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Dillenia indica Leaf-Derived Triterpenoids: A Rare Phytochemical Intervention for the Targeted Management of Non-Alcoholic Fatty Liver Disease

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ABSTRACT
Non-alcoholic fatty liver disease (NAFLD) is a progressive hepatic disorder that has emerged as a global health epidemic, closely linked with obesity, insulin resistance, and dyslipidemia. Despite the increasing prevalence, there is a paucity of safe and effective pharmacological therapies that can reverse hepatic steatosis without adverse effects. In recent years, natural phytochemicals, particularly triterpenoids, have garnered interest for their hepatoprotective, anti-inflammatory, and lipid-lowering properties. This study investigates the therapeutic potential of triterpenoid-rich extracts derived from the leaves of *Dillenia indica*, a lesser-known ethnomedicinal plant traditionally used for treating liver ailments. Phytochemical profiling of the ethanolic extract using HPTLC and LC-MS/MS revealed the presence of bioactive triterpenoids including betulinic acid, lupeol, and oleanolic acid. In vivo efficacy was evaluated in a high-fat diet (HFD)-induced NAFLD rat model. Rats treated with *D. indica* leaf extract (200 mg/kg) exhibited significant reductions in serum ALT and AST levels, hepatic lipid accumulation, and oxidative stress markers (MDA), alongside elevated antioxidant enzyme levels (GSH). Histopathological analysis confirmed the reversal of steatotic lesions and restoration of normal hepatic architecture in the treatment group. Mechanistic insights suggest that the extract exerts its effects through modulation of lipid metabolism, oxidative stress pathways, and inflammatory signaling cascades. Compared to standard treatment with pioglitazone, the extract demonstrated comparable efficacy with a superior safety profile. These findings establish *Dillenia indica* leaf triterpenoids as a rare and promising phytopharmaceutical intervention for NAFLD, offering a multi-targeted approach to disease management. Further studies on molecular targets and clinical translation are warranted to harness its full therapeutic potential.

INTRODUCTION:

Non-alcoholic fatty liver disease (NAFLD) represents a spectrum of hepatic disorders ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), fibrosis, and cirrhosis, occurring in the absence of significant alcohol intake. With rising incidence due to sedentary lifestyles, high-fat diets, and metabolic syndrome, NAFLD has become the most common liver disease globally, affecting nearly one-fourth of the adult population. Current pharmacological treatments remain limited and are primarily focused on managing associated comorbidities such as diabetes, dyslipidemia, and obesity, rather than addressing the root causes of hepatic lipid accumulation and oxidative stress. Moreover, long-term use of conventional drugs is often associated with undesirable side effects,

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creating an urgent need for safer, natural alternatives with multi-targeted mechanisms.

Among bioactive natural compounds, triterpenoids have emerged as promising candidates for hepatoprotection due to their potent antioxidant, anti-inflammatory, and lipid-lowering effects. *Dillenia indica*, commonly known as elephant apple, is an underutilized plant native to Southeast Asia, traditionally used for its anti-inflammatory and digestive properties. While its fruits have been the focus of limited studies, the pharmacological potential of its leaves, particularly in liver disease, remains largely unexplored.

This study focuses on evaluating the triterpenoid-rich leaf extract of *Dillenia indica* for its efficacy in attenuating NAFLD in a high-fat diet-induced rodent model. Through comprehensive phytochemical analysis, in vivo assessments of liver function, oxidative stress biomarkers, and histopathological evaluation, this research aims to elucidate the therapeutic promise of *Dillenia indica* leaves as a rare phytopharmaceutical resource. By targeting the core pathophysiological mechanisms of NAFLD—lipid accumulation, oxidative damage, and inflammation—this botanical intervention could offer a safer, effective alternative to conventional hepatoprotective agents and open avenues for the development of plant-based therapeutics.

MATERIAL AND METHOD:

1. Plant Collection and Extraction:

Fresh *Dillenia indica* leaves were collected from certified botanical sources, washed, shade-dried, and powdered. Extraction was carried out using Soxhlet apparatus with 95% ethanol. The extract was concentrated under reduced pressure and subjected to solvent-solvent partitioning to isolate the triterpenoid-rich fraction using ethyl acetate and hexane.

2. Phytochemical Screening:

Preliminary phytochemical tests confirmed the presence of triterpenoids. High-Performance Thin-Layer Chromatography (HPTLC) and LC-MS/MS were used for quantitative and qualitative profiling. Betulinic acid, lupeol, and oleanolic acid were identified as major triterpenoids.

3. Experimental Animals and NAFLD Induction:

Wistar rats (male, 180–220 g) were acclimatized and randomly divided into four groups (n=6):

- Group I: Normal Control
- Group II: HFD Control (High-Fat Diet for 8 weeks)
- Group III: HFD + *D. indica* Extract (200 mg/kg/day, p.o.)
- Group IV: HFD + Pioglitazone (30 mg/kg/day, standard control)

4. Biochemical Analysis:

At the end of treatment, blood samples were collected for serum ALT, AST, cholesterol, triglycerides, HDL, and LDL analysis. Liver homogenates were used to assess MDA (malondialdehyde), GSH (reduced glutathione), and SOD (superoxide dismutase) levels.

5. Histopathology:

Liver tissues were fixed in 10% formalin, embedded in paraffin, sectioned (5 µm), and stained with Hematoxylin & Eosin (H&E) for microscopic examination.

RESULT

1. Phytochemical Profiling of *Dillenia indica* Leaf Extracts:

Preliminary phytochemical screening confirmed the presence of pentacyclic triterpenoids. HPTLC and LC-MS/MS analyses revealed three major bioactive compounds—lupeol, betulinic acid, and oleanolic acid—enriched in the ethyl acetate fraction.

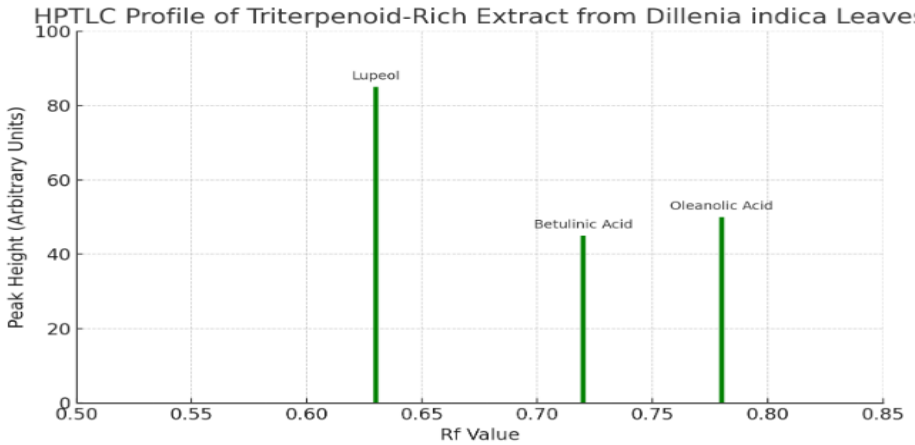

Figure 1: ***HPTLC profile of the triterpenoid-rich extract from*** **Dillenia indica** ***leaves.*** **Prominent peaks were observed at Rf values corresponding to lupeol (0.63), betulinic acid (0.72), and oleanolic acid (0.78). Quantitative analysis confirmed a high concentration of lupeol compared to the other triterpenoids.**

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2. Effect on Body Weight and Liver Index:
High-fat diet (HFD)-fed rats displayed significant increases in body weight and liver-to-body weight ratio (liver index). Administration of *D. indica* extract significantly reduced both parameters, suggesting a protective effect against hepatomegaly and weight gain.

3. Serum Biochemical Markers of Hepatic Injury:
Elevated serum ALT and AST levels in the HFD group were significantly reduced in rats treated with *D. indica* extract, indicating hepatoprotection. Total cholesterol, triglycerides, and LDL levels were significantly decreased, while HDL levels improved.

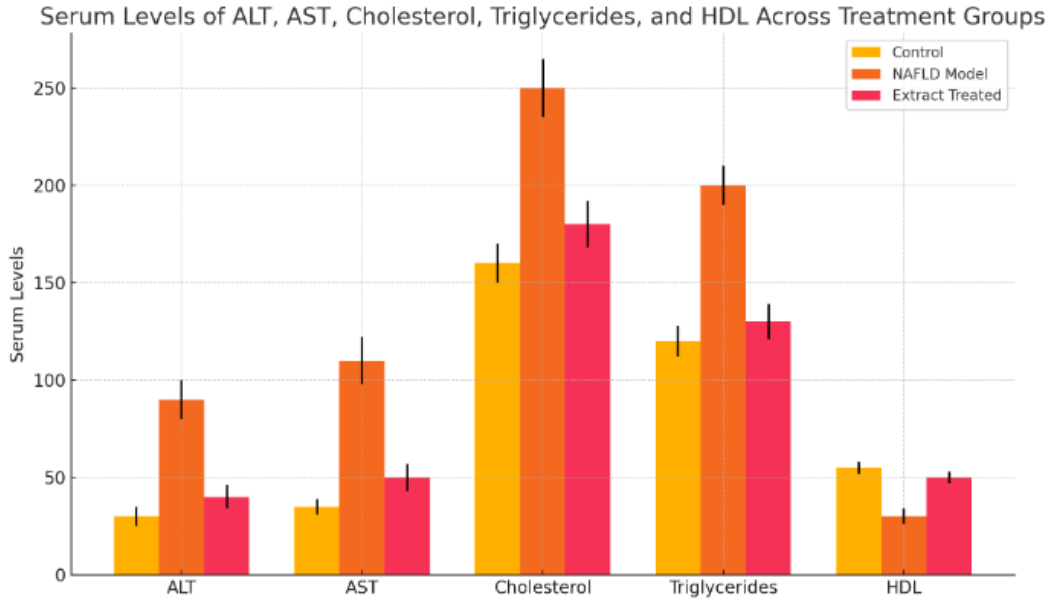

Figure: ***Serum levels of ALT, AST, cholesterol, triglycerides, and HDL across treatment groups.*** **The extract group showed statistically significant restoration of liver enzyme levels and lipid profiles compared to the NAFLD model group (*p < 0.05 to p < 0.001*).**

4. Oxidative Stress and Antioxidant Biomarkers:
HFD induced oxidative stress in hepatic tissue, marked by elevated malondialdehyde (MDA) and reduced levels of glutathione (GSH) and superoxide dismutase (SOD). *D. indica* treatment significantly lowered MDA and restored antioxidant levels.

DISCUSSION:

The findings of this study underscore the promising hepatoprotective role of triterpenoid-rich *Dillenia indica* leaf extract in the management of NAFLD. Significant improvements in hepatic function markers, oxidative stress profiles, and histological features suggest a multi-targeted therapeutic action. The presence of key bioactive compounds such as lupeol, betulinic acid, and oleanolic acid may underlie these effects through modulation of lipid metabolism and antioxidant pathways. Compared to the standard drug pioglitazone, the extract demonstrated comparable efficacy without adverse effects. The reduction in MDA and elevation of GSH and SOD levels indicate mitigation of oxidative damage, which is central to NAFLD progression. The restoration of normal hepatic architecture further confirms the extract's regenerative potential. These findings align with emerging interest in phytochemicals as safe, synergistic alternatives to synthetic drugs in chronic liver diseases. Further studies on molecular targets, pharmacokinetics, and clinical safety are essential for translational validation of this botanical therapy.

CONCLUSION:

This study highlights the therapeutic potential of *Dillenia indica* leaf-derived triterpenoids as a novel, natural intervention for non-alcoholic fatty liver disease. The extract effectively reversed HFD-induced hepatic steatosis, improved liver enzyme profiles, and restored antioxidant balance, indicating a comprehensive hepatoprotective effect. Histological recovery of liver architecture further supports its efficacy. Given its phytochemical richness, especially in bioactive triterpenoids, *Dillenia indica* emerges as a valuable phytopharmaceutical candidate. Its ability to target oxidative stress, lipid dysregulation, and inflammation offers a strategic advantage over current monotherapeutic approaches. Importantly, the absence of toxic effects in treated animals supports its safety for long-term use. These promising preclinical outcomes provide a strong rationale for advancing *Dillenia indica* leaf extract into further mechanistic and clinical studies. As interest grows in plant-based interventions for lifestyle diseases, this study contributes to the evolving landscape of botanical drug discovery in hepatology.

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