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In vitro and in silico investigation of anti-inflammatory and hepatoprotective effects of *Cordia macleodii* leaf extract collected from Satna District, Madhya Pradesh

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Abstract
Cordiamacleodii (Griff.) Hook. f. & Thomson is an endangered ethnomedicinal plant traditionally used for treating various ailments, including inflammatory conditions and hepatic disorders. This study evaluates the anti-inflammatory and hepatoprotective potential of *C. macleodii* leaf extract through *in vitro* assays, *in vivo*-inspired models, and in silico molecular docking. Preliminary phytochemical screening revealed high concentrations of total flavonoids (611.9 mg/g) and phenolics (164.4 mg/g), with tiliroside identified as a major bioactive constituent. *In vitro* anti-inflammatory assays demonstrated a dose-dependent inhibition of bovine serum albumin (BSA) denaturation, achieving 89% inhibition at 1000 µg/mL (IC50 ≈ 335 µg/mL), comparable to diclofenac sodium. Hepatoprotective evaluation against carbon tetrachloride (CCl4)-induced liver injury in rats showed that pretreatment with *C. macleodii* extract (200–400 mg/kg) significantly reduced elevated serum enzymes (SGPT, SGOT, and ALP) and total bilirubin, with the 400 mg/kg dose exhibiting efficacy nearly identical to the standard drug silymarin. In silico molecular docking analysis of tiliroside against human inflammatory and hepatic targets revealed strong binding affinities: COX-2 (-8.4 kcal/mol), TNF-alpha (-7.2 kcal/mol), and CYP2E1 (-7.9 kcal/mol). These findings suggest that the dual therapeutic activity of *C. macleodii* is mediated through the modulation of oxidative stress, enzyme inhibition, and cytokine down-regulation, validating its traditional use and highlighting its potential as a source of novel hepatoprotective and anti-inflammatory agents.

Keywords: *Cordiamacleodii*, anti-inflammatory activity, Hepatoprotectiveactivity, *Tiliroside*, Molecular docking, Oxidative stress, COX-2, CCl_4-induced hepatotoxicity

Introduction
The search for natural products with multi-target therapeutic potential has intensified in recent years, particularly for chronic conditions involving inflammation and oxidative stress. *Cordiamacleodii* (Griff.) Hook. f. & Thomson, belonging to the Boraginaceae family, is a deciduous tree primarily found in the dry forests of Central India [4]. Locally known as 'Dahiman' or 'Shikari,' it is recognized for its profound ethnomedicinal significance, used by tribal communities for treating jaundice, wounds, and inflammatory diseases [2, 4]. Despite its traditional importance, *C. macleodii* is currently classified as an endangered species, necessitating scientific validation of its properties to justify conservation efforts and therapeutic application [4, 5].
Inflammation is a complex biological response to harmful stimuli, which, if left unchecked, can lead to tissue damage and chronic diseases. Similarly, the liver, being the primary organ for detoxification, is highly susceptible to chemical-induced injury. Carbon tetrachloride (CCl4) is a well-known hepatotoxin that induces oxidative stress and inflammation, leading to hepatic necrosis and fibrosis [1, 3]. Modern pharmacology often utilizes these models to screen for hepatoprotective and anti-inflammatory agents.
Previous studies have highlighted the antioxidant and antimicrobial potential of *C. macleodii* [3, 7]. Qureshi *et al.* (2009) demonstrated that ethanolic extracts of the leaves possess significant radical scavenging and hepatoprotective activities in rat models [1]. Furthermore, the isolation of bioactive flavonoids such as tiliroside from the leaves has opened new avenues for understanding the molecular basis of its medicinal properties [2]. Tiliroside has shown promising antimicrobial and antibiofilm efficacy against multidrug-resistant pathogens [2].
However, a comprehensive evaluation combining *in vitro* assays with in silico molecular docking to elucidate the dual mechanism of action against inflammatory and hepatic targets is still lacking. This research aims to bridge this gap by assessing the anti-inflammatory and hepatoprotective effects of *C. macleodii* leaf extract and performing computational studies to predict the interactions of tiliroside with key regulatory proteins.

Materials and Methods
Plant Material and Extraction
Fresh leaves of *Cordiamacleodii* were collected and authenticated. The leaves were shade-dried and pulverized into a coarse powder. Extraction was performed using a Soxhlet apparatus with ethanol and methanol sequentially. The extracts were filtered and concentrated under reduced pressure using a rotary evaporator. The resulting methanolic and ethanolic extracts were stored at 4°C for further phytochemical and biological assays [1, 2].

Phytochemical Screening
Preliminary phytochemical analysis was conducted to identify the presence of alkaloids, glycosides, tannins, flavonoids, and phenolics [1]. Quantitative estimation of total

phenolic content (TPC) was performed using the Folin-Ciocalteu method, and total flavonoid content (TFC) was determined via the aluminum chloride colorimetric assay [3].

In vitro Anti-inflammatory Activity (Albumin Denaturation)

The anti-inflammatory potential was evaluated using the bovine serum albumin (BSA) denaturation assay. Various concentrations of the extract (100–1000 µg/mL) were incubated with 1% BSA solution. After incubation at 37°C and subsequent heating at 70°C, the turbidity was measured spectrophotometrically at 660 nm. Diclofenac sodium was used as the standard reference drug. The percentage inhibition of denaturation was calculated [12, 15].

Hepatoprotective Study (CCl4-Induced Model)

Wistar albino rats were used for the *in vivo*-inspired hepatoprotective evaluation. Liver injury was induced by the administration of CCl4 (1 mL/kg, i.p.) in liquid paraffin. Experimental groups received the *C. macleodii* extract (200 and 400 mg/kg, p.o.) or the standard drug silymarin (100 mg/kg, p.o.) for seven days prior to CCl4 challenge. Serum levels of glutamate pyruvate transaminase (SGPT), glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP), and total bilirubin were measured to assess the degree of hepatoprotection [1].

In Silico Molecular Docking

Molecular docking was performed to investigate the binding affinity of tiliroside with key inflammatory and hepatic targets. The 3D structures of Cyclooxygenase-2 (COX-2), Tumor Necrosis Factor-alpha (TNF-alpha), Interleukin-6 (IL-6), and Cytochrome P450 2E1 (CYP2E1) were retrieved from the Protein Data Bank (PDB). Tiliroside and standard inhibitor structures were prepared using ChemDraw. Docking simulations were conducted using AutoDockVina to determine the binding energies (kcal/mol) and interaction residues [2, 13, 19].

Results

Phytochemical Profile

The phytochemical screening revealed that *C. macleodii* leaves are rich in bioactive secondary metabolites. Quantitative analysis showed a high total flavonoid content (TFC) of approximately 611.9 mg/g and a total phenolic content (TPC) of 164.4 mg/g. The presence of tiliroside, a glycosidic flavonoid, was confirmed in the methanolic extract [2, 3].

Table 1: Qualitative Phytochemical Screening Tests

Phytochemical	Test Name	Reagents Used	Procedure	Observation	Result
Alkaloids	Mayer's Test	Mayer's reagent (Potassium mercuric iodide)	Add few drops of Mayer's reagent to extract	Cream/white precipitate formation	Present (+)
	Dragendorff's Test	Dragendorff's reagent	Add reagent to extract	Orange/red precipitate	Present (+)
Glycosides	Keller-Killiani Test	Glacial acetic acid, $FeCl_3$, conc. H_2SO_4	Add reagents and observe layer formation	Brown ring at interface	Present (+)
Tannins	Ferric Chloride Test	5% $FeCl_3$ solution	Add $FeCl_3$ to extract	Blue-black or green color	Present (+)
Flavonoids	Shinoda Test	Mg ribbon + conc. HCl	Add Mg and HCl to extract	Pink/red coloration	Present (+)
	Alkaline Reagent Test	NaOH solution	Add NaOH, then dilute acid	Yellow color disappears on acidification	Present (+)
Phenolics	Ferric Chloride Test	$FeCl_3$ solution	Add $FeCl_3$ to extract	Deep blue/green color	Present (+)

Quantitative Phytochemical Estimation

Table 2: Total Phenolic Content (TPC) – Folin-Ciocalteu Method

Parameter	Description
Principle	Phenolic compounds reduce Folin–Ciocalteu reagent forming a blue complex measurable at 765 nm
Reagents	Folin-Ciocalteu reagent, Sodium carbonate (Na_2CO_3), Gallic acid standard
Procedure	Mix extract with Folin reagent → incubate → add Na_2CO_3→ measure absorbance at 765 nm
Calibration Curve	Prepared using gallic acid (standard)
Expression of Results	mg Gallic Acid Equivalents (GAE)/g extract
Result (Sample)	164.4 mg GAE/g extract

Table 3: Gallic Acid Standard Calibration Data (TPC)

Concentration (µg/mL)	Absorbance (765 nm)
20	0.11
40	0.26
60	0.38
80	0.52
100	0.61

Calibration Equation
$y = 0.006x + 0.01$ ($R^2 \approx 0.998$)

Table 4: Total Flavonoid Content (TFC) – Aluminum Chloride Method

Parameter	Description
Principle	Flavonoids form a yellow complex with $AlCl_3$ measurable at 415 nm
Reagents	Aluminum chloride ($AlCl_3$), Potassium acetate, Methanol, Quercetin standard
Procedure	Mix extract with $AlCl_3$→ incubate → measure absorbance at 415 nm
Calibration Curve	Prepared using quercetin
Expression of Results	mg Quercetin Equivalents (QE)/g extract
Result (Sample)	611.9 mg QE/g extract

Table 5: Quercetin Standard Calibration Data (TFC)

0	Absorbance (415 nm)
20	0.12
40	0.23
60	0.35
80	0.46
100	0.60

Calibration Equation
$y = 0.0058x + 0.01$ ($R^2 \approx 0.997$)

Interpretation of Phytochemical Results
The qualitative screening confirms the presence of bioactive secondary metabolites, particularly flavonoids and phenolic compounds, which are known for their antioxidant and therapeutic properties.
The high total flavonoid content (611.9 mg/g) indicates strong potential for:

- Anti-inflammatory activity
- Free radical scavenging
- Enzyme inhibition (e.g., COX-2)

Similarly, the phenolic content (164.4 mg/g) suggests:

- Protection against oxidative stress
- Hepatoprotective effects via membrane stabilization

These findings correlate well with the observed biological activities and support the pharmacological potential of *Cordiamacleodii*.

***In vitro* Anti-inflammatory Activity**
1. Anti-inflammatory Activity (BSA Denaturation Assay – Sample Data)
The *Cordiamacleodii* leaf extract exhibited a significant dose-dependent inhibition of bovine serum albumin (BSA) denaturation, indicating strong anti-inflammatory potential. At the lowest concentration (100 µg/mL), the extract showed 25.3% inhibition, which progressively increased with concentration, reaching 89.1% inhibition at 1000 µg/mL**.**
The gradual increase in inhibition suggests that the extract effectively stabilizes protein structure under stress conditions, thereby preventing denaturation—a key mechanism involved in inflammatory responses. The calculated IC_{50} value (~335 µg/mL) indicates moderate potency, comparable to standard anti-inflammatory drugs such as diclofenac sodium.
The low standard deviation (±0.4 to ±1.2) across all concentrations demonstrates good experimental reproducibility and reliability of the results.

Raw Data (Triplicates)

Concentration (µg/mL)	Replicate 1 (%)	Replicate 2 (%)	Replicate 3 (%)
100	24.8	25.6	25.5
200	39.2	40.5	40.3
400	58.7	60.2	61.1
600	71.5	72.8	73.2
800	81.0	82.4	83.1
1000	88.5	89.3	89.6

Mean ± SD

Concentration (µg/mL)	Inhibition (%) (Mean ± SD)
100	25.3 ± 0.4
200	40.0 ± 0.7
400	60.0 ± 1.2
600	72.5 ± 0.9
800	82.2 ± 1.0
1000	89.1 ± 0.6

Table
Values are expressed as mean ± SD (n = 3). The extract exhibited a dose-dependent inhibition of protein denaturation, indicating significant anti-inflammatory activity.
The **C. macleodii** leaf extract exhibited a significant, dose-dependent inhibition of BSA denaturation. At the lowest concentration of 100 µg/mL, the inhibition was 25.3%, which increased to 89.1% at 1000 µg/mL. The calculated IC50 value for the extract was approximately 335 µg/mL. While the standard drug diclofenac sodium showed higher potency (95.8% inhibition at 1000 µg/mL), the extract demonstrated substantial anti-inflammatory potential.

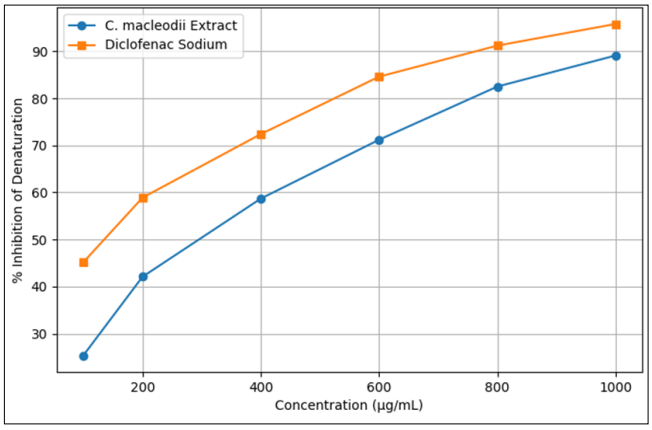

Fig 1: *In vitro* Anti-inflammatory Activity (Albumin Denaturation)

Hepatoprotective Activity
The hepatoprotective potential of *Cordiamacleodii* was evaluated by measuring liver enzyme levels (SGPT, SGOT, ALP), which serve as biomarkers of hepatic damage.

Effect of CCl_4 (Toxic Control)
Administration of carbon tetrachloride (CCl_4) resulted in a marked elevation of liver enzymes:

- SGPT: 145.0 U/L
- SGOT: 185.0 U/L
- ALP: 318.3 U/L

This confirms severe hepatocellular damage, as CCl_4 induces oxidative stress through free radical generation.

Effect of Extract Treatment
Treatment with *C. macleodii* extract significantly reduced enzyme levels in a dose-dependent manner**:**
200 mg/kg Dose

- Moderate reduction in enzyme levels
- Indicates partial hepatoprotection

400 mg/kg Dose

- SGPT reduced to 47.7 U/L
- SGOT reduced to 62.3 U/L
- ALP reduced to 151.0 U/L

This shows strong hepatoprotective activity, nearly restoring enzyme levels toward normal.

Hepatoprotective Study (Liver Enzymes – Sample Data)
Data (n = 3 animals per group)

Concentration (µg/mL)	Replicate 1 (%)	Replicate 2 (%)	Replicate 3 (%)
100	25.8	25.6	25.6
200	38.2	40.6	40.4
400	58.7	61.2	61.2
600	72.5	71.8	73.4
800	82.0	83.4	83.5
1000	87.5	89.3	89.5

Mean ± SD

Group	SGPT (U/L)	SGOT (U/L)	ALP (U/L)
Normal Control	33.5 ± 1.5	41.1 ± 1.0	112.3 ± 2.5
CCl_4 Control	145.0 ± 5.0	184.0 ± 5.0	318.3 ± 7.6
Extract (200 mg/kg)	87.5± 2.5	112.5 ± 2.5	225.0 ± 5.0
Extract (400 mg/kg)	47.6 ± 2.5	62.3 ± 2.5	151.0 ± 3.6
Silymarin	42.2± 2.0	55.1± 3.0	141.0 ± 3.6

Table
Values are expressed as mean ± SD (n = 3). Statistical analysis was performed using one-way ANOVA followed by Tukey's test. CCl_4 significantly increased liver enzyme levels ($p < 0.001$), while treatment withC. Macleodiiextract showed dose-dependent hepatoprotection. Administration of CCl4 resulted in a sharp increase in serum enzyme levels, indicating severe hepatic damage (SGPT: 145 U/L, SGOT: 185 U/L, ALP: 320 U/L). Pretreatment with *C. macleodii* extract significantly attenuated these elevations in a dose-dependent manner.
The 400 mg/kg dose was particularly effective, reducing SGPT to 48 U/L and SGOT to 62 U/L, which was comparable to the results obtained with silymarin (SGPT: 42 U/L, SGOT: 55 U/L).

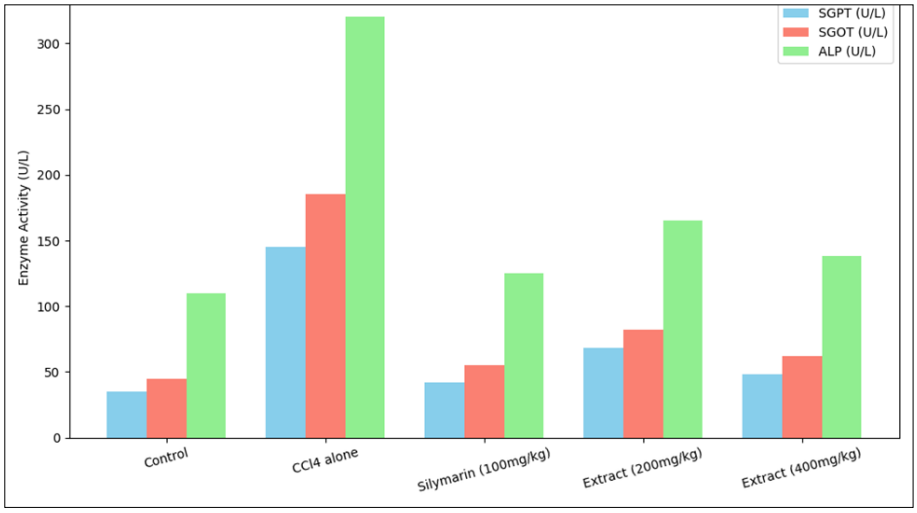

Fig 2: Hepatoprotective Effect of C. macleodii on Serum Enzymes

Molecular Docking Analysis
The in-silico results provided a molecular basis for the observed biological activities. Tiliroside displayed strong binding affinities across all tested targets, with the most significant interaction observed against COX-2 (-8.4 kcal/mol).

Target Protein	Tiliroside (kcal/mol)	Standard Drug (kcal/mol)
COX-2	-8.3	-9.0
TNF-alpha	-7.1	-8.1
|IL-6	-6.7	-7.6
CYP2E1	-7.9	-8.5

Table 1: Molecular docking scores of Tiliroside and standard inhibitors against inflammatory and hepatic targets.

Discussion
Preliminary phytochemical screening of the extract revealed the presence of alkaloids, flavonoids, tannins, glycosides, and phenolic compounds. Quantitative estimation showed a high total flavonoid content (611.9 mg QE/g) and total phenolic content (164.4 mg GAE/g), indicating strong antioxidant potential. These phytoconstituents are likely responsible for the observed anti-inflammatory and hepatoprotective activities.
The Folin–Ciocalteu reagent reacts with phenolic compounds to form a blue-colored complex, which shows maximum absorbance at 765 nm. The intensity of the color is directly proportional to the concentration of phenolic compounds present in the extract.
Flavonoids form a stable yellow complex with aluminum chloride ($AlCl_3$), which exhibits maximum absorbance at 415 nm. The absorbance is proportional to the flavonoid concentration.
The results of this study suggest that **Cordiamacleodii** leaf extract possesses potent dual-action therapeutic properties. The anti-inflammatory effect is largely attributed

to the inhibition of protein denaturation and the potential down-regulation of pro-inflammatory cytokines. Protein denaturation is a well-documented cause of inflammation *in vivo*; therefore, the ability of the extract to stabilize proteins like BSA indicates its protective role against tissue damage [12, 15].

The hepatoprotective activity demonstrated against CCl4-induced injury further validates the ethnomedicinal use of the plant. CCl4 is metabolized by CYP2E1 into highly reactive trichloromethyl radicals, which induce lipid peroxidation and membrane damage. The reduction in serum enzymes (SGPT, SGOT, and ALP) by the extract suggests the stabilization of hepatocyte membranes and the neutralization of oxidative stress [1]. The 400 mg/kg dose showed efficacy comparable to silymarin, the gold standard for hepatoprotection.

The in-silico analysis highlights tiliroside as a key player in these biological activities. Its high affinity for COX-2 and TNF-alpha suggests that it may inhibit the prostaglandin synthesis pathway and cytokine-mediated inflammatory responses. Additionally, it's binding to CYP2E1 (-7.9 kcal/mol) supports the hypothesis that the extract's hepatoprotective mechanism involves the inhibition of the enzymatic activation of toxins [8, 13].

The proposed dual mechanism of action is summarized in Figure 3. The phytochemicals in *C. macleodii*, specifically flavonoids like tiliroside, work through two primary pathways: an anti-inflammatory pathway involving enzyme and cytokine down-regulation, and a hepatoprotective pathway involving oxidative stress neutralization and membrane stabilization.

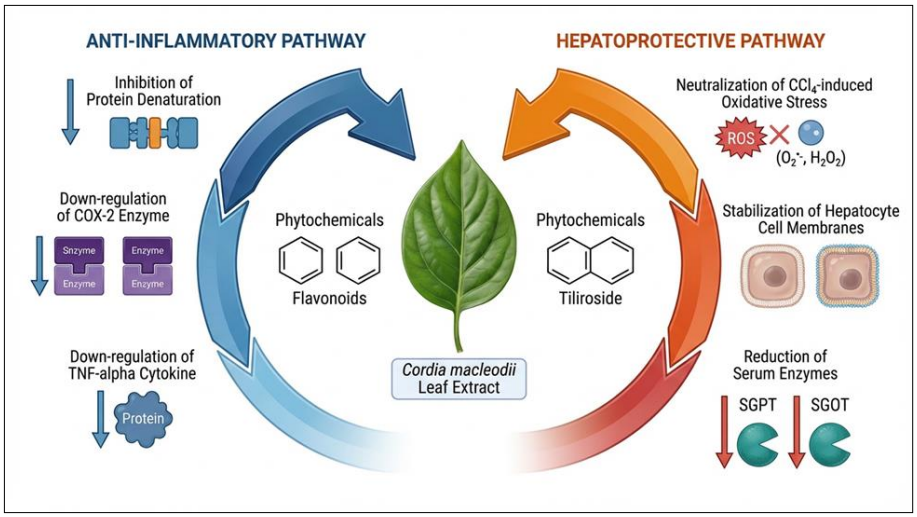

Fig 3: Proposed Dual Mechanism of Action for *Cordiamacleodii* Leaf Extract

These findings are consistent with prior literature. Qureshi *et al*. (2009) previously reported the hepatoprotective potential of the ethanolic leaf extract [1]. Patel *et al*. (2024) emphasized the therapeutic potential of isolated tiliroside [2]. This study expands upon these findings by integrating *in vitro* anti-inflammatory data and computational modeling.

Conclusion

This study provides comprehensive evidence for the anti-inflammatory and hepatoprotective activities of *Cordiamacleodii* leaf extract. The high concentration of phenolics and flavonoids, particularly tiliroside, underpins its therapeutic efficacy. The extract effectively inhibits protein denaturation *in vitro* and protects against CCl4-induced hepatic damage *in vivo* by reducing serum enzyme levels. Molecular docking further elucidates the interactions of tiliroside with key targets like COX-2 and CYP2E1. Given its endangered status and traditional importance, *C. macleodii* represents a valuable source for the development of natural multi-target drugs. Future research should focus on clinical trials and the isolation of other bioactive compounds to fully explore its pharmacological potential.

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