

EXTRACTION AND PHYTOCHEMICAL ANALYSIS OF *PHYLLANTHUS ROTUNDIFOLIUS* EFFECT ON ANTI-HYPERLIPIDEMIC ACTIVITY

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ABSTRACT:

Background: Hyperlipidaemia is a major metabolic disorder associated with abnormal lipid metabolism and an increased risk of cardiovascular complications. Plant-derived bioactive constituents may offer potential therapeutic benefits in its management.

Objective: To evaluate the anti-hyperlipidaemic, antioxidant, and protein-denaturation inhibitory activities of *Phyllanthus rotundifolius* leaf extract using in-vitro and in-vivo experimental models.

Methods: Leaves of *P. rotundifolius* were collected, authenticated, and extracted with methanol. Preliminary phytochemical screening and acute oral toxicity studies were performed according to OECD Guideline 423. Hyperlipidaemia was induced in rats using a high-fat diet, and the extract was administered at 100 and 200 mg/kg. Serum lipid parameters were evaluated, while protein-denaturation, hydrogen peroxide, and nitric oxide scavenging assays were performed in vitro. Data were analysed using one-way ANOVA followed by Tukey's post-hoc test.

Results: The extract contained alkaloids, flavonoids, saponins, tannins, and phenolic compounds. No mortality or observable toxicity occurred at 2000 mg/kg. Extract treatment produced favourable changes in serum lipid parameters. Protein-denaturation inhibition increased from 55.04% to 90.13%, while nitric oxide scavenging increased from 2.61% to 35.07% with increasing concentrations.

Conclusion: *P. rotundifolius* leaf extract demonstrated promising anti-hyperlipidaemic, antioxidant, and protein-denaturation inhibitory activities, warranting further pharmacological investigation.

Keywords: antioxidant; anti-inflammatory; hyperlipidaemia; *Phyllanthus rotundifolius*; phytochemicals; lipid profile.

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INTRODUCTION:

Hyperlipidaemia, a metabolic disorder caused by the accumulation of lipids in the blood, is characterized by high lipid levels in the blood. This condition may manifest as raised total cholesterol, triglycerides, and/or bad cholesterol (LDL) levels, or as a drop in good cholesterol (HDL) levels. The metabolic process of lipids is dysfunctional, thus resulting in atherosclerosis, which makes hyperlipidaemia one of the leading causes of cardiovascular diseases (CVD). It is believed that atherosclerosis, in some way or the other, happens due to CVD risk factors and is prevalent in everyone¹. Primary Hyperlipidaemia Familial Hyperlipidaemia has been called a medical condition that is primarily related to blood, hence the name. The root cause is genetic

disorders that are inherited and transmitted from the parent to the child. The condition of high blood lipids indicates not only a problem within the body but also a failure of the body to metabolise the lipids properly; that is, lipids such as cholesterol and triglycerides are not getting broken down completely. Congenital conditions usually denote that the diagnosis happens at birth, but the other scenario is that it could manifest even after several years of being unnoticed. In the majority of cases of primary hyperlipidaemia, the patient or patients concerned have one or both parents with a history of hyperlipidaemia or related cardiovascular diseases. This is a genetic disorder that parents might not even be aware of and thus unknowingly pass it down to

their children. Moreover, an unhealthy lifestyle consisting of poor diet and lack of physical activity can aggravate this condition. However, hunger can be a counterproductive factor for the body as well. The person may be subjected to a high risk of heart and vascular diseases if not treated. One can make changes to their lifestyle and also take medicines to a considerable extent, but still, the risk of heart attack or stroke, etc. cannot be eliminated. Early treatment might also help in avoiding a serious health issue². Acquired hyperlipidaemia (otherwise known as "secondary hyperlipidaemia") is the same as the non-hereditary form of hyperlipidaemia (as described in the first part of this article), yet it is triggered by other factors that influence the health of the individual who is mostly due to poor lifestyle decisions. In the possible explanation for this type of acquired form of hyperlipidaemia is a medical condition of diabetes, hypothyroidism, or a combination of a sedentary lifestyle and/or a diet high in carbs (carbohydrates)³. Hyperlipidaemias have risk factors that fall into two general categories: Modifiable and non-modifiable. Both types of risk factors are used for planning, Prevention, and managing increased lipids in the body. Most risk factors are non-modifiable; however, many of the same risk factors can be modified, altered, or influenced to help prevent excessive lipid levels in the body⁴. There are several modifiable risk factors associated with hyperlipidaemia, and many of these factors are related to lifestyle habits and many conditions or illnesses which can be managed or treated. For example, a diet high in saturated and trans fat and low in fruits, vegetables, and fibre is a main reason people develop elevated lipid levels. Issues such as the use of prescription medications, excessive alcohol intake, and smoking are lifestyle-based reasons for a person to have elevated blood lipid levels. Some individuals have elevated or lowered lipid levels because they have poor management of certain metabolic disorders such as Diabetes Mellitus or Polycystic Ovary Syndrome (PCOS). In addition to the items discussed above, being overweight, gaining excess weight, and low physical activity are also risk factors that increase blood lipid levels. High blood pressure and sleep issues may disrupt the normal balance within the individual, lead to high blood lipid levels, and are therefore other causes of hyperlipidaemia⁵.

METHODOLOGY:

Collection and extraction of *Phyllanthus rotundifolius* plant leaves

Collection and Authentication of Plant leaves

The whole plant of *Phyllanthus rotundifolius* was gathered from Pammal, Chennai, India, in JUNE-SEPT 2025. The Plant specimen was authenticated by Dr P. Santhan, PhD (Bot), taxonomist, at Druva Herbal Centre, Chennai, India.

Plant leaves extract preparation

Fresh plant material is collected, authenticated, washed with tap water and then distilled water, shade-dried until brittle, and ground to a coarse powder (about 40-60 mesh). About 100 g of the powder is macerated in 500-1000 mL of methanol (1:5 to 1:10 w/v) on an orbital shaker for 48-72 hours at room temperature, protected from light. The mixture is filtered through muslin and Whatman No. 1 paper; the residue is re-extracted with fresh methanol two to three times, and the pooled filtrates are concentrated in a rotary evaporator at 40-50 °C to obtain the crude extract⁶.

Qualitative Phytochemical Analysis

Acute toxicity study:

Sl. no	Phytochemicals	Test Description	Observation
7	Phenolic Compounds	2 ml extract + 2-3 drops of Ferric chloride solution (1%)	Bluish black or dark green colour indicated the presence of phenols

The acute toxicity study of *Phyllanthus rotundifolius* was carried out in accordance with OECD Guideline 423. The extract was administered orally at a dose of 2000 mg/kg body weight, and the animals were observed for a period of 14 days. No mortality or signs of toxicity were observed in the treated animals during the observation period. Therefore, the median lethal dose (LD₅₀) of *Phyllanthus rotundifolius* was considered to be greater than 2000 mg/kg body weight. Based on the acute toxicity findings, doses of 100 mg/kg and 200 mg/kg body weight were selected for subsequent pharmacological evaluation^{7,8}.

In vivo methods:

Induction of Hyperlipidemic activity:

Rats weighing 150-200 g were used after one week of acclimatisation to induce hyperlipidemic conditions. Hyperlipidemia was induced in the rats by administering a hyperlipidemic diet (HFD). Each rat received 12 g of HFD per day with no significant variation in the daily quantity. The rats subsequently underwent intragastric administration for 28 days and were divided into five groups, with six rats in each group⁹.

Group A: normal diet and water (control).

Group B: High-fat diet and cholesterol (25 mg/kg b. w./day).

Group C: Atorvastatin (4 mg/kg b.w./day).

Group D: Normal diet cholesterol (25 mg/kg b.w./day) *Phyllanthus rotundifolius* (Low dose) up to 28 days.

Group E: Normal diet cholesterol (25 mg/kg b.w./day) *Phyllanthus rotundifolius* (High dose) up to 28 days

Anti-inflammatory markers:

Protein Denaturation:

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The protein denaturation assay was performed using an albumin denaturation method. The reaction mixture consisted of the protein solution and different concentrations of the plant extract. The pH of the reaction mixture was adjusted appropriately using phosphatebuffered saline. The samples were incubated at 37°C for approximately 20 minutes, followed by heating at 70°C for 5 minutes to induce protein denaturation. After cooling, the absorbance of each reaction mixture was measured spectrophotometrically at approximately 660 nm. The control contained all assay components except the plant extract. Diclofenac sodium was used as the reference standard. The percentage inhibition of protein denaturation was calculated using the following equation^{10,11,12}:

Protein Denaturation Inhibition (%) = [(Absorbance of Control – Absorbance of Sample) / Absorbance of Control] × 100.

Anti-oxidative markers:

Hydrogen peroxide:

A 40 mM solution of hydrogen peroxide is prepared in phosphate buffer (50 mM, pH 7.4). The extract is dissolved in methanol or distilled water and diluted to a series of concentrations (for example 20-100 µg/mL). To 1 mL of each extract concentration, 0.6 mL of the H₂O₂ solution is added, and the mixture is incubated for 10 minutes at room temperature. The absorbance is read at 230 nm against a blank containing the extract and phosphate buffer without H₂O₂. Ascorbic acid at the same concentrations is used as the standard, and the control contains the H₂O₂ solution and buffer without extract (1, 2). The percentage of H₂O₂ scavenged is calculated as^{13,14}:

% Scavenging = [(A₀ – A₁) ÷ A₀] × 100

Nitric oxide:

Sodium nitroprusside (10 mM) is prepared in phosphate-buffered saline (pH 7.4). To 2 mL of this solution, 0.5 mL of the plant extract at different concentrations (for example 20-100 µg/mL) is added, and the mixture is incubated at 25 °C for 150 minutes, during which the nitroprusside generates nitric oxide that reacts with oxygen to form nitrite. After incubation, 0.5 mL of the reaction mixture is mixed with 1 mL of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and left for 5 minutes for diazotization. Then 1 mL of naphthylethylenediamine dihydrochloride (0.1% w/v) is added, and the mixture is incubated for 30 minutes at 25 °C, which produces a pink chromophore. The absorbance is read at 540 nm. The control contains all components except the extract, and ascorbic acid (or gallic acid) is used as the standard (1-3). The percentage of nitric oxide scavenged is calculated as^{15,16,17}:

% Scavenging = [(A₀ – A₁) ÷ A₀] × 100

Statistical Analysis:

GraphPad Prism version 10.0 was employed for the statistical analysis of the data. The results were expressed as mean ± SEM. Statistical comparisons

among the groups were performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc multiple comparisons test.

Results:

Extraction:

The leaves of *Phyllanthus rotundifolius* were successfully processed and subjected to Soxhlet extraction using 95% methanol as the solvent. The extraction was carried out at 60–70°C under continuous reflux conditions, followed by concentration of the extract under reduced pressure to remove the solvent. A dark brown, semisolid ethanolic extract was obtained with a characteristic Odor. The extract was collected, weighed, and the percentage yield was calculated based on the initial weight of the dried powder. The obtained extract was stored in an airtight container under refrigerated conditions until further phytochemical analysis.

Phytochemical Analysis:

Preliminary phytochemical screening of the ethanolic fruit of *Phyllanthus rotundifolius* Leaves revealed the presence of alkaloids, flavonoids, saponins, tannins, and phenolic compounds (+), while steroids and glycosides (–) were absent. The presence of flavonoids and phenolic compounds suggests significant antioxidant potential, whereas alkaloids may contribute to the anti-hyperlipidemic activity by modulating lipid metabolism, reducing cholesterol and triglyceride levels, and enhancing antioxidant defence. Mentioned below (Table No: 02)

Table No 02: Indication of presence or absence of phytochemical constituents

Sl. no	Phytochemicals	Test Description	Observation
1	Alkaloid	2 ml of extract + 2 ml HCl → boil and cool → add 2 drops of Wagner's reagent	Brownish red coloration indicated the presence of an alkaloid
2	Tannin	2 ml extract + 2 ml water + 2–3 drops of Ferric chloride (5%)	Green precipitate indicated the presence of tannin

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3	Flavonoid	3 ml extract → evaporated to dryness → Residue + 1–2 ml 50% CH ₃ CH ₂ OH + heat + Mg ribbon + 4–5 drops HCl	Red or orange color indicated the presence of flavonoid
4	Steroid	5 ml extract + 2 ml CHCl ₃ + 3 ml conc. H ₂ SO ₄	Reddish-brown precipitate at the interface indicated the presence of steroid
5	Glycoside	2 ml extract + 2 ml CHCl ₃ + 1–2 ml CH ₃ COOH	Violet, blue, or green coloration indicated the presence of glycoside
6	Saponin	2 ml extract → evaporated to dryness → Residue + 1 ml water → shaken vigorously	Persistent foam (1 cm in the test tube) confirmed the presence of saponin
7	Phenolic Compounds	2 ml extract + 2–3 drops of Ferric chloride solution (1%)	Bluish black or dark green colour indicated the presence of phenols

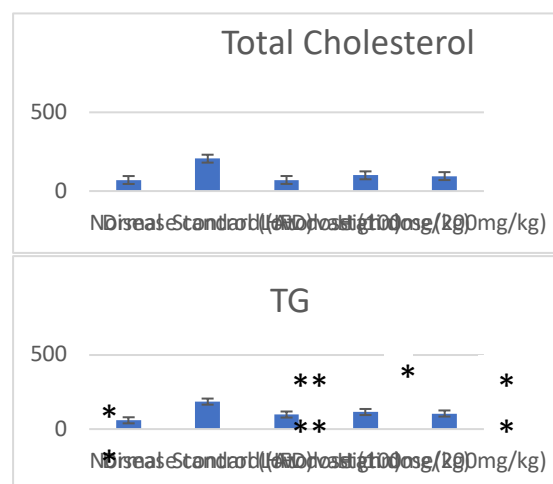
IN VIVO STUDIES

Establishment and Validation of the Experimental Hyperlipidemia Model

Hyperlipidemia was successfully induced in the experimental rats following administration of a high-cholesterol/high-fat diet for a 28-day induction period. At the end of the induction period, the hyperlipidemic control group showed increased serum lipid parameters, including total cholesterol, triglycerides, and LDL-C, compared with the normal control group, whereas HDL-C levels were altered. These findings confirmed the successful establishment of the experimental hyperlipidemia model. (Table 03 and figures 01)

Table:03 Effect of *Phyllanthus rotundifolius* Leaves extract on serum lipid profile in hyperlipidemic rats.

Group no	Treatment	Lipid levels			
		Total cholesterol	TG	HDL	LDL
Normal	Normal saline	70.2 ±11.16	59.3 ±9.33	60.3 ±10.62	15.1 ±6.22
Diseased control	HFD	205.8 ±19.25	185.5 ±40.30	35.8 ±2.94	140.3 ±19.41
Standard	Atorvastatin 4mg/kg	70.4 ±4.77	100.3 ±10.21	45.1 ±4.20	34.3 ±9.26
Low dose	100 mg/kg	100.0 ±5.34	101.5 ±5.89	58.6 ±4.10	102.8 ±8.85
High dose	200 mg/kg	95.1 ±2.58	108.5 ±4.97	53.1 ±3.32	98.9 ±8.74



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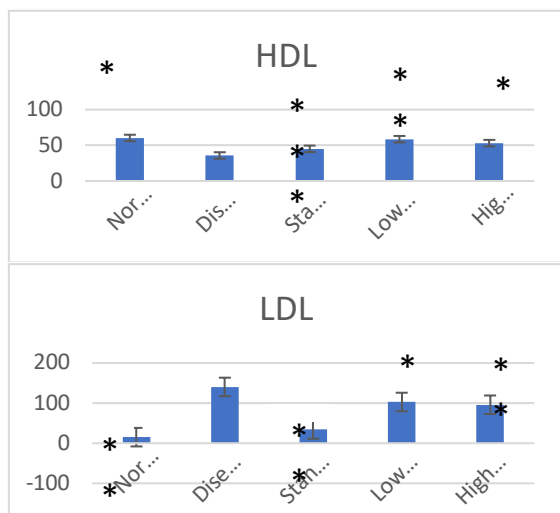


Figure No 01: Effect of treatment on serum lipid profile in HFD-induced dyslipidaemic rats. Values are expressed as mean $\pm$ SEM, showing the levels of total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) in normal, diseased control, atorvastatin-treated, and test-drug-treated groups.

IN Vitro models:

Protein Denaturation: The test sample showed concentration-dependent inhibition of protein denaturation, with the percentage inhibition increasing with increasing concentrations. The highest concentration exhibited the maximum inhibition of protein denaturation, indicating the potential anti-inflammatory activity of the test sample. (Table 04 and Figure 02)

Table No: 04 values of protein denaturation

Concentration (ug/ml)	Sample OD	Sample % Inhibition	Vitamin C OD	Vitamin C % Inhibition
10	0.820	55.04 %	0.780	57.24 %
20	0.610	66.56 %	0.560	69.30 %
30	0.390	78.62 %	0.320	82.46 %
40	0.180	90.13 %	0.120	93.46 %

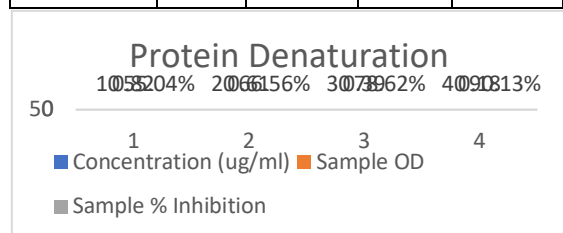


Figure No 02: Concentration-dependent free radical scavenging activity of the test sample and Vitamin C. The percentage inhibition was evaluated at different concentrations (10–40 μ g/mL) based on the optical density (OD), with Vitamin C used as the standard antioxidant

Hydrogen peroxide:

The sample exhibited concentration-dependent hydrogen peroxide scavenging activity, with the percentage inhibition increasing with increasing concentration. The maximum scavenging activity was observed at the highest tested concentration, indicating the antioxidant potential of the sample.

Table No 05: Values of hydrogen peroxide

Catalase

Replicate	OD at 620nm	H ₂ O ₂ Conc (um)	Activity(um/mg)
1	0.085	6.50	65.0
2	0.080	6.10	61.0
3	0.076	5.80	58.0

Test sample

Replicate	OD at 620nm	H ₂ O ₂ Conc (um)	Activity(um/mg)
1	0.095	7.20	72.0
2	0.090	6.80	68.0
3	0.086	6.40	64.0

Nitrogen peroxide:

The sample exhibited concentration-dependent nitric oxide scavenging activity, with the percentage inhibition increasing with increasing concentration. The maximum nitric oxide scavenging activity was observed at the highest tested concentration, indicating the antioxidant potential of the sample. (Table 06 and Figure 03)

Table No: 06 Values of Nitrogen peroxide

Concentration(ug/ml)	Sample OD	% Inhibition
10	2.280	2.61
20	2.120	9.44
30	1.930	17.56
40	1.710	26.95
50	1.520	35.07

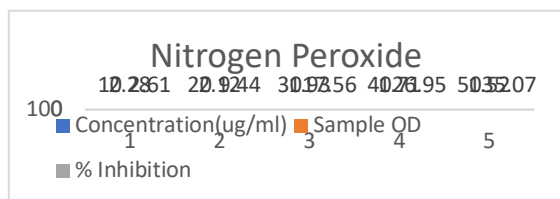


Figure No 03: Concentration-dependent inhibitory activity of the test sample. The percentage inhibition was determined at concentrations ranging from 10–50 µg/mL based on the corresponding optical density (OD) values.

DISCUSSION:

The present study evaluated the pharmacological potential of *Phyllanthus rotundifolius* leaf extract using in-vitro and in vivo models of hyperlipidaemia. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, tannins, and phenolic compounds, whereas steroids and glycosides were absent. The presence of flavonoids and phenolic compounds may have contributed to the antioxidant potential of the extract, while other phytoconstituents may have contributed to its observed lipid-modulating activity.

The acute toxicity study demonstrated that oral administration of the extract at 2000 mg/kg did not produce mortality or observable signs of toxicity during the 14-day observation period. The LD₅₀ was therefore considered to be greater than 2000 mg/kg, and doses of 100 and 200 mg/kg were selected for subsequent pharmacological evaluation.

The high-fat diet successfully established the experimental hyperlipidaemic condition, as indicated by increased total cholesterol, triglycerides, and LDL-C levels in the diseased control group compared with the normal control group. Treatment with *P. rotundifolius* extract produced changes in the serum lipid profile, with the extract-treated groups showing lower total cholesterol and LDL-C values compared with the hyperlipidaemic control. The observed lipid-lowering effect suggested that the extract may have exerted a beneficial influence on lipid metabolism.

The in-vitro protein denaturation assay demonstrated concentration-dependent activity of the test sample. The percentage inhibition increased from 55.04% at 10 µg/mL to 90.13% at 40 µg/mL, while Vitamin C showed 57.24–93.46% inhibition over the same concentration range. These findings indicated that the extract possessed an ability to inhibit protein denaturation, supporting its potential anti-inflammatory activity.

The antioxidant evaluation also demonstrated concentration-dependent activity. In the hydrogen peroxide-related assay, the reported activity increased with increasing concentration, indicating the ability of the test sample to interact with reactive oxygen species. The catalase-related measurements further demonstrated measurable H₂O₂-associated activity across the tested replicates.

Similarly, the NO₂ assay showed increasing inhibition with increasing concentration. The reported sample inhibition increased from 2.61% at 10 µg/mL to 35.07% at 50 µg/mL, indicating concentration-dependent nitric oxide/nitrite scavenging activity. These findings, together with the presence of phenolic and flavonoid constituents, supported the antioxidant potential of *P. rotundifolius* extract.

Overall, the findings suggested that the biological activity of *P. rotundifolius* extract may have been associated with the combined effects of its phytochemical constituents. The observed antioxidant and anti-inflammatory activities may have contributed to the improvement in lipid-related parameters observed in the experimental hyperlipidaemia model.

CONCLUSION:

The present study demonstrated that the methanolic extract of *Phyllanthus rotundifolius* leaves possessed anti-hyperlipidaemic, antioxidant, and protein-denaturation inhibitory activities. The extract was found to be well tolerated at the tested acute dose, with an LD₅₀ greater than 2000 mg/kg. The extract produced beneficial changes in serum lipid parameters in hyperlipidaemic rats and showed concentration-dependent activity in the protein denaturation and antioxidant assays. The presence of flavonoids, phenolics, alkaloids, tannins, and saponins may have contributed to these biological effects. Therefore, *P. rotundifolius* leaf extract showed promising pharmacological potential for further investigation as a plant-derived candidate for management of hyperlipidaemia and associated oxidative stress.

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