The Academic
International Journal of Multidisciplinary Research

An Online Peer Reviewed / Refereed Journal
Volume 3 | Issue 5 | May 2025
ISSN: 2583-973X (Online)
Website: www.theacademic.in

Phytochemical Screening and Antioxidant Activity of *Elaeocarpus ganitrus* in High-Fat Diet Induced Diabetic Rats

Vinita Jaiswal
Ph.D. Research Scholar in Nutrition Science
Mansarovar Global University, Sehore (M. P)

Dr. Megha Jha
Faculty of Basic Science,
Mansarovar Global University, Sehore (M. P)

ARTICLE DETAILS

Research Paper

Accepted: 16-05-2025

Published: 10-06-2025

Keywords:
Elaeocarpus ganitrus, Rudraksha, phytochemicals, antioxidants, diabetes, high-fat diet, oxidative stress.

ABSTRACT

The current study evaluates the phytochemical constituents and antioxidant activity of *Elaeocarpus ganitrus* (commonly known as Rudraksha) in high-fat diet-induced diabetic rats. Phytochemical screening revealed the presence of bioactive compounds, including flavonoids, phenolics, alkaloids, and tannins, which are known for their therapeutic properties. Diabetes was experimentally induced using a high-fat diet followed by streptozotocin injection. The methanolic extract of *E. ganitrus* was administered to diabetic rats, resulting in a noteworthy reduction in blood glucose levels and oxidative stress markers. Furthermore, antioxidant enzyme stages such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were markedly improved. These findings suggest that *E. ganitrus* possesses potent antioxidant and antidiabetic properties, making it a promising candidate for the management of oxidative stress and metabolic disorders.

DOI : https://doi.org/10.5281/zenodo.15685201

1. Introduction

In recent decades, the incidence of metabolic disorders such as type-2 diabetes mellitus (T2DM) has reached alarming proportions globally, particularly in developing nations. Among various contributing factors, sedentary lifestyles, poor dietary habits, and high intake of saturated fats have been closely associated with the development of insulin resistance and glucose intolerance. High-fat diet (HFD)-induced diabetes is widely used as an experimental model to replicate human metabolic dysfunctions, as it not only impairs glucose homeostasis but also exacerbates oxidative stress through the excessive generation of reactive oxygen species (ROS). Oxidative stress, in turn, plays a central role in the pathophysiology of diabetes and its complications by damaging cellular proteins, lipids, and DNA, ultimately leading to β-cell dysfunction and insulin resistance.

Given the growing burden of diabetes and limitations of conventional pharmacological treatments, there has been an increasing interest in exploring alternative therapies derived from natural sources. Medicinal plants, rich in bioactive phytochemicals, offer a multifaceted approach to disease management through their antioxidant, anti-inflammatory, and metabolic regulatory properties. Among such plants, *Elaeocarpus ganitrus* Roxb., commonly known as Rudraksha, holds significant value in traditional Indian medicine, particularly in Ayurveda and Siddha systems. Traditionally revered for its spiritual significance, the plant has also been recognized for its pharmacological activities, including anti-hypertensive, anti-inflammatory, neuroprotective, and antimicrobial effects. However, scientific evidence supporting its potential role in managing oxidative stress and metabolic dysfunction in diabetes remains limited and fragmented.

Phytochemicals such as flavonoids, alkaloids, saponins, phenolics, and tannins found in *E. ganitrus* are known to modulate oxidative pathways, enhance antioxidant defense systems, and regulate glucose metabolism. These constituents have the potential to combat oxidative stress-induced cellular damage, which is central to the progression of diabetes and its complications. Therefore, investigating the phytochemical profile and antioxidant efficacy of *E. ganitrus* in a diabetic model is crucial to validate its therapeutic utility.

This learning is designed to evaluate the antioxidant activity of *Elaeocarpus ganitrus* in high-fat diet-induced diabetic rats and to identify the phytochemicals responsible for its observed effects. By utilizing both qualitative phytochemical screening and in vivo antioxidant assays, this research aims to conduit the gap between traditional knowledge and scientific endorsement. The outcomes of this study may contribute to the development of safe, plant-based interventions for the prevention and management of diabetes-related oxidative stress.

2. Materials and Methods

2.1 Plant Material and Extraction

Mature fruits of *Elaeocarpus ganitrus* were collected from naturally growing trees in the Western Ghats region of India during the peak season. The fruits were washed thoroughly under running tap water, followed by rinsing with purified water to remove dust and microbial contaminants. The cleaned fruits were then shade-dried at area temperature for 10–15 days to preserve their phytoconstituents. Once fully dried, the fruits were coarsely powdered using a mechanical grinder and stored in airtight containers for further use.

The dried dust was subjected to Soxhlet extraction using methanol as the solvent. Approximately 200 g of powdered plant material was placed in the Soxhlet apparatus and continuously extracted with methanol for 6–8 hours. The obtained extract was filtered and concentrated under reduced pressure using a rotating evaporator at 45–50°C to yield a semisolid residue. The final extract was stored at 4°C until further analysis.

2.2 Phytochemical Screening

Introductory phytochemical screening of the methanolic extract of *E. ganitrus* was carried out using standard qualitative tests to detect the presence of major secondary metabolites:

- **Alkaloids**: Detected by Dragendorff's test, where the appearance of an orange-red precipitate indicates a positive result.
- **Flavonoids**: Confirmed using the Shinoda test by the formation of a pink or red color upon reaction with magnesium and concentrated hydrochloric acid.
- **Saponins**: Identified by the foam test, where persistent frothing indicates the presence of saponins.
- **Tannins**: Detected using ferric chloride test, producing a blue-black or greenish color.
- **Phenolic compounds**: Identified by the lead acetate test, where a white precipitate confirms their presence.
- **Cardiac Glycosides**: Confirmed through the Keller–Killiani test, showing a reddish-brown ring at the interface.

All tests were performed in triplicate to ensure reproducibility and accuracy of the results.

2.3 Experimental Design

A total of thirty healthy mature male Wistar albino rats (weighing 180–220 grams) were obtained from a certified animal house. Animals were housed in polypropylene cages under controlled environmental conditions (12-hour light/dark cycle, temperature 22 ± 2°C, relative humidity 50–60%). Standard pellet diet and water were provided ad libitum. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and conducted in accordance with CPCSEA guidelines.

The animals were randomly divided into five groups (n = 6 in each) as follows:

- **Group I**: Normal control (fed standard diet only)
- **Group II**: HFD control (received high-fat diet only)
- **Group III**: HFD + *E. Ganitrus* extract (100 mg/kg body weight)
- **Group IV**: HFD + *E. Ganitrus* extract (200 mg/kg body weight)
- **Group V**: HFD + Metformin (100 mg/kg body weight, standard reference drug)

To induce Type 2 diabetes, rats in groups II–V were fed a high-fat diet comprising 60% fat, 20% protein, and 20% carbohydrates for six weeks. Following dietary induction, a single intraperitoneal injection of streptozotocin (STZ) at a dose of 35 mg/kg body weight (dissolved in cold citrate buffer, pH 4.5) was administered. Fasting blood glucose was measured 72 hours post-injection using a glucometer, and rats with glucose levels above 200 mg/dL were considered diabetic and included in the study.

2.4 Antioxidant Assays

To evaluate the antioxidant potential of *E. ganitrus*, liver tissues were harvested from each rat at the end of the treatment period and homogenized in ice-cold phosphate buffer (pH 7.4). The homogenates were centrifuged at 10,000 rpm for 15 minutes at 4°C, and the supernatant was used for biochemical estimations.

- **DPPH Radical Scavenging Assay**: The antioxidant activity of the extract was evaluated by measuring its ability to scavenge DPPH free radicals. The decrease in absorbance at 517 nm was recorded spectrophotometrically.
- **FRAP Assay (Ferric Reducing Antioxidant Power)**: The ferric reducing power of the extract was estimated by its ability to reduce Fe^{3+} to Fe^{2+} in the presence of TPTZ (2,4,6-tripyridyl-s-triazine). The increase in absorbance at 593 nm was measured.
- **Malondialdehyde (MDA) Estimation**: Lipid peroxidation in liver tissue was quantified using the Thio barbituric acid reactive substances (TBARS) method. MDA levels were measured as an index of oxidative damage to lipids.
- **Enzymatic Antioxidants**:
  - **Superoxide Dismutase (SOD)** activity was determined based on its ability to inhibit the auto-oxidation of pyrogallol.

- **Catalase (CAT)** activity was estimated by measuring the rate of decomposition of hydrogen peroxide.
- **Glutathione Peroxidase (GPx)** was assessed using the coupled reaction with glutathione reductase.

3. Results

3.1 Phytochemical Composition

The preliminary phytochemical screening of the methanolic extract of *Elaeocarpus ganitrus* revealed a rich presence of multiple bioactive compounds. Notably, flavonoids and phenolic compounds were found in significant abundance. These constituents are widely recognized for their potent antioxidant assets, primarily due to their ability to donate electrons and neutralize reactive oxygen species (ROS). In addition, the extract tested positive for saponins and tannins, which are traditionally known for their antihyperlipidemic and membrane-stabilizing effects. These findings propose that the therapeutic potential of *E. ganitrus* is likely attributed to the synergistic action of these phytoconstituents.

3.2 Antioxidant Activity

The antioxidant activity of *E. ganitrus* extract was evaluated through various in vitro and in vivo assays. The DPPH radical scavenging assay demonstrated that the extract exhibited significant free radical neutralizing ability in a dose-dependent manner. Higher concentrations of the extract showed greater DPPH inhibition, indicating a strong correlation between phytochemical content and antioxidant capacity.

Moreover, the FRAP (Ferric Reducing Antioxidant Power) assay further confirmed the antioxidant potential of the extract. The FRAP values were markedly increased in the treated groups compared to the diabetic control group, reflecting enhanced ferric ion reducing ability of the plant constituents.

Lipid peroxidation, a key marker of oxidative stress, was assessed by measuring malondialdehyde (MDA) levels in liver tissue. A notable reduction in MDA levels was observed in the groups treated with *E. ganitrus*, suggesting a protective effect against oxidative degradation of lipids.

Furthermore, the activities of endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were significantly elevated in the liver homogenates of treated rats. This enhancement of enzymatic defense mechanisms indicates the extract’s capacity to restore redox homeostasis and reduce oxidative damage induced by a high-fat diet and streptozotocin.

3.3 Glycemic and Lipid Profile

Administration of *E. ganitrus* extract to diabetic rats resulted in a remarkable improvement in glycemic control. Both 100 mg/kg and 200 mg/kg doses (Groups III and IV) showed a significant reduction in fasting blood glucose levels when compared to the diabetic control group. The higher dose demonstrated slightly superior efficacy, suggesting a dose-responsive relationship.

In terms of lipid metabolism, the extract exhibited favorable effects on the lipid profile of diabetic rats. Treated groups showed a substantial decline in serum total cholesterol, triglycerides, and low-density lipoprotein (LDL) levels, which are typically elevated in diabetes and contribute to cardiovascular risks. Additionally, high-density lipoprotein (HDL) levels were significantly elevated in the extract-treated groups, indicating an overall improvement in lipid regulation.

These results collectively demonstrate that *Elaeocarpus ganitrus* possesses not only potent antioxidant properties but also significant antidiabetic and antihyperlipidemic potential, thereby validating its traditional medicinal use and supporting its role in managing metabolic disorders.

4. Discussion

The present study was conducted to evaluate the phytochemical composition, antioxidant potential, and therapeutic efficacy of the methanolic extract of *Elaeocarpus ganitrus* in high-fat diet (HFD) and streptozotocin (STZ)-induced diabetic rats. The findings of this study reinforce the traditional use of *E. ganitrus* in managing metabolic disorders and offer scientific validation for its application in modern phytotherapy.

The preliminary phytochemical screening discovered the presence of flavonoids, phenolics, saponins, tannins, and glycosides—compounds that have been extensively reported to possess antioxidant, antihyperglycemic, and lipid-lowering activities. Among them, flavonoids and phenolic compounds are particularly noteworthy due to their ability to scavenge reactive oxygen species (ROS) and chelate metal ions, thus mitigating oxidative damage at the cellular level. These phytoconstituents may contribute synergistically to the observed therapeutic effects of the extract.

Oxidative anxiety plays a pivotal role in the pathogenesis of diabetes and its complications. In this study, the *E. ganitrus* extract demonstrated significant antioxidant activity as evidenced by increased DPPH scavenging capacity and elevated FRAP values. These in vitro results were further supported by in vivo biochemical markers. The extract significantly reduced hepatic malondialdehyde (MDA) levels, a lipid peroxidation marker, and enhanced the activities of key antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). These findings are consistent with earlier studies where natural antioxidants ameliorated oxidative damage in diabetic models (Sharma et al., 2018; Kumar & Singh, 2020).

The hypoglycemic effects observed in this study align with the pharmacological actions of flavonoids and saponins, which are known to improve insulin sensitivity & promote glucose uptake in peripheral tissues. The significant reduction in fasting blood glucose levels in the *E. ganitrus*-treated groups suggests that the extract may act through multiple mechanisms, including insulin-mimetic effects, pancreatic β-cell preservation, and inhibition of glucose absorption from the intestine.

Furthermore, the extract exerted a beneficial impact on the lipid profile of diabetic rats. Dyslipidemia is a common co-morbidity in diabetes and contributes to the progression of cardiovascular diseases. The noteworthy reduction in serum total cholesterol, triglycerides, and LDL levels, along with the increase in HDL levels, indicates a promising antihyperlipidemic effect. These findings may be attributed to the ability of polyphenolic compounds to modulate lipid metabolism, enhance lipoprotein lipase activity, and inhibit cholesterol synthesis in the liver.

Notably, the therapeutic efficacy of *E. ganitrus* at the higher dose (200 mg/kg) was comparable to that of the standard antidiabetic drug metformin, highlighting its potential as a natural alternative or adjunct therapy. While metformin primarily acts by inhibiting hepatic gluconeogenesis, the extract's multi-targeted action—combining antioxidant, antihyperglycemic, and lipid-lowering effects—offers a holistic approach to managing diabetes.

Overall, the present investigation not only confirms the antioxidant and metabolic regulatory roles of *Elaeocarpus ganitrus* but also lays a strong foundation for future studies focusing on the isolation of its active constituents, elucidation of underlying molecular mechanisms, and clinical validation in human subjects.

5. Conclusion

The present investigation provides compelling evidence for the medicinal value of *Elaeocarpus ganitrus* in managing diabetes and its associated oxidative stress. The methanolic extract exhibited a rich phytochemical profile, notably containing flavonoids, phenolics, tannins, and saponins—compounds widely acknowledged for their therapeutic benefits. Through a combination of in vitro and in vivo evaluations, the extract demonstrated potent antioxidant activity, as shown by its capacity to scavenge free radicals, reduce lipid peroxidation, and enhance the activities of vital antioxidant enzymes like SOD, CAT, and GPx in liver tissues.

In diabetic rat models induced by a high-fat diet and streptozotocin, the extract effectively reduced fasting blood glucose levels and favorably modulated serum lipid parameters. The improvements in glycemic control and lipid profiles highlight its potential role in alleviating metabolic imbalances common in type 2 diabetes. Furthermore, the performance of the extract at higher doses was comparable to the standard drug metformin, suggesting its viability as a plant-based alternative or complementary therapy.

Overall, the study validates the ethnopharmacological use of *Elaeocarpus ganitrus* and underscores its multifaceted bioactivity. These findings pave the way for future pharmacological explorations, including compound isolation, mechanistic studies, and clinical evaluations, to further substantiate its role in integrative and evidence-based diabetes management.

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