants*, *9*(1), 2.
33. Xia, W., Wang, Y., Bi, R., & Wu, J. (2022). High yield synthesis of nigerooligosaccharides by transglycosylation catalyzed by α-glucosidase TaAglA from Thermoplasma acidophilum. *Food Bioscience*, *47*, 101582.

34. Quazi, A., Patwekar, M., Patwekar, F., Alghamdi, S., Rajab, B. S., Babalghith, A. O., & Islam, F. (2022). In vitro alpha-amylase enzyme assay of hydroalcoholic polyherbal extract: proof of concept for the development of polyherbal teabag formulation for the treatment of diabetes. *Evidence-based Complementary and Alternative Medicine*, *2022*.
35. Rahman, K., Khan, S. U., Fahad, S., Shinwari, Z. K., Khan, D., Kamal, S., ... & Noor, M. (2018). In vitro biological screening of a critically endangered medicinal plant, Atropa acuminata Royle Ex Lindl of north western Himalaya. *Scientific Reports*, *8*(1), 11028.
36. Bhat, M. N., Singh, B., Surmal, O., Singh, B., Shivgotra, V., & Musarella, C. M. (2021). Ethnobotany of the Himalayas: Safeguarding medical practices and traditional uses of Kashmir regions. *Biology*, *10*(9), 851.
37. Pandey, V., Bhatt, I. D., & Nandi, S. K. (2019). Environmental stresses in Himalayan medicinal plants: research needs and future priorities. *Biodiversity and Conservation*, *28*, 2431-2455.
38. Kai, G., Zhao, W., Shi, M., & Wang, Y. (2018). Biotechnological interventions of hairy roots of Tropane alkaloid-bearing plants. *Hairy Roots: An Effective Tool of Plant Biotechnology*, 71-93.
39. Tungmunnithum, D., Thongboonyou, A., Pholboon, A., & Yangsabai, A. (2018). Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. *Medicines*, *5*(3), 93.
40. Castillo-Henríquez, L., Alfaro-Aguilar, K., Ugalde-Álvarez, J., Vega-Fernández, L., Montes de Oca- Vásquez, G., & Vega-Baudrit, J. R. (2020). Green synthesis of gold and silver nanoparticles from plant extracts and their possible applications as antimicrobial agents in the agricultural area. *Nanomaterials*, *10*(9), 1763.
41. Sre, P. R., Reka, M., Poovazhagi, R., Kumar, M. A., & Murugesan, K. (2015). Antibacterial and cytotoxic effect of biologically synthesized silver nanoparticles using aqueous root extract of Erythrina indica lam. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *135*, 1137-1144. [42] Tietze, R., Zaloga, J., Unterweger, H., Lyer, S., Friedrich, R. P., Janko, C., ... & Alexiou, C. (2015). Magnetic nanoparticle-based drug delivery for cancer therapy. *Biochemical and biophysical research communications*, *468*(3), 463-470.
42. Mudalige, T., Qu, H., Van Haute, D., Ansar, S. M., Paredes, A., & Ingle, T. (2019).
43. Characterization of nanomaterials: Tools and challenges. *Nanomaterials for food applications*, 313353. 44.Levingstone, T. J., Herbaj, S., & Dunne, N. J. (2019). Calcium phosphate nanoparticles for therapeutic applications in bone regeneration. *Nanomaterials*, *9*(11), 1570.
45. Arif, M., Ullah, R., Ahmad, M., Ali, A., Ullah, Z., Ali, M., ... & Sher, H. (2022). Green synthesis of silver nanoparticles using Euphorbia wallichii leaf extract: Its antibacterial action against citrus canker causal agent and antioxidant potential. *Molecules*, *27*(11), 3525.
46. Borchers, T. E., Seid, A., Babu, S. S., Shafer, P., & Zhang, W. (2015). Effect of filler metal and post-weld friction stir processing on stress corrosion cracking susceptibility of Al–Zn–Mg arc welds. *Science and Technology of Welding and Joining*, *20*(6), 460-467.
47. Ramos, K., Gómez-Gómez, M. M., Cámara, C., & Ramos, L. (2016). Silver speciation and characterization of nanoparticles released from plastic food containers by single particle ICPMS. *Talanta*, *151*, 83-90.
48. Tanase, C., Berta, L., Coman, N. A., Roșca, I., Man, A., Toma, F., ... & Mare, A. (2019). Antibacterial and antioxidant potential of silver nanoparticles biosynthesized using the spruce bark extract. *Nanomaterials*, *9*(11), 1541.
49. Connolly, M., Fernandez-Cruz, M. L., Quesada-Garcia, A., Alte, L., Segner, H., & Navas, J. M. (2015). Comparative cytotoxicity study of silver nanoparticles (AgNPs) in a variety of rainbow trout cell lines (RTL-W1, RTH-149, RTG-2) and primary hepatocytes. *International journal of environmental research and public health*, *12*(5), 5386-5405.
50. Zafar, M., & Iqbal, T. (2022). Green synthesis of silver and zinc oxide nanoparticles for novel application to enhance shelf life of fruits. *Biomass Conversion and Biorefinery*, 1-16.
51. Ukwatta, K. M., Lawrence, J. L., & Wijayarathna, C. D. (2019). The study of antimicrobial, anticancer, anti-inflammatory and α-glucosidase inhibitory activities of Nigronapthaphenyl, isolated from an extract of Nigrospora sphaerica. *Mycology*, *10*(4), 222-228.
52. Gomathi, A. C., Rajarathinam, S. X., Sadiq, A. M., & Rajeshkumar, S. (2020). Anticancer activity of silver nanoparticles synthesized using aqueous fruit shell extract of Tamarindus indica on MCF-7 human breast cancer cell line. *Journal of drug delivery science and technology*, *55*, 101376.
53. Zou, T. B., He, T. P., Li, H. B., Tang, H. W., & Xia, E. Q. (2016). The structure-activity relationship of the antioxidant peptides from natural proteins. *Molecules*, *21*(1), 72.
54. Zhang, H., Jung, J., & Zhao, Y. (2016). Preparation, characterization and evaluation of antibacterial activity of catechins and catechins–Zn complex loaded β-chitosan nanoparticles of different particle sizes. *Carbohydrate Polymers*, *137*, 82-91.

55. Reddy, N. J., Vali, D. N., Rani, M., & Rani, S. S. (2014). Evaluation of antioxidant, antibacterial and cytotoxic effects of green synthesized silver nanoparticles by Piper longum fruit. *Materials Science and Engineering: C*, *34*, 115-122.
56. Kalidindi, N., Thimmaiah, N. V., Jagadeesh, N. V., Nandeep, R., Swetha, S., & Kalidindi, B. (2015). Antifungal and antioxidant activities of organic and aqueous extracts of Annona squamosa Linn. leaves. *Journal of food and drug analysis*, *23*(4), 795-802.
57. Ganjali, M., Ganjali, M., Aljabali, A. A., & Barhoum, A. (2022). Drug delivery systems based on nano- herbal medicine. In *Bionanotechnology: Emerging Applications of Bionanomaterials* (pp. 491530). Elsevier.