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Investigation of *in-vitro* Anti-inflammatory and Lipid-modulating Activities of *Catharanthus roseus* Flower Extract in Experimental Model of Hyperlipidaemia

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Abstract

Catharanthus roseus (Periwinkle), a perennial medicinal plant of the Apocynaceae family, contains a variety of bioactive compounds distributed throughout the plant. Traditionally, it has been used by communities in the East Godavari region for treating menstrual disorders, wound healing, bacterial infections, ulcers, and diarrhoea. However, scientific evidence supporting its anti-inflammatory and lipid-lowering properties, particularly from its flowers, remains limited. The present study aimed to evaluate the anti-inflammatory and hypolipidemic effects of *C. roseus* flower extract using both in vitro and in vivo models. In vitro anti-inflammatory activity was assessed by protein denaturation and HRBC membrane stabilization methods. For in vivo studies, hyperlipidaemia was induced in rats using a high-fat diet supplemented with cholesterol for 3–4 weeks. Body weight was monitored weekly, and blood samples were analysed for lipid profile parameters including Total Cholesterol (TC), Triglycerides (TG), Low-Density Lipoprotein Cholesterol (LDL-C), and High-Density Lipoprotein Cholesterol (HDL-C). Treatment groups received graded doses of the flower extract and were compared with hyperlipidaemic controls. The extract showed significant reductions in TC (25–35%), TG (28–40%), and LDL-C (30–45%), along with an increase in HDL-C (12–20%). In vitro studies demonstrated dose-dependent inhibition of protein denaturation (40–70%) and HRBC membrane stabilization (45–72%), indicating strong anti-inflammatory activity.

Major Findings: The findings suggest that *C. roseus* flower extract possesses significant anti-inflammatory and hypolipidemic effects, supporting its potential therapeutic application in managing inflammation and hyperlipidaemia.

Keywords: Anti-inflammatory, *C. roseus*, FTIR, HRBC, Hypolipidemic, Lipid Profiles, Protein Denaturation

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1. Introduction

Inflammation is a fundamental protective response of the body triggered by harmful physical, chemical or biological stimuli. It is clinically manifested through classical signs such as redness, heat, swelling, pain and in severe cases loss of function[1]. Although essential for host defence and tissue repair, uncontrolled or persistent inflammation contributes to the progression of several chronic diseases. Among these, metabolic disorders such as hyperlipidaemia remain closely interlinked with systemic inflammatory processes.

Hyperlipidaemia refers to an abnormal elevation of circulating lipids, including total cholesterol, triglycerides and Low-Density Lipoproteins (LDL). It is widely recognised as a major predisposing factor for cardiovascular diseases, which remain the leading cause of morbidity and mortality worldwide[2]. Chronic elevation of lipid levels promotes oxidative stress and endothelial dysfunction, which subsequently accelerate

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the development of atherosclerotic plagues and other metabolic complications. Despite the availability of effective synthetic hypolipidemic drugs, particularly statins and fibrates, their prolonged use is frequently associated with adverse effects such as hepatotoxicity, gastrointestinal disturbances, myalgia and myopathy, along with poor patient compliance. These limitations have intensified research interest in plant-derived therapeutic agents that offer greater safety, accessibility and multifaceted biological actions.

Catharanthus roseus of family *Apocynacea,* commonly known as Madagascar periwinkle, is a perennial herb with a long history of traditional medicinal use across various cultures[3]. In different regions, it has been widely employed for the management of bacterial infections, menstrual irregularities and other ailments. The plant is enriched with diverse classes of saponins and phenolic derivatives which collectively contribute to its broad spectrum of pharmacological properties[4]. Although extensive investigations have been conducted on the leaves, stems and roots of *C. roseus*, comparatively little scientific attention has been directed toward its flowers, despite their potential reservoir of bioactive compounds[5].

Recent findings suggest that floral extracts of medicinal plants often possess unique phytoconstituents with potent anti-inflammatory, antioxidant and lipid-modulating activities[6,7]. However, the specific role of *C. roseus* flower extract in ameliorating inflammation and dyslipidemia remains underexplored[8,9]. Addressing this gap is critical, giving the increasing global demand for safer natural therapeutics capable of targeting multiple pathways involved in metabolic and inflammatory disease.

The present study is therefore designed to investigate the dual therapeutic potential of *C. roseus* flower extract in modulating inflammatory responses ad correcting lipid abnormalities. By integrating both *in-vitro* and *in-vivo* experimental approaches, the study aims to provide scientific validation for the traditional use of the plant and establish its suitability as a promising natural agent for managing hyperlipidaemia and its associated inflammatory complications.

2. Materials and Methods

2.1 Extraction

Freshly collected flowers were subjected to shade drying to remove moisture. The dried flowers were then finely powdered using a grinder to obtain a uniform powder (40-80 mesh) weighing approximately 400 g [10]. This powdered material was soaked in ethanol and kept for maceration at room temperature (25°C) for 3 days with occasional stirring. After the maceration period, the mixture was filtered through Whatman No.1 filter paper to obtain the filtrate. The filtrate was then concentrated under reduced pressure using a rotary evaporator at a temperature below 50°C until a deep-green coloured ethanolic extract was obtained.

2.2 Chemicals and Glassware

All the chemicals and glassware utilised in the study were of analytical grade and standard quality.

2.3 FTIR Studies

FTIR spectroscopy was performed to characterise the functional groups present in the *C. roseus* ethanolic flower extract. Solid samples were finely ground with potassium bromide (KBr) and compressed into a thin pellet. The prepared pellet was then placed in the sample holder of the FTIR spectrometer. Upon exposure to infrared radiation, the instrument recorded the absorption spectrum of the sample. The spectra were recorded in the range of 4000-400cm^{-1} at a resolution of 4 cm^{-1} using an FTIR spectrometer. The obtained spectrum revealed the molecular vibrations and functional groups present in the sample.

2.4 Animals Used

Healthy adult Wistar albino rats (150-200 g) of either sex were selected for study. Animals were procured from NIN (National Institute of Hyderabad) and maintained under standard laboratory conditions (Temperature 25° C, 12-hour light/dark cycle). The animals were fed with high-fat diet supplemented with cholesterol for 3-4 weeks and water ad libitum. Body weight was recorded weekly[11,12]. All the selected animals are healthy and they were acclimatised to laboratory conditions.

2.5 Anti Inflammatory Activity

2.5.1 HRBC Method

2.5.1.1 Preparation of Test Extract

For experimental studies, a stock solution of the extract was prepared in Dimethyl Sulfoxide (DMSO) and subsequently diluted with phosphate buffered saline (PBS

pH 7.4) to obtain final working concentrations of 100 µg/ml and 200 µg/ml. The final concentration of DMSO in all assay tubes did not exceed 1% (v/v).

2.5.1.2 Collection of Human Blood Sample

Fresh human blood was collected from healthy volunteers who have abstained from any anti-inflammatory medications for at least two weeks prior to sample collection. The collected blood was mixed with an equal volume of sterile alsever's solution to prevent coagulation and stored at 4°C until use.

2.5.1.3 Preparation of Human Red Blood Cell (HRBC) Suspension

The blood samples were centrifuged at 3000 rpm for 10 minutes, and the supernatant was carefully removed. The packed erythrocytes were washed three times with isotonic saline (0.9% Nacl). A 10% (v/v) suspension of washed erythrocytes was prepared by mixing 0.5 ml of packed cells with 4.5 ml of isotonic saline. The suspension was used immediately for *in vitro* membrane stabilisation assay.

2.5.1.4 Evaluation of Anti-inflammatory Activity by HRBC Membrane Stabilisation Method

The *in vitro* anti-inflammatory potential of the ethanolic flower extract of *C. roseus* was assessed using the Human Red Blood Cell (HRBC) membrane stabilisation assay.

Test samples: 1.0 ml of phosphate buffer (PH 7.4) 1.0 ml of hypotonic solution (distilled water) 0.5 ml of 10 % HRBC suspension

Standard: 0.5 ml of Diclofenac (100 µg/ml) in place of extract

Negative control: 0.5 ml of vehicle (PBS containing 1 % DMSO) instead of extract.

Isotonic control: Isotonic saline (0.5 ml) instead of extract.

All the mixtures were incubated at 37°C for 30 minutes in a water bath and subsequently centrifuged at 3000 rpm for 10 minutes. The absorbance of the supernatant was measured spectrophotometrically at 560 nm against appropriate blank. The percentage of membrane stabilisation, indicating protection against hypotonic-induced haemolysis, was calculated using equation.

$$\% \text{ Membrane stabilisation} = \frac{\text{Abs of control-Abs of treated}}{\text{Abs of treated}}$$

2.5.2 Protein Denaturation Method

The *in-vitro* anti-inflammatory activity of the ethanolic flower extract of *C. roseus* was evaluated using the protein denaturation method.

2.5.2.1 Preparation of Test Samples

The ethanolic flower extract of *C. roseus* was dissolved in Dimethyl Sulfoxide (DMSO) to prepare test solutions of 100 µg/ml and 200 µg/ml. Diclofenac sodium (100 µg/ml) served as standard drug. While the control contains only distilled water and buffer without extract or standard.

2.5.2.2 Experimental Procedure

The reaction mixture comprised 0.2 ml of egg albumin, 2.8 ml of the test extract or standard drug solution. The mixtures were incubated at 37°C for 15 minutes, followed by heating at 70°C for 5 minutes to induce protein denaturation. After cooling to room temperature, the turbidity was measured spectrophotometrically at 660nm. The percentage inhibition of protein denaturation was calculated using the following formula.

$$\% \text{ Inhibition of protein} = \frac{\text{(Abs of control-Abs of treated)}}{\text{denaturation Abs of control}}$$

2.6 *In-vivo* Hypolipidemic Activity

2.6.1 Experimental Design

Hyperlipidaemia was induced by feeding the rats with a High-fat Diet (HFD) for 30 days[13]. The animals were divided into 5 groups each containing 3 animals (n=3 per group)

Group 1 - (Normal) = Standard diet

Group 2 - (Obese control) = High fat diet

Group 3 - HFD+ *C. roseus* flower extract (200 mg/kg)

Group 4 - HFD+ *C. roseus* flower extract (400 mg/kg)

Group 5 - Standard (HFD+ Atorvastatin 10 mg/kg)

Following a week of administration, every rat initial body weight was noted[14]. The treatment was administered to each group orally once daily for 30 days[15].

2.6.2 Biochemical Estimation

Rats that have been fasting for the entire night had a 1 ml amount of blood extracted *via* the retro orbital method[16]. After allowing the sample to coagulate for 30 minutes at room temperature, the plasma was separated by centrifuging it for 15 minutes at a rate of 2000rpm[17]. The samples of the serum were estimated for total cholesterol,

LDL-C, HDL-C, VLDL-C, and triglycerides[18]. These were estimated by using ROBONIK biochemical analyser.

2.6.3 Statistical Analysis

Using one-way Analysis of Variance (ANOVA) followed by Dunnet's multiple tests. Results are mainly expressed as Mean ± SEM, differences among groups consider as significant at $p<0.001$ level.

3. Results and Discussion

3.1 FTIR Analysis

Figure 1 explains the FTIR analysis of the sample extract revealed the presence of various functional groups, indicating the complex phytochemical composition of the extract. A broad absorption band observed at 3264.10 cm^{-1} corresponds to O–H stretching vibrations of hydroxyl groups, suggesting the presence of alcohols or phenolic compounds, while the peak at 2924.13 cm^{-1} is attributed to C–H stretching of aliphatic groups. A distinct band at 1712.31 cm^{-1} represents the C=O stretching vibration of carbonyl groups, confirming the presence of esters, aldehydes, ketones, or carboxylic acids. The absorption peak at 1611.86 cm^{-1} indicates C=C stretching of aromatic rings or C=N stretching, suggesting the presence of aromatic or conjugated compounds. Peaks at 1376.31 cm^{-1} and 1245.61 cm^{-1} correspond to C–H bending and C–O stretching vibrations, respectively, indicating the presence of methyl and ether linkages. The band observed at 1020.21 cm^{-1} is associated with C–O–C or C–O stretching of alcohols or polysaccharides, while multiple peaks between 922.09 and 719.68 cm^{-1} correspond to C–H out-of-plane bending of aromatic compounds. Overall, the FTIR spectrum confirms the presence of hydroxyl, carbonyl, aromatic, and ether functional groups, suggesting that the extract contains bioactive constituents such as phenols, flavonoids, and other oxygenated compounds responsible for its potential therapeutic properties.

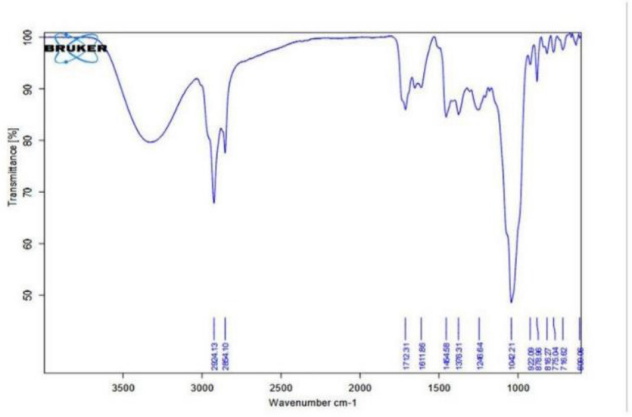


Figure 1. FTIR spectrometry of *C. roseus* ethanolic flower extract.

3.2 *In-vitro* Anti-inflammatory Effect of *C. roseus* Flower Extract

The anti-inflammatory property of *C. roseus* flower extract was assessed *in-vitro* Human Red Blood Cell (HRBC) membrane stabilisation method. The obtained results probably indicate a concentration dependent inhibition of analysis and protein denaturation demonstrating that the extract possesses membrane stabilisation and anti-inflammatory activity. In the HRBC membrane stabilisation assay at lower concentrations 100µg/ml, the extract showed moderate inhibition of 43% of analysis while at 200µg/ml concentration the percentage inhibition significantly increased to 68% membrane as shown in Figure 3, lysis indicating that the anti-inflammatory activity compared to standard reference drug Diclofenac sodium exhibiting 65.50 % of membrane lysis at 10µg/ml concentration as shown in the Table 2. In protein denaturation method, the *C. roseus* exhibited 44.50% of inhibition at 100µg/ml concentration but whereas higher concentration of 200µg/ml positively increased the inhibition to 65 % as shown in the Figure 2 which is compared to that of Diclofenac sodium which produced 75.50% of inhibition at 10µg/ml as shown in the Table 1. The anti-inflammatory activity exhibited a concentration dependent manner.

3.3 Effect of *C. roseus* Ethanolic Flower Extract on Hyperlipidaemia Induced Rats

The anti-inflammatory property of C. roseus flower extract was assessed in-vitro Human Red Blood Cell

Table 1. *In vitro* anti-inflammatory effect of *C. roseus* flower extract by protein denaturation method

S. No	Concentration (µg/ml)	% Inhibition *C. roseus* Diclofenac Sodium Flower Extract
1	10µg/ml	74.50±0.165.50±0.7
2	100 µg/ml	44.50±0.6------
3	200 µg/ml	65±1.6------

Values are expressed as mean ±SEM, (n=3 each), $p<0.01$

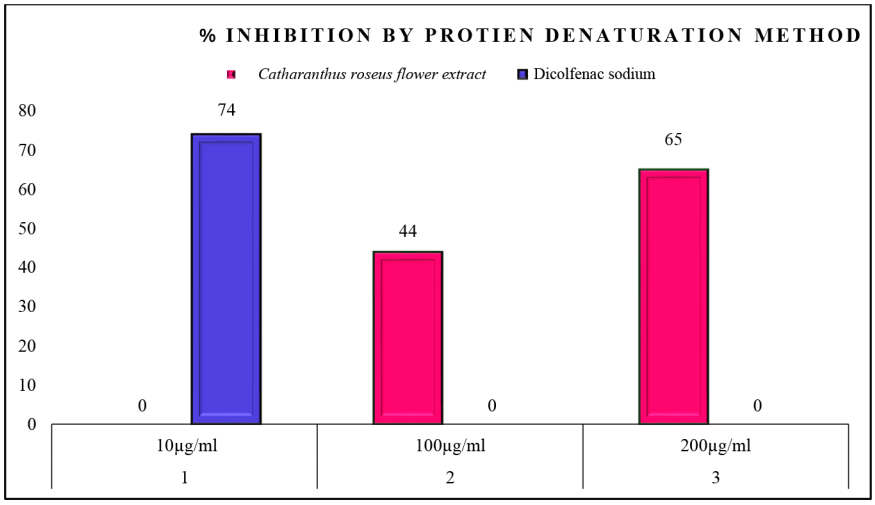


Figure 2. % Inhibition of inflammation by protein denaturation method.

Table 2. *In vitro* anti-inflammatory effect of *C. roseus* flower extract by HRBC method

S. No	Concentration (µg/ml)	% of Membrane Lysis *C. roseus* Diclofenac Sodium Flower Extract
1	10µg/ml	65.50±0.7 -----
2	100 µg/ml	43±1.5 ------
3	200 µg/ml	68±1.2 ------

Values are expressed as mean ±SEM, (n=3 each), p<0.01

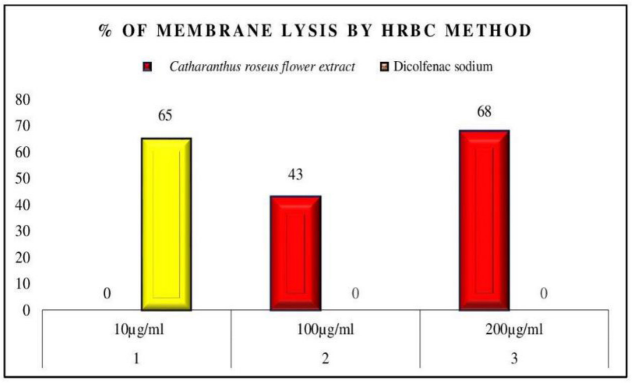


Figure 3. % of membrane lysis by HRBC method.

Table 4. Effect of *C. roseus* flower extract on serum lipid profile (% reduction)

Parameter	Standard (%)	Test 1 (200 mg/kg)	Test 2 (400 mg/kg)
TC	25.75	20.14	24.77
LDL-C	62.4	53.8	57.9
VLDL-C	31.7	16.5	26.9
TG	35.4	26.8	30.9

Table 5. Effect of *C. roseus* flower extract on HDL (% elevation)

Parameter	Standard (%)	Test 1 (200 mg/kg)	Test 2 (400 mg/kg)
HDL	8.99	11.85	25.80

(HRBC) membrane stabilisation method. The obtained results probably indicate a concentration dependent inhibition of analysis and protein denaturation demonstrating that the extract possesses membrane stabilisation and anti-inflammatory activity. In the HRBC

Table 3. Effect of ethanolic flower extract of *C. roseus* on lipid profile parameters in high fat diet induced hyperlipidaemic rats

Treatment Group		TC	HDL -C	LDL-C	VLDL-C	TG
Normal		175.6±1.62	36.72±1.62	70.62±2.25	36.36±0.90	168.7±2.93
Obese control		197±0.44	12.09±0.12	141.9±0.17	41.24±0.28	210.8±0.36
Ethanolic flowers extract	Test-1 (200mg/kg)	157.3±1.67	39.6±1.54	65.52±1.25	34.54±1.6	154.37±1.5
	Test -2 (400mg/kg)	148.2±1.41	42.02±1.7	59.73±2.1	30.16±1.51	145.6±1.67
Standard (10mg/kg) (Atorvastatin)	146.33±6.5	46.17±1.6	53.33±0.1	28.17±2.7	136.33±5.6	136.33±1.67

Values are indicated in terms of Mean ±SD; n=3 in each group *p< 0.05, statistically significant difference in comparison with obese control group, **p < 0.01. Statistically significant difference in comparison with obese control group.

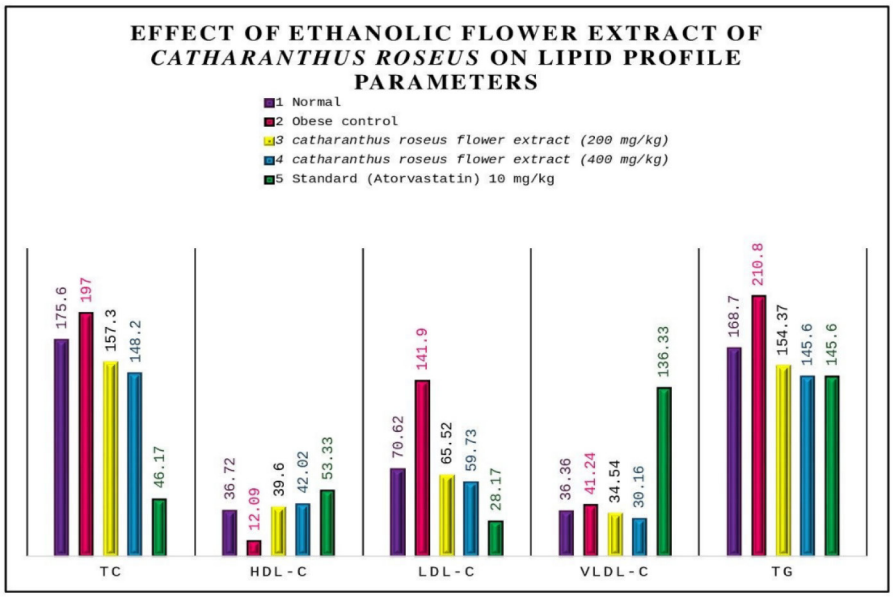


Figure 4. Effect of ethanolic flower extract of *C. roseus* on hyperlipidaemia induced rats.

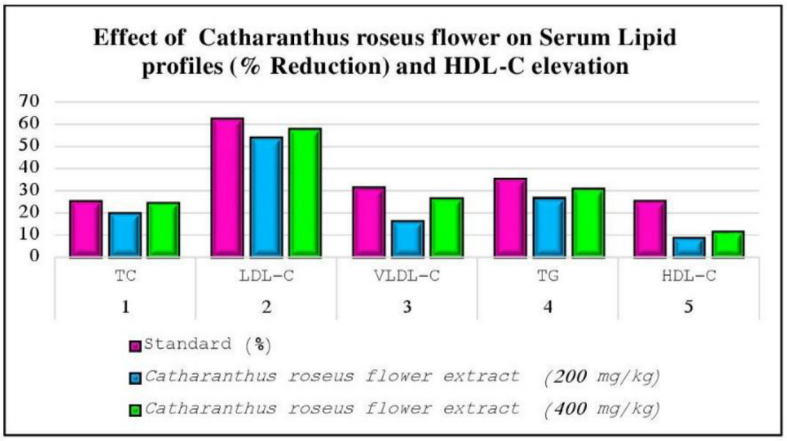


Figure 5. Effect of *C. roseus* flower extract on serum lipid profile.

membrane stabilisation assay at lower concentrations 100µg/ml, the extract showed moderate inhibition of 43% of analysis while at 200µg/ml concentration the percentage inhibition significantly increased to 68% membrane as shown in (Figure 3) lysis indicating that the anti-inflammatory activity compared to standard reference drug Diclofenac sodium exhibiting 65.50 % of membrane lysis at 10µg/ml concentration as shown in the (Table 2). In protein denaturation method, the C. roseus exhibited 44.50% of inhibition at 100µg/ml concentration but whereas higher concentration of 200µg/ml positively increased the inhibition to 65 % as shown in the Figure 2 which is compared to that of Diclofenac sodium which produced 75.50% of inhibition at 10µg/ml as shown in the (Table 1). The anti-inflammatory activity exhibited a concentration dependent manner.

4. Discussion

Prior to induction of hyperlipidaemia with high fat diet, the initial lipid profile parameters were assessed and the normal cholesterol, triglycerides, LDL-C, VLDL-C, and HDL-C were found to be normal. The high fat diet produced marked increased in serum lipid concentration

along with the increase in body weight and it was found that all the experimental rats exhibited significant elevated levels of Total cholesterol, Triglycerides, LDL-C, VLDL-C along with drastic decline in High Density Lipoprotein (HDL-C), these disturbances in lipid profile parameters clearly indicates there is a successful induction of hyperlipidaemia. After 7 days of treatment, the test extract *C. roseus* flower extract treated groups indicated good improvement in lipid profiles. The ethanolic flower extract of *C. roseus* at dose of 200 mg/kg produced significant reduction in percentage of lipid profiles as shown in the Figure 4. The % values ranging between 16-53%, the higher dose of *C. roseus* (400 mg/kg) pronounced significant lower values of lipid profiles as shown in the Table 3. The % reductions are 24-58% of serum lipids which is a clear indication as shown in the Figure 5 of hyperlipidaemic activity as shown in the Table 4. The very good significant observation is elevation of HDL-C levels, indicating improved reverse cholesterol transport lipid metabolism as indicated in Table 5.

4.1 Possible Mechanism of Action Based on FTIR Studies, *in-vitro* and *in-vivo* Experimental Findings

Based on the overall experimental findings, it is suggested that *C. roseus* ethanolic flower extract exhibits its anti-inflammatory and hypolipidemic effects through multiple phytochemical mediated pathways. The mechanistic insights of FTIR analysis indicate the presence of several functional groups corresponding to phenols, flavonoids, tannins, carbonyl compounds, aromatic derivatives and ether linkages, which are well-known for anti-oxidant activity wherein O-H and C=O groups suggest a high concentration of phenolic and flavonoid compounds, which can donate hydrogen and neutralise reactive oxygen species. Such antioxidant action reduces lipid peroxidation, a key trigger of both inflammation and hyperlipidaemia. The compounds with aromatic and phenolic rings stabilise proteins and lipid membranes, thereby preventing protein denaturation, erythrocyte membrane damage *etc*, the modulation of lipid metabolism is based on oxygenated groups such as C-O, C=O and C-O-C are common in phytochemicals known to inhibit HMG-CoA reductase that may enhance bile acid excretion and improve lipid catabolism. The phytochemical composition which is identified supports anti-inflammatory and lipid-lowering properties.

The probable and possible mechanistic statement that can be established is that the ethanolic flower extract of *C. roseus* likely exhibits its anti-inflammatory activity through protein stabilisation, lysosomal membrane protection and suppression of inflammatory mediator release, mediated by phenolic and flavonoid constituents. Its hypolipidemic effect appears to involve inhibition of HMG-CoA reductase, enhancement of reverse cholesterol transport, reduction in triglyceride synthesis and increased lipid catabolism, supported by elevated HDL-C and lowered TC, LDL-C and TG levels. The FTIR identified functional groups confirm the presence of bioactive compounds capable of mediating antioxidant, anti-inflammatory and lipid-lowering actions, establishing a strong biochemical basis for the extract's dual therapeutic potential.

5. Conclusion

The present research study was carried out in a systematic manner and based on the results obtained, the ethanolic flower extract of *C. roseus* exhibited significant anti-inflammatory and hypolipidemic activity where, the lipid profile parameters were markedly lowered and subsequently, evidenced with increased level of HDL-C which can be marked as significant. The phytochemical constituents like flavonoids, polyphenols, glycosides may be responsible for these two important biological activities, in folkloric usage, *C. roseus* flower extract be used as a native medicine to reduce the lipid profiles particularly in patients suffering with cardiovascular complaints. More research needs to be emphasised to investigate other pharmacological actions and possible mechanism of actions responsible for these pharmacological investigations.

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Author Contributions

Conceptualisation. Tony Priyanka; Methodology, V. Keerthana; Formal analysis, S. Amala; Resources, Aditya college of pharmacy; and Data curation, Dr, K. Ravi Shankar, Gnaneshwari; writing original article preparation, V. Keerthana; writing-review and editing, Dr, K. Ravi Shankar, V. Keerthana, K. Gnaneshwari; supervision, Dr, K. Ravi Shankar. All authors have read and agreed to the published version of the manuscript.

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