Orbital
The electronic journal of chemistry

the electronic journal of chemistry

Full Paper | http://dx.doi.org/10.17807/orbital.v17i6.22299

Anti-Inflammatory, Anti-leishmanial, Catalytic, Anticoagulant and Thrombolytic Effects of Silver Nanoparticles (AgNPs) Synthesized from *Datura stramonium* Extracts

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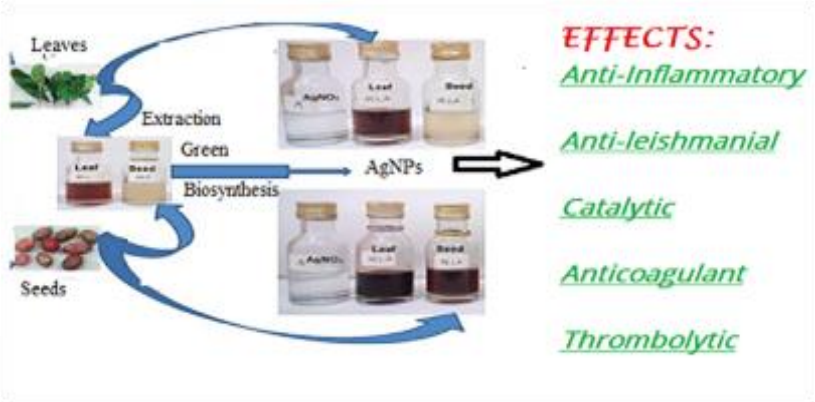
Datura stramonium, commonly known as thorn apple, jimson weed, devil's snare, or devil's trumpet is an important medicinal plant. It belongs to Kingdom: Plantae, Order Solanales, Family: Solanaceae, Genus Datura, and Species: *D. stramonium*. It is used in traditional medicine to treat many ailments, including skin eruptions, abscesses, inflammation, boils and acne. Leishmaniasis is an important parasitic problem and is in focus for development of new drugs for it all over the world. Anti-leishmanial activity was performed against *Leishmania tropica* KWH23 promastigote. Potent anti-leishmanial activity was observed for *D. stramonium* methanol extract (IC50 = 10.9 ± 1.1 μg/mL) in comparison to other plant extracts. However, *D. stramonium* "ethyl acetate fraction" was more active (IC50 = 5.3 ± 0.2 μg/mL) against *Leishmania tropica* KWH23 among all plant fractions as well as standard Glucantime drug (6.0 ± 0.1 μg/mL). In the present work, we report the phytosynthesis of AgNPs mediated by seed and leaf extracts of *D. stramonium*. The extracts catalyzed the formation of brown colloidal AgNPs, which stabilized in 10 min. The seed and leaf AgNPs yielded surface plasmon resonance at 440 and 438.5 nm, respectively. Prominent peaks at 3408, 2357, 2089, and 1639 cm^{-1} were recorded for seed AgNPs, whereas 3404, 2368, 2081, and 1641 cm^{-1} were revealed for leaf-mediated AgNPs from Fourier transform infrared data. The particles were fairly spherical and crystalline in nature having size of 4–26 nm, with prominence of silver in the colloidal solutions. Using 20 µg/mL of AgNPs, malachite green was degraded by approximately 80% in 24 h. Similarly, the particles displayed blood anticoagulant activities as well as achieved thrombolysis. The AgNPs can be explored for biomedical and catalytic applications.

Graphical abstract



Keywords

Cytokines
Anti-inflammation
Anti-leishmanial
Silver nanoparticles
Thrombolysis

Article history

Received 19 Nov 2024
Revised 18 Marc 2025
Accepted 27 May 2025
Available online 18 Feb 2026

Handling Editor: Adilson Beatriz

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1. Introduction

Datura stramonium (*D. stramonium*) is a widespread annual plant from the Solanaceae family. It is one of the widely well known folklore medicinal herb. It is a wild growing flowering plant and was investigated as a local source for tropane alkaloids which contain a methylated nitrogen atom (N-CH_3) and include the anti-cholinergic drugs atropine, and scopolamine. Studies have shown that *D. stramonium* have many biological activities, which include anti-inflammatory, anti-leishmanial, catalic, anti-coagulant and thrombolytic activities [1]. About ten species of *Datura* are found, of which *Datura anoxia* and *D. stromonium* are most important drug plants. *Datura* has long been known as a medicinal plant and as a plant hallucinogen all over the world. Pre-historic use of *Datura* in medicinal and ceremonial rituals could be observed in aboriginal in Indian sub-continent [2]. The therapeutic activities of most plants are due to the presence of one or more of such components like alkaloids, tannins, saponins and cardiac glycosides. The phytochemical screening revealed the presence of saponins, tannins, steroids, alkaloids, flavonoids, phenols and glycosides [3]. Atropine and scopolamine are competitive antagonists of muscarinic cholinergic receptors and are central nervous system depressants. All parts of the plant are toxic, but the highest amount of alkaloids is contained in the ripe seeds [4]. Leishmaniasis is an important protozoal disease caused by parasite belonging to genus Leishmania. Sand fly is the responsible vector for its transmission. Leishmaniasis is a major health risk and a danger to 350 million populations throughout the world. Approximately, 12 million peoples are currently infected and every year around 1-2 million new cases are appearing [5]. This disease may vary in its presentation; it can be self-healing or fatal. Various infectious types of leishmaniasis can be categorized as (a) cutaneous leishmaniasis (b) mucocutaneous leishmaniasis and (c) visceral leishmaniasis. Hereby, about 0.5 and 1.5 million cases of visceral and cutaneous leishmaniasis are reported, respectively.

All body parts especially the exposed ones are mainly targeted by cutaneous leishmaniasis. The nascent lesion may give rise to an ulcer. Systemic leishmaniasis affects internal organs of a body, that is, spleen and liver, rare in India but can prove to be fatal. The multiple ulcers resulting from multiple bites of sand fly are not a rare case in India. On the other hand, it is rapidly spreading out creating an alarming situation. Cutaneous and visceral leishmaniasis is the major threats to India, despite of mucocutaneous leishmaniasis which is rarely reported [1]. The treatments currently in use as prime therapy include meglumine antimoniate (Glucantime) in addition to penta-valent antimonials sodium stibogluconate (Pentostam) but they are harmful to some extent, require prolong parenteral administration courses, and have virulent side effects [3]. In some cases, pentamidine as well as amphotericin B are used as second line treatment which may also have lethal affects [6]. There is a need to develop novel drugs to counter leishmaniasis due to the existence of various hazards which include high cost of current medicines [7] along with their possible toxic effects [8] and resistance development in parasites [9]. Because of these problems, scientists are in continuous search to find new drugs against these infections. Naturally existing medicinal herbs and plants are considered to be the rich supply of a variety of useful organic substances. As natural compounds are considered good and affluent source of bioactive compounds having anti-leishmanial ability [10], there is a need for the identification and analysis of various natural products originated from different medicinal plants so that new drugs can be synthesized [11].

The fabrication of nanoparticles through green route has attracted special attention of scientists in the growing area of nanotechnology because of the simplicity of procedure, the non use of hazardous chemicals or techniques, the low consumption of energy, and the increased level of biocompatibility for applications in the living systems. In addition, the large-scale production of nanoparticles can be easily achieved through this means, whereas the abundance of biomolecules that can serve as bioreductants in the green synthesis of nanoparticles in diverse living things also contributes enormously to the growing trend in green nanotechnology. In this regard, biomolecules obtained from bacteria [12], fungi [13], macro- and microalgae [14], arthropods [15], and different parts of green plants [16] have been used to synthesize a wide range of nanoparticles under ambient conditions. We have documented the use of Colanitida seeds, seed shell and pod [17, 18], cocoa pod husk [19], spider cobweb [20] to synthesize AgNPs. Among diverse metallic nanoparticles that have been studied, AgNPs occupy prime position because of several applications alluded to them. Their optical, electronic, catalytic, antimicrobial, and electrochemical attributes have greatly influenced their relevance in diverse areas such as in food, health care, agriculture, biomedical, environmental, textile, and catalytic applications [21]. Suffice to say that the list of applications of AgNPs seems endless as new areas of relevance emerge very often. The green synthesis of AgNPs also contributes to the upsurge in the applications of AgNPs, as the improved compatibility with biological systems through the avoidance of hazardous procedures is achieved. Therefore, newer sources of biomolecules from diverse living entities are continually sought to catalyze the biological synthesis of nanoparticles for various applications in different areas of human endeavors. An important emerging area of application of biosynthesized AgNPs is in the management of blood coagulation disorders, whereby nanoparticles can be used to prevent aggregation of platelets [22] to inhibit blood coagulation. Similarly, nanoparticles can be used alone or as carriers of active drugs for the efficient and timely dissolution of blood clots, thereby preventing the untold outcome of the formation of blood clot (thrombus), which may include ischemia or stroke arising from blood coagulation and cardiovascular disorders. The fruit as the source of miraculin can be used for the control of diabetes, whereas several other active principles, including pigment and phytochemicals, can be formulated into pharmaceutical products [23]. Owing to richness in biologically active phytochemicals, different parts of the plant have been used in folklore medicine. The leaves have been reportedly used to treat diabetes, malaria, hyperthermia, hemorrhoids, and enuresis, and the seeds can be used for the treatment of stomach ache, anemia, and obesity [24,25]. Similarly, the roots of the plant have been used in the treatment of cough and tuberculosis, and the bark is used to manage prostate problems [24]. In this work, the usefulness of leaf and seed extracts of *Datura Stramonium* to synthesize AgNPs as well as the evaluation of the anti-inflammatory, anti-leishmanial, catalytic anti-coagulant and thrombolytic actions of the biosynthesized nanoparticles were conducted.

2. Material and Methods

The plant is easily cultivated, growing well in open, sunny situation. It flourishes in most moderately good soil but grows best in calcareous rich soil, or in a good sandy loam, with leaf mould added. Seeds are sown in open in May, in drill 3 feet apart, barely covered. In August the plant reaches to a height of 1 meter and bears flowers to give fruits. In the end of August stems with leaves and flowering tops were collected and shade dried under 45 °C to 50 °C. The leaves and seeds obtained from these dried stems were again dried at room temperature (30°C ± 2°C) for 5 days, after which dried samples were blended into powder.

Extract Preparation

After collection, plant samples were shade dried till the complete removal of moisture and samples were made to mesh sized powder by using plant grinder. Powders (5kg) of each sample were soaked in crude methanol (10L) for extraction for 72 hours. Both the samples were processed two times repeating above procedure. For the purpose of filtration, Whatman's No. 1 filter was used and methanol was evaporated on a rotary evaporator at 40◦C under reduced pressure.

***Fractionation*:** For finding the compounds in the crude extract with increasing polarity, crude extract (6g) was suspended in distilled water (250mL) and passed to liquid liquid partition by using solvents in order of n-hexane, chloroform, ethylacetate and n-butanol. The residue left behind after n-butanol fraction was termed as aqueous fraction (Figure 1). Rotary evaporator was used to concentrate the fraction by evaporating the solvent under reduced pressure at 40◦C. Fractions were further dried under dark and weighed and stored at 4◦C for phytochemical and pharmacological evaluation.

***Phytochemical Analysis*:** Qualitative phytochemical investigation of extracts and fractions for the existence of alkaloids, saponins, terpenoids [26], anthraquinones, cardiac glycosides, coumarins, phlobatannins [27], flavonoids, phenolics, and tannins [28] was performed.

Extracts obtained from the leaf and seeds of *D. stramonium* were used for the phytosynthesis of AgNPs using previously described procedures [27, 28] by allowing plant extract to react with silver nitrate (Merck) under ambient condition. The timely development and stabilization of color as function of the formation of AgNPs were monitored. The colloidal AgNPs were subjected to UV-Vis spectroscopy by scanning the absorbance within 240–850 nm on spectrophotometer, whereas Fourier transform infrared (FTIR) spectra were obtained on IR Affinity-1S Spectrometer. TEM analysis was conducted using JEM-1400 operated at 200 kV as previously described [19].

Cell Culture

The human monocytic leukaemia cellline, THP-1 was obtained. THP-1 cells were cultured in RPMI (1640) medium containing 10 % FBS and 1 % Pen-strep. The cells were incubated at 37◦C in a humidified atmosphere of 5% CO_2 in a SL SHEL LAB incubator (Sheldon manufacturing, Cornelius, OR, USA). THP-1 monocytes were differentiated into macrophages using PMA. The THP-1 monocytes were seeded in 24-well plates (500 µL) at a density of 2×10^5 cells/mL and were treated with 100 nM PMA. These cells were incubated at 37◦C in a humidified atmosphere of 5 % CO_2. After 3 days of incubation, the culture media was replaced with PMA-free media (resting phase) for another 24 h.

Cell viability was evaluated using the WST-1 assay, according to the manufacturer's protocol, this assay was conducted to determine the toxicity of the prepared AgNPs on the differentiated THP-1 macrophages. Using 96-well cell culture plates, the macrophages were treated with different concentrations of the *D. stramonium*-AgNPs (20, 10, 5, 2.5, 1.3, 0.6 and 0.3 µg/mL). The cells were incubated for 24 h at 37 ◦C in a humidified atmosphere of 5 % CO_2. After incubation, the medium was removed from the wells and replaced with a medium containing 10% WST-1 reagent. The cells were incubated for another 3 h, and the absorbance of the culture media was measured at 440 nm using the POLAR star Omega plate reader. Cell viability was expressed as a percentage of the absorbance of treated cells to control (untreated) cells.

Stock solution for anti-leishmanial assay was prepared by dissolving 5mg/ml of each plant extract and derived fractions in 1 mL of DMSO (dimethyl sulfoxide). Stock solutions were further diluted serially (2500, 1250, 625, 312.5, 156.3, 78.1, 39.1, and 19.5μg/mL) using DMSO to obtain the concentration from 333.3 to 1.3 μg/mL in the wells. Samples were filtered by using a 0.45μm syringe filter. *Leishmania tropica* KWH23 was previously isolated from a patient in medical college Bhopal, M.P, and was characterized (data not shown). The promastigotes form of the Leishmania were grown in M199 medium with 10% fetal calf serum (FCS), HEPES buffer, streptomycin and penicillin. Log phase promastigotes at 1×10^6/100 μL were used for the entire assay. About 90μL of 199 media, 50μL of *Leishmania tropica* KWH23 log phase culture, and 10μL of each plant dilution were dispensed to different wells of microtitter plate. Here, DMSO was used as a negative control while Glucantime as positive control. Afterwards, microtitter plate was incubated at 24◦C for72h. After incubation, about15 μL of each dilution was pipetted on a neubauer counting chamber and was counted under a microscope.

Degradation of dye

The degradation of malachite green was undertaken by mixing 20 µg/mL of AgNPs with malachite green (40 ppm) in a 1:10 ratio. Mixtures of the dye and extracts alone were set up as control samples. The mixtures were kept on rotary shaker at 100 rpm for up to 24 h. At periodic intervals, samples were taken, and absorbance readings were obtained. The degradation of malachite green was determined; thus,

$$\text{Percentage dye degradation} == \frac{A\ \text{control} - A\ test}{A\ control} \text{x } 100$$

Where, A is the absorbance at 619 nm.

Blood anti-coagulation and thrombolytic activities

AgNPs were investigated for their anti-coagulant properties using the blood sample freely given by a healthy donor. Using AgNPs of 100µg/mL, 0.5mL was dispensed in clean, grease-free bottle, to which fresh human blood (5 mL) was added. A series of control experiments were set up, and these included a collection of blood into an ethylenediaminetetraacetic acid (EDTA) container (positive control) and an ordinary clean bottle (negative control). All the experiments were conducted at 30°C ± 2°C and monitored for the formation of blood clot. Microscopic investigation of the samples was undertaken by smearing blood samples on clean, grease-free slides and observed under the Olympus

microscope. The determination of thrombolytic activities of AgNPs was conducted as previously described [29] by treating preformed blood clots with 0.2 mL of 100 µg/mL AgNPs on the slide. Similarly, control samples were set up by treating blood clots with the extracts of *S. dulcificum* and $AgNO_3$ solution. All the experiments were conducted at 30°C ± 2°C, and thrombolysis (dissolution of blood clot) was monitored. Microscopic images of the contents were also obtained.

3. Results and Discussion

The human monocytes THP-1 cells, used herein to evaluate immunomodulatory activity, were cultured in RPMI and differentiated into macrophages using 100 nM of PMA. PMA, acognate of diacylglycerol, is an activator of protein kinase that is used in cell differentiation [30]. Upon differentiation, there are morphological changes that occur within the cells. In comparison to undifferentiated THP-1, differentiated THP-1 cells are larger, adherent and show expansion of the cytoplasm and of cytoplasmic organelles such as mitochondria and lysosomes [31]. Other differentiation stimuli, such as 1.25-dihydroxyvitamin D3, have been reported. However, the differentiation of monocytes with PMA was shown to have more resemblance to the phenotype of human tissue macrophages [32].

Cell viability assays are crucial for the determination of the cytotoxic effects of compounds on different cell lines in vitro. In this study, a WST-1 assay was used to determine the cytotoxicity of *D. Stramonium*-AgNPs on the differentiated THP-1 macrophages. This assay is based on the conversion of a water-soluble tetrazolium salt into formazan by the mitochondrial dehydrogenases of the viable cells. Therefore, the number of viable cells can be quantified by detecting the amount of formazan produced. *D. Stramonium*-AgNPs showed no toxicity to THP-1 cells at concentrations below 10 µg/mL (Figure1.A). The percentage viability of the differentiated THP-1 macrophages was 84 %, 52 % and 2 % when the cells were treated with 5, 10 and 20 µg/mL of *D. Stramonium*-AgNPs, respectively. Thus, 5 µg/mL of *D. Stramonium*-AgNPs was the least toxic concentration and was used to evaluate the immune modulatory effects of the nanoparticles.

ELISA was used to determine the effects of D. Stramonium-AgNPs on cytokine production in the differentiated THP-1 cells. After differentiation with PMA, the macrophages were stimulated with LPS before exposure to 5 µg/mL *D. Stramonium*-AgNPs. LPS are biologically active substances found in the outer membrane of Gram-negative bacteria [33]. Exposure of THP-1 macrophages to LPS stimulates the production of pro-inflammatory cytokines, which are essential for antibacterial defense when produced in appropriate amounts [34]. The levels of cytokine production by the macrophages in the presence of *D. Stramonium*-AgNPs are shown in Figure 1(B). LPS treatment of the THP-1 macrophages resulted in the production of pro-inflammatory cytokines (TNF-α, IL-6 and IL-1β). However, the addition of *D. Stramonium*-AgNPs (5 µg/mL) to the macrophages reduced the production levels of these cytokines. The levels of cytokine production in the cells treated with *D. Stramonium*-AgNPs was significantly decreased by approximately 3.5-, 7- and 10.5-fold for TNF-α, IL-1β and IL-6, respectively, when compared to the LPS treated cells (Figure 1(B). Therefore, the results obtained in this study indicate that *D. Stramonium*-AgNPs have anti-inflammatory properties, as they decreased the production levels of pro-inflammatory cytokines in LPSstimulatedTHP-1macrophages.

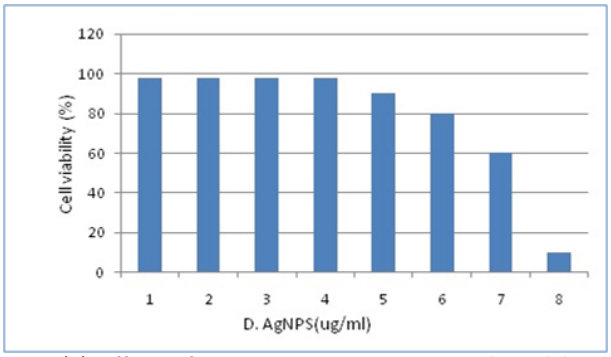

Fig. 1(A). Effects of *Datura stramonium*-AgNPs on the viability of differentiated THP-1 macrophages. Each value represents mean ± SEM; statistical significance of the *D stramonium*-AgNPs-treated cells when compared to the untreated control cells is indicated with****for p<0.0001.

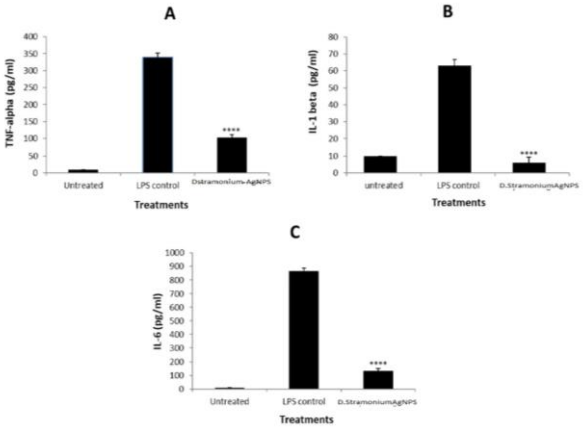

Fig. 1(B). Effects of *D Stramonium*-AgNPs on cytokine secretion in LPS stimulated THP-1 macrophages, differentiated using PMA. (**A**) represents TNF-α, (**B**) represents IL-1β, and (**C**) represents IL-6. Each value represents mean ± SEM; statistical significance of the *D. Stramonium*-AgNPs-treated cells to the LPS control is indicated with****for$p$<0.0001.

Anti-leishmanial Activity

Table 1 displays IC50 values of plant studied for anti-leishmanial activity. Datura stramonium fraction exhibited the best activity in terms of IC50 value (5.3 ± 0.2 μg/mL) comparably low than standard drug Glucantime (5.6 ± 0.25) against *Leishmania tropica* promastigotes. Highest IC50 was expressed by DSCE. IC50 values of DSME, DSEE, DSHE and DSAE fall in the arrange with minor differences. Regressions square(R^2) value ranged from 0.81– 0.9.

Brine Shrimp Toxicity

Brine shrimp toxicity is an easy and economical in vitro assay to determine toxicity and safety of crude extract. Brine shrimp in vitro assay was performed to evaluate the safety assessment extracts and its derived fractions. Table 1 displays toxicity data of plants. DSME extract showed LC50 of 733.0 ± 15.1 μg/mL. In derived fractions, LC50 ranged from 569.5 ± 7.4 to 1593 ± 20.2 μg/mL. Lowest LC50 was shown by DSEE while higher by DSAE. Regression R^2 ranged from 0.92–1.0.

Table 1. Anti-leishmanial and toxicity activity of *D. Stramonium* methanol extract and its derived fractions.

Sample D. Stramonium	Anti-leishmanial R^2 IC50 (μg/mL)		Toxicity R^2 LC50 (μg/mL)	
DSME	0.84	10.9±1.1	1.00	733.0±15.1
DSHE	0.95	7.2±0.5	0.98	982.5±12.3
DSCE	0.81	47.7±2.3	0.98	834.5±8.5
DSEE	0.95	5.3±0.2	0.98	569.5±7.4
DSBE	0.97	21.8±2.4	0.95	958.3±10.8
DSAE	0.97	6.0±0.1	0.92	159.3±20.2

Biosynthesized AgNPs and their characteristics

Both leaf and seed extracts of *D. Stramonium* catalyzed the formation of AgNPs (Figure 2), leading to the development of brown colloidal solution within 5min.The color stabilized in 10 min., with more intense color recorded in leaf AgNPs. The change in color of metallic salt solutions is a manifestation of the formation of nanoparticles. The nature of bio-reductant molecules largely influences the excitation of reduced particles, thereby leading to the development of different shades of color. The nanoparticles absorbed maximally at wavelengths of 440 and 438.5nm for leaf and seed AgNPs, respectively; however, higher absorbance was obtained for leaf AgNPs as a function of intense color formation (Figure 3). The broadness of the spectra may be indicative of fair incidence of polydispersed particles. The absorbance values are within the range of 431,436,454.5, and 457.5 nm obtained for AgNPs biosynthesized using *D. Stramonium* leaf, seed shell, and seed extracts, respectively [17, 18, 20]. The reddish brown colloidal AgNPs produced by the leaf extract of pine apple also yielded maximum absorption between 440 and 460 nm [35]. The higher absorbance readings obtained for leaf AgNPs clearly indicated the formation of more nanoparticles than in the seed AgNPs and this may be attributed to the abundance of bioreductant molecules in the leaf of *D. Stramonium*.

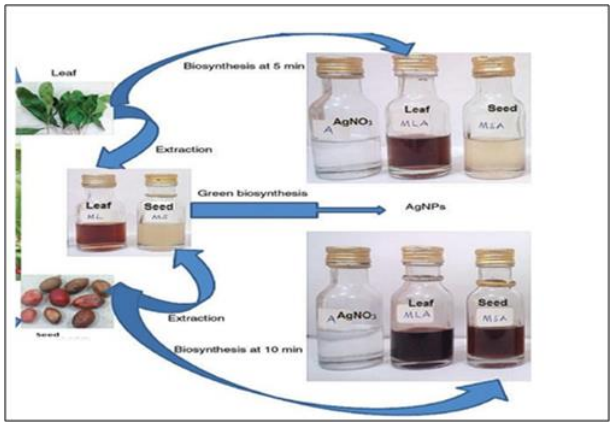

Fig. 2. Schematic view of the biosynthesis of AgNPs using leaf and seed extracts of *D.Stramonium*.

The FTIR analysis revealed prominent peaks at 3408, 2357, 2089, and 1639 cm^{-1} for leaf AgNPs, whereas peaks at 3404, 2368, 2081, and 1641 cm−1 were obtained for seed AgNPs (Figure 4). In addition, smaller peaks at 1483, 1465, 1117, 517, 487, 437, and 417cm^{-1} were recorded. The peaks 3404 and 3408cm^{-1} are attributed to the O-H [36] or H-bonded bands of phenolics or alcoholic compounds. The peaks 2357 and 2368 m^{-1} are related to vibrations of NH_2/NH_3 in the peptide bonds of protein molecules [37] or nitrogen-rich compounds for instance nitriles (C-N) and cyanates (O-CN) [38], where as 1639 and 1641 cm^{-1} are assigned N-H bend in primary amine [39]. Similarly, peaks occurring at 1465–1483 cm^{-1} are indicative of C-C stretch in aromatic compounds, whereas the peak 1117cm^{-1} found only in the seed AgNPs is attributed to C-N stretch present in aliphatic amines. All these indicate that the hydroxyl group of phenolic compounds and proteins in the extracts might have formed layers on the particles as capping molecules to prevent aggregation of the AgNPs. Leaves of *D. Stramonium* have been reported to contain several bioactive materials such as sitosterol, stigmasterol, pheophytin-a and b, lupeol, lupenone, tocopherol, and quinine [40], whereas the seed is abundantly rich in phenolics and proteins [41, 42]. It is evident from the foregoing that the leaves and seeds of *D. Stramonium* are rich in biomolecules that served as bioreductants to synthesize AgNPs.

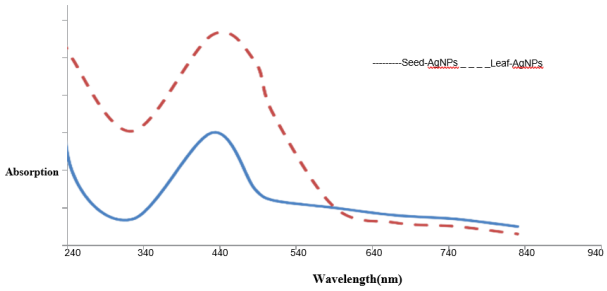

Fig. 3. UV-Vis absorption of the biosynthesized AgNPs using leaf and seed extracts of *D. Stramonium*.

Transmission electron micrographs (Figure 5) showed the formation fairly spherical AgNPs having size of 5–22 and 4–26 nm for leaf AgNPs and seed AgNPs, respectively. The leaf AgNPs (Figure 5A1) were well dispersed, where as little agglomeration was noticed in seed AgNPs (Figure 5B1), which might be as a result of sedimentation at the latter stage of synthesis as similarly observed by Ahmad et al. [38]. The agglomeration brought about few incidences of non spheri-cal and bigger particles. The selected electron area diffraction showed that the AgNPs had crystalline nature (Figure 5A2 and B2) typical of a gas shown by the ring like patterns [43], where as EDX indicated prominence of Ag (Figure 5A3 and B3).

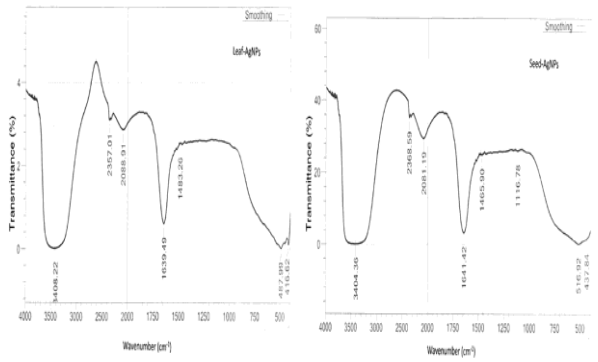

Fig. 4. FTIR spectra of the biosynthesized AgNPs using leaf and seed extracts of *D. Stramonium*.

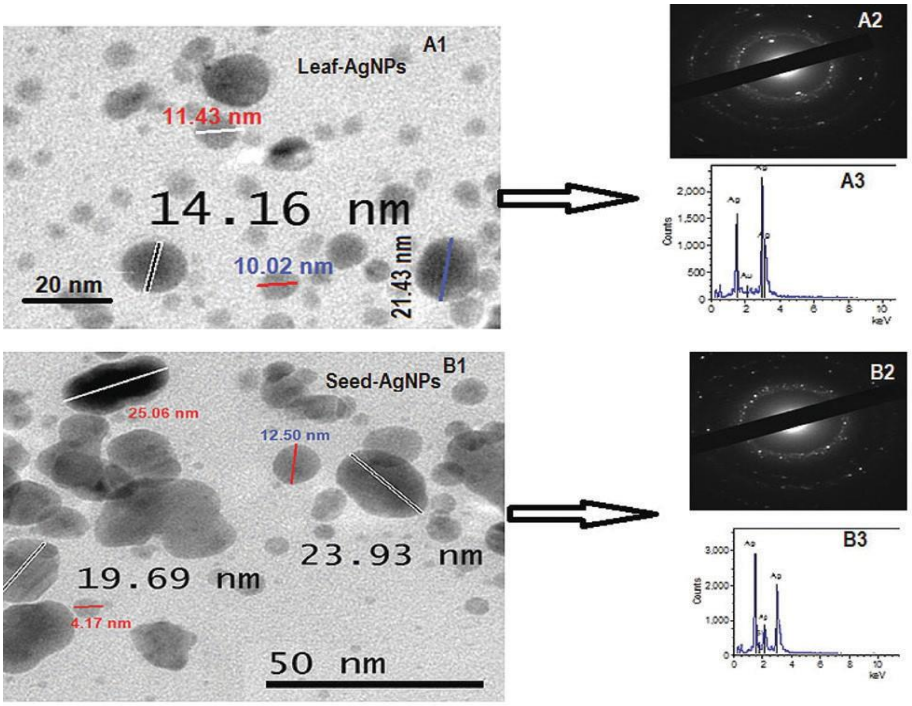

Fig. 5. TEM images (A1, B1), selected electron area diffraction (A2, B2), and energy-dispersive X-ray spectroscopic analysis (EDX) patterns (A3, B3) of the biosynthesized AgNPs using leaf (A) and seed (B) extracts of *D. Stramonium*.

Degradation of malachite green by the biosynthesized AgNPs

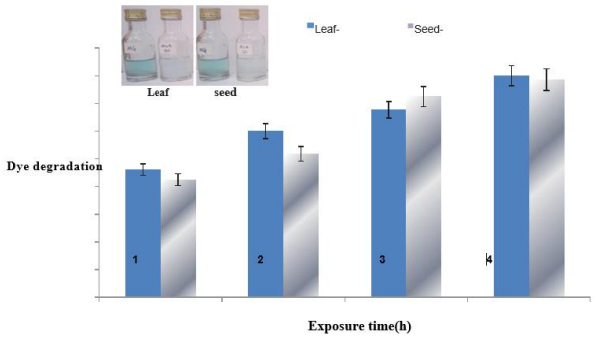

Fig. 6. Degradation of malachite green by biosynthesized AgNPs using leaf and seed extracts of *D. Stramonium*.

The AgNPs degraded malachite green at working concentration of 20 mg/mL, producing approximately 80% reduction within 24 h (Figure 6). The degradation was steady and concentration dependent in both leaf and seed AgNPs, whereas exposure to extracts alone did not lead to degradation of malachite green (data not shown). The use of nanoparticles as obtained in this study for the degradation of dyes is reported in literature [44, 45], with some advantages over conventional techniques of absorption, adsorption, coagulation, flocculation, ultrafiltration, reverse osmosis, and membrane technologies that only involve concentration or transferring of organic compounds from one form to another [46]. Using $NaBH_4$, 100% catalytic reduction was achieved with 29.4 g/ml of *Sterculia acuminata* fruit extract-mediated gold nanoparticles in 12 min [44], whereas Kumari and Philip [45] reported 83% and 95% degradation of methylene blue within 12 min, using *Punica granatum* fruit extract mediated AgNPs and AuNPs, respectively, under the influence of $NaBH_4$. However, in the present study, the catalytic degradation of malachite green was achieved without the use of $NaBH_4$ and exposure to light. Within 3 h, the degradation of malachite green at 67.7% and 72.5% was achieved by the leaf and seed-mediated AgNPs, respectively. The degradation showed little improvements to 80% and 78.6% in the next 21 h, which may be attributed to the early attainment of equilibrium. The underlying mechanism of dye degradation of dyes by nanoparticles had been captured in an electron relay effect [47], where by nanoparticles mediate in transferring electron between biomolecules borne on nanoparticles and the dye for the catalytic degradation of dye. Results obtained in this work have further shown the practicability of the applications of biosynthesized AgNPs for the catalytic degradation of malachite green.

Anti-coagulant and thrombolytic activities of biosynthesized AgNPs

Both leaf and seed AgNPs displayed excellent blood anti-coagulant activities (Figure 7), which prevented the blood samples from clotting. The microscopic examination revealed the presence of well-dispersed red blood cells, which are comparable with those obtained using the conventional EDTA blood anticoagulant. The observed structural change of red blood cells in blood treated with AgNPs as against the biconcave forms in EDTA-treated blood may be attributed to the nature of the biosynthesized AgNPs, particularly the concentration used and the pH level of the colloidal solution. We are currently focusing on the optimization of these parameters to produce combinations that can prevent blood coagulation and maintain the morphology of blood cells and biochemical attributes of the blood. The essence of blood coagulation system in the maintenance of steady blood flow, the prevention of bleeding, and the prevention of the spread of infectious agents [48] by assisting the innate immune system is well established.

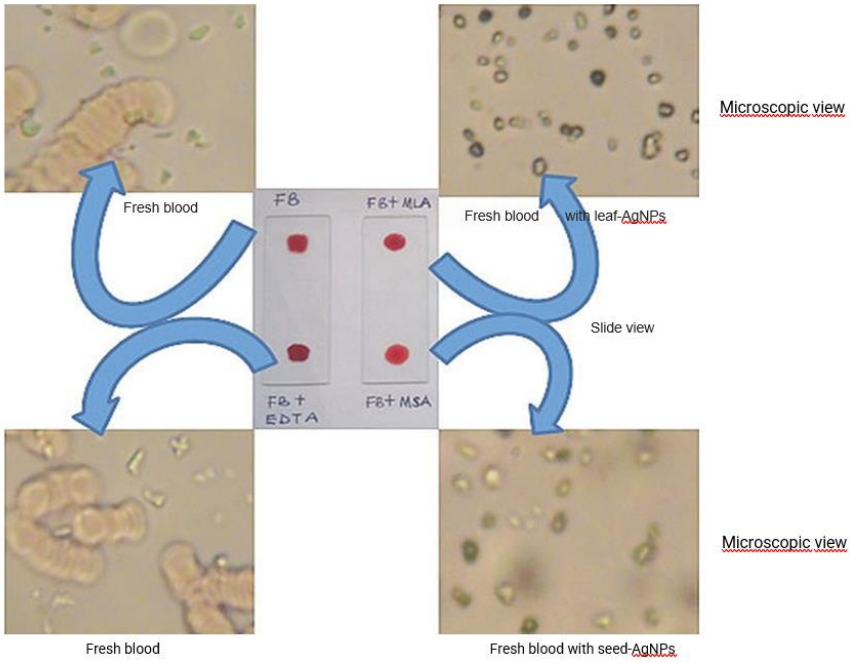

Fig. 7. Blood anticoagulant activities of the biosynthesized AgNPs using leaf and seed extracts of *D. Stramonium*.

However, the system can also portend some troubles as the blood clots arising from infections are capable of destroying tissues, which may ultimately affects organs [49]. This can be seen in cardiovascular diseases, immunological disorders, wounds, and onset of tumor [50]. Also, developed cancer cells can initiate the formation of blood clot by stimulating pro-inflammatory cytokines, the production of pro-coagulants, and the interaction with blood platelets [51]. These problems have shown the necessity to control blood coagulation disorders, which can be achieved through nanotechnology. In a previous study, Shrivasta vaetal. [22] used AgNPs to prevent the aggregation of platelets, thereby forestalling the formation of blood clot (thrombus). The study also established that AgNPs were not toxic to the blood platelets. Therefore, the non-toxic nature of AgNPs to platelets and its established actions against microbes can represent a paradigm shift in preventing blood coagulation. Most recently, Kim et al. [52], reported improvement in the anti-coagulant activities of heparin coupled with gold nanoparticles biosynthesized using the earth worm extract. The heparin AuNPs produced a 118.9% improvement on the clotting time of heparin alone. The potential of AgNPs as a potential anticoagulant is further established in the present study.

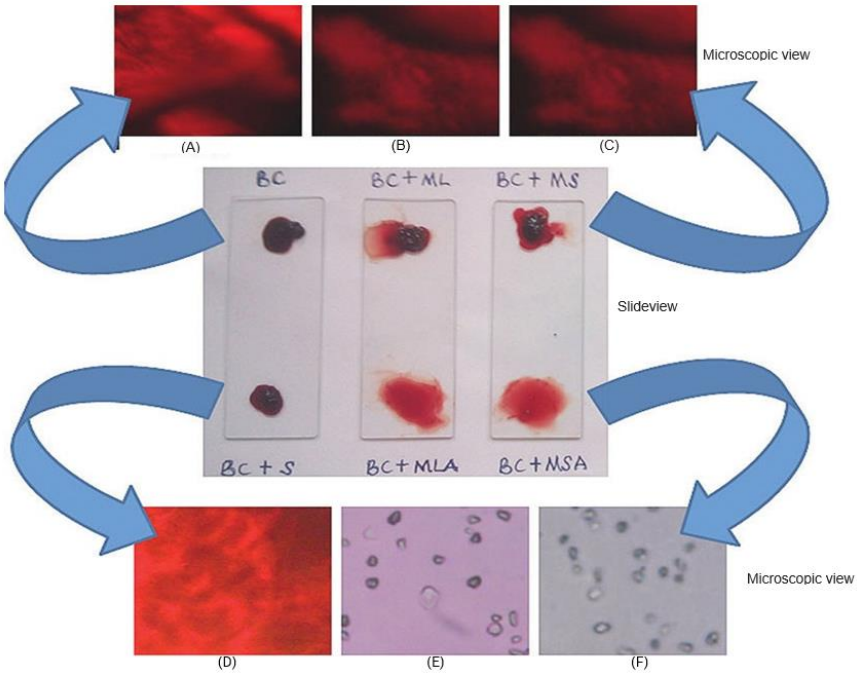

Fig. 8. Thrombolytic activities of the biosynthesized AgNPs using leaf and seed extracts of *D. Stramonium*. (A) Blood clot. (B) Blood clot treated with leaf extract. (C) Blood clot treated with seed extract. (D) Blood clot treated with $AgNO_3$ solution. (E) Blood clot treated with leaf-AgNPs. (F) Blood clot treated with seed-AgNPs.

The AgNPs dissolved preformed blood clots within 2 min, providing clear blood fluid on the slides (Figure 8).When viewed under the microscope, the dispersion of red blood cells was seen in AgNP-treated blood clots as opposed to the negative controls, where the $AgNO_3$ solution and leaf and seed extracts of *D. Stramonium* failed to dissolve the blood clots. The results showed clearly the thrombolytic activities of both leaf and seed AgNPs, which are similar to the report of Harish et al. [29]. Blood clotting is nature's antidote to excessive bleeding, but its timely dissolution is needed to prevent thrombosis and maintain homeostasis [53], and this can be aptly achieved by optimizing treatment through the application of nanomaterials. The long-established anticoagulant management strategy, for instance, the use of streptokinase, is plagued with problems of short half-life, neutralization of the foreign agents through the activities of antibodies, and danger of extreme bleeding. These problems can be solved through the applications of nano technology, where by non-toxic and biocompatible nanoparticles can be used as either stand-alone thrombolytic agents or carriers of active drugs. The possible mechanisms of thrombolysis by AgNPs is presented in Figure 10, where by the nanoparticles can act directly on fibrin (mechanism 2) to break it down as evidenced from the plate assay of Harishet al. [29]. By contrast, AgNPs can act on plasminogen, causing its activation to release plasmin that then breaks the blood clot (mechanism 1). In addition, the nanoparticles can inhibit the activities of inhibitors that may prevent the activation of plasminogen and plasmin. It is envisaged that the two mechanisms could occur together, there by leading to the pronounced thrombolytic activities as obtained in this study. Although there is limited report on the exploitation of AgNPs as a thrombolytic material, the present investigation showed that it can serve as a thrombolytic nano agent in nanomedicine. Most recently, we have shown that Ag, Au and Ag-AuNPs possessed anti-coagulant and thrombolytic activities [43, 54-56], with potential for biomedical applications.

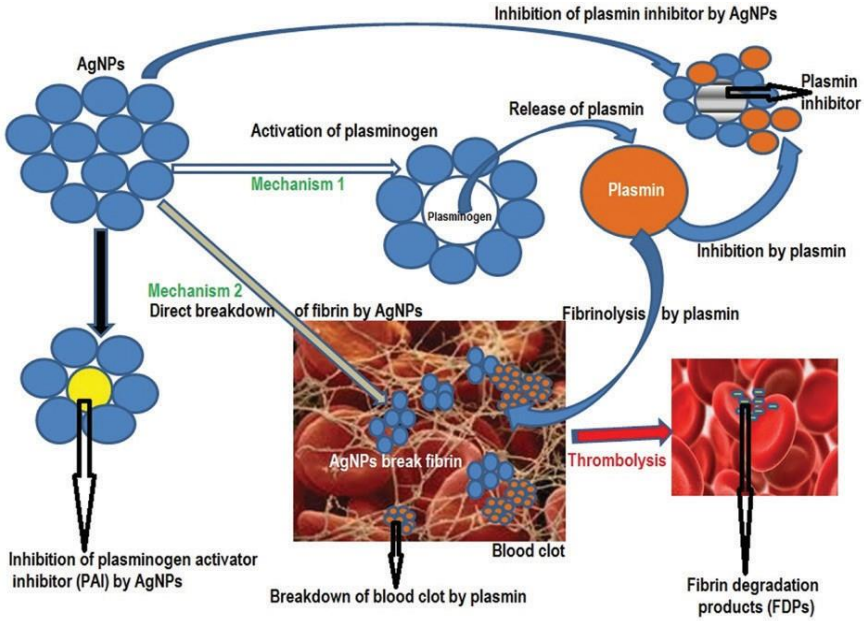

Fig. 9. The possible mechanisms of thrombolytic activities of the biosynthesized AgNPs.

4. Conclusions

AgNPs were successfully synthesized using *D. Stramonium* extract. *D. Stramonium*-AgNPs also exhibited anti-inflammatory effects by suppressing the production of pro-inflammatory cytokines (IL-1β, IL-6 andTNF-α) in macrophages. Therefore, this study demonstrates the potential of *D. Stramonium*-AgNPs as agents to control inflammatory disorders that cause chronic inflammation. The results depict that the plant described above has highly potent compounds involving in anti-leishmanial activity and can be used as an effective mean against Leishmania. This work has demonstrated the phytosynthesis of AgNPs using leaf and seed extracts of *D. stramonium* leading to the production of fairly spherical particles of 4–26 nm in size. The particles showed maximum absorption at wavelengths of 438.5 and 440 nm for the leaf and seed AgNPs, respectively. The rich phytochemicals in the extracts, particularly phenolics and proteins, took part in the synthesis of particles as evidenced from the FTIR spectra. The particles achieved approximately 80% degradation of malachite green under ambient conditions, whereas potent blood anticoagulant and thrombolytic activities were displayed by the AgNPs. It is therefore evident that AgNPs could be applied as antimicrobial agents, as nanocatalysts to degrade dyes in wastewater/effluents, and in nanomedicine for the management of blood coagulation disorders.

Acknowledgments

The authors are grateful to the mentioned affiliated reputed institutes for providing the necessary facilities and environment for the present work.

Author Contributions

Mushtaq Ahmad Bhat: Investigation, Formal analysis, Writing-original draft, conceptualization; Fairooz Ahmad Khan: Conceptualization, supervision, software, Writing- review & editing.

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How to cite this article

Bhat, M. A.; Khan, F. A. *Orbital: Electron. J. Chem*. **2025**, *17*, 560. DOI: http://dx.doi.org/10.17807/orbital.v17i6.22299