

GREEN SYNTHESIS OF SILVER NANOPARTICLES USING POLYHERBAL EXTRACTS AND THEIR INCORPORATION INTO A HYDROGEL FOR ANTI-INFLAMMATORY APPLICATIONS

¹Mohammed Afnan Aquib U*, ¹Maheswari M, ¹Mahasri R, ¹Mohan Kumar G, ¹Mahadoma Harisha I.

²R. Ramasubramania Raja, ³S.Sriram,

¹Department of Pharmacognosy, Sri Ramakrishna Institute of Paramedical Sciences, College of Pharmacy, Coimbatore - 641044, Tamil Nadu, India

²Associate Professor, Department of Pharmacognosy, Sri Ramakrishna Institute of Paramedical Sciences, College of Pharmacy, Coimbatore - 641044, Tamil Nadu, India

³Principal, Sri Ramakrishna Institute of Paramedical Sciences, College of Pharmacy, Coimbatore - 641044, Tamil Nadu, India

ABSTRACT

The current investigation explores the green synthesis of silver nanoparticles (AgNPs) using polyherbal hydroalcoholic extracts of *Curcuma longa* (rhizomes), *Vitex negundo* (leaves), and *Cardiospermum halicacabum* (leaves), with subsequent incorporation into a Carbopol 934-based hydrogel for potential topical anti-inflammatory application. The plant materials were authenticated by the Botanical Survey of India, Coimbatore. Extraction by cold maceration with 70% ethanol afforded percentage yields of 6.5%, 4.5%, and 4.0% (w/w) for *C. longa*, *V. negundo*, and *C. halicacabum*, respectively. Qualitative phytochemical screening confirmed the presence of flavonoids, phenols, tannins, and saponins in all three extracts, with additional carbohydrates in *C. longa* and alkaloids in *C. halicacabum* and terpenoids in *V. negundo*. UV-Visible and FTIR spectroscopic analyses corroborated these phytochemical findings. In vitro anti-inflammatory activity, assessed by the egg albumin protein denaturation inhibition assay, yielded IC₅₀ values of 132, 165, and 217 µg/mL for *C. longa*, *V. negundo*, and *C. halicacabum*, respectively, compared with 79 µg/mL for diclofenac sodium. Silver nanoparticles were synthesised by reacting plant extract with 1 mM AgNO₃ at extract: AgNO₃ volume ratios of 9:1, 8:2, and 7:3. The 8:2 formulation was selected as optimal based on a sharp Surface Plasmon Resonance (SPR) peak at 423 nm. FTIR analysis confirmed that phenolic and flavonoid moieties served as reducing and stabilising agents. Scanning Electron Microscopy (SEM) revealed quasi-spherical nanoparticles predominantly ranging from 61-120 nm, and entrapment efficiency was 74.3%. Six hydrogel formulations (F1-F6) were prepared and evaluated; all exhibited homogeneity, pH 5.09-5.57, spreadability 86.90-93.75 g·cm/sec, and viscosity 4456-5190 cP—properties consistent with safe and effective topical application. These findings demonstrate that the selected medicinal plants possess significant anti-inflammatory activity and that their phytoconstituents successfully mediate stable AgNP synthesis. The resultant nanoparticle-loaded hydrogel represents a promising candidate as a natural topical anti-inflammatory formulation warranting further in-vivo evaluation.

Keywords: *Curcuma longa*, *Vitex negundo*, *Cardiospermum halicacabum*, green synthesis, silver nanoparticles, anti-inflammatory, protein denaturation assay, topical hydrogel.

1. INTRODUCTION

Inflammation is a fundamental protective response of living tissues against injury caused by harmful stimuli such as pathogens, toxins, physical damage, or chemical agents. It serves as a defence mechanism aimed at eliminating the initial cause of cell injury, clearing out necrotic cells, and initiating tissue repair [1]. Although inflammation is essential for survival, excessive or prolonged inflammatory responses can lead to tissue damage and contribute to various pathological conditions such as rheumatoid arthritis, atherosclerosis, and hypersensitivity reactions [1].

The classical signs of inflammation - redness (rubor), swelling (tumor), heat (calor), pain (dolor), and loss of function (functio laesa) - reflect underlying vascular and cellular events [1]. Inflammation is broadly classified into acute and chronic types. Acute inflammation is a rapid and short-term response characterized by increased vascular permeability, fluid exudation, and predominance of neutrophils. It involves two major processes: vascular changes such as vasodilation and increased permeability, and cellular events including leukocyte migration and phagocytosis [1]. In contrast, chronic inflammation is a prolonged response marked by mononuclear cell infiltration, tissue destruction, and fibrosis, often resulting from persistent infection or repeated injury [1].

Chemical mediators play a crucial role in regulating inflammation. These include cell-derived mediators such as histamine, prostaglandins, leukotrienes, cytokines, and reactive oxygen species, as well as plasma-derived mediators like complement proteins and kinins [1]. These mediators control vascular changes, leukocyte recruitment, pain, and tissue injury, thereby influencing the overall inflammatory response.

Due to the involvement of inflammation in many diseases, the discovery of effective anti-inflammatory agents is of great importance. In-vitro models are widely used as preliminary screening methods for evaluating anti-inflammatory activity. Assays such as protein denaturation inhibition, membrane stabilization, and proteinase inhibitory methods are simple, cost-effective, and reproducible techniques for identifying bioactive compounds [2-4]. These methods are based on the ability of compounds to prevent protein denaturation, stabilize biological membranes, and inhibit proteolytic enzymes involved in inflammation [3-4]. Natural products, especially plant-derived phytochemicals such as flavonoids and phenolic compounds, have demonstrated significant anti-inflammatory potential in such models [2].

Despite the availability of conventional anti-inflammatory drugs, limitations such as poor bioavailability, lack of target specificity, and adverse effects necessitate the development of improved delivery systems. Novel Drug Delivery Systems (NDDS) have been developed to overcome these challenges by enhancing drug solubility, stability, and targeted delivery [5,6]. These systems enable controlled and sustained drug release, improving therapeutic efficacy while minimizing side effects.

Nanotechnology has emerged as a promising approach in drug delivery, particularly using nanoparticles. Nanoparticles are small particles ranging from 1 to 1000 nm, possessing a high surface area-to-volume ratio that enhances drug solubility, bioavailability, and interaction

with biological systems [7]. They are widely used in targeted drug delivery, controlled release formulations, and biomedical applications.

Among various nanoparticles, silver nanoparticles (AgNPs) have gained significant attention due to their potent antimicrobial, antioxidant, and anti-inflammatory properties [8]. AgNPs exert their biological effects by interacting with microbial cell membranes, generating reactive oxygen species, and modulating inflammatory mediators [10]. Conventional methods for nanoparticle synthesis often involve toxic chemicals; therefore, green synthesis using plant extracts has emerged as an eco-friendly and sustainable alternative [9]. In this approach, phytochemicals act as reducing and stabilizing agents, producing biocompatible nanoparticles with enhanced therapeutic potential [9].

The integration of nanoparticles into advanced delivery systems, particularly topical formulations such as hydrogels, has further improved their therapeutic effectiveness. These systems provide controlled drug release, enhanced skin penetration, and prolonged retention at the site of action, making them highly suitable for the treatment of inflammatory conditions.

In conclusion, the combination of anti-inflammatory phytochemicals, nanotechnology, and novel drug delivery systems offers a promising strategy for developing effective and targeted therapies with improved safety and efficacy.

2. MATERIAL AND METHODOLOGIES

2.1 Collection of Plant Materials

Fresh rhizomes of *Curcuma longa*, whole plant of *Cardiospermum halicacabum*, and leaves of *Vitex negundo* were collected from Tamil Nadu, India, during November 2025 under similar environmental conditions.

2.2 Herbarium Preparation

Herbarium specimens of all three plant species were successfully prepared following standard botanical techniques.

2.3 Authentication of Plants

The plant materials were authenticated by a qualified botanist from the Botanical Survey of India (BSI), Coimbatore, and voucher numbers were assigned for reference.

2.4 Preparation of Extracts

Plant materials were washed, shade-dried ($\leq 40^{\circ}\text{C}$), coarsely powdered, and extracted using 70% ethanol by cold maceration. Extracts were filtered, concentrated at 40°C , and stored at 4°C until use [11,12,13].

2.5 Preliminary Phytochemical Screening

Qualitative phytochemical screening was carried out to detect major secondary metabolites such as alkaloids, flavonoids, phenolics, tannins, saponins, glycosides, and terpenoids using standard methods [14].

2.6UV-Vis Spectral Analysis

UV-Visible analysis of the extracts was performed in the wavelength range of 200-800 nm to identify characteristic absorption peaks corresponding to phytoconstituents [15-17].

2.7FTIR Analysis

FTIR spectroscopy was carried out using the KBr pellet method in the range of 4000-400 cm^{-1} to identify functional groups present in the extracts [16].

2.8In-Vitro Anti-inflammatory Activity

Anti-inflammatory activity was evaluated using the egg albumin denaturation assay at concentrations of 31.25-500 $\mu\text{g/mL}$. Diclofenac sodium was used as the standard. Percentage inhibition and IC_{50} values were calculated using standard procedures [18].

2.9Green Synthesis of Silver Nanoparticles

Silver nanoparticles were synthesized using polyherbal extracts with 1 mM AgNO_3 at different ratios (9:1, 8:2 and 7:3) under continuous stirring in dark conditions. Formation of nanoparticles was confirmed by a colour change after 24 h [19].

2.10Characterization of Silver Nanoparticles

2.10.1UV-Visible Spectroscopy

The synthesized AgNPs were analysed in the range of 300-800 nm, and formation was confirmed by a surface plasmon resonance peak at 400-450 nm [20].

2.10.2 FTIR Analysis

FTIR spectra (4000-400 cm^{-1}) were recorded using the KBr pellet method to identify functional groups responsible for nanoparticle stabilization [21].

2.10.3 Percentage Entrapment Efficiency (%EE)

Entrapment efficiency was determined by centrifugation followed by UV-Visible analysis of the supernatant at 275 nm using a calibration curve [22].

2.10.4 Scanning Electron Microscopy

SEM analysis was performed to evaluate the morphology and particle size distribution of AgNPs [23].

2.11Preparation of Silver Nanoparticles loaded Hydrogel

AgNP-loaded hydrogels were prepared using Carbopol 934 along with parabens and propylene glycol and neutralized with triethanolamine to obtain suitable pH and consistency [24]. Six formulations (F1-F6) were prepared with escalating AgNP concentrations (25, 50, 75, 100, 125, and 150 mg) while keeping excipient quantities constant as mentioned in **Table 1**.

Table 1: Composition of AgNP-loaded hydrogel formulations (F1-F6).

Parameter	F1	F2	F3	F4	F5	F6
AgNPs (mg)	25	50	75	100	125	150
Carbopol 934 (g)	0.2	0.2	0.2	0.2	0.2	0.2
Propylene Glycol (mL)	1.15	1.15	1.15	1.15	1.15	1.15
Propyl Paraben 0.2% (mL)	0.02	0.02	0.02	0.02	0.02	0.02
Methyl Paraben 0.5% (mL)	0.04	0.04	0.04	0.04	0.04	0.04
Triethanolamine (mL)	0.2	0.2	0.2	0.2	0.2	0.2
Vehicle (q.s. mL)	20	20	20	20	20	20

2.12 Evaluation of Silver Nanoparticles loaded Hydrogel

All six formulations were evaluated for: (i) appearance - visual assessment of colour, texture, and phase separation; (ii) pH - 1 g gel dispersed in 10 mL distilled water, measured with a calibrated digital pH meter at $25 \pm 1^\circ\text{C}$; (iii) homogeneity - gentle rubbing between fingers to detect grittiness; (iv) washability - ease of removal with running water; (v) spreadability - glass slide method ($S = m \times l / t$, where m = weight applied, l = length moved, t = time); and (vi) viscosity - Brookfield viscometer, spindle 4, 75 rpm [24,25].

3. RESULT AND DISCUSSION

3.1 Collection of Plants

Fresh rhizomes of *Curcuma longa* were procured from Pattuviduthi, Pudukottai district, Tamil Nadu, in November 2025. *Cardiospermum halicacabum* and *Vitex negundo* were simultaneously collected from Thanjavur, Tamil Nadu, ensuring uniform seasonal conditions for all plant materials.

3.2 Herbarium Preparation

Herbarium specimens of all three plant species were successfully prepared following standard botanical techniques. The dried and mounted herbarium sheets preserved key macroscopic features: rhizome morphology and characteristic, orange-coloured interior section of *C. longa*; compound, trifoliate leaf structure of *V. negundo*; and inflated, balloon-like fruit capsules with heart-marked seeds of *C. halicacabum*. These specimens serve as permanent voucher references for future verification.

3.3 Authentication

All three plant species were formally authenticated by a qualified botanist from the Botanical Survey of India (BSI), Southern Regional Centre, Coimbatore. Voucher numbers are **BSI/SRC/5/23/2025-26/Tech./712** (*C. longa*), **BSI/SRC/5/23/2025-26/Tech./711** (*V. negundo*), and **BSI/SRC/5/23/2025-26/Tech./710** (*C. halicacabum*). BSI authentication provides a traceable quality assurance step critical for the reproducibility of herbal research.

3.4 Extraction Yields

The percentage yields of the hydroalcoholic extracts were: *C. longa*: 6.5% w/w (dark yellow, semi-solid, aromatic); *V. negundo*: 4.5% w/w (greenish-brown, semi-solid, characteristic herbal odour); and *C. halicacabum*: 4.0% w/w (dark green, viscous, mild odour). The relatively higher yield of *C. longa* may reflect the abundance of curcuminoids, volatile oils, and polysaccharides in the rhizome matrix. **Table 2** shows % yield of extract with their characteristic colours.

Table 2: Percentage yield and physical characteristics of hydroalcoholic extracts.

Plant Species	Plant Part	Yield (% w/w)	Extract Characteristics
<i>Curcuma longa</i>	Rhizome	6.5	Dark yellow; aromatic; semi-solid
<i>Vitex negundo</i>	Leaves	4.5	Greenish brown; herbal odour; semi-solid
<i>Cardiospermum halicacabum</i>	Leaves	4.0	Dark green; viscous; mild odour

3.5 Preliminary Phytochemical Screening

Qualitative phytochemical screening revealed a diverse secondary metabolite profile in all three extracts is presented in **Table 3**. Flavonoids, phenols, tannins, and saponins were detected in all three species. Carbohydrates were specifically detected in *C. longa* consistent with the high polysaccharide content of turmeric rhizomes. Alkaloids were identified in *C. halicacabum* and *V. negundo*. Terpenoids were detected in *V. negundo*. The presence of these bioactive classes - particularly flavonoids and phenolic compounds is consistent with the established anti-inflammatory mechanisms of these plants.

Table 3: Qualitative phytochemical screening of hydroalcoholic extracts. (+) present; (–) absent.

Secondary Metabolite	<i>Curcuma longa</i>	<i>Vitex negundo</i>	<i>Cardiospermum halicacabum</i>
Alkaloids	–	+	+
Carbohydrates	+	–	–
Flavonoids	+	+	+
Phenols	+	+	+
Tannins	+	+	+
Saponins	+	+	+
Terpenoids	–	+	–
Glycosides	–	–	–

3.6 UV-Visible Spectral Analysis

Curcuma longa displayed absorption peaks at 202 nm, 237 nm, and 426 nm as displayed in **Figure 1**. The peak at 237 nm corresponds to the 230-290 nm flavonoid absorption band, while the prominent peak at 426 nm is characteristic of conjugated polyphenols, particularly curcuminoids (curcumin I, II, and III), which represent the primary bioactive constituents of the rhizome.

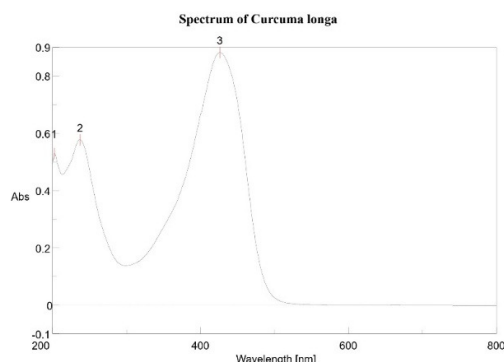


Figure 1: UV-Visible absorption spectrum of *Curcuma longa* extract showing characteristic peaks at 202 nm, 237 nm, and 426 nm.

Vitex negundo showed peaks at 205, 249, 332, and 665 nm as displayed in **Figure 2**. The band at 249 nm confirms flavonoid-class compounds (casticin, luteolin, artemetin); the 332 nm peak is consistent with conjugated flavonoid derivatives; and the 665 nm absorption corresponds to chlorophyll pigments in the leaf extract.

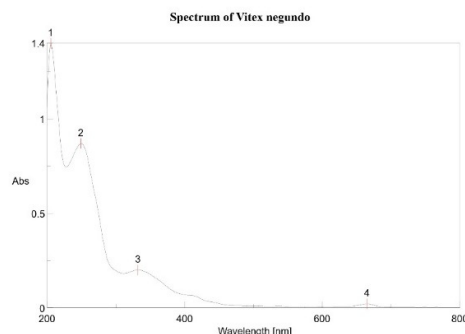


Figure 2: UV-Visible absorption spectrum of *Vitex negundo* extract showing characteristic peaks at 205 nm, 249 nm, 332 nm, and 665 nm.

Cardiospermum halicacabum exhibited peaks at 206, 268, and 328 nm as displayed in **Figure 3**. The 268 nm and 328 nm peaks are diagnostic for flavonoids (apigenin, luteolin, chrysoeriol) and their glucuronide derivatives. The UV-Visible spectral data collectively corroborate the phytochemical screening results.

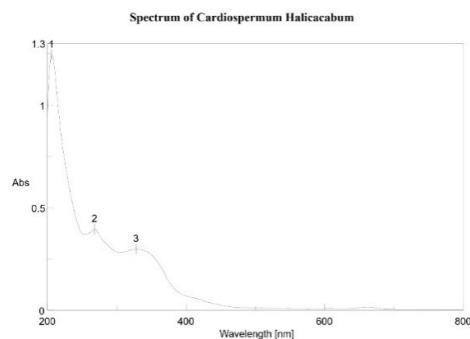


Figure 3: UV-Visible absorption spectrum of *Cardiospermum halicacabum* extract showing characteristic peaks at 206 nm, 268 nm, and 328 nm.

3.7 FTIR Spectral Analysis of Herbal Extracts

Curcuma longa

The FTIR spectra of the herbal extracts were recorded in the region of $4000\text{--}400\text{ cm}^{-1}$ to identify the functional groups present in the phytochemical constituents.

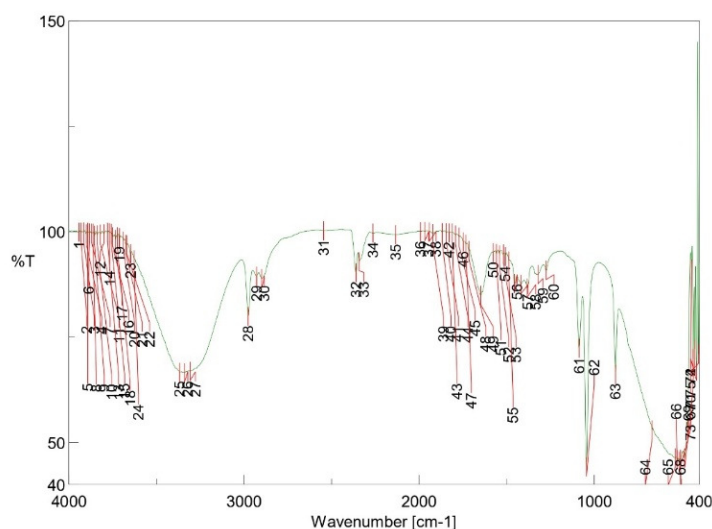


Figure 4: FTIR spectrum of *Curcuma longa* extract recorded in the range of $4000\text{--}400\text{ cm}^{-1}$, showing characteristic absorption peaks corresponding to different functional groups present in the extract.

The FTIR spectrum of *Curcuma longa* as presented in **Figure 4** exhibited a broad absorption band at 3339 cm^{-1} , corresponding to **O-H stretching vibrations**, indicating the presence of phenolic and alcoholic groups. Peaks observed at 2975 and 2928 cm^{-1} correspond to **aliphatic C-H stretching vibrations**. A prominent peak around 1647 cm^{-1} indicates **C=C stretching vibrations of aromatic rings**, suggesting the presence of phenolic compounds. Bands observed at 1558 and 1507 cm^{-1} correspond to **aromatic skeletal vibrations**, while peaks around 1273 and 1044 cm^{-1} correspond to **C-O stretching vibrations**. Additional peaks in the region 878 cm^{-1} indicate **aromatic C-H bending vibrations**. The characteristic FTIR peaks and their functional group assignments are summarized in **Table 4**.

Table 4: FTIR peak assignments of *Curcuma longa* extract showing the observed wavenumbers and corresponding functional group transitions.

Wavenumber (cm ⁻¹)	Transition	Functional group indicated
3339	O-H stretching	Phenols / alcohols
2975, 2928	C-H stretching	Aliphatic hydrocarbons
1647	C=C stretching	Aromatic compounds
1558, 1507	Aromatic skeletal vibration	Aromatic rings / flavonoids
1273	C-N stretching	Amines
1044	C-O stretching	Alcohols / phenols
878	Aromatic C-H bending	Aromatic compounds
536-403	Fingerprint region vibrations	Complex organic structures

Vitex negundo

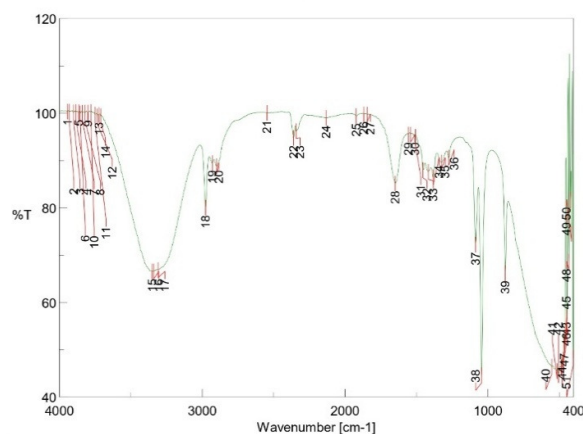


Figure 5: FTIR spectrum of *Vitex negundo* extract recorded in the range of 4000-400 cm⁻¹, indicating the presence of functional groups associated with phytochemical constituents.

The FTIR spectrum of *Vitex negundo* is shown in **Figure 5** showed a broad absorption band around **3350-3339 cm⁻¹**, corresponding to **O-H stretching vibrations**, indicating phenolic compounds. Peaks observed at **2975 and 2929 cm⁻¹** correspond to **aliphatic C-H stretching vibrations**. A prominent absorption peak at **1647 cm⁻¹** indicates **C=C stretching vibrations of aromatic rings**. Bands appearing around **1557 and 1507 cm⁻¹** correspond to **aromatic skeletal vibrations**, while peaks around **1273 and 1044 cm⁻¹** correspond to **C-O stretching vibrations**. The characteristic FTIR peaks are summarized in **Table 5**.

Table 5: FTIR peak assignments of *Vitex negundo* extract indicating functional groups present in the phytochemical constituents.

Wavenumber (cm ⁻¹)	Transition	Functional group indicated
3350, 3339	O-H stretching	Phenols / alcohols
2975, 2929	C-H stretching	Aliphatic hydrocarbons
1647	C=C stretching	Aromatic compounds
1557, 1507	Aromatic skeletal vibration	Aromatic rings
1273	C-N stretching	Amines
1044	C-O stretching	Alcohols / phenols
878	Aromatic C-H bending	Aromatic compounds
552-403	Fingerprint region vibrations	Complex organic structures

Cardiospermum halicacabum

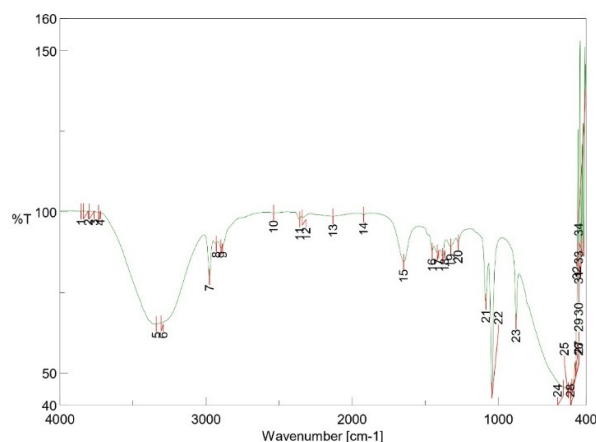


Figure 6: FTIR spectrum of *Vitex negundo* extract recorded in the range of 4000-400 cm⁻¹, indicating the presence of functional groups associated with phytochemical constituents.

The FTIR spectrum of *Cardiospermum halicacabumas* shown in **Figure 6** exhibited a broad peak around **3306 cm⁻¹**, corresponding to **O-H stretching vibrations**, indicating phenolic compounds. Peaks observed at **2976 and 2930 cm⁻¹** correspond to **aliphatic C-H stretching vibrations**. A strong absorption peak around **1647 cm⁻¹** indicates **C=C stretching vibrations of aromatic compounds**. Bands appearing at **1453 and 1382 cm⁻¹** correspond to **C-H bending vibrations**, while peaks around **1273 and 1044 cm⁻¹** correspond to **C-O stretching vibrations**. The characteristic peaks are summarized in **Table 6**.

Table 6: FTIR peak assignments of *Cardiospermum halicacabum* extract showing the characteristic functional groups identified from the spectrum.

Wavenumber (cm ⁻¹)	Transition	Functional group indicated
3306	O-H stretching	Phenols / alcohols
2976, 2930	C-H stretching	Aliphatic hydrocarbons
1647	C=C stretching	Aromatic compounds
1453, 1382	C-H bending	Alkyl groups
1273	C-N stretching	Amines
1044	C-O stretching	Alcohols / phenols
878	Aromatic C-H bending	Aromatic compounds
554-404	Fingerprint region vibrations	Complex organic structures

3.8 Anti-Inflammatory Activity

All three extracts demonstrated concentration-dependent inhibition of egg albumin protein denaturation across the tested concentration range of 31.25-500 µg/mL is presented in **Table 7**. The dose-response curves for all extracts and the standard showed a progressive sigmoidal increase in inhibition with increasing concentration as depicted in **Figure 7**

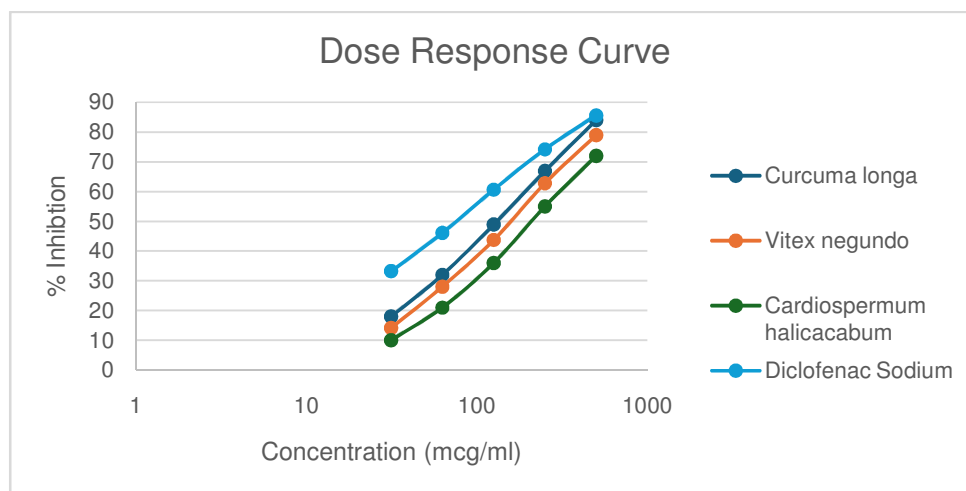


Figure 7: Dose-response curve showing concentration-dependent inhibition of protein denaturation by *Curcuma longa*, *Vitex negundo*, *Cardiospermum halicacabum*, and diclofenac sodium.

C. longa exhibited the highest anti-inflammatory activity with an IC₅₀ of **132 µg/mL**, attributable to its high curcuminoid content — particularly curcumin I, which is established as a potent inhibitor of NF-κB, COX-2, and pro-inflammatory cytokines (IL-1β, IL-6, TNF-α). *V. negundo* showed moderate-high activity (IC₅₀ = **165 µg/mL**), consistent with the anti-inflammatory effects of casticin, luteolin, and iridoid glycosides reported in the literature. *C.*

halicacabum demonstrated comparatively moderate activity ($IC_{50} = 217 \mu\text{g/mL}$), attributed to apigenin, luteolin, and β -amyrin, which are known suppressors of prostaglandin and cytokine production. Diclofenac sodium demonstrated the highest inhibitory potential with an IC_{50} of $79 \mu\text{g/mL}$, consistent with its established COX-inhibitory mechanism of action.

The order of anti-inflammatory activity was Diclofenac sodium > *C. longa* > *V. negundo* > *C. halicacabum*.

Table 7: Percentage inhibition of protein denaturation at concentrations 31.25-500 $\mu\text{g/mL}$ and IC_{50} values.

Conc. ($\mu\text{g/mL}$)	<i>C. longa</i>	<i>V. negundo</i>	<i>C. halicacabum</i>	Diclofenac Na
31.25	18.0	14.1	10.0	33.2
62.50	32.0	28.0	21.0	46.1
125	48.9	43.7	36.0	60.6
250	66.9	62.8	55.0	74.2
500	84.0	79.0	72.0	85.6
IC_{50} ($\mu\text{g/mL}$)	132	165	217	79

3.9 Green Synthesis of Silver Nanoparticles

Silver nanoparticles were successfully biosynthesized using polyherbal extract and AgNO_3 at ratios of 9:1, 8:2, and 7:3. Prior to incubation, all reaction mixtures were colourless. Following magnetic stirring at 600-700 RPM in dark conditions, a characteristic colour change was observed in all formulations as shown in **Figure 8**:

- 9:1 ratio: Colour change from colourless to yellowish-brown within 10 minutes, stabilising to colloidal brown after 24 hours.
- 8:2 ratio: Similar transition within 20 minutes, yielding a stable, uniformly brown colloid.
- 7:3 ratio: Colour development after 45 minutes; deeper brown coloration with slight opacity suggesting higher extract concentration influenced synthesis kinetics.

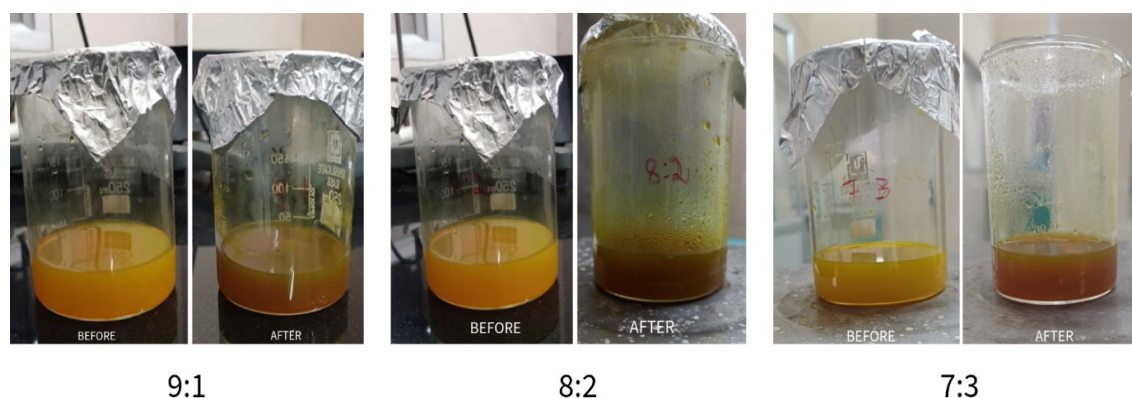


Figure 8: Characteristics colour change of all formulation.

The brown coloration results from the excitation of surface plasmon resonance in AgNPs and is widely used as a primary indicator of nanoparticle formation. The variation in reaction time across ratios indicates that extract concentration modulates reduction kinetics: higher extract

proportions provided more reducing equivalents, though at the expense of stability in the 7:3 system, as confirmed by UV-Vis spectroscopy.

3.10 Characterization of AgNPs

3.10.1 UV-Visible Spectroscopy

UV-Visible spectroscopy was employed to confirm AgNP formation and identify the optimal ratio. All three formulations exhibited characteristic SPR absorption bands in the visible region as illustrated in Figures 9-11

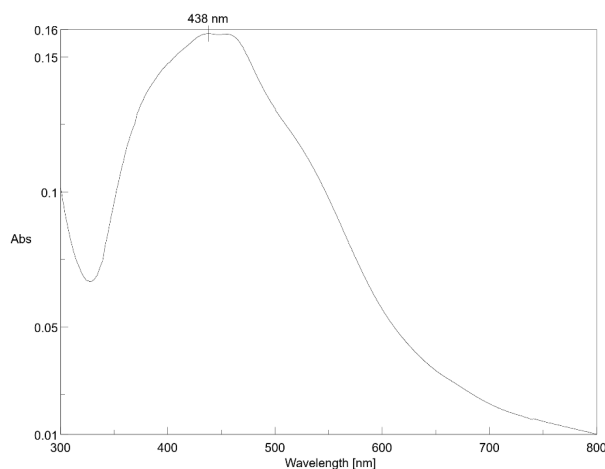


Figure 9: UV-Vis spectrum of AgNPs (9:1 extract:AgNO₃) showing an SPR peak at 438 nm, confirming nanoparticle formation.

- 9:1 ratio: SPR peak at 438 nm - acceptable AgNP formation; moderate spectral sharpness.

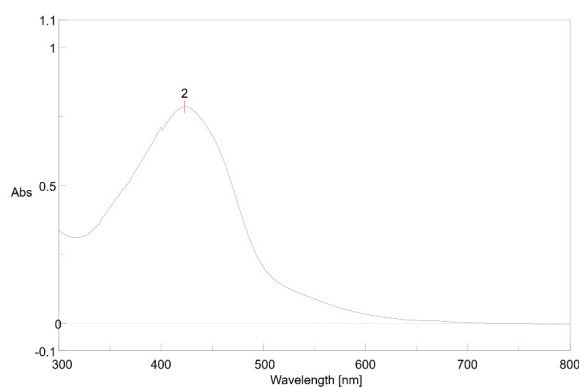


Figure 10: UV-Vis spectrum of AgNPs (8:2 extract:AgNO₃) showing an SPR peak at 423 nm, confirming nanoparticle formation.

- 8:2 ratio: Sharp, well-defined SPR peak at 423 nm, within the 400-450 nm range typical of stable, monodisperse AgNPs.

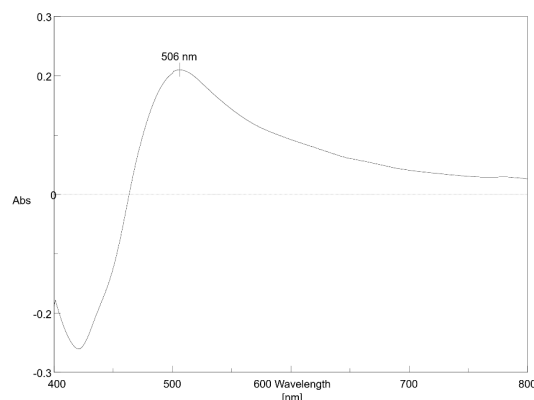


Figure 11: UV-Vis spectrum of AgNPs (7:3 extract:AgNO₃) showing an SPR peak at 506 nm, confirming nanoparticle formation.

- 7:3 ratio: Broadened SPR band at 506 nm - a red shift indicating larger particle dimensions and probable aggregation due to excess phytoconstituents.

The narrow, symmetrical SPR peak at 423 nm in the 8:2 formulation is consistent with the formation of small, well-dispersed spherical AgNPs. The 8:2 ratio was selected as the optimized formulation for further characterization based on SPR characteristics.

3.10.2 FTIR Analysis of Synthesized AgNPs

FTIR analysis of the 8:2 AgNP formulation was performed to elucidate the functional groups of phytoconstituents involved in nanoparticle reduction and stabilization as presented in **Table 8; Figure 12**). The spectrum of AgNPs exhibited a broad O-H stretching peak at 3293-3306 cm⁻¹, confirming the involvement of phenolic hydroxyl groups from the plant extract in reducing Ag⁺ to Ag⁰. A C=C aromatic stretching band at 1638 cm⁻¹ and aromatic skeletal vibrations at 1515 cm⁻¹ indicate the presence of flavonoid-based capping agents on the nanoparticle surface. C-H bending at 1452 cm⁻¹ and C-O stretching at 1045 cm⁻¹ further confirm the adsorption of phenolic and flavonoid constituents on the AgNP surface.

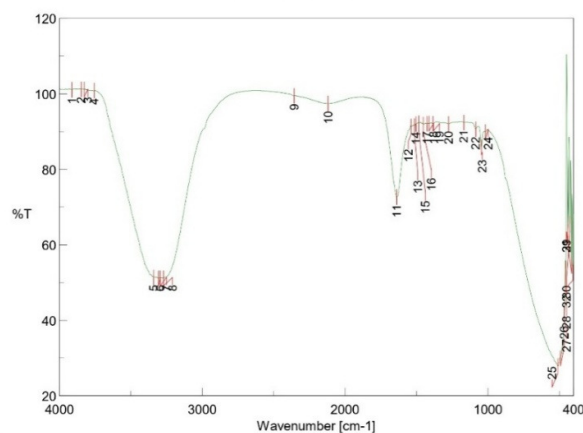


Figure 12: FTIR spectrum of synthesized silver nanoparticles (AgNPs) recorded in the range of 4000-400 cm⁻¹, indicating the functional groups involved in the reduction and stabilization of nanoparticles.

Notably, the O-H stretching peak in the AgNP spectrum shifted to a slightly lower wavenumber (3293 cm^{-1}) compared to the parent extracts ($3306\text{--}3350\text{ cm}^{-1}$), indicating coordinative interaction between the phenolic groups and the silver surface. The fingerprint region ($400\text{--}510\text{ cm}^{-1}$) showed absorption peaks consistent with Ag-O vibrational modes, confirming successful silver nanoparticle formation. These results collectively demonstrate that polyphenols and flavonoids in the polyherbal extract act as dual reducing and stabilizing agents during green synthesis.

Table 8: FTIR peak assignments for synthesized AgNPs (8:2 ratio), indicating functional groups involved in reduction and stabilization.

Wavenumber (cm ⁻¹)	Transition	Role in Nanoparticle Formation
3293, 3306	O-H stretching	Phenolic reduction agents; capping layer
1638	C=C stretching	Aromatic stabilizing agents (flavonoids)
1515	Aromatic skeletal vibration	Flavonoid framework on AgNP surface
1452	C-H bending	Alkyl groups of capping phytoconstituents
1274	C-N stretching	Amine groups from protein/enzyme fraction
1045	C-O stretching	Alcohol/phenol oxygen coordinated to Ag
400-510	Fingerprint vibrations	Ag-O / Ag-O-Ag vibrational modes

3.10.3 Percentage Entrapment Efficiency

The calibration curve of polyherbal extract at 275 nm displayed excellent linearity ($R^2 = 0.998$) over the range of $100\text{--}600\text{ }\mu\text{g/mL}$, with the regression equation $A = 0.0048C$ as presented in **Figure 13**. Beer-Lambert's law was satisfied, validating the method for quantification of free phytoconstituents.

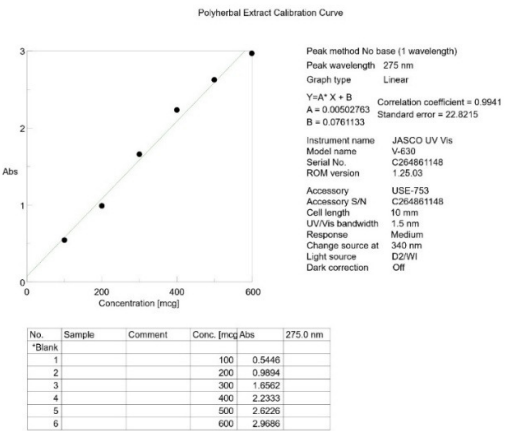


Figure 13: Calibration curve of polyherbal extract showing the relationship between concentration ($\mu\text{g/mL}$) and absorbance at 275 nm.

Calculation: Absorbance of supernatant = 1.97 AU. Free extract concentration: $C = 1.97/0.0048 = 410.4 \mu\text{g/mL}$. Amount of free extract in 20 mL supernatant: $410.4 \times 20 = 8208 \mu\text{g} = 8.21 \text{ mg}$. Total extract used (T) = 32 mg; Free extract (F) = 8.21 mg.

$$\%EE = [(32 - 8.21) / 32] \times 100 = 74.3\%$$

An entrapment efficiency of 74.3% for the 8:2 AgNP formulation indicates efficient incorporation of phytoconstituents into the nanoparticle matrix.

3.10.4 SEM Analysis

SEM analysis of the 8:2 AgNP formulation revealed the formation of quasi-spherical nanoparticles distributed across the sample surface as presented in **Figure 14**. Some particles exhibited slight cuboidal character, and mild aggregation was observable, consistent with the tendency of phytoconstituent-capped nanoparticles to cluster during drying on the substrate. The particle size, determined by ImageJ analysis from SEM micrographs, ranged from 41 to 180 nm. The histogram was plotted by using Microsoft excel to determine particle size distribution as shown in **Figure 15** indicates that the majority of particles were distributed in the 61-120 nm range, with a modal size class of 80-100 nm.

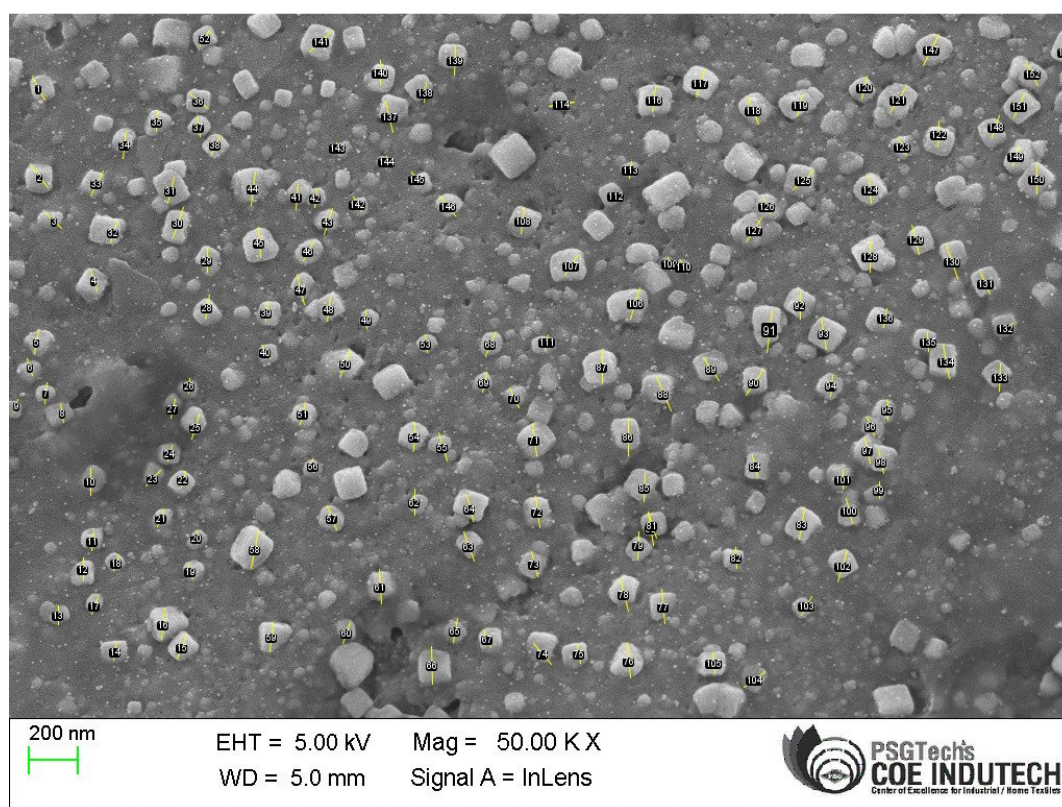


Figure 14: SEM micrograph showing the morphology and particle size of silver nanoparticles.

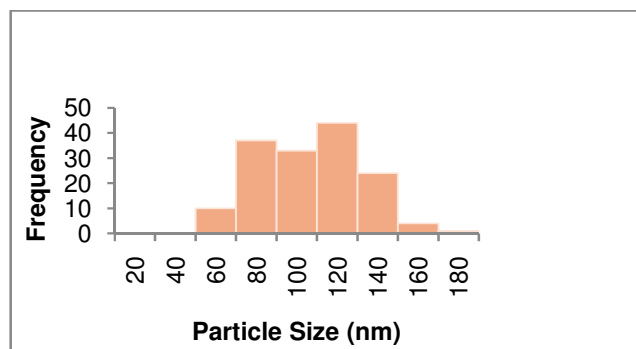


Figure 15: Histogram representing the particle size distribution of biosynthesized silver nanoparticles obtained from SEM analysis.

The nanoscale dimensions (40-180 nm) are appropriate for topical drug delivery, facilitating penetration through skin appendages and providing a large surface area for biological interaction. The slight polydispersity observed is characteristic of biogenic nanoparticle systems, where the heterogeneous composition of plant extracts leads to variable reduction and capping dynamics.

Table 9: Particle size distribution of biosynthesized AgNPs from SEM analysis ($n = 50$ particles).

Size Range (nm)	Number of Particles	Frequency (%)
41-60	6	12
61-80	12	24
81-100	15	30
101-120	11	22
121-140	4	8
141-180	2	4

3.11 Formulation of AgNP-Loaded Hydrogel

Six Carbopol 934-based hydrogel formulations (F1-F6) incorporating increasing concentrations of AgNPs (25-150 mg/20 mL) were successfully prepared. Carbopol 934 swelled completely in distilled water, forming a homogeneous gel base. Gradual addition of the AgNP suspension with parabens and propylene glycol, followed by neutralisation with triethanolamine, produced uniformly smooth gels with no phase separation. A progressive colour gradient from slight orange (F1) to deep orange (F6) was observed, reflecting the increasing AgNP concentration. All formulations showed excellent gel consistency upon visual and tactile examination as presented in **Figure 16**.



Figure 16: Prepared silver nanoparticle-loaded hydrogel formulations (F1-F6)

3.12 Evaluation of AgNP-Loaded Hydrogel

3.12.1 Physical Appearance

All six formulations exhibited a smooth, jelly-like texture with pleasant odour, appropriate consistency, and absence of grittiness. Colour progressively intensified from slight orange (F1-F2) to moderate orange (F3-F4) and deep orange (F5-F6), directly reflecting the increasing AgNP content. No phase separation or sedimentation was observed in any formulation, indicating good physical stability and uniform nanoparticle distribution.

3.12.2 pH

The pH of all formulations was measured to be within the acceptable range for topical skin application (5.0-7.0). The recorded values are presented in **Table 10** fell within the physiological skin pH range of 4.5-6.5, ensuring minimal risk of skin irritation. Minor pH variations among formulations may reflect slight differences in the ionic contributions of varying AgNP concentrations.

Table 10: pH values of AgNP-loaded hydrogel formulations (F1-F6).

Formulation	pH
F1	5.55
F2	5.09
F3	5.56
F4	5.57
F5	5.09
F6	5.54

3.12.3 Homogeneity and Washability

All formulations demonstrated complete homogeneity with smooth, uniform texture and absence of grittiness upon inter-digital rubbing. No aggregates or undispersed particles were detected in any formulation, confirming uniform nanoparticle distribution within the Carbopol matrix. All formulations were readily removed from the skin surface under running tap water, confirming satisfactory washability - an important requirement for patient compliance in topical formulations.

3.12.4 Spreadability

Spreadability values for all formulations ranged from 86.90 to 93.75 g·cm/sec as shown in **Table 11**. F1 exhibited the highest spreadability (93.75 g·cm/sec), while F5 showed the lowest (86.90 g·cm/sec). A mild inverse trend between increasing AgNP concentration and spreadability was observed, likely reflecting the incremental increase in viscosity with higher nanoparticle content. All values were within the acceptable range for topical gels, indicating ease of application

Table 11: Spreadability values of AgNP-loaded hydrogel formulations (F1-F6).

Formulation	Spreadability (g·cm/sec)
F1	93.75
F2	88.45
F3	89.50
F4	87.45
F5	86.90
F6	89.98

3.12.5 Viscosity

Viscosity values of all formulations ranged from 4456 to 5190 cP as presented in **Table 12**, indicating suitable gel-like consistency for topical application. F6 exhibited the highest viscosity (5190 cP) and F5 the lowest (4456 cP). All viscosity values fall within the clinically acceptable range for topical gels (1000-5500 cP), ensuring optimal spreadability and skin retention.

Table 12: Viscosity of AgNP-loaded hydrogel formulations (F1-F6) measured by Brookfield viscometer (spindle 4, 75 rpm).

Formulation	Viscosity (cP)
F1	4908
F2	4878
F3	4508
F4	4710
F5	4456
F6	5190

4. CONCLUSION

The present study successfully demonstrated the phytochemical characterization, anti-inflammatory evaluation, green synthesis of silver nanoparticles, and formulation of an AgNP-loaded topical hydrogel using three traditionally significant medicinal plants — *Curcuma longa*, *Vitex negundo*, and *Cardiospermum halicacabum*. Hydroalcoholic extraction yielded 6.5%, 4.5%, and 4.0% w/w for the three species respectively, with phytochemical screening confirming a rich secondary metabolite profile including flavonoids, phenols, tannins, and saponins. UV-Visible and FTIR spectroscopy corroborated these findings, identifying characteristic absorption bands consistent with curcuminoids, flavonoid derivatives, and phenolic compounds.

In vitro anti-inflammatory evaluation using the egg albumin protein denaturation assay demonstrated significant concentration-dependent inhibition, with IC_{50} values of 132 $\mu\text{g/mL}$ (*C. longa*), 165 $\mu\text{g/mL}$ (*V. negundo*), and 217 $\mu\text{g/mL}$ (*C. halicacabum*), compared to 79 $\mu\text{g/mL}$ for the standard diclofenac sodium.

The 8:2 (extract:AgNO₃) ratio was identified as optimal for AgNP synthesis, exhibiting a sharp SPR peak at 423 nm indicative of stable, monodisperse nanoparticles. FTIR analysis confirmed the involvement of phenolic and flavonoid functional groups in reduction and stabilization. Entrapment efficiency of 74.3% and SEM-confirmed quasi-spherical particle sizes of 41-180 nm (predominantly 61-120 nm) validated the physicochemical quality of the synthesized AgNPs.

AgNP-loaded Carbopol 934 hydrogel formulations (F1-F6) exhibited acceptable physicochemical properties: smooth homogeneous appearance, pH 5.09-5.57, spreadability 86.90-93.75 g·cm/sec, and viscosity 4456-5190 cP.

Overall, this study validates the use of polyherbal extracts as dual anti-inflammatory agents and nanoparticle-mediated systems for topical drug delivery. Future studies should focus on in vivo anti-inflammatory evaluation, dermal safety profiling, skin permeation studies, drug release kinetics, and long-term stability assessment to confirm the clinical potential of this formulation.

5. REFERENCES

1. Zhang H, et al. Inflammation-modulating polymeric nanoparticles: design strategies and therapeutic applications. *Mater Today Bio*. 2025.
2. Ramesh MM, et al. Pharmacological enhancement of anti-inflammatory drug delivery using solid lipid nanoparticles. *Inflammopharmacology*. 2026.
3. Zhao X, et al. Nanotechnology-based regulation of inflammatory pathways in disease. *Front Immunol*. 2025.
4. Yang J, et al. Anti-inflammatory effects of natural polysaccharides and their mechanisms. *Front Immunol*. 2025.
5. Mishra AK, et al. Nanotechnology approaches in anti-inflammatory drug development. *Eur J Pharm Sci*. 2024.
6. Yuan F, et al. Designing nanoparticles to minimize inflammatory responses in nanomedicine. *Nanotoxicology*. 2025.
7. Oselusi SO, et al. Green synthesized silver nanoparticles and their anti-inflammatory and wound healing potential. *Sci Rep*. 2025.
8. Eker F, et al. Green synthesis of silver nanoparticles: physicochemical properties and biomedical applications. *Int J Mol Sci*. 2025.
9. Hano C, Abbasi BH. Plant-based green synthesis of nanoparticles and biomedical applications. *Biomolecules*. 2022.
10. Morakul B, et al. Nanostructured lipid carriers for dermal delivery and anti-inflammatory activity. *Pharmaceutics*. 2023.
11. Abraham M, Nambiar S, Charagannavar VC, Augustine D, Sowmya SV, Babu A, et al. Histologic comparison between curcumin stain and synthetic eosin. *J Clin Diagn Res*. 2017;11(11):ZC05-ZC08. doi:10.7860/JCDR/2017/26024.10809.
12. Dowlath MJH, Karuppannan SK, Darul RGI, Mohamed KSB, Subramanian S, Arunachalam KD. Effect of solvents on phytochemical composition and antioxidant activity of *Cardiospermum halicacabum* (L.) extracts. *Pharmacogn J*. 2020;12(6):1241-1251. doi:10.5530/pj.2020.12.173.
13. Patil SP, Laddha KS. Extraction efficiency of agnuside from *Vitex negundo* leaves using different techniques and its quantitative determination by HPLC. *Int J Health Sci Res*. 2018;8(8):129-135.
14. Shaikh JR, Patil MK. Qualitative tests for preliminary phytochemical screening: an overview. *Int J Chem Stud*. 2020;8(2):603-608. doi:10.22271/chemi.2020.v8.i2i.8834.
15. Patle TK, Shrivastava K, Kurrey R, Upadhyay S, Jangde R, Chauhan R. Phytochemical screening and determination of phenolics and flavonoids in *Dillenia pentagyna* using UV-Vis and FTIR spectroscopy. *Spectrochim Acta A Mol Biomol Spectrosc*. 2020;242:118717.
16. Saxena M, Saxena J. Evaluation of phytoconstituents of *Acorus calamus* by FTIR and UV-Vis spectroscopic analysis. *Int J Biol Pharm Res*. 2012;3(3):498-501.
17. Johnson M, Syed Ali Fathima M. Spectroscopic studies on *Pouzolzia wightii* Benn. *Int J Pharm Pharm Sci*. 2018;10(3):124-132.

18. Aidoo DB, Konja D, Henneh IT, Ekor M. Protective effect of bergapten against human erythrocyte hemolysis and protein denaturation in vitro. *Int J Inflamm.* 2021;2021:1279359. doi:10.1155/2021/1279359.
19. Ghani S, Rafiee B, Bahrami S, Mokhtari A, Aghamiri S, Yarian F. Green synthesis of silver nanoparticles using plant extracts of *Vitex agnus-castus* L.: an ecofriendly approach to overcome antibiotic resistance. *Int J Prev Med.* 2022;13:133. doi:10.4103/ijpvm.ijpvm_140_22.
20. Dalir SJB, Djahaniani H, Nabati F, Hekmati M. Characterization and evaluation of antimicrobial activities of silver nanoparticles biosynthesized from *Carya illinoensis* leaf extract. *Heliyon.* 2020;6(3):e03624. doi:10.1016/j.heliyon.2020.e03624.
21. Kayed K, Issa M, Al-Ourabi H. The FTIR spectra of Ag/Ag₂O composites doped with silver nanoparticles. *J Exp Nanosci.* 2024;19(1):2336227. doi:10.1080/17458080.2024.2336227.
22. Jamous YF, Al-Sawalha NA, Al-Damen MA. Formulation and characterization of solid lipid nanoparticles for improved drug delivery: evaluation of drug loading and entrapment efficiency using UV-Vis spectrophotometry. *Processes.* 2023;11(10):3039.
23. Fahim M, Shahzaib A, Nishat N, Jahan A, Bhat TA, Inam A. Green synthesis of silver nanoparticles: a comprehensive review of methods, influencing factors, and applications. *JCIS Open.* 2024;16:100125.
24. Atpadkar KD, Mote AN, Shewale SA, Pandhare AV, Pawar VH. Formulation and evaluation of *Aloe vera* herbal hydrogel. *Int J Sci Res Eng Manag.* 2025;9(2):1-21. doi:10.55041/IJSREM41718.
25. Patel G, Patel B, Parmar R. A quality by design approach: development and evaluation of herbal hydrogel. *Int J Pharm Sci Res.* 2023;14(10):4810-4816. doi:10.13040/IJPSR.0975-8232.14(10).4810-16.