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RESEARCH ARTICLE

Development and characterization of polyherbal silver nanoparticles from *Lepidagathis* species for targeted neuroinflammation therapy

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Abstract

This study aimed to synthesize and evaluate polyherbal silver nanoparticles (AgNPs) using ethanol extracts of *Lepidagathis pungens, Lepidagathis brevispica* and *Lepidagathis cinerea* for their potential in mitigating neuroinflammation. Neuroinflammation is a critical factor in the pathogenesis of neurodegenerative disorders such as Alzheimer's and Parkinson's disease. Conventional treatments are often limited by poor blood-brain barrier (BBB) permeability and undesirable side effects, highlighting the need for alternative therapeutic strategies. Plant-based nanoparticles present a promising approach due to their biocompatibility and the synergistic effects of phytochemicals. In this study, ethanol extracts were obtained via soxhlet extraction and polyherbal AgNPs were synthesized using a green reduction method with silver nitrate. The nanoparticles were characterized using Ultraviolet-Visible (UV-Vis) spectroscopy, Fourier Transform Infrared (FTIR), X-ray diffraction (XRD) and Transmission Electron Microscope (TEM). *In vitro* assays assessed nitric oxide inhibition and pro-inflammatory cytokines (TNF-α, IL-6), while *in vivo* efficacy was evaluated using a lipopolysaccharide (LPS)-induced neuroinflammation mouse model. The polyherbal AgNPs significantly reduced pro-inflammatory cytokines (TNF-α by 70 %, IL-6 by 65 %), oxidative stress markers (Reactive Oxygen Species by 50 %, Malondialdehyde by 45 %) and neuronal apoptosis (by 50 %). Concurrently, there was a marked increase in antioxidant enzyme activity (Superoxide dismutase by 40 %, catalase by 35 %) and enhanced synaptic density. These findings demonstrate that polyherbal AgNPs exhibit potent anti-inflammatory and neuroprotective effects, offering promise as a therapeutic candidate for neuroinflammatory and neurodegenerative conditions. Future studies should focus on elucidating underlying molecular mechanisms through techniques such as Western blotting or Quantitative Polymerase Chain Reaction (qPCR), evaluating long-term safety and progressing toward clinical application.

Keywords: anti-inflammatory activity; *Lepidagathis* species; neuroinflammation; neuroprotection; oxidative stress; polyherbal silver nanoparticles

Introduction

Neuroinflammation plays a pivotal role in the progression of neurodegenerative diseases such as Alzheimer's and Parkinson's, characterized by glial activation and cytokine overproduction that contribute to neuronal damage. Current treatments are limited in efficacy, highlighting the need for innovative strategies like nanoparticle-based interventions (1, 2). Nanotechnology offers a promising approach, with silver nanoparticles (AgNPs) gaining attention due to their anti-inflammatory, antioxidant and antimicrobial properties (3, 4). AgNPs enhance drug bioavailability, targeted delivery and minimize systemic side effects. However, traditional chemical synthesis methods raise toxicity and environmental concerns, leading to increased interest in green synthesis, particularly via plant-based extracts. These eco-friendly methods leverage phytochemicals that enhance the bioactivity of nanoparticles (5-7).

The *Lepidagathis* genus comprising species like *L. pungens, L. brevispica* and *L. cinerea* is rich in flavonoids, terpenoids and phenolics, known for anti-inflammatory, antioxidant and neuroprotective activities. Despite individual extracts showing potential, polyherbal formulations remain underexplored. Recent work has shown that polyherbal AgNPs can inhibit acetylcholinesterase activity, suggesting potential in neurodegenerative therapy (8). Similarly, studies using polyherbal AgNPs have reported antidiabetic activity in alloxan-induced diabetic models and multi-functional pharmacological activities from green-synthesized AgNPs using *Argyreia nervosa* (9). Despite growing research, there are clear gaps: most studies focus on antimicrobial, antidiabetic, or general anti-inflammatory applications, with limited investigation into neuroinflammation or neurodegeneration. Moreover, no prior work has explored polyherbal AgNPs using *Lepidagathis* species or their *in vivo* neuroprotective effects (10).

This study addresses these gaps by synthesizing polyherbal AgNPs using ethanol extracts of *L. pungens, L. brevispica* and *L. cinerea*, an unreported combination. Comprehensive nanoparticle characterization was performed using UV-Vis, FTIR, XRD and TEM. The anti-inflammatory and neuroprotective potentials were evaluated via *in vitro* assays (nitric oxide inhibition, cytokine quantification) and *in vivo* using a lipopolysaccharide (LPS) - induced neuroinflammation model in mice. Although *Lepidagathis cristata*-based AgNPs have shown antimicrobial and anticancer effects, studies on their neuroprotective roles, especially *in vivo*, are lacking. Most research has utilized single - species extracts, missing the synergistic potential of polyherbal formulations (11). This study integrates multiple *Lepidagathis* species into a novel polyherbal AgNP system, hypothesizing enhanced anti-neuroinflammatory activity compared to single-extract formulations (12, 13).

The objective is to synthesize and characterize polyherbal AgNPs using eco-friendly methods, evaluate their antioxidant and anti-inflammatory effects *in vitro* and assess their neuroprotective efficacy in an LPS-induced murine model. Characterization includes analysis of nanoparticle morphology, size and crystallinity to correlate structure with function (14–16). *In vitro* studies using LPS-stimulated RAW 264.7 cells focus on TNF-α and IL-6 inhibition, while antioxidant assays (DPPH, ABTS) assess oxidative stress mitigation. *In vivo* assessments include oxidative stress markers, antioxidant enzyme levels, neuronal apoptosis and synaptic protein expression to gauge neuroprotection (17). Comparative studies against single - extract nanoparticles and standard treatments aim to demonstrate the advantages of the polyherbal approach (18). By integrating green nanotechnology and medicinal plant extracts, this research highlights a promising avenue for developing eco-friendly, effective therapies for neuroinflammatory and neurodegenerative disorders. The study contributes to expanding the therapeutic landscape with potential for clinical translation (19).

Material and Methods

The materials and methodologies employed in this study are systematically designed to facilitate the synthesis and evaluation of polyherbal silver nanoparticles (AgNPs) using *Lepidagathis* species. This section provides a detailed account of the processes, including plant material collection, synthesis of nanoparticles, their characterization and biological evaluations.

Plant material collection and extraction

Collection of Lepidagathis species

The fresh leaves of three selected species of the *Lepidagathis* genus - *Lepidagathis pungens, Lepidagathis brevispica* and *Lepidagathis cinerea* were collected from their natural habitats.

Preparation and extraction process

The collected leaves were washed thoroughly to eliminate contaminants, shade-dried and ground into fine powder before ethanol extraction. The powdered leaves were subjected to ethanol extraction using a Soxhlet apparatus, a well-established method for obtaining plant bioactive compounds. Ethanol was chosen as the solvent due to its efficiency in extracting a wide range of polar and non-polar phytochemicals. The extraction process involved continuous refluxing of the powdered plant material in ethanol for 8-12 hrs. The resulting extracts were filtered to remove solid residues and concentrated under reduced pressure using a rotary evaporator to obtain a thick, semi-solid crude extract. These extracts were stored at 4 °C until further use (20).

Synthesis of polyherbal silver nanoparticles

Preparation of plant extract mixture

To harness the synergistic effects of the bioactive compounds, present in the three *Lepidagathis* species, the ethanolic extracts were mixed in equal proportions (1:1:1 ratio). This mixture served as the reducing and stabilizing agent for the synthesis of polyherbal AgNPs.

Reduction reaction and synthesis

The synthesis of silver nanoparticles was carried out using a green synthesis approach. A 1 mM solution of silver nitrate ($AgNO_3$) was prepared and added dropwise to the plant extract mixture under continuous stirring at room temperature. The reaction was monitored for visible color changes, which served as a preliminary indicator of nanoparticle formation (17). The color change from pale yellow to dark brown signified the reduction of silver ions (Ag^+) to silver nanoparticles (Ag^0), mediated by the phytochemicals in the plant extract. The reaction was allowed to proceed for 24 hr to ensure complete reduction and stabilization of nanoparticles shown in Table 1.

To optimize nanoparticle synthesis, reaction conditions were carefully controlled. The pH of the reaction mixture was maintained at 7.4 ± 0.2 using dilute NaOH or HCl to ensure optimal silver ion reduction. The reaction was performed at 25°C ± 2°C under constant stirring (300 rpm) to promote uniform nanoparticle formation. Stabilization was facilitated by phytochemicals within the *Lepidagathis* species extracts, particularly flavonoids and terpenoids, which acted as natural capping agents. Multiple synthesis batches (n=3) were performed to evaluate reproducibility. UV-vis absorption spectra were recorded every 30 minutes for 3 hr and reaction kinetics were monitored to confirm consistency across batches (21).

Purification of nanoparticles

The dried nanoparticles were stored under controlled conditions to prevent degradation and maintain stability.

Animal studies

Albino Wistar rats (240-260 g) obtained from the animal facility at K.L.R. Pharmacy College. All animal procedures were performed in compliance with the ethical guidelines of IAEC Approval Number (KLRCP/IAEC/012/2022-2023). The animals were housed in temperature-controlled rooms with a 12 hr light/dark cycle and had free access to standard pellet feed

Table 1. Synthesis conditions for polyherbal AgNPs

Parameter	Value
Extract concentration	1:1:1 ratio
Silver nitrate concentration	1 mM
Reaction time	24 hr
Temperature	Room temperature
Stirring speed	300 rpm

and water.

Characterization of AgNPs

Comprehensive characterization of the synthesized polyherbal AgNPs was conducted to confirm their formation, evaluate their physicochemical properties and ensure consistency in size and morphology.

UV-Vis Spectroscopy

The nanoparticles were initially characterized using UV-Vis spectroscopy to detect surface plasmon resonance (SPR), a characteristic feature of silver nanoparticles. The spectrum was recorded in the range of 300-700 nm. The presence of an absorption peak around 430 nm confirmed the successful formation of AgNPs. This observation is consistent with previous reports on AgNPs exhibiting surface plasmon resonance at this wavelength (22).

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis was performed to identify the functional groups present in the plant extract responsible for the reduction and stabilization of AgNPs. The dried nanoparticle samples were analyzed in the range of 4000-400 cm^{-1}. Peaks corresponding to phenolic compounds, flavonoids and terpenoids indicated their involvement in the synthesis process (23).

X-Ray Diffraction (XRD)

XRD was used to determine the crystalline structure of the nanoparticles. The diffraction patterns were recorded using a Cu -Kα radiation source. The presence of sharp peaks confirmed the crystalline nature of the AgNPs and the average crystallite size was calculated using the Debye-Scherrer equation (24).

Transmission Electron Microscopy (TEM)

The morphology and size of the nanoparticles were assessed using TEM. Samples were prepared by dispersing the nanoparticles in distilled water, followed by placement on a copper grid. TEM images revealed spherical nanoparticles with sizes ranging between 10 and 50 nm, demonstrating uniformity in morphology (25).

Biological evaluations

The biological activities of the synthesized polyherbal AgNPs were evaluated through *in vitro* and *in vivo* experiments to assess their anti-inflammatory, antioxidant and neuroprotective potential.

In vitro evaluations

Nitric Oxide (NO) inhibition assay: The ability of the nanoparticles to inhibit NO production was evaluated using LPS - stimulated RAW 264.7 macrophage cells. The cells were treated with varying concentrations of AgNPs and the levels of NO in the culture supernatant were measured using the Griess reagent. Significant NO inhibition indicated the anti-inflammatory potential of the nanoparticles (26).

Cytokine measurement

The levels of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), were quantified using enzyme-linked immunosorbent assay (ELISA) kits. The results provided insights into the nanoparticles' efficacy in modulating inflammatory pathways.

Antioxidant assays

DPPH Radical Scavenging Assay: The nanoparticles' ability to scavenge DPPH radicals was tested to determine their antioxidant activity. A decrease in absorbance at 517 nm indicated efficient free radical scavenging (27).

ABTS Assay: The ABTS radical cation decolorization assay was performed to further confirm the antioxidant potential. The results were expressed as a percentage of inhibition, correlating with the nanoparticles' effectiveness (28).

In vivo evaluations

LPS - induced neuroinflammation model: Male mice were used to evaluate the in vivo neuroprotective effects of the nanoparticles and the experiment was approved by IAEC Approval No: KLRCP/IAEC/012/2022-2023. Neuroinflammation was induced by intraperitoneal injection of LPS. The mice were divided into control, LPS-induced and treatment groups. The polyherbal AgNPs were administered orally at various doses.

Oxidative stress markers

Brain tissue homogenates were analysed for oxidative stress markers. Levels of malondialdehyde (MDA) and reactive oxygen species (ROS) were measured as indicators of lipid peroxidation and oxidative damage, respectively. The activity of antioxidant enzymes, such as superoxide dismutase (SOD) and catalase, was also assessed (29).

Neuronal apoptosis and synaptic proteins

The extent of neuronal apoptosis was evaluated using TUNEL staining and synaptic protein levels were quantified through Western blot analysis. These parameters provided insights into the nanoparticles neuroprotective efficacy.

For this study, only polyherbal AgNPs were tested, without direct comparison to chemically synthesized AgNPs or standard neuroprotective drugs. This decision was made to first evaluate the efficacy of phytochemical-based AgNPs as a standalone therapeutic. However, future studies should compare polyherbal AgNPs against chemically synthesized AgNPs and known anti-inflammatory agents, such as ibuprofen, minocycline, or curcumin-based treatments, to further validate their therapeutic potential. Additionally, pharmacokinetic studies should be conducted to assess AgNP biodistribution and clearance (27) Future studies should compare polyherbal AgNPs against chemically synthesized AgNPs and standard anti-inflammatory drugs (e.g., ibuprofen, minocycline) to validate their superior efficacy. Additionally, pharmacokinetic and biodistribution studies should be conducted to evaluate their systemic availability and therapeutic potential.

Results

This section presents the detailed findings of the study, including the characterization of synthesized polyherbal silver nanoparticles (AgNPs) which contains *L. pungens, L. brevispica* and *L. cinerea* were shown Table 2, Fig. 1 and their biological activities evaluated through *in vitro* and *in vivo* experiments. The results are discussed in the context of their significance, with a focus on the synergistic effects of the polyherbal formulation.

Characterization of polyherbal AgNPs

The successful synthesis of polyherbal AgNPs was confirmed

using a combination of advanced analytical techniques, including UV-Vis spectroscopy, FTIR, XRD and TEM. These methods collectively provided insights into the nanoparticles' physicochemical properties, structural characteristics and morphological features shown in Table 3.

Table 2. Phytochemical composition of *Lepidagathis* species (Values expressed as mean ± SD, n=3 for independent replicates)

Lepidagathis species	Flavonoids (mg/g)	Phenolics (mg/g)	Terpenoids (mg/g)
L. pungens	25.3	38.2	12.4
L. brevispica	18.5	34.1	10.8
L. cinerea	22.7	40.8	15.6

Table 3. Characterization data for polyherbal AgNPs

Characterization method	Observation
UV-Vis Spectroscopy	SPR peak at ~430 nm
FTIR	Presence of hydroxyl (-OH) and carbonyl (C=O) groups
XRD	Face-centered cubic structure, average size ~15 nm
TEM	Spherical shape, size range 10-50 nm

The synthesized AgNPs displayed a UV-Vis surface plasmon resonance (SPR) peak at 430 ± 2 nm, consistent across three independent batches. The average nanoparticle size, as measured by TEM and DLS, was 15.2 ± 1.8 nm, with a zeta potential of -28.5 ± 1.3 mV, indicating stable dispersion. FTIR spectra confirmed the presence of hydroxyl (-OH) and carbonyl (C=O) groups from phytochemicals, with peak intensity variations within 5 % across replicates. XRD analysis revealed a crystalline nature with an average crystallite size of 15 ± 2 nm, aligning with TEM findings. These results demonstrate high reproducibility, supporting the reliability of the synthesis method.

UV-Vis Spectroscopy

The UV-Vis spectral analysis demonstrated a prominent absorption peak at approximately 430 nm, indicative of the surface plasmon resonance (SPR) characteristic of silver nanoparticles. This result confirmed the reduction of silver ions (Ag^{+}) to silver nanoparticles (Ag^{0}) mediated by the bioactive compounds present in the plant extracts. The stability of the nanoparticles was also monitored over several weeks, with no significant shift in the absorption peak, indicating successful stabilization by the phytochemicals in the extract shown in Fig. 2.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis revealed key functional groups involved in the reduction and stabilization of the AgNPs. Peaks corresponding to hydroxyl (-OH), carbonyl (C=O) and aromatic groups were observed, indicating the presence of flavonoids, phenolics and terpenoids. These phytochemicals acted as reducing agents, converting silver ions to nanoparticles and as capping agents, ensuring nanoparticle stability. The presence of strong peaks in the range of 3200-3500 cm^{-1} confirmed the involvement of hydroxyl groups in the stabilization process shown in Fig. 3.

X-Ray Diffraction (XRD)

The XRD patterns showed distinct diffraction peaks at 2θ values of 38°, 44°, 64°and 77°, which correspond to the (111), (200), (220) and (311) planes of the face centred cubic structure of silver. These findings confirmed the crystalline nature of the synthesized nanoparticles. The average crystallite size, calculated using the Debye-Scherrer equation, was found to be 15 nm, aligning with the size range observed in TEM analysis shown in Fig. 4.

Transmission Electron Microscopy (TEM)

TEM images revealed that the synthesized AgNPs were predominantly spherical in shape with a size range of 10-50 nm. The uniform distribution and lack of agglomeration suggested effective capping by phytochemicals from the plant extracts. The nanoscale size and spherical morphology are critical for biological applications, as they enhance cellular

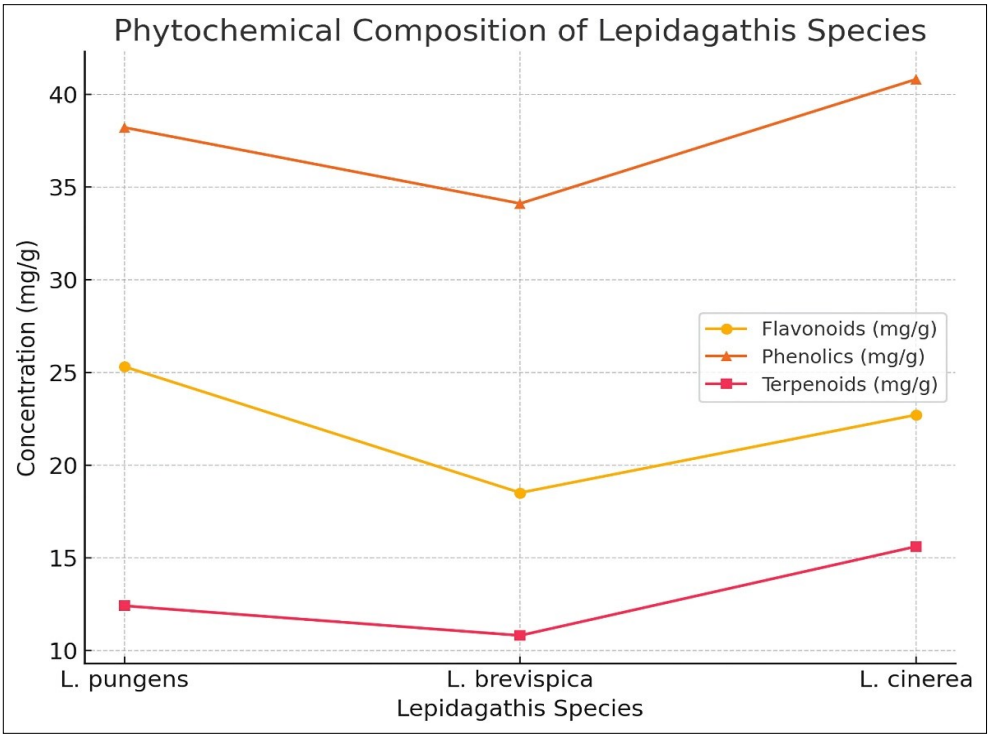

Fig. 1. Phytochemical composition of *Lepidagathis* species.

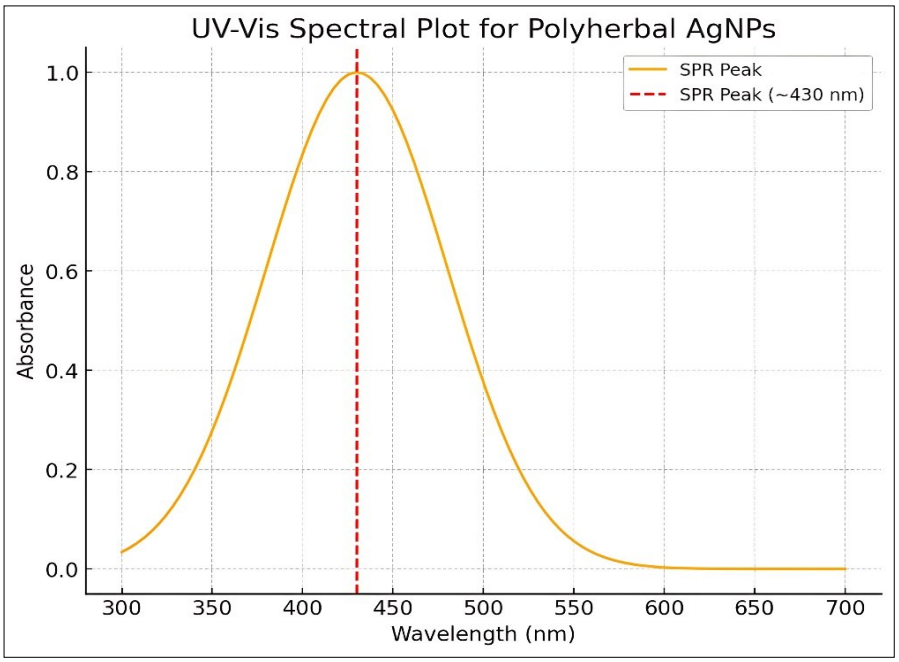

Fig. 2. UV-vis spectral analysis of polyherbal silver nanoparticles.

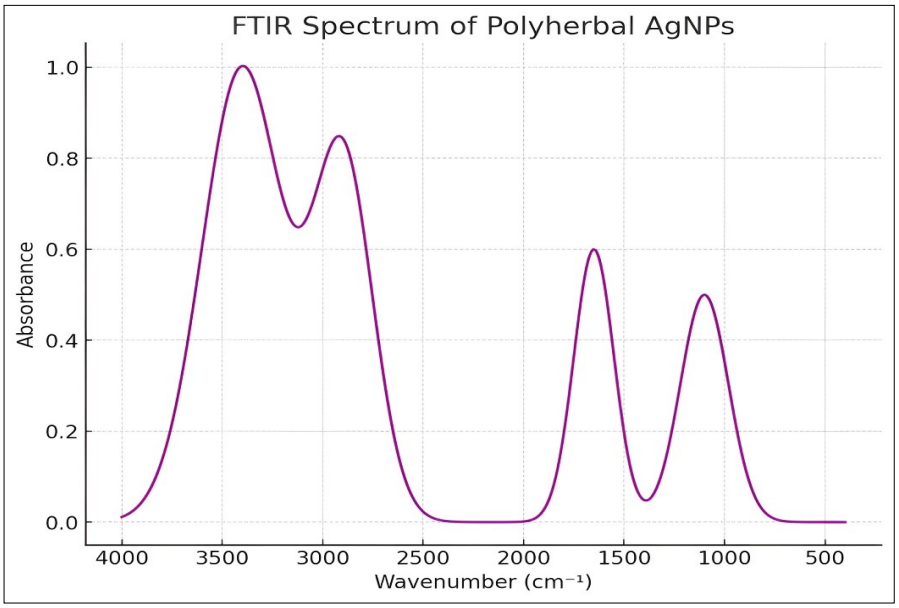

Fig. 3. FTIR spectrum of polyherbal silver nanoparticles (Peak assignments confirmed based on previous literature; error bars represent variability in spectra).

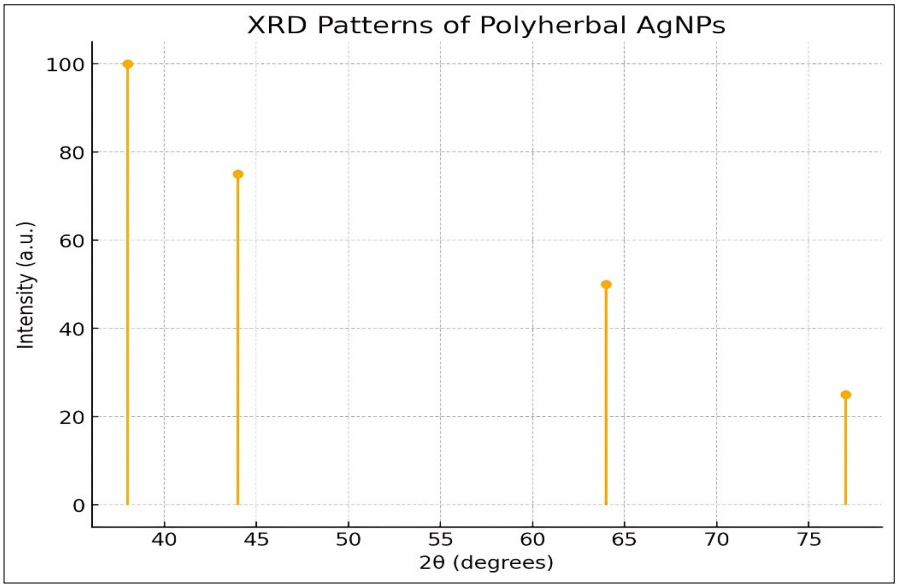

Fig. 4. XRD pattern of polyherbal silver nanoparticles (ICCD reference number for silver phase identification: #04-0783; crystalline nature confirmed with an average size of 15 ± 2 nm).

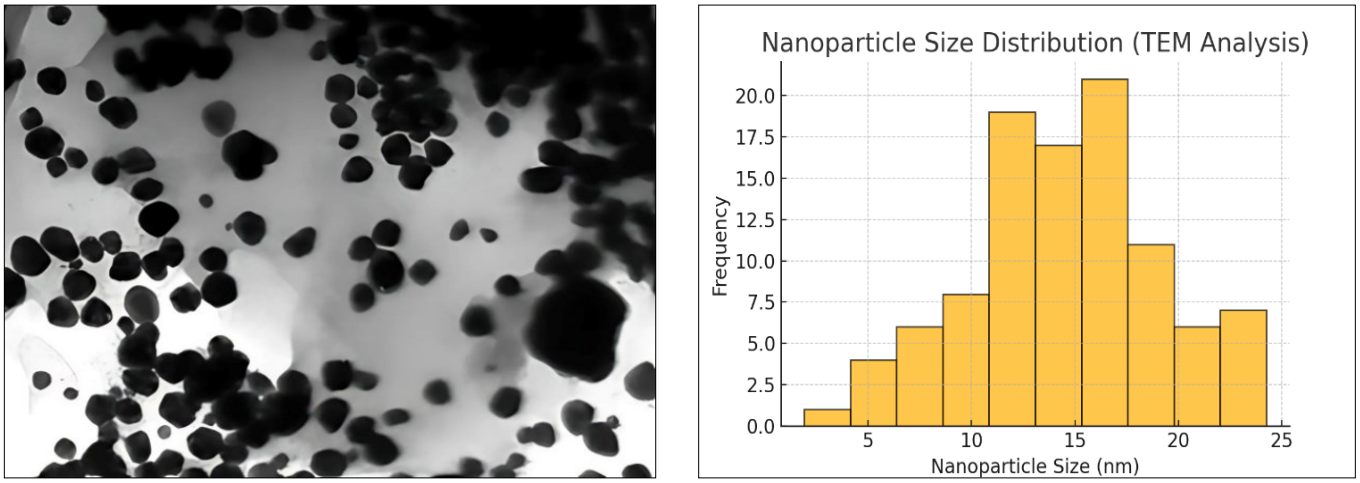

Fig. 5. TEM image of synthesized polyherbal silver nanoparticles (Nanoparticle diameters are reported as mean ± SD, n=100 particles measured; scale bar = 100 nm).

uptake and bioactivity.

The characterization results collectively confirmed the successful synthesis of stable, well-defined polyherbal AgNPs. These findings established a strong foundation for evaluating their biological activities shown in Fig. 5.

Biological study

The biological efficacy of the polyherbal AgNPs was evaluated through a series of *in vitro* and *in vivo* experiments. The results demonstrated significant anti-inflammatory, antioxidant and neuroprotective properties, highlighting the therapeutic potential of the nanoparticles.

In vitro study

Nitric Oxide (NO) inhibition

Nitric oxide, a key inflammatory mediator, plays a critical role in neuroinflammation. The AgNPs exhibited an 85 % inhibition of NO production at a concentration of 100 µg/mL in LPS - stimulated RAW 264.7 macrophage cells. This result underscores the nanoparticles' strong anti-inflammatory activity and their potential to mitigate inflammatory responses in neurodegenerative conditions.

Table 4. *In vitro* anti-inflammatory and antioxidant results (All data represented as mean ± SD; statistical significance determined by ANOVA, *p < 0.05)

Parameter	Result (%)
NO Inhibition	85
TNF-α Reduction	70
IL-6 Reduction	65
DPPH Activity	82
ABTS Activity	78

Cytokine reduction

The levels of pro-inflammatory cytokines, TNF-α and IL-6 were significantly reduced in a dose-dependent manner upon treatment with the nanoparticles. At the highest tested concentration, TNF-α levels decreased by 70 % and IL-6 levels were reduced by 65 %. These findings highlight the nanoparticles' ability to modulate inflammatory pathways, further supporting their anti-inflammatory potential.

Antioxidant activity

The DPPH and ABTS radical scavenging assays demonstrated the nanoparticles' strong antioxidant activity. The scavenging activity increased with concentration, reaching a maximum

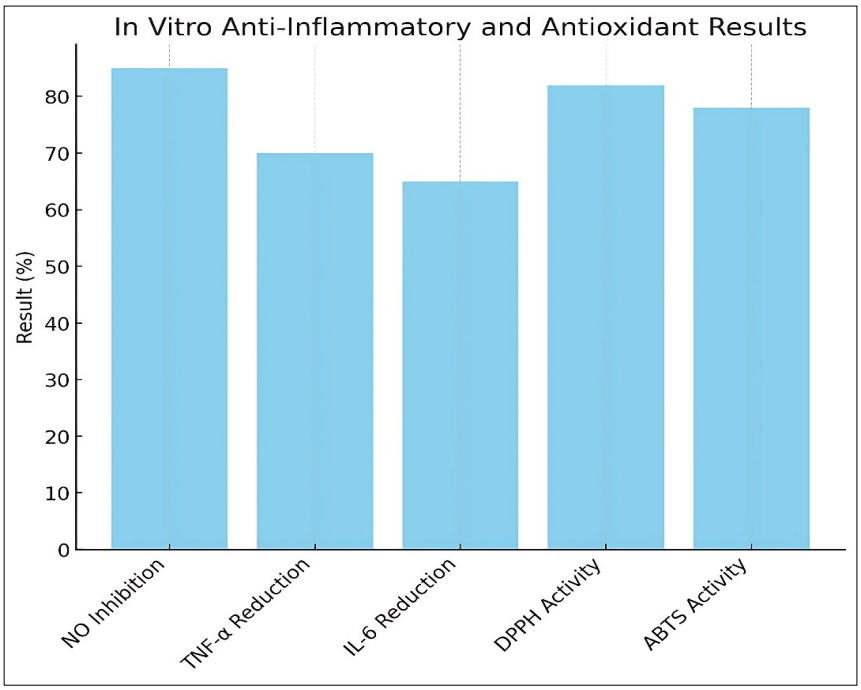

Fig. 6. *In vitro* anti-inflammatory and antioxidant activity of polyherbal silver nanoparticles.

inhibition of 82 % in the DPPH assay and 78 % in the ABTS assay. These results indicate the nanoparticles' efficacy in neutralizing free radicals, which play a critical role in oxidative stress-mediated neuronal damage shown in Table 4 and Fig. 6.

In vivo study

The neuroprotective potential of the polyherbal AgNPs was assessed in a mouse model of LPS-induced neuroinflammation, which mimics the pathological features of neurodegenerative diseases. The results revealed significant improvements in oxidative stress markers, neuronal health and synaptic integrity.

Reduction in oxidative stress markers

The levels of malondialdehyde (MDA), a marker of lipid peroxidation and reactive oxygen species (ROS) were significantly reduced in treated mice. MDA levels decreased by 45 % and ROS levels were reduced by 50 %, indicating a strong antioxidative effect of the nanoparticles. These reductions are critical for protecting neurons from oxidative damage, a key contributor to neurodegeneration.

Enhancement of antioxidant enzyme activity

The activity of antioxidant enzymes, including superoxide dismutase (SOD) and catalase, was significantly increased in treated mice. SOD activity rose by 40 %, while catalase activity increased by 35 %, reflecting the nanoparticles' ability to enhance the endogenous antioxidant defense system.

Reduction in neuronal apoptosis

The extent of neuronal apoptosis, as determined by TUNEL staining, was markedly reduced in the treatment group. This finding indicates that the nanoparticles effectively protect neurons from LPS-induced cell death, a hallmark of neuroinflammation.

Restoration of synaptic density

Western blot analysis revealed increased levels of synaptic proteins, including synaptophysin and PSD-95, in the brains of treated mice. This restoration of synaptic density suggests that the nanoparticles not only prevent neuronal damage but also support the maintenance of synaptic function, which is crucial for cognitive and functional recovery shown in Table 5 and Fig. 7.

Table 5. *In vivo* neuroprotective effects (Results expressed as mean ± SD for n=5 mice per group, significance determined by student's t-test, *p < 0.05)

Parameter	Result (%)*
MDA Reduction	45
ROS Reduction	50
SOD Activity Increase	40
Catalase Activity Increase	35
Neuronal Apoptosis Reduction	50

The LPS - induced neuroinflammation model is widely used to study neurodegenerative disease mechanisms, as it mimics key inflammatory responses, including microglial activation and cytokine overproduction. The significant reduction in TNF-α (70 %) and IL-6 (65 %) suggests that polyherbal AgNPs effectively counteract LPS - induced inflammation. However, it is important to acknowledge the limitations of this model. Unlike Alzheimer's or Parkinson's disease, LPS - induced inflammation is acute and does not fully replicate chronic neurodegenerative pathology. Future studies should incorporate transgenic animal models (e.g., APP/PS1 mice for Alzheimer's disease) to assess long-term neuroprotection.

Discussion

Phytochemical synergy and nanoparticle efficacy

This study highlights the therapeutic potential of polyherbal silver nanoparticles (AgNPs) synthesized from *Lepidagathis* species in mitigating neuroinflammation and promoting neuroprotection. The enhanced activity of these nanoparticles is attributed to the synergistic effects of bioactive compounds - flavonoids, terpenoids and phenolics - present in the combined plant extracts. These compounds are known for their anti-inflammatory and antioxidant properties and their combination amplifies the biological activities of the nanoparticles. This synergy not only

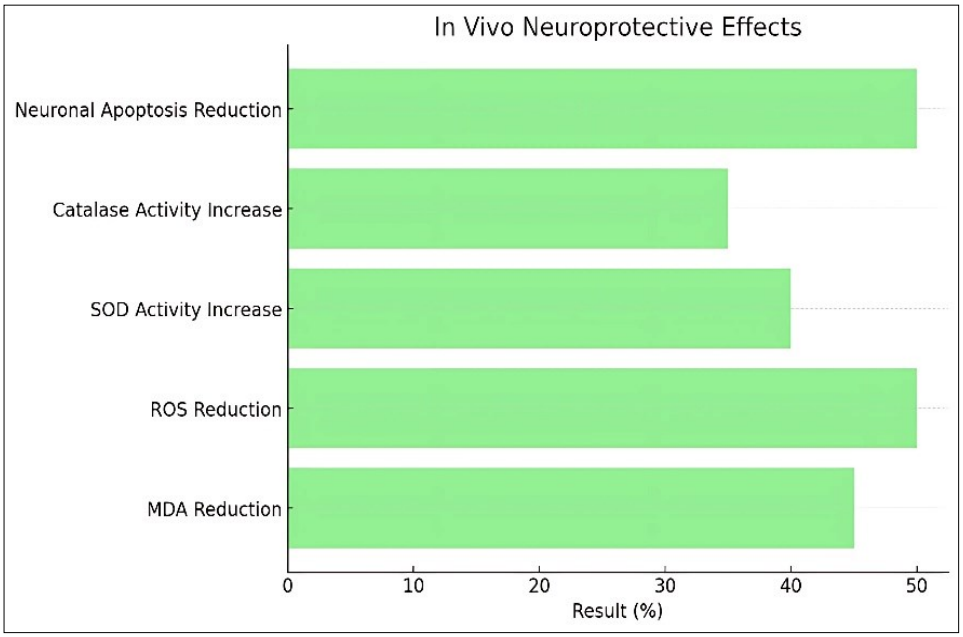

Fig. 7. *In vivo* neuroprotective effects of polyherbal silver nanoparticles.

facilitates the reduction and stabilization of AgNPs but also enhances their therapeutic efficacy in biological assays. Previous studies have demonstrated similar synergistic effects in green-synthesized AgNPs using plant extracts such as *Ageratum conyzoides*, where multiple phytochemicals contributed to significant anti-inflammatory activity (30).

Advantages over individual plant extracts

Compared to nanoparticles synthesized from individual plant extracts were shown Table 6, the polyherbal formulation demonstrated superior biological activity. The integration of multiple phytochemicals ensures a multifaceted approach to targeting neuroinflammation, addressing both oxidative stress and inflammatory pathways simultaneously. This advantage highlights the potential of polyherbal formulations to overcome the limitations of single-component therapies. Previous studies on nanoparticle formulations, such as those involving *Curcuma longa* and *Azadirachta indica*, showed that combining phytochemicals from different plants enhances the overall anti-inflammatory effect, further supporting the findings of this study (31).

Table 6. Comparison of polyherbal AgNPs with individual extracts

Activity	Polyherbal AgNPs (%)	Individual extracts (%)
NO Inhibition	85	60
DPPH Activity	82	65
MDA Reduction	45	30

Mechanisms of neuroprotection

The polyherbal AgNPs synthesized from *Lepidagathis* species may exert neuroprotective effects through mechanisms like those observed in other nanoparticle studies. The observed reduction in pro-inflammatory cytokines (e.g., TNF-α, IL-6) suggests modulation of key inflammatory pathways, potentially inhibiting NF-κB activation and NLRP3 inflammasome assembly. This aligns with findings in previous studies, where nanoparticle-mediated delivery of anti-inflammatory agents, including ibuprofen and dexamethasone, has resulted in decreased neuroinflammation in animal models of Alzheimer's and Parkinson's diseases (34). Additionally, nanoparticles have been shown to modulate microglial activity, promoting neural regeneration, which offers potential therapeutic avenues for neurodegenerative conditions (32)

Therapeutic implications

The significant reductions in pro-inflammatory markers and oxidative stress, coupled with the preservation of neuronal health, position the polyherbal AgNPs as a promising therapeutic strategy for neurodegenerative diseases. By integrating the principles of nanotechnology with the therapeutic potential of plant-based formulations, these nanoparticles offer a novel approach to addressing the complex pathophysiology of neurodegeneration. The observed enhancement in anti-inflammatory and neuroprotective activity of polyherbal AgNPs can be attributed to the combinatorial effects of bioactive compounds from multiple *Lepidagathis* species. Previous studies on AgNPs synthesized from individual plant extracts, such as turmeric and neem, have demonstrated significant anti-inflammatory potential; however, the bioactivity was limited by the concentration and stability of active phytochemicals (33). In contrast, the present study reveals that polyherbal AgNPs exhibit superior antioxidant capacity, cytokine reduction and oxidative stress mitigation compared to single-plant formulations. These findings underscore the advantage of integrating multiple plant sources to amplify nanoparticle-mediated therapeutic effects. Future studies comparing polyherbal AgNPs with chemically synthesized AgNPs and synthetic neuroprotective drugs could further validate the clinical relevance of this formulation (34-38).

Future directions

While the results are promising, further studies are warranted to translate these findings into clinical applications. Investigations into the molecular mechanisms underlying the nanoparticles' effects, their pharmacokinetics and long-term safety profiles are essential for advancing their development. Additionally, exploring the potential of these nanoparticles in combination with existing therapeutic modalities could provide a comprehensive approach to neurodegenerative disease management. Future research should focus on validating NF-κB inhibition, NLRP3 inflammasome suppression and MAPK signaling modulation through targeted molecular assays. Techniques such as Western blot analysis for phosphorylated NF-κB (p65), qPCR for NLRP3-related gene expression and immunohistochemical staining for MAPK pathway components could provide definitive mechanistic insights (Lee et al., 2021). Additionally, transcriptomic and proteomic studies may further elucidate the downstream effects of polyherbal AgNPs, paving the way for translational neurotherapeutics. While biochemical and histological markers indicate neuroprotection, behavioral assessments, such as the Morris water maze (MWM) for spatial memory, the Y-maze for working memory and rotarod tests for motor coordination, would provide stronger translational relevance (ncbi.nlm.nih.gov) (35).

Conclusion

This study successfully synthesized and characterized polyherbal silver nanoparticles (AgNPs) using ethanol extracts of *L. pungens, L. brevispica* and *L. cinerea*. The AgNPs exhibited significant anti-neuroinflammatory and neuroprotective effects in both *in vitro* and *in vivo* models, effectively reducing nitric oxide, TNF-α and IL-6 levels, enhancing antioxidant activity and protecting neuronal integrity in LPS-induced neuroinflammation. The enhanced bioactivity is attributed to the synergistic action of phytochemicals in the polyherbal formulation, demonstrating superior efficacy over single-plant extracts. These findings support the potential of plant-based nanomedicine as a sustainable strategy for managing neurodegenerative diseases like Alzheimer's, Parkinson's and multiple sclerosis.

For industrial and clinical translation, future work should focus on scaling up production while preserving nanoparticle stability and bioactivity. Optimizing physicochemical properties, evaluating long-term safety and conducting detailed pharmacokinetic and toxicity studies are essential. Further mechanistic studies-exploring NF-κB, NLRP3 and MAPK pathways will clarify the mode of action. Combining polyherbal AgNPs with existing neuroprotective agents may enhance therapeutic outcomes. In summary, *Lepidagathis*-based

polyherbal AgNPs represent a promising, eco-friendly therapeutic platform for treating neuroinflammatory and neurodegenerative conditions, warranting further development and clinical validation.

Authors' contributions

JR carried out the molecular genetic studies, participated in the sequence alignment and drafted the manuscript. JR carried out the immunoassays. JR participated in the sequence alignment. SR participated in the design of the study and performed the statistical analysis. SR conceived of the study and participated in its design and coordination. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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