

Pharmacognostic Standardization, Phytochemical Profiling, Antioxidant, Antidiabetic and Hepatoprotective Evaluation of *Clerodendrum colebrookianum* Walp. Ethanolic Extract

Samuel Mukiri^{1*}, Eswar Kumar Kilari²

^{1*}Research Scholar, Department of Pharmacology, AU College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, India. E-mail: samuelmukiri77@gmail.com, Mob: +91-8790422917.

²Professor, Pharmacology Division, AU College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, India.

Abstract

Background: The present study evaluated the standardization, preliminary phytochemical profile, antioxidant activity, acute oral safety, antidiabetic activity, hepatoprotective activity and chromatographic characterization of *Clerodendrum colebrookianum* Walp.

Methods: Aqueous, ethanolic and petroleum ether extracts were evaluated for physicochemical parameters and preliminary phytochemical constituents. DPPH radical-scavenging activity was assessed against BHT. Ethanolic extract was further evaluated in rats for acute oral toxicity, glucose tolerance, alloxan-induced diabetes and CCl₄-induced hepatotoxicity, followed by histopathology. Column fractions were examined by TLC.

Results: The ethanolic extract showed the lowest DPPH IC₅₀ among the three solvents (211 µg/mL) and produced substantial reductions in blood glucose during the 15-day alloxan study, with a 15-day value of 122.6 mg/dL. It also reduced serum SGPT, SGOT, ALP and bilirubin in CCl₄-treated rats.

Conclusion: The reported findings support further investigation of the ethanolic extract of *Clerodendrum colebrookianum* Walp., while the present data should be validated in appropriately powered and independently replicated studies.

Keywords: *Clerodendrum*, pharmacognostic standardization, phytochemical screening, DPPH, antidiabetic activity, hepatoprotective activity, TLC.

How to cite this article: Mukiri S, Kilari EK., Pharmacognostic Standardization, Phytochemical Profiling, Antioxidant, Antidiabetic and Hepatoprotective Evaluation of *Clerodendrum colebrookianum* Walp. Ethanolic Extract. Int J Drug Deliv Technol. 2026;16(77s): 891-897; DOI: 10.25258/ijddt.16.77s.105

Introduction

Clerodendrum colebrookianum Walp. is a medicinal plant belonging to the family Lamiaceae and is traditionally used in several parts of South and Southeast Asia. Different parts of the plant have been employed in traditional systems of medicine for conditions associated with inflammation, metabolic disturbances, and other common ailments. The therapeutic relevance of the species has been linked to the presence of diverse secondary metabolites, including phenolics, flavonoids, terpenoids, alkaloids, and other bioactive constituents [1,2]. However, variations in geographical origin, environmental conditions, harvesting practices, and processing can influence the quality and phytochemical composition of medicinal plant materials. Pharmacognostical and physicochemical evaluation therefore provides an important basis for establishing the identity and quality of crude plant material [3]. Preliminary phytochemical screening can further provide an indication of the major groups of constituents present in an extract [4]. In addition, biological evaluation is useful for determining whether the traditionally reported medicinal potential is supported by measurable experimental activity. Although *C. colebrookianum* has received attention for its ethnomedicinal importance, integrated information combining quality-control characteristics, phytochemical observations, and biological responses remains comparatively limited.

Establishing such a profile may assist in the scientific standardization of the plant and provide a basis for further investigation. The present study was therefore designed to evaluate selected quality-control parameters of *C. colebrookianum* and to characterize its preliminary phytochemical profile. Selected biological assays were also undertaken to obtain an initial indication of its pharmacological potential. The investigation focused on generating reproducible experimental data that could support the medicinal relevance of the species. Particular attention was given to parameters that are practical for routine evaluation of herbal raw materials and extracts. The findings were considered collectively rather than as isolated observations, allowing the chemical and biological characteristics of the plant to be viewed in relation to its quality attributes. Such an approach may contribute to the development of reliable standards for future research and formulation studies. It may also help identify areas requiring more detailed phytochemical and mechanistic investigations. Overall, the study aimed to provide a preliminary scientific framework for the quality assessment and biological characterization of *C. colebrookianum* Walp. and to strengthen the evidence supporting its potential as a medicinal plant.

2. Materials and Methods

2.1 Plant Material and Authentication

Fresh leaves of *Clerodendrum colebrookianum* Walp. were collected on 20 April 2022 from the forest vegetation along the Brahmaputra River bank, Jorhat, Assam, India. The plant material was authenticated by Prof. Madhav Setty, Sri Venkateswara University, Tirupati, Andhra Pradesh, India. A representative voucher specimen was prepared and deposited for future reference under [Voucher number 0559 dated 24-4-2022]. The collected leaves were carefully examined and freed from foreign matter and damaged portions.

2.2 Preparation of Plant Material

The collected leaves were washed thoroughly with water to remove adhering dust and extraneous matter. The cleaned material was shade-dried under suitable conditions with protection from direct sunlight. The dried leaves were powdered using a mechanical grinder and stored in a suitable airtight container until further extraction.

2.3 Extraction Procedure

The powdered leaves of *C. colebrookianum* were extracted separately using water, ethanol, and petroleum ether. Following extraction, the individual extracts were filtered and concentrated to obtain the respective crude extracts. The concentrated extracts were stored under suitable conditions until further phytochemical and biological investigations.

The percentage yield of each extract was calculated using the following equation:

Percentage yield (%) = (Weight of dried extract / Weight of powdered plant material) × 100

2.4 Preliminary Phytochemical Screening

The aqueous, ethanolic, and petroleum ether extracts were subjected to preliminary phytochemical screening using standard qualitative chemical tests [5]. The extracts were examined for major classes of phytoconstituents, including carbohydrates, steroids, glycosides, flavonoids, proteins, amino acids, alkaloids, tannins, and triterpenoids.

2.5 DPPH Radical-Scavenging Assay

The antioxidant activity of the aqueous, ethanolic, and petroleum ether extracts was evaluated by the DPPH radical-scavenging method. The assay was performed spectrophotometrically at 517 nm. Butylated hydroxytoluene (BHT) was used as the reference standard [6,7]. The assay was performed in triplicate, and radical-scavenging activity was determined from the reduction in absorbance of the DPPH reaction mixture. The IC₅₀ value was determined from the concentration–response relationship.

2.6 Experimental Animals

Healthy albino Wistar rats were used for the in vivo pharmacological investigations. The experimental work was ethically approved and conducted at Narasaraopeta Institute of Pharmaceutical Sciences, Narasaraopet, Andhra Pradesh, India (IAEC/1414/A/11/CPCSEA/25/563/2010-AWD). The animals were maintained under standard laboratory conditions and provided standard laboratory feed and water ad libitum.

The animals were acclimatized to the laboratory environment before commencement of the experiments.

The exact animal number, sex, age, body-weight range, housing temperature, relative humidity, light–dark cycle, and acclimatization period should be inserted from the original animal-house record if required by the journal.

2.7 Acute Oral Toxicity Study

The acute oral toxicity of the ethanolic extract of *C. colebrookianum* was evaluated in albino Wistar rats according to the applicable OECD guideline [8]. The ethanolic extract was administered orally, and the animals were observed following administration for any apparent signs of toxicity or abnormal behavioural changes.

The observations included general behaviour and visible signs of toxicity during the observation period. The dose selected for subsequent pharmacological investigations was based on the toxicity evaluation. The exact OECD guideline number and observation schedule should be inserted from the original experimental record.

2.8 Oral Glucose Tolerance Test

An oral glucose tolerance test (OGTT) was performed in albino Wistar rats to evaluate the effect of the ethanolic extract on glucose handling. Animals were administered the test treatment according to the experimental protocol, followed by oral glucose administration. Blood glucose concentrations were determined at fasting, 30, 60, 120, and 240 min following glucose administration.

Blood glucose was estimated using the appropriate biochemical method, and the glucose response over the observation period was recorded for comparison among the experimental groups.

2.9 Alloxan-Induced Antidiabetic Study

Diabetes was experimentally induced in albino Wistar rats using alloxan according to the established experimental protocol [9,10]. Following confirmation of the diabetic state, the animals were allocated to the respective experimental groups.

The ethanolic extract of *C. colebrookianum* was administered orally at the selected experimental dose. Blood glucose concentrations were monitored during the study. In the single-dose experiment, blood glucose was assessed at fasting and at 30, 60, 120, 240, and 360 min after treatment.

For the multidose experiment, the ethanolic extract was administered for 15 days. Blood glucose and body weight were monitored during the treatment period. Blood glucose was assessed on days 0, 5, 10, and 15. Serum lipid parameters and pancreatic histopathology were also evaluated at the end of the experimental period.

2.10 Serum Lipid Profile

At the completion of the antidiabetic experiment, blood samples were collected for serum biochemical analysis. Serum was separated and used for estimation of triglycerides, HDL, VLDL, LDL, and total cholesterol using standard biochemical procedures [11].

2.11 CCl₄-Induced Hepatotoxicity and Hepatoprotective Study

Hepatotoxicity was induced in albino Wistar rats using carbon tetrachloride (CCl₄) according to the experimental protocol. The ethanolic extract of *C. colebrookianum* was administered to the designated treatment group.

At the end of the experiment, blood samples were collected and serum was separated for biochemical assessment. Hepatic injury and the protective effect of the plant extract were evaluated by estimating SGPT, SGOT, ALP, and total bilirubin [12,13,14].

2.12 Histopathological Examination

Following completion of the respective pharmacological experiments, the relevant tissues were collected for histopathological evaluation. Pancreatic tissue was examined in the antidiabetic experiment, while liver tissue was examined in the hepatoprotective experiment. Tissue specimens were processed according to standard histopathological procedures and examined microscopically for pathological alterations and treatment-associated changes [15].

2.13 Column Chromatography and TLC

Selected fractions obtained from the ethanolic extract were subjected to qualitative chemical testing and thin-layer chromatography (TLC). The fractions were applied to TLC plates and developed using appropriate solvent systems [16].

For the flavonol glycoside fraction, toluene:dioxane:acetic acid (90:25:5) was used as the developing solvent system and anisaldehyde-H₂SO₄ as the visualising reagent. For the sterol fraction, petroleum ether : acetone (85:15) was used as the solvent system and Liebermann-Burchard reagent for visualisation. The developed chromatograms were examined and the R_f

values were determined by comparison with reference standards.

3. Results and Discussion

3.1 Plant Material and Extraction

Clerodendrum colebrookianum Walp. was processed as described in the source study. The powdered plant was extracted with water, ethanol and petroleum ether. After concentration, the yields were 14.8% for water, 11.6% for ethanol and 4.5% for petroleum ether. All extracts were semisolid, viscous and brown. The ethanolic extract was chosen for the main biological tests because of its initial phytochemical profile and antioxidant activity.

The use of multiple solvents provided extracts with different polarity characteristics and therefore allowed preliminary comparison of the constituent classes recovered from the plant material.

3.2 Pharmacognostic and Physicochemical Standardisation

The powdered plant was checked for organoleptic and physicochemical properties. It was coarse and brown, with no noticeable odour or taste. Extractive values were 23% for water, 12% for alcohol and 4% for ether. Loss on drying was 10%. Total ash was 11% and acid-insoluble ash was 1%. The water-soluble ash value was not reported. These parameters are routinely used in the quality assessment and standardization of herbal materials the values are showed in atable.1

Table 1. Organoleptic and physicochemical characteristics of *C. colebrookianum* powder

Parameter	Observation
Nature	Coarse powder
Colour	Brown
Odour	No particular odour
Taste	No taste
Aqueous extractive value	23%
Alcohol extractive value	12%
Ether extractive value	4%
Loss on drying	10%
Total ash	11%
Acid-insoluble ash	1%
Water-soluble ash	Not reported

Table 2. Percentage yield and physical characteristics of extracts

Extract	Yield (% w/w)	Nature/Colour
Aqueous	14.8	Semisolid, viscous; brown
Ethanolic	11.6	Semisolid, viscous; brown
Petroleum ether	4.5	Semisolid, viscous; brown

3.3 Preliminary Phytochemical Screening

Preliminary phytochemical tests were performed on the water, ethanol and petroleum ether extracts. The water and ethanol extracts contained carbohydrates, steroids, glycosides and flavonoids. Proteins, amino acids, alkaloids, tannins and triterpenoids were not detected. The petroleum ether extract tested positive for steroids only. These findings represent qualitative screening observations and should not be interpreted as quantitative determinations of individual compounds.

3.4 DPPH Radical-Scavenging Activity

The antioxidant activity of the aqueous, ethanolic and petroleum ether extracts was evaluated using the DPPH radical-scavenging assay. Absorbance was measured at 517 nm, with BHT as the reference. Tests were conducted in triplicate, and percentage inhibition and IC₅₀ values were calculated. The ethanolic extract showed the lowest reported IC₅₀ among the three extracts, indicating the strongest radical-scavenging activity under the reported assay conditions [6,7].

Table 4. DPPH radical-scavenging activity of *C. colebrookianum* extracts

Extract	IC ₅₀ (µg/mL)
Aqueous	490
Ethanolic	211
Petroleum ether	920

Note: Lower IC₅₀ indicates stronger radical-scavenging activity under the reported assay conditions. BHT was used as the reference standard.

Table 5. DPPH radical-scavenging activity of ethanolic extract at different concentrations

Concentration (µg/mL)	DPPH inhibition (%)
10	2.96
50	8.34
100	19.10
250	26.34
500	50.27
1000	67.90

3.5 Acute Oral Toxicity

Acute oral toxicity was tested in rats using the ethanolic extract at 200 mg/kg body weight, following OECD guidelines. The animals were observed for toxicity. This dose was also used for the main antidiabetic and hepatoprotective studies. The reported procedure is consistent with the general framework of OECD Test Guideline 423 for acute oral toxicity [8].

Table 6. Acute oral toxicity and dose used for pharmacological evaluation

Parameter	Reported value
Experimental animal	Albino Wistar rats
Test extract	Ethanolic extract
Acute oral toxicity dose	200 mg/kg body weight
Dose used for principal pharmacological studies	200 mg/kg body weight
Guideline	OECD guideline
Main evaluations	Antidiabetic and hepatoprotective studies

3.6 Antidiabetic Evaluation

The antidiabetic effect of the ethanolic extract was tested using an oral glucose tolerance test, a single-dose study in alloxan-induced diabetic rats and a 15-day multidose study. The extract was given at 200 mg/kg. Blood glucose, body weight, serum lipids and pancreatic histology were measured. The use of OGTT and alloxan-induced diabetes provides complementary observations on glucose handling and experimental diabetic status.

Table 7. Oral glucose tolerance test

Time	Blood glucose (mg/dL)
Fasting	91.0
30 min	137.0
60 min	136.5
120 min	117.5
240 min	97.83

Over 15 days of treatment, blood glucose dropped from 275.5 mg/dL on day 0 to 194.2 mg/dL on day 5, 164.4 mg/dL on day 10 and 122.6 mg/dL on day 15. Body weight fell from 206.5 g on day 0 to 200.0 g, 194.0 g and 187.0 g on days 5, 10 and 15, representing a reported 9.44% decrease over 15 days. These observations are descriptive of the reported experimental group and should be interpreted together with the missing group size, variance and statistical significance information before making inferential claims.

Table 8. Effect of ethanolic extract on blood glucose in alloxan-induced diabetic rats

Treatment period	Blood glucose (mg/dL)
Day 0	275.5
Day 5	194.2
Day 10	164.4
Day 15	122.6

Table 9. Effect on body weight during 15-day treatment

Treatment period	Body weight (g)
Day 0	206.5
Day 5	200.0
Day 10	194.0
Day 15	187.0
Percentage change	−9.44%

Table 10. Single-dose effect on blood glucose in alloxan-induced diabetic rats

Time	Blood glucose (mg/dL)
Fasting	275.5
30 min	270.0
60 min	263.0
120 min	262.0
240 min	248.3
360 min	233.8

3.7 Serum Lipid Profile

Serum lipids were measured after the treatment period. The reported values for triglycerides, HDL, VLDL, LDL and total cholesterol were 94.0, 45.66, 22.0, 23.0 and 60.0 mg/dL, respectively. Serum biochemical measurements should be interpreted with the corresponding control and treatment groups and the statistical analysis from the original experimental record [10].

Table 11. Serum lipid profile after treatment

Lipid parameter	Value (mg/dL)
Triglycerides (TGL)	94.0
HDL	45.66
VLDL	22.0
LDL	23.0
Total cholesterol	60.0

3.8 Hepatoprotective Evaluation

The hepatoprotective effect of the ethanolic extract was tested in rats treated with carbon tetrachloride (CCl₄). On day 10, serum SGPT, SGOT, ALP and total bilirubin were measured. Liver tissue was also examined for histopathological changes. In the group treated with the plant extract, SGPT, SGOT, ALP and total bilirubin were 149.0, 198.5, 292.0 and 0.856, respectively. Histopathology was used to assess liver changes and treatment effects. The biochemical endpoints are established indicators used in experimental assessment of hepatic injury and treatment-associated changes [13–15,18].

Table 12. Hepatoprotective effect of ethanolic extract in CCl₄-induced hepatotoxicity

Biochemical parameter	Plant extract group
SGPT	149.0
SGOT	198.5
ALP	292.0
Total bilirubin	0.856

3.9 Column Chromatography and TLC

Selected column fractions obtained from the ethanolic extract were subjected to qualitative chemical testing and TLC. For the flavonol glycoside fraction, fraction 3 was developed in toluene:dioxane:acetic acid (90:25:5) and visualised with anisaldehyde–H₂SO₄.

The sample had an R_f of 0.83 (yellow spot), compared with 0.85 for quercetin. For the sterol fraction, fraction 7 was developed in petroleum ether:acetone (85:15) and visualised with Liebermann–Burchard reagent. The sample showed an R_f value of 0.47 with a brown spot, compared with an R_f value of 0.45 for β-sitosterol. R_f comparison provides preliminary chromatographic evidence of similarity but does not by itself establish compound identity; confirmation requires appropriate spectroscopic or chromatographic standards.

3.10 Histopathological Studie for Alloxaninduce diabetic and Ccl4 induced hepatoprotective model (Fig.1)

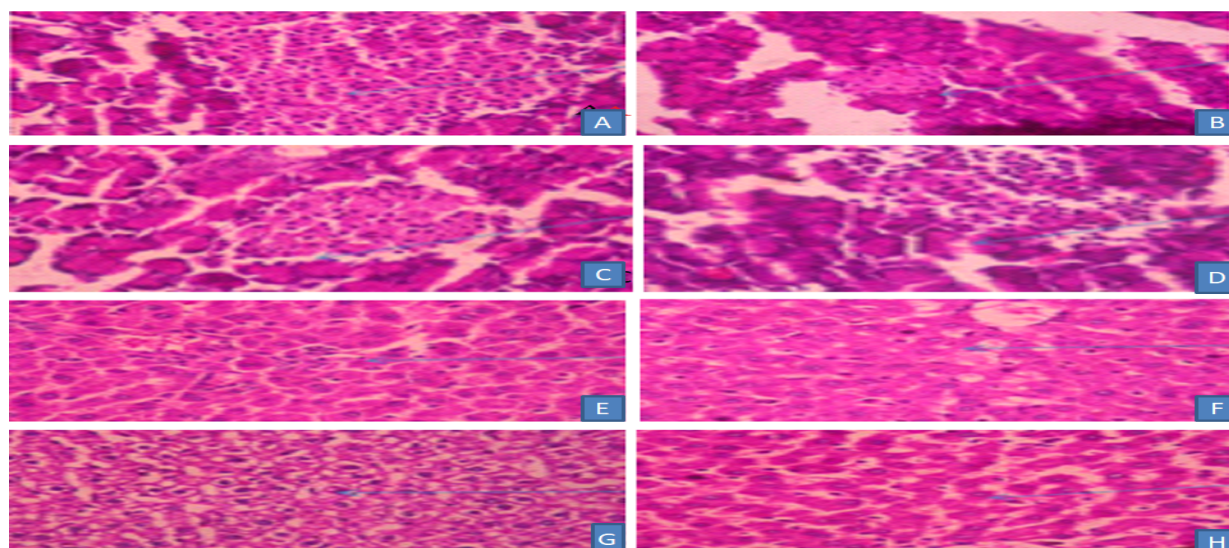


Figure 1. A - Normal control diabetic model, B - Deceased control in diabetic model, C- cell recovery by standard drug in diabetic model, D- cell recovery after extracted drug treatment in diabetic model, E- Normal control for hepatoprotective model , F- Disease control, G-Standard natural drug, H- cell recovery after treatment with extacted drug

Table 13. TLC profile of selected column fractions

Constituent class	Fraction	Solvent system	Visualising reagent	Sample Rf	Reference Rf
Flavonol glycosides	Fraction 3	Toluene:dioxane:acetic acid (90:25:5)	Anisaldehyde–H ₂ SO ₄	0.83 (yellow)	Quercetin: 0.85 (yellow)
Sterols	Fraction 7	Petroleum ether:acetone (85:15)	Liebermann–Burchard	0.47 (brown)	β-Sitosterol: 0.45 (brown)

3.10 Statistical Analysis

Results were reported as mean $\pm$ SEM where noted. Statistical analysis followed the methods in the original study. Details on sample size, software, statistical tests, post-hoc methods and significance thresholds were not provided and should be added from the thesis or laboratory records before submission of the manuscript. No additional statistical values have been introduced in this rewritten version.

4. Conclusion:

The proposed study is designed to provide a comprehensive scientific evaluation of bioactive phytochemicals obtained from *Oroxylum indicum*, *Schleichera oleosa*, and *Buchanania lanzan*. By combining pharmacognostical standardization, phytochemical investigation, advanced chromatographic and spectroscopic techniques, computational approaches, pharmacological studies, mechanistic validation, and toxicological assessment, the research aims to establish a clear link between the chemical constituents of these medicinal plants and their biological effects. The integration of network pharmacology, molecular docking, molecular dynamics, and ADMET prediction with experimental investigations will help identify promising phytochemicals and clarify their possible multitarget mechanisms of action. Subsequent in vitro

and in vivo studies will provide biological validation of the predicted activities, while molecular studies will help explain the pathways involved. Toxicological evaluation will further establish the safety profile of the selected extracts or compounds. Overall, the study is expected to generate reliable scientific evidence supporting the therapeutic potential of these traditionally important medicinal plants. The findings may contribute to the identification of promising lead molecules, development of quality-control markers, and future formulation of standardized plant-based therapeutic products. The integrated approach may therefore provide a useful foundation for translating traditional medicinal knowledge into evidence-based and scientifically validated phytopharmaceutical development.

References

1. Sun W, Shahrajabian MH. Therapeutic Potential of Phenolic Compounds in Medicinal Plants-Natural Health Products for Human Health. *Molecules*. 2023;28(4):1845. <https://doi.org/10.3390/molecules28041845>.
2. Baruah J, Lal M, Begum T, Dutta D. *Clerodendrum colebrookianum* (Walp.) A wonder plant and its ethno medicinal importance and pharmacological activities: A review. *Pharmacological Research – Natural*

- Products. 2024;4:100080. <https://doi.org/10.1016/j.prenap.2024.100080>.
3. Bruce SO, Onyegbule FA & Ezugwu CO. Pharmacognostic, physicochemical and phytochemical evaluation of the leaves of *Fadogia cienkowski Schweinf* (Rubiaceae). *Journal of Pharmacognosy and Phytotherapy*. 2019; 11(3): 52-60. doi.org/10.5897/JPP2019.0552.
4. Kibiti CM, Afolayan AJ. Preliminary Phytochemical Screening and Biological Activities of *Bulbine abyssinica* Used in the Folk Medicine in the Eastern Cape Province, South Africa. *Evid Based Complement Alternat Med*. 2015;2015:617607. <https://doi.org/10.1155/2015/617607>.
5. Chennaithody, M. P., & Shankar, R. K. (2025). Evaluation of Preliminary Phytochemical Screening, Estimation of Total Phenolic Content, and Antioxidant Potential of *Amoora rohituka* Roxb. *Pharmacognosy Research*. 2025;18(1): 184–190. <https://doi.org/10.5530/pres.20260042>
6. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. *LWT – Food Science and Technology*. 1995;28(1):25–30.
7. Blois MS. Antioxidant determinations by the use of a stable free radical. *Nature*. 1958;181:1199–1200.
8. Organisation for Economic Co-operation and Development (OECD). Test No. 423: Acute Oral Toxicity—Acute Toxic Class Method. OECD Guidelines for the Testing of Chemicals, Section 4. Paris: OECD Publishing; 2002. <https://doi.org/10.1787/9789264071001-en>.
9. Etuk EU. Animals models for studying diabetes mellitus. *Agriculture and Biology Journal of North America*. 2010;1(2):130–134.
10. Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia*. 2008;51:216–226.
11. Dong J, Guo H, Yang R, et al. Serum LDL- and HDL-cholesterol determined by ultracentrifugation and HPLC. *J Lipid Res*. 2011;52(2):383–388. <https://doi.org/10.1194/jlr.D008979>.
12. Reitman S, Frankel S. A colorimetric method for determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology*. 1957;28(1):56–63.
13. King EJ, Armstrong AR. A convenient method for determining serum and bile phosphatase activity. *Canadian Medical Association Journal*. 1934;31:376–381.
14. Jendrassik L, Grof P. Vereinfachte photometrische Methoden zur Bestimmung des Blutbilirubins. *Biochemische Zeitschrift*. 1938;297:81–89.
15. Gurina TS, Simms L. Histology, Staining. [Updated 2023 May 1]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2026 Jan-. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK557663/>
16. Kowalska T, Sajewicz M. Thin-Layer Chromatography (TLC) in the Screening of Botanicals-Its Versatile Potential and Selected Applications. *Molecules*. 2022;27(19):6607. <https://doi.org/10.3390/molecules27196607>.