

Original Research

Received 23 May 2026

Revised 18 July 2026

Accepted 01 Aug 2026

Natural Resources for Human Health 2026, Vol. 6 (13s): 1445–1461

KEYWORDS:

Ficus racemosa, Antioxidant enzyme, Box-Behnken Design, Total phenolic content, DPPH, Hepatoprotective efficacy, biochemical markers.

Evaluation of *Ficus Racemosa* as A Hepatoprotective Agent and Its Nano-Enabled Drug Delivery Strategy

Mishra S*, Bhandarkar S¹, Kokate B², Pakmode D³, Sidam P⁴, Mahajan U⁵, More S⁶, Riyaz A⁷

Assistant Professor, Dadasaheb Balpande College of Pharmacy, Besa, Nagpur. 440037

^{1, 3, 4} Researchers- Dadasaheb Balpande College of Pharmacy, Besa, Nagpur. 440037

⁵ Professor, Dadasaheb Balpande College of Pharmacy, Besa, Nagpur. 440037

⁶ Associate Professor, Dadasaheb Balpande College of Pharmacy, Besa, Nagpur. 440037

² Assistant Professor, Tulsiramji Gaikwad Patil College of Pharmacy, Mohgaon, Nagpur

⁷ Principal, Vivekanand College of Pharmacy, Sitasangli.

*Corresponding Author: Dr. Suchitra Mishra

ABSTRACT: *Ficus racemosa*, traditionally recognized in Ayurveda for its hepatoprotective properties, lacks substantial validation in contemporary scientific literature. This study aimed to evaluate the hepatoprotective potential of ethyl acetate leaf extract of *Ficus racemosa* and its biosynthesized silver nanoparticles (AgNPs) against carbon tetrachloride (CCl₄)-induced hepatotoxicity in male Sprague-Dawley rats. Plant leaves and bark were extracted using Soxhlet reflux with ethyl acetate and methanol over three days. Phytochemical profiling confirmed the presence of flavonoids, phenols, steroids, alkaloids, and tannins. Total phenolic content was estimated via Folin-Ciocalteu assay, and flavonoids quantified by aluminum chloride colorimetric method at 415 nm. Antioxidant activity was assessed using DPPH and nitric oxide radical scavenging assays, demonstrating strong radical inhibition with low IC₅₀ values. Silver nanoparticles were synthesized from ethyl acetate leaf extract and optimized using Box-Behnken Design in Design Expert software. Rats were administered 400 mg/kg of the ethyl acetate extract and AgNPs orally for seven consecutive days. Hepatoprotective efficacy was evaluated via serum biochemical markers (ALT, AST, ALP, bilirubin, total protein) and antioxidant enzymes, alongside histopathological examination of liver tissues. Both treatment groups demonstrated significant reversal of CCl₄-induced liver damage, with silver nanoparticles showing superior efficacy in normalizing elevated enzyme levels and improving antioxidant defenses. Histology confirmed reduced hepatic necrosis and inflammation in treated groups. The study concludes that the ethyl acetate extract and its AgNP formulation possess potent hepatoprotective and antioxidant properties, likely attributed to their rich phytochemical content.

1. INTRODUCTION

The liver is the second largest organ in the human body, playing a crucial role in more than 5,000 physiological functions. It is responsible for detoxifying harmful substances, regulating metabolism of glucose, iron, and cholesterol, synthesizing clotting factors, processing nutrients, and maintaining hormonal balance [Krishnan 2019, Zhang et al 2019].

Its regenerative capacity is remarkable, allowing it to heal itself after injury. Despite this resilience, the liver is susceptible to various diseases caused by genetic disorders, viral infections, toxic substances like drugs or chemicals, autoimmune reactions,

obesity, and diabetes. If left untreated, these hepatic conditions can lead to severe complications, including irreversible damage to liver tissues or bile ducts, ultimately threatening life [Phong et al 2021, Yadav et al 2015].

Throughout history, humans have relied on medicinal plants as a therapeutic resource, using roots, leaves, flowers, or stems of various species. These botanicals are rich in antioxidant compounds such as flavonoids, phenolics, tannins, vitamins, quinones, and lignans, which combat oxidative stress and cellular damage. *Ficus racemosa* (Linn), also known as the cluster fig or Indian fig tree, is one such plant belonging to the Moraceae family. Widely distributed across India, Southeast Asia, Australia, and Malaysia, it grows in forests and hilly regions. Its traditional usage in Ayurveda and other systems of natural medicine underscores its potential in treating liver disorders, largely due to its abundance of bioactive phytochemicals [Wahid et al 2016].

Carbon tetrachloride (CCl_4) is widely used to experimentally induce liver damage in animal models, particularly rats. Once metabolized by the liver's cytochrome P450 system, CCl_4 generates trichloromethyl free radicals and reactive oxygen species (ROS), initiating a cascade of lipid peroxidation. This leads to extensive hepatic injury, including inflammation, necrosis, and impaired liver function. Such models are invaluable for evaluating the hepatoprotective efficacy of medicinal plants like *Ficus racemosa*, allowing researchers to study their antioxidant activity and therapeutic mechanisms in a controlled and reproducible setting [Gopalasathees et al 2018].

2. MATERIALS AND METHODS

Materials

All chemical reagents and solvents used in the study were of analytical grade, with a minimum purity of 99%, ensuring experimental reliability and reproducibility. Petroleum ether (60–80 °C), methanol, ethyl acetate, DPPH, Folin-Ciocalteu reagent, silver nitrate, chloroform, and n-butanol were procured from Loba Chemicals, New Delhi, while additional reagents were sourced from Himedia, Mumbai. These high-purity chemicals were integral to various procedures, including solvent extraction, phytochemical screening, antioxidant assays, and synthesis of silver nanoparticles, ensuring accurate assessment of bioactive components and hepatoprotective efficacy.

Plant collection and extraction

Leaves and bark of *Ficus racemosa* were collected from the botanical garden of Dadasaheb Balpande College of Pharmacy, Nagpur, and authenticated by a qualified botanist. The plant materials were washed with tap water and shade-dried before undergoing a continuous hot extraction using a Soxhlet apparatus. Approximately 100–150 g of each sample was successively extracted with petroleum ether, ethyl acetate, and methanol, following a sequential solvent extraction protocol. After each solvent cycle, the plant residue was dried and re-extracted with the next solvent. The resulting extracts were filtered and evaporated to obtain crude dried residues, with final yields recorded as follows: ethyl acetate leaf extract (1.66%), ethyl acetate bark extract (1.33%), methanol leaf extract (2.22%), and methanol bark extract (3.75%). This method ensured comprehensive recovery of phytoconstituents from both leaf and bark materials [Parbuntari et al 2019].

Phytochemical screening

Phytochemical screening of *Ficus racemosa* leaf and bark extracts was performed to identify the presence of key secondary metabolites known for their therapeutic relevance. The analysis included tests for alkaloids, saponins, flavonoids, tannins, phenolics, and steroids/terpenoids. Multiple solvent extracts—ethyl acetate, methanol, and petroleum ether—were evaluated separately for both leaf and bark samples. This comprehensive profiling revealed varying concentrations of bioactive constituents across different extracts, with ethyl acetate and methanol-based extracts showing pronounced presence of flavonoids, phenolics, and tannins, while petroleum ether extracts displayed lower polarity constituents such as steroids and terpenoids. These results provide foundational insights into the antioxidant and hepatoprotective potential of the plant [Parbuntari et al 2019].

Determination of Total Flavonoid Content

The total flavonoid content of *Ficus racemosa* ethyl acetate extract was quantified using the aluminum chloride colorimetric assay. A methanolic solution of the extract (1 mg/mL) was prepared and subjected to a sequence of reagent additions—including sodium nitrite, aluminum chloride, and sodium hydroxide—with incubation intervals to facilitate complex formation. The final reaction volume was adjusted to 10 mL and left to stabilize for 15 minutes before measuring absorbance at 510 nm. Using a quercetin

calibration curve, the flavonoid content was calculated and expressed as milligrams of quercetin equivalent per gram of dry extract [Pandey et al 2015]

Determination of Total Phenolic Content

The total phenolic content of *Ficus racemosa* ethyl acetate extract was determined using the Folin-Ciocalteu method at a concentration of 1 mg/mL. In the assay, 1 mL of the extract was mixed with 2.5 mL of 10% Folin-Ciocalteu reagent and 2.5 mL of 7.5% sodium bicarbonate (NaHCO_3), with a blank prepared simultaneously using water instead of extract. The mixture was allowed to stand for 45 minutes to develop coloration, and absorbance was measured spectrophotometrically at 765 nm. Each sample was analyzed in triplicate to ensure precision, and results were compared against a gallic acid standard curve (25–125 $\mu\text{g/mL}$) constructed via linear regression. The phenolic content was quantified as milligrams of gallic acid equivalent (GAE) per gram of dry extract, providing a reliable estimate of the extract's antioxidant potential [Abeyasinghe et al 2021]

Antioxidant Activity Study

A. Evaluation of scavenging activity on DPPH Radicals

The antioxidant activity of *Ficus racemosa* ethyl acetate extract was evaluated using the DPPH free radical scavenging assay. Extract concentrations ranging from 25–125 $\mu\text{g/mL}$ were prepared in ethanol and added to freshly made DPPH solution (2.9 mL of 100 $\mu\text{g/mL}$), followed by incubation in the dark for 30 minutes to facilitate the reaction. The absorbance of each reaction mixture was measured at 517 nm using a Shimadzu 1600 UV-Visible spectrophotometer, with a control containing only ethanol and DPPH. Ascorbic acid served as the standard reference. The percent scavenging activity was calculated by comparing absorbance values of the test and control using the formula: % Scavenging = $[(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$, where A_{control} is the absorbance of the control and A_{sample} is the absorbance of the test extract. This method provides an effective measure of the extract's ability to neutralize free radicals [Alara et al 2018]

$$\% \text{ scavenging of DPPH} = (A_{\text{control}} - A_{\text{test}}) / A_{\text{control}} \times 100$$

B. Determination of reducing power assay

The reducing power of *Ficus racemosa* ethyl acetate extract was determined using a phosphate buffer–ferricyanide system, where increasing concentrations (25–125 $\mu\text{g/mL}$) were incubated with potassium ferricyanide, followed by reaction termination with tricarboxylic acid and treatment with ferric chloride; absorbance measured at 700 nm indicated the extract's electron-donating ability and antioxidant potential, with higher absorbance reflecting stronger free radical neutralization [Singhal et al 2011]

C. Determination of ferrous metal ion chelating activity (FRAP)

The iron chelating activity of *Ficus racemosa* ethyl acetate extract was assessed by mixing varying concentrations (25–125 $\mu\text{g/mL}$) with FeCl_2 (5 mM), allowing the mixture to equilibrate for 10 minutes at room temperature; absorbance was measured at 562 nm, and the percentage inhibition of the ferrozine– Fe^{2+} complex was calculated to determine the extract's metal chelating potential, indicating its ability to reduce oxidative damage by limiting iron-induced free radical formation [Langley-Evans 2000]

$$\% \text{ Inhibition} = [(A_0 - A_1) / A_0] \times 100$$

Where, A_0 - absorbance of the control solution,

A_1 - absorbance of the test extracts or standard drug

In-vitro Anti-inflammatory Activity

The *in-vitro* anti-inflammatory activity of *Ficus racemosa* extract was evaluated using the Human Red Blood Corpuscles (HRBC) membrane stabilizing method, where blood from a healthy volunteer was processed to prepare a 10% HRBC suspension; the test mixture containing hypotonic saline, phosphate buffer, extract (1000 $\mu\text{g/mL}$), and HRBC was incubated at 56 °C for 30 minutes, then cooled and centrifuged, and the absorbance of the supernatant was measured at 560 nm to assess membrane stabilization, with higher percentage indicating better anti-inflammatory potential by preventing hemolysis under hypotonic stress [Djuichou et al 2019]

$$\% \text{ Inhibition of haemolysis} = \frac{[\text{Absorbance of control} - \text{Absorbance of test}] \times 100}{\text{Absorbance of control}}$$

Synthesis of Silver Nanoparticles by Green Method.

Silver nanoparticles were synthesized using *Ficus racemosa* leaf extract by dissolving 1 g of extract in 10 ml ethyl acetate, adjusting the pH to 9–11, and heating the solution to 60–80 °C; varying concentrations of AgNO₃ (0.01–0.10 M) were added dropwise with continuous magnetic stirring, and nanoparticle formation was indicated by a color change from yellow to brownish-yellow or deep brown, with UV spectroscopy (400–800 nm) confirming the presence of silver nanoparticles through characteristic surface plasmon resonance peaks [Santhosh et al 2021].

Optimization of Silver Nanoparticles

The synthesis of silver nanoparticles using *Ficus racemosa* extract was systematically optimized through Box-Behnken design (BBD) using Design Expert v13.0, selecting silver nitrate concentration, temperature, and pH as key independent variables at three levels (-1, 0, +1) as shown in table 1. Experimental trials were conducted to evaluate particle size and absorbance intensity as responses, and the data were fitted to a quadratic second-order model analysed via ANOVA, correlation coefficients (r^2 , adjusted r^2 , predicted r^2), and predicted residual sum of squares, with optimal synthesis conditions determined using numerical desirability and graphical optimization methods [Nikaeen et al 2020].

Table No. 1: Experimental factors and levels for synthesis of Silver Nanoparticles

Independent variables	Variable Levels			Dependant variables
	Low (-1)	Medium (0)	High (+)	
Concentration of silver nitrate (M)	0.01	0.05	0.1	Particle Size
Temperature (°C)	60	70	80	Absorbance Intensity
pH	9	10	11	

Characterization of Silver Nanoparticles

Surface Plasmon Resonance

UV-Visible spectrophotometry is a rapid, reliable, and sensitive technique for the primary characterization of synthesized silver nanoparticles, offering a simple method to monitor their formation and stability by measuring absorbance in the 200–800 nm range using distilled water as reference; the Shimadzu UV-1800 spectrophotometer was employed to detect characteristic surface plasmon resonance peaks, which vary based on particle size, dielectric medium, and chemical environment, making this technique highly selective and efficient without requiring calibration for colloidal suspensions [Ider et al 2017].

Percentage Entrapment Efficiency

To determine the entrapment efficiency of flavonoids in silver nanoparticles, the reaction mixture was centrifuged at 8000 rpm for 10 minutes at room temperature across three cycles; the supernatant was analyzed using a UV-Visible spectrophotometer to quantify the untrapped flavonoid content, and entrapment efficiency was calculated by referencing a standard calibration curve of flavonoids, indicating the proportion of flavonoid successfully incorporated into the nanoparticles [Jackson et al 2019].

Particle Size and Polydispersity Index

Dynamic Light Scattering (DLS) was employed to determine the particle size and polydispersity index (PDI) of silver nanoparticles using an Anton Paar instrument; the colloidal suspension was diluted with distilled water, and measurements revealed the size distribution, where PDI values ranging from 0.0 to 1.0 indicated the uniformity of particle sizes—lower values representing monodispersity and higher values indicating a broader size range within the sample [Danaei et al 2018].

Zeta Potential Measurement

The electrophoretic mobility of nanoparticles in a U-type tube at 25°C was used to calculate zeta potential using a zetasizer (Anton Paar) [Khoshnevisan et al 2015].

Fourier transform infrared spectroscopy (FTIR)

FTIR spectroscopy was conducted using the IR Affinity model from Shimadzu to evaluate the compatibility and potential interactions between *Ficus racemosa* leaf extract and synthesized silver nanoparticles; the analysis involved 45 scans at a resolution of 4 cm⁻¹ across the spectral range of 4000–400 cm⁻¹, enabling identification of functional groups and chemical bonds involved in nanoparticle formation and stabilization [Wongsa et al 2022].

Differential scanning calorimetry (DSC)

Differential Scanning Calorimetry (DSC) was employed to investigate the thermal properties, stability, and potential interactions between *Ficus racemosa* leaf extract and its optimized silver nanoparticle formulation; using the DSC-1 821e model from Mettler-Toledo, samples were heated from 20 °C to 300 °C at a constant rate of 10 °C/min in a nitrogen atmosphere with dried nitrogen as purge gas, and thermograms revealed changes in enthalpy, peak patterns, and onset temperatures, providing insights into melting behavior, degradation, compatibility, and possible formation of new entities [Chiu et al 2011]

X-Ray Diffraction

X-ray diffraction (XRD) analysis was performed using the Bruker D2 Phaser (second generation, Germany) to investigate the crystalline nature and structural changes during the formation of silver nanoparticles from *Ficus racemosa* extract; the scan was conducted over a 2θ range of 10°–80°, enabling detection of polymorphs, crystal habit modifications, and the emergence of new crystalline phases, thereby confirming nanoparticle formation and assessing their crystallinity [Latha et al 2020].

Field Emission Scanning Electron Microscopy

Field Emission Scanning Electron Microscopy (FESEM) was utilized to analyze the surface morphology of optimized silver nanoparticles using the JSM-7610F microscope (Jeol, Tokyo, Japan) at an accelerating voltage of 200 kV under high vacuum; 1–2 drops of nanoparticle dispersion were mounted on a glass grid with double carbon adhesive tape, sputter-coated with gold-palladium, and covered with a round coverslip lined with silver paint for conductivity, allowing detailed observation of particle shape, size, surface features like pores and pits, and any aggregation [Moond et al 2023].

Stability Study

An accelerated stability study of the formulated silver nanoparticles was conducted in accordance with ICH guidelines by storing samples at 40 °C ± 0.5 °C and 75% ± 5% relative humidity for 90 days; evaluations were performed on day 0 and day 30 to assess changes in surface plasmon resonance, particle size, and colour, providing insights into the formulation's physical stability and consistency under stress conditions [Sadeghi et al 2015].

In-vivo Study

Sprague dawley rats weighing 150 to 300 g were housed in standard laboratory conditions of temperature [25±2 °C], 12 h light and dark places and with food and water ad libitum [Ferreira et al 2010].

Acute oral Toxicity

The acute oral toxicity study was carried out as per the guidelines set by OECD, revised draft guidelines 423, received from CPCSEA, Ministry of Social Justice and empowerment, Govt. of India [Gupta et al 2010].

Experimental Protocol

In a hepatoprotective study, 18 rats were randomly divided into three groups of six: Group I served as the normal control receiving 1 ml/kg/day of normal saline orally; Group II was treated with CCl₄ (0.5 ml/kg i.p., 1:1 in olive oil) and *Ficus racemosa* ethyl acetate leaf extract (400 mg/kg p.o.) for 7 days; Group III received silver nanoparticles of the extract (400 mg/kg p.o.) along with CCl₄ (0.5 ml/kg i.p.) for 7 days. After 24 hours of the final dose, animals were sacrificed, blood was collected for serum separation at 2500 rpm for 15 minutes, and liver tissues were harvested for biochemical and histopathological analysis [Ranawat et al 2011].

Biochemical studies

Biochemical parameters including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), direct bilirubin (Dbil), total bilirubin (Tbil), and total protein (TP) were quantitatively estimated using standardized assay kits from Lab Care Diagnostics (India) Pvt. Ltd, providing essential markers for evaluating liver function and assessing the hepatoprotective effects of the treatments [Chandrashekhar et al 2010].

Histopathology

Liver tissues were excised and fixed in 10% formalin, followed by dehydration through graded ethanol (50–100%), clearing in xylene, and embedding in paraffin wax; thin sections (5–6 µm) were prepared and stained with hematoxylin and eosin for photomicroscopic examination, with histopathological changes evaluated and scored on a scale of 1–4 to assess the extent of liver damage and therapeutic efficacy of the treatments [Lu et al 2017].

2.14 Statistical analysis

The data were expressed as mean $\pm$ S.E.M. Statistical differences at $P < 0.05$ between the groups were analyzed by one-way ANOVA followed by Dunnett's multiple comparison test using SPSS 15.0 software [Pareek et al 2013].

Preparation of Herbal syrup

An ethyl acetate extract of *Ficus racemosa* was prepared using the Soxhlet extraction method to isolate specific active constituents; the extract was filtered, and measured aliquots were combined as per the formulation table to produce 50 ml of syrup, with added coloring and flavoring agents for palatability. The final syrup was transferred into an amber-colored bottle, sealed tightly, and stored in a cool, dry place to preserve its stability and efficacy [Ghosh et al 2023].

3. RESULT AND DISCUSSION

Total flavonoid content

Ethyl acetate and methanol extracts containing key phytoconstituents were standardized for total flavonoid content using quercetin (QE) as a reference, calculated from the linear regression equation ($R^2 = 0.998$), $y = 0.0149x + 0.0168$; at 10 µg/ml concentration, ethyl acetate leaf and bark extracts showed higher flavonoid levels (82.6 mg/g and 75.26 mg/g QE/g) compared to methanol extracts (69.26 mg/g and 65.26 mg/g QE/g), leading to the selection of ethyl acetate leaf extract for further investigation due to its superior flavonoid content.

Total Phenolic Content

Ethyl acetate and methanol extracts, identified to contain specific phytoconstituents, were subjected to standardization for total polyphenolic content using gallic acid (GAE) as a reference, calculated from the linear regression equation ($R^2 = 0.9948$), $y = 0.0096x + 0.0381$; at a concentration of 10 µg/ml, the ethyl acetate extract exhibited a polyphenolic content of 18 mg/g GAE, confirming its richness in phenolic compounds and supporting its selection for further studies.

Antioxidant activity study

A. Evaluation of scavenging activity on DPPH radicals:

DPPH assay is a widely used method to assess the free radical scavenging activity of compounds, where the stable DPPH radical is reduced upon receiving an electron or hydrogen atom, indicating antioxidant potential; the reduction is measured by a decrease in absorbance, and a concentration-dependent response is typically observed, with greater scavenging activity at higher compound concentrations (as shown in table 2), reflecting their hydrogen-donating ability and primary antioxidant strength.

$$\% \text{ scavenging of DPPH} = (A_{\text{control}} - A_{\text{test}}) / A_{\text{control}} \times 100$$

Table No. 2: Effects of test extracts on % DPPH inhibition

Conc. (µg/ml)	% scavenging activity on DPPH radicals	
	Ascorbic acid	Ethyl acetate leaf
25	14.34%	12.79%

50	33.95%	23.56%
75	57.82%	34.10%
100	62.09%	44.96%
125	72.71%	55.11%

The ethyl acetate leaf extract of *Ficus racemosa* showed significant antioxidant activity as compared to the ascorbic acid and IC₅₀ for ethyl acetate extract was found to be 105 µg mL⁻¹

B. In-vitro Anti-Inflammatory Activity:

The in-vitro anti-inflammatory activity of *Ficus racemosa* ethyl acetate leaf extract was evaluated using the HRBC membrane stabilizing method, where the extract at 1000 µg/ml demonstrated notable membrane stabilization compared to Diclofenac potassium; since lysosomal enzymes released during inflammation contribute to various disorders, and HRBC membranes resemble lysosomal membranes, the extract's ability to prevent hypotonicity-induced hemolysis indicates its potential to inhibit enzyme release or stabilize membranes, reflecting its anti-inflammatory efficacy as shown in table 3.

$$\% \text{ Inhibition of haemolysis} = \frac{[\text{Absorbance of control} - \text{Absorbance of test}] \times 100}{\text{Absorbance of control}}$$

Table No. 3: In-vitro anti-inflammatory activity of extracts of *Ficus racemosa* leaf

Treatment	Absorbance	% Inhibition
Control	0.256	-
Extract	0.165	35.54
Diclofenac	0.155	65.16

The results have found that ethyl acetate leaf extract showed significant anti-inflammatory activity.

Synthesis of Silver Nanoparticles

Silver nanoparticles were biologically synthesized using the ethyl acetate extract of *Ficus racemosa* leaves, leveraging the extract's rich flavonoid content, which not only contributes to hepatoprotective activity but also facilitates the reduction of silver ions to nanoparticles; this green synthesis approach utilizes the natural reducing and stabilizing properties of phytoconstituents present in the extract, making it an effective and eco-friendly method for nanoparticle formation.

Optimization and Validation of Method for the Preparation of Silver Nanoparticles

Table No. 4: Batches for synthesis of silver nanoparticles by Box Behnken Design

Runs	Conc of AgNO ₃ (M)	Temp (°C)	pH	Particle Size (nm)	Absorbance Intensity
1	0.05	60	9	216	0.588
2	0.05	80	11	267	0.512
3	0.1	80	10	350	0.443
4	0.01	80	10	155	0.639
5	0.01	70	11	165	0.698
6	0.01	60	10	171	0.690
7	0.05	70	10	229	0.573
8	0.1	70	9	313	0.502

9	0.05	60	11	229	0.542
10	0.1	70	11	315	0.486
11	0.1	60	10	355	0.423
12	0.05	70	10	228	0.573
13	0.05	80	9	216	0.553
14	0.01	70	9	150.32	0.715
15	0.05	70	10	237	0.573

Particle size = + 240.13 A + 85.75 B +2.12 C Equation 1

Absorbance intensity = + 0.5730 – 0.1105 A – 0.0120 B – 0.0145 C + 0.0178 AB -0.0008 AC + 0.0012 BC + 0.0131 A² – 0.0374 B² + 0.0131 C².....Equation 2

Factor-Response Relationship and Response Surface Mapping

Response surface analysis using 3D plots revealed significant interactions among independent variables—silver nitrate concentration, temperature, and pH—and their effects on response variables such as particle size and absorbance intensity; Fig 1 showed that increasing silver nitrate concentration and temperature led to larger particle sizes, while Figs 2, 3, and 4 demonstrated that decreasing silver nitrate concentration, temperature, and pH resulted in higher absorbance intensity, indicating more efficient nanoparticle formation under milder synthesis conditions.

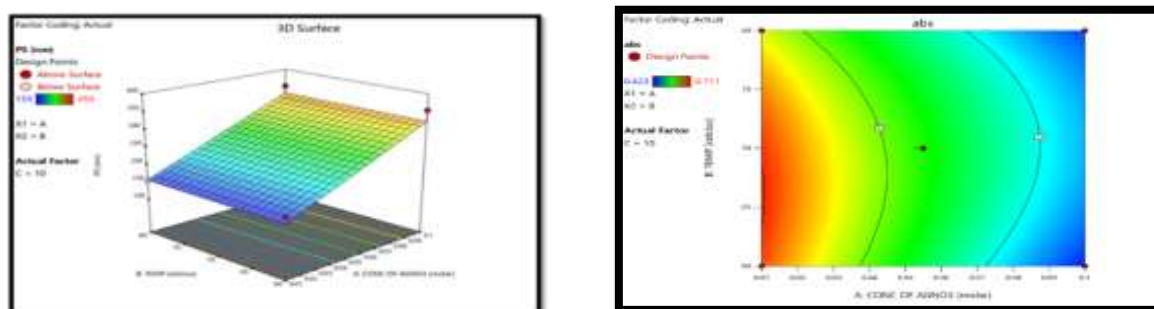


Fig No. 1: Counter plot and 3D Response surface plot showing effect of concentration of silver nitrate and temperature on Particle size



Fig No. 2: Contour plot and 3D-Response surface plot showing effect of concentration of AgNO₃ and Temperature on Absorbance intensity (abs)

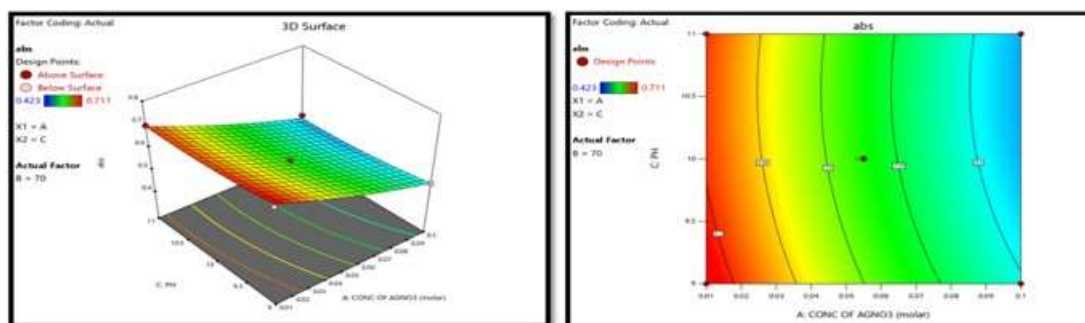


Fig No. 3 Contour plot and 3D-Response surface plot showing effect of concentration of AgNO_3 and pH on Absorbance intensity

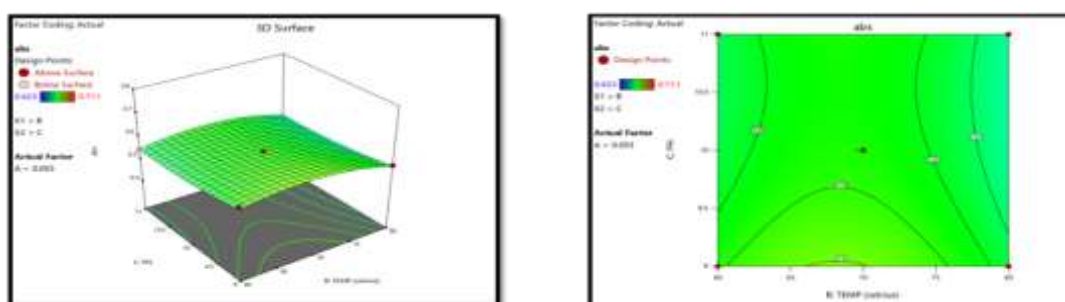


Fig No. 4: Contour plot and 3D-Response surface plot showing effect of pH and Temperature on Absorbance intensity

Validation of silver nanoparticle

Checkpoint batches C1 and C2 were prepared based on the optimized conditions derived from the overlay plot, and evaluated for particle size and absorbance intensity as shown in Table 1.9; when the actual experimental values were compared with the predicted values from the response surface model, all responses showed less than 5% deviation as shown in table 5, confirming the accuracy and reliability of the optimization process.

Table No. 5: Predicted and actual response of checkpoint batch

Evaluation parameters	Batch C1			Batch C2		
	Predicted value	Actual value	% Error	Predicted value	Actual value	% Error
Particle size	156.242	158.365	1.35 %	156.04	161.08	3.22 %
Absorbance intensity	0.6303	0.6491	2.98 %	0.695	0.711	2.30 %

3.6.3 Optimization of silver nanoparticle

The overlay plot indicated the yellow colour region as the optimized region along with the flagged point representing concentration of silver nitrate 0.01M, temperature at 70 °C and at pH 10, as the optimized silver nanoparticle synthesis as shown in figure 5. This also indicated predicted response for the synthesized silver nanoparticle having particle size and absorbance intensity.

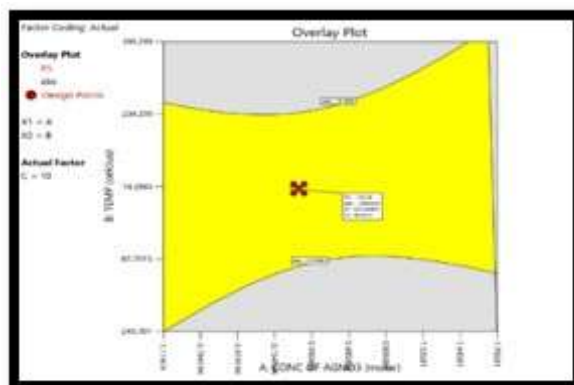


Fig No. 5: Overlay plot for optimization

Table No. 6: Results of optimized batch

Batch	Concentration of AgNO ₃ (M)	Temperature (°C)	pH	Absorbance Intensity	Particle Size
Optimized batch	0.01M	69	10	0.725 ± 0.02	151.32 ± 1.32 nm

Above are the results of the optimized batch obtained practically, which is the same as suggested by DoE software.

Characterization of Silver Nanoparticles

UV-Visible Spectrophotometry

The optimized silver nanoparticles were subjected to UV-Visible spectrophotometer, which produced absorption spectra of the nanoparticles depicted in (Fig No. 6). At the optimal setting of concentration of silver nitrate, temperature and pH, optimized silver nanoparticles were synthesized. The spectrum depicting the peak was observed at 400-430 nm with yellowish brown colour of the silver nanoparticle dispersion owing to the surface plasmon resonance of synthesized silver nanoparticles.

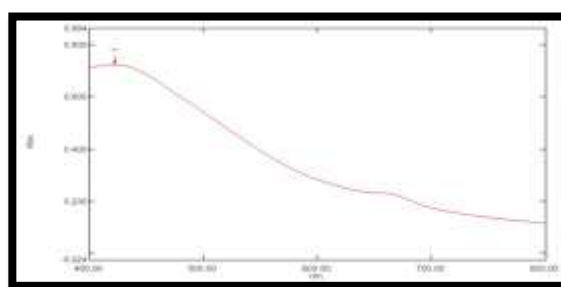


Fig No. 6: Absorption spectra of the silver nanoparticles

Percentage Entrapment Efficiency

The optimum entrapment efficiency was found to be **75.45%** for silver nanoparticles, optimized batch formulation was enough to entrapped *Ficus racemosa* leaf extract.

Particle Size and Polydispersity Index

Particle size distribution of optimized silver nanoparticles as per DLS was found to be having average diameter of **151.32 nm** and **0.238** polydispersity index (Fig No. 7). This indicated nanosized range of the silver nanoparticles, which is required for the

desired activity. The optimum particle size and polydispersity index was based on the concentration of silver nitrate, temperature and pH.

Zeta Potential Measurement

The zeta potential of optimized silver nanoparticles was found to be **-12.6 mV** (Fig No. 8). The observed zeta potential was in the stability range i.e., -30 mV to +30 mV depicting the stability of the formulation.

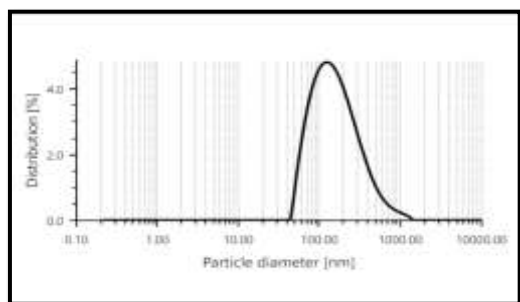


Fig. No. 7: Particle Size of Silver Nanoparticles

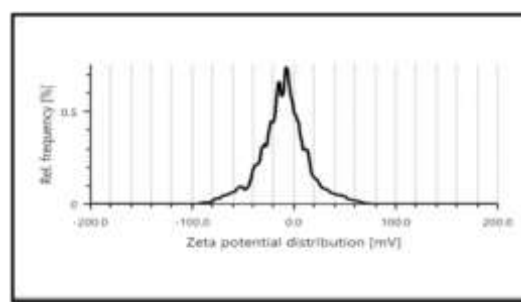


Fig. No.8: Zeta potential of Silver Nanoparticles

Differential Scanning Calorimetry



Fig. No. 9: DSC Thermogram of *Ficus racemosa* leaf Extract

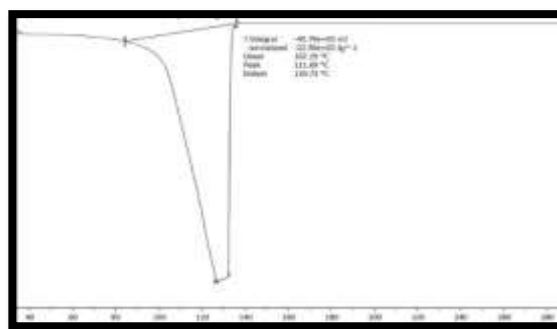


Fig No. 10: DSC Thermogram of Silver nanoparticle of *Ficus racemosa* leaf extract

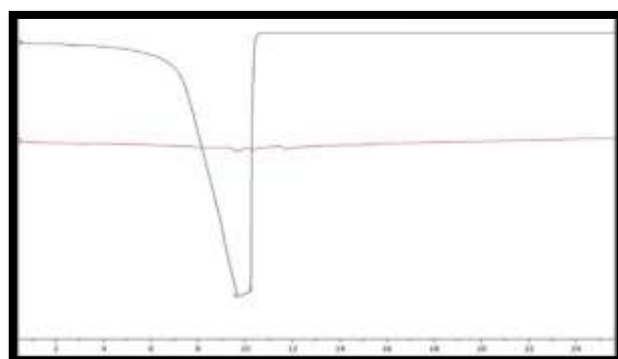


Fig No. 11: DSC Thermogram Overlay of *Ficus racemosa* Extract and Silver Nanoparticles

DSC study demonstrated that *Ficus racemosa* ethyl acetate leaf extract showed the presence of sharp endothermic peak at 126.15°C as shown in (fig.no.9). DSC thermogram of *Ficus racemosa* ethyl acetate leaf extract silver nanoparticles as shown in (fig.no. 10) the broad endothermic peak at 111.69°C which is shifted due to the formation of nanoparticles. The endothermic

peak of the silver nanoparticle was well observed with slight change in terms of broadening of the peak and shifting toward lower temperature. Thus, these minor changes in the melting peak of the silver nanoparticle could be due to the encapsulation of the extract and silver nanoparticles. (fig. no. 11) show overlay of extract and silver nanoparticles.

X-Ray Diffraction

The diffractogram (Fig No. 12) of ethyl acetate extract of *Ficus racemosa* leaf peak patterns showed broad peaks which confirmed that the extract was amorphous in nature at 2θ. However, broad peaks with reduced intensity (Fig No. 13) of silver nanoparticles of ethyl acetate extract of *Ficus racemosa* indicating the complete amorphous form of the silver nanoparticle. Overlay of extract and silver nanoparticles (Fig No. 14) showing extract encapsulation inside the nanoparticles.

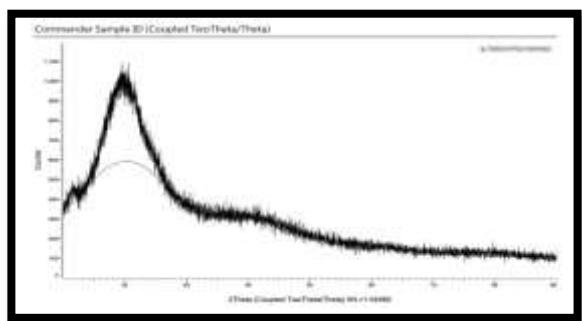


Fig No. 12: XRD pattern of *Ficus racemosa* leaf extract

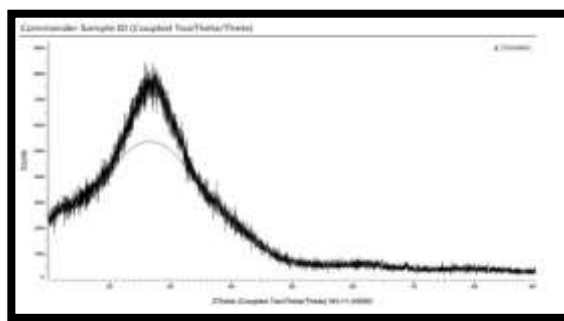


Fig. No. 13: XRD pattern of Silver nanoparticles

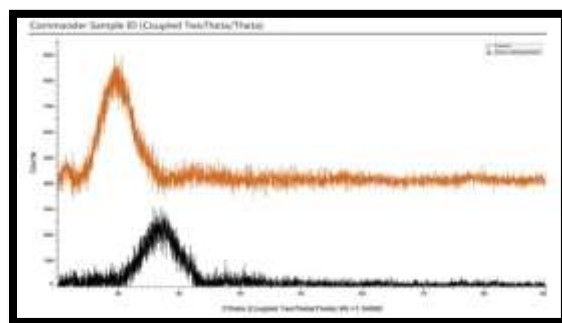


Fig No. 14: XRD Analysis of overlay of extract and silver nanoparticle

Field Emission Scanning Electron Microscopy

Shape analysis and surface morphology of silver nanoparticles of optimized batch were carried out by FE-SEM. FE-SEM images of silver nanoparticles revealed that the surface was smooth and slightly oval in shape (Fig No. 15 and 16). The images were taken at 70,000X and 50,000X in which some of the silver nanoparticles were aggregated, and some individual particles were also observed.

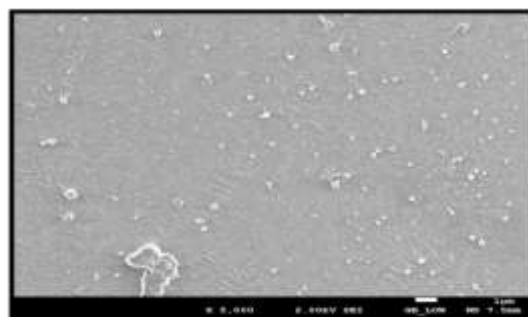
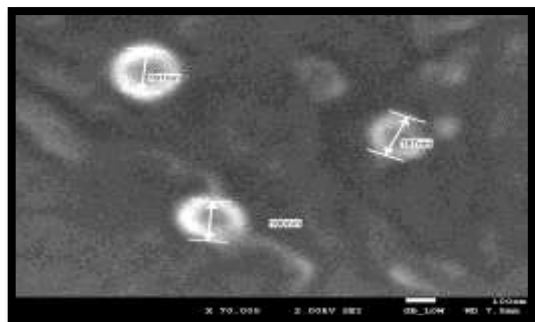


Fig No. 15: FESEM 70,000X of Silver nanoparticles

Fig No. 16: FESEM 5,000X of Silver nanoparticles

Stability Study

Stability testing assesses how the quality of a product (including its packaging) varies with time under the influence of environmental factors, including temperature, humidity and light. Stability study was performed on silver nanoparticle of *Ficus racemosa* and there was no major change in the parameters like drug content which indicated stability of the formulation at 40°C/75% RH for a period of 3 months and it was found that the formulation justifies all the characterization parameters even after 30 days, 60 days, and 90 days. The observations obtained showed that the formulation does not change in any parameters. The results have been compiled in Table No. 9.

Table No. 9: Stability data of final optimized batch

Evaluation Parameters	0 day	30 day	60 day	90 day
Colour	Brown	Brown	Brown	Brown
Absorbances Intensity	0.725 ± 0.011	0.733 ± 0.016	0.803 ± 0.014	0.812 ± 0.018
Particle Size	151.32 ± 2.34 nm	156.52 ± 2.67 nm	165.87 ± 3.12 nm	170.12 ± 3.18 nm

In-Vivo Study

Biochemical study

CCl₄-induced hepatotoxicity resulted in marked elevation of liver biomarkers including AST, ALT, ALP, total and direct bilirubin, indirect bilirubin, total protein, albumin, and globulin, as presented in Table 10; post-treatment with 400 mg/kg of crude ethyl acetate leaf extract and silver nanoparticles of *Ficus racemosa* significantly attenuated these elevated levels, indicating strong hepatoprotective effects and restoration of liver function.

Table No. 10: Effect of CCl₄, Extract and Silver nanoparticles on Rat Liver

Group	Total Bilirubin (mg/dL)	Direct Bilirubin (mg/dL)	Indirect Bilirubin (mg/dL)	SCOT (AST) (U/L)	SGPT (ALT) (U/L)	Alkaline Phosphatase (U/L)	Total Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)
Group I Normal Control	0.23 ± 0.013	0.10 ± 0.004	0.13 ± 0.005	212.9 ± 4.27	160.1 ± 2.12	224.0 ± 2.15	6.32 ± 0.46	3.56 ± 0.13	2.76 ± 0.11

Group II CCl₄ Control	0.48 ± 0.02	0.55 ± 0.021	0.38 ± 0.017	751.2 ± 513	418.3 ± 2.97	364 ± 2.18	7.81 ± 0.53	5.20 ± 0.12	4.61 ± 0.17
Group III <i>Ficus racemosa</i> Leaf Extract	0.34 ± 0.013	0.25 ± 0.014	0.20 ± 0.011	267.3 ± 3.36	187.9 ± 1.98	246 ± 2.14	6.49 ± 0.39	3.98 ± 0.34	3.05 ± 0.13
Group VI Silver nanoparticle of extract	0.21 ± 0.011	0.13 ± 0.007	0.15 ± 0.008	223.8 ± 2.98	163.2 ± 2.16	223.0 ± 2.19	6.25 ± 0.28	3.62 ± 0.28	2.89 ± 0.11

Histopathology

Histopathological evaluation of liver tissues presented in Fig. 17 demonstrated the protective effects of *Ficus racemosa* ethyl acetate leaf extract and its silver nanoparticle formulation against CCl₄-induced liver damage. The normal control group (Fig. 17 A) exhibited intact hepatic architecture with well-preserved hepatocytes and normal central veins. In contrast, the CCl₄-treated group (Fig. 17 B) showed extensive hepatic injury, including fatty infiltration, hepatocyte degeneration, congested veins and sinusoids, damaged biliary ducts, and bridging necrosis—indicative of severe hepatotoxicity. Treatment with 400 mg/kg of *Ficus racemosa* ethyl acetate extract (Fig. 17 C) resulted in noticeable histological improvement, with only mild fatty degeneration, reduced inflammation, and interstitial edema, while maintaining normal vein structure. Remarkably, rats treated with 400 mg/kg of silver nanoparticles of the extract (Fig. 17 D) displayed nearly normal hepatic histology, with restored hepatocytes and vein integrity, suggesting superior hepatoprotective efficacy of the nanoparticle formulation over the crude extract.

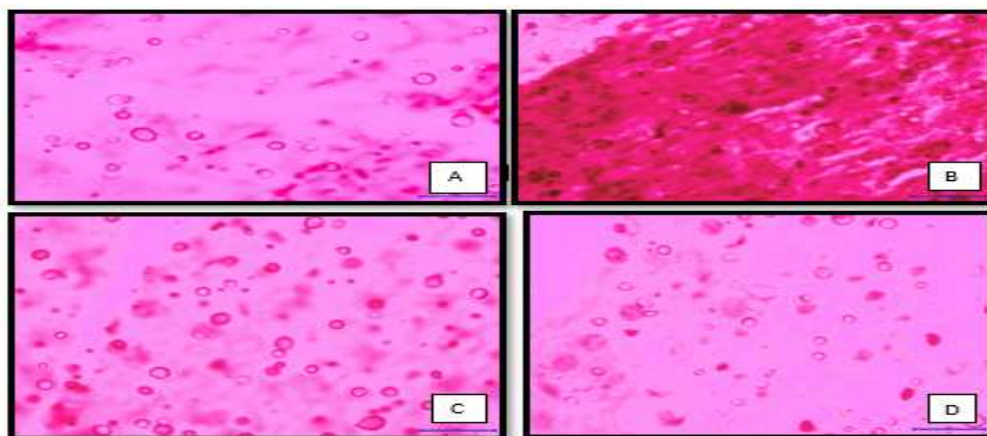


Fig. No. 17 Illustrates the histopathological changes in liver sections across different treatment groups, highlighting the protective effects of *Ficus racemosa* extracts.

Panel A shows the liver section from the normal control rat, displaying well-organized hepatic architecture with healthy hepatocytes and intact central veins, indicative of normal liver function.

Panel B reveals the liver damage induced by CCl₄, characterized by extensive fatty infiltration, degeneration of hepatocytes, congestion in veins and sinusoids, degeneration of the biliary duct, and bridging necrosis—hallmarks of severe hepatotoxicity.

Panel C, representing rats treated with CCl_4 and 400 mg/kg of *Ficus racemosa* ethyl acetate leaf extract, shows significant histological improvement, with only mild fatty degeneration, reduced inflammation, and interstitial edema, while the vein structure remains largely preserved.

Panel D demonstrates the most pronounced recovery in rats treated with CCl_4 and 400 mg/kg of silver nanoparticles of the extract, where liver histology closely resembles that of the normal control, with nearly normal hepatocytes and vein architecture, suggesting that the nanoparticle formulation offers enhanced hepatoprotective efficacy compared to the crude extract.

4. CONCLUSION

The ethyl acetate leaf extract of *Ficus racemosa* demonstrated high levels of flavonoids and polyphenols, which are known for their potent antioxidant and hepatoprotective properties. *Ex-vivo* studies confirmed strong anti-inflammatory effects, supporting its therapeutic potential in liver disorders. Animal trials further revealed that silver nanoparticles synthesized from this extract offered superior hepatoprotection compared to the crude extract and its syrup formulation, indicating that nanoparticle-enhanced herbal preparations could provide a more effective intervention for hepatotoxic conditions.

ACKNOWLEDGEMENTS

The authors acknowledge the complete assistance provided by the Dadasaheb Balpande Collge of Pharmacy, Besa, Nagpur.

AUTHOR CONTRIBUTIONS

Dr. Suchitra Mishra: Conceptualization, Supervision

Dr. Ujwal Mahajan: Guide

Dr. Bhagyashree Kokate and Mr. Aslam Riaz: Data Curation, Formal Analysis

Snehal Bhandarkar: Methodology, Laboratory work, Investigation

Dr. Sachin More: Animal Study support

Divya Pakmode and Prerna Sidam: Writing & Editing.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICS STATEMENT

Ethical approval was obtained from Animal Ethical Committee.

DATA AVAILABILITY STATEMENT

Dr. Suchitra Mishra: Data supporting the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

- [1] Abeysinghe, D.T., Kumara, K.A, Kaushalya, K.A., Chandrika, U.G., and Alwis, D.D. 2021. Phytochemical screening, total polyphenol, flavonoid content, in vitro antioxidant and antibacterial activities of Sri Lankan varieties of *Murraya koenigii* and *Micromelum minutum* leaves. *Heliyon*. 7(7):e07449. Available from: <https://doi.org/10.1016/j.heliyon.2021.e07449>.
- [2] Alara, O.R, Abdurahman, N.H., Ukaegbu, C.I., and Azhari, N.H.2018. *Vernonia cinerea* leaves as the source of phenolic compounds, antioxidants, and anti-diabetic activity using microwave-assisted extraction technique. *Indian Crops Production*. 122:533–44. Available from: <https://doi.org/10.1016/j.indcrop.2018.06.034>.
- [3] Chandrashekhar, V.M., Muchandi, A.A., Sudi, S. V., and Ganapthy, S. 2010. Hepatoprotective activity of *Stereospermum suaveolens* against CCl_4 -induced liver damage in albino rats. *Pharmaceutical Biology*. 48(5):524–8.
- [4] Chiu, M., and Prenner, E. 2011. Differential scanning calorimetry: An invaluable tool for a detailed thermodynamic characterization of macromolecules and their interactions. *Journal of Pharmaceutical Bioallied Science*. 3(1):39–59.
- [5] Danaei, M., Dehghankhold, M., Ataie, S., Hasanzadeh, Davarani, F., Javanmard, R., and Dokhani, A. 2018. Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. *Pharmaceutics*.10(2).

- [6] Djuichou Nguemnang, F., Tsafack, E.G, Mbiatcha, M., Gilbert, A., Atsamo, A.D, and Yousseu Nana W. 2019. *In Vitro* Anti-Inflammatory and in Vivo Antiarthritic Activities of Aqueous and Ethanolic Extracts of *Dissotis thollonii* Cogn. (Melastomataceae) in Rats. Evidence-based Complement Alternative Medicine. 2019.
- [7] Ferreira, E.A, Gris, E.F., Felipe, K.B., Correia, J., Cargnin-Ferreira, E., and Filho, D.W. 2010 Potent hepatoprotective effect in CCl₄-induced hepatic injury in mice of phloracetophenone from *Myrcia multiflora*. Libyan Journal of Medicine. 5(1):1–10.
- [8] Ghosh, A.K. 2023. Formulation and Evaluation of Herbal Syrup as Bronchodilator. International Journal for research in applied science and engineering technology.11(6):2762–72.
- [9] Gopalasatheeskumar, K. 2018. Significant Role of Soxhlet Extraction Process in Phytochemical Research Development of Herbal Formulations for Diabetes View Project. Mintage Journal of Pharmaceutical Medical Sciences.7(1):43–7.: www.mintagejournals.com.
- [10] Gupta, R.K., Hussain, T., Panigrahi, G., Das, A., Singh, G.N., and Sweetey K. 2011. Hepatoprotective effect of *Solanum xanthocarpum* fruit extract against CCl₄ induced acute liver toxicity in experimental animals. Asian Pacific Journal of Tropical Medicines. 4(12):964–8. doi.org/10.1016/S1995-7645(11)60227-7.
- [11] Ider, M., Abderrafi, K., Eddahbi, A., Ouaskit, S., and Kassiba, A. 2017. Silver Metallic Nanoparticles with Surface Plasmon Resonance: Synthesis and Characterizations. Journal of Cluster Science. 28(3):1051–69.
- [12] Jackson, T.C., Agboke, A.A., Udofa, E.J., Ucheokoro, A.S., Udo, B.E., and Ifekpolugo, N.L. 2019. Characterization and Release Kinetics of Metronidazole Loaded Silver Nanoparticles Prepared from *Carica papaya* Leaf Extract. Advance Nanoparticles. 08(03):47–54.
- [13] Khoshnevisan, K., and Barkhi, M. 2015 Information about Zeta Potential. Encycl Membr. 2063–4.
- [14] Krishnan, S. 2019. Liver Diseases-An Overview. 2019. World Journal of Pharmacy and Pharmaceutical Sciences 8(1):1385-1395. DOI: [10.20959/wjpps20191-13036](https://doi.org/10.20959/wjpps20191-13036)
- [15] Langley-Evans, S. C. 2000. Antioxidant potential of green and black tea determined using the ferric reducing power (FRAP) assay. International Journal Food Science Nutrition. 51(3):181–8.
- [16] Latha, S., Shilpa, M, Parushuram, N., and Sangappa, Y. 2020. Green synthesis of silver nanoparticles and their characterization. AIP Conference Proceedings. 2220.
- [17] Lu, Y., Chen, J., Ren, D., Yang, X., and Zhao, Y. 2017. Hepatoprotective effects of phloretin against CCl₄-induced liver injury in mice. Food Agriculture Immunology. 28(2):211–22.
- [18] Moond, M., Singh, S., Sangwan, S., Devi, P., Beniwal, A., and Rani, J. 2023. Biosynthesis of Silver Nanoparticles Utilizing Leaf Extract of *Trigonella foenum-graecum* L. for Catalytic Dyes Degradation and Colorimetric Sensing of Fe³⁺/Hg²⁺. Molecules. 28(3).
- [19] Nikaeen, G., Yousefinejad, S., Rahmdel, S., Samari, F., and Mahdavinia S. 2020. Central Composite Design for Optimizing the Biosynthesis of Silver Nanoparticles using *Plantago major* Extract and Investigating Antibacterial, Antifungal and Antioxidant Activity. Scientific Report. 10(1):1–16.
- [20] Pandey, B., and Rajbhandari, M. 2015. Estimation of Total Phenolic and Flavonoid Contents in Some Medicinal Plants and Their Antioxidant Activities. Nepal Journal of Science and Technology. 15(1):53–60.
- [21] Parbuntari, H., Etika, S.B., Mulia, M., and Delvia, E. 2019. A Preliminary Screening of the Different of Secondary Metabolites Ruku-Ruku Leaves (*Ocimum tenuiflorum* Linn) in West Sumatera. Eksakta Berkala Ilmiah Bidang MIPA. 20(2):17–24.
- [22] Pareek, A., Godavarthi, A., Issarani, R., and Nagori, B.P. 2013. Antioxidant and hepatoprotective activity of *Fagonia schweinfurthii* (Hadidi) Hadidi extract in carbon tetrachloride induced hepatotoxicity in HepG2 cell line and rats. Journal of Ethnopharmacol. 150(3):973–81. doi.org/10.1016/j.jep.2013.09.048
- [23] Phong, H.X., Viet, N.T., Quyen, N.T., Van Thinh, P., Trung, N.M., and Ngan, T.T. 2022. Phytochemical screening, total phenolic, flavonoid contents, and antioxidant activities of four spices commonly used in Vietnamese traditional medicine. Mater Today Proc. 56:A1–5. doi.org/10.1016/j.matpr.2021.12.142.
- [24] Ranawat, L., Bhatt, J., and Patel, J. 2010. Hepatoprotective activity of ethanolic extracts of bark of *Zanthoxylum armatum* DC in CCl₄ induced hepatic damage in rats. Journal of Ethnopharmacol. 127(3):777–80.
- [25] Sadeghi, B., and Gholamhoseinpoor, F. 2015. A study on the stability and green synthesis of silver nanoparticles using *Ziziphora tenuior* (Zt) extract at room temperature. Spectrochim Acta - Part A Molecular and Biomolecular Spectroscopy. 134:310–5.

- [26] Santhosh, A.S., Sandeep, S., Manukumar, H.M., Mahesh, B., and Kumara Swamy, N. 2021. Green synthesis of silver nanoparticles using cow urine: Antimicrobial and blood biocompatibility studies. *Journal of Colloid and Interface Science Open*. 3:100023. doi.org/10.1016/j.jciso.2021.100023
- [27] Singhal, M., Paul, A., Singh, H.P., Dubey, S.K., and Gaur, K. 2011. Evaluation of reducing power assay of chalcone semicarbazones. *Journal of Chemical Pharmaceutical Res*. 3(3):639–45.
- [28] Wongsu, P., Phatikulrungsun, P., and Prathumthong, S. 2022. FT-IR characteristics, phenolic profiles and inhibitory potential against digestive enzymes of 25 herbal infusions. *Scientific Report*. 12(1):1–11. doi.org/10.1038/s41598-022-10669-z
- [29] Wahid, A., Hamed, A.N., Eltahir, H.M., and Abouzied, M.M. 2016. Hepatoprotective activity of ethanolic extract of *Salix subserrata* against CCl₄-induced chronic hepatotoxicity in rats. *BMC Complementary Alternative Medicine*. 16(1):1–10. doi.org/10.1186/s12906-016-1238-2.
- [30] Yadav, R.K., Nandy, B.C., Maity, S., Sarkar, S., and Saha, S. 2015. Phytochemistry, pharmacology, toxicology, and clinical trial of *Ficus racemosa*. *Pharmacognosy Review*. 9(17):73–80.
- [31] Zhang, H., Jacob, J.A., Jiang, Z., Xu, S., Sun, K., and Zhong, Z. 2019. Hepatoprotective effect of silver nanoparticles synthesized using aqueous leaf extract of *Rhizophora apiculata*. *International Journal of Nanomedicine*. 14:3517–24.