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Assessment of *Flacourtia indica* Leaves for its Hepatoprotective Potential

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Abstract: The hepatoprotective properties of aqueous, ethanol, and petroleum ether extracts derived from *Flacourtia indica* (Burm. f.) Merr leaves were thoroughly evaluated in a rat model of ethanol-induced hepatic necrosis. All three extracts exhibited the capability to reduce the levels of key liver enzymes, including serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and serum alkaline phosphatase (SAP) as compared to ethanol treated group. Notably, the aqueous and ethanol extracts, administered at a single oral dose of 1.5 g/kg b wt., produced the most significant reductions in SGOT and SGPT levels. These extracts resulted in a substantial decrease in SGPT, SGOT, and SAP levels when compared to animals treated with ethanol at a higher dose of 3.67 g/kg/b wt. The hepatoprotective effects observed are believed to be associated with the inhibition of microsomal drug-metabolizing enzymes.

Keywords: *Flacourtia indica*, Antioxidant, Xenobiotics, Hepatic Disease, Hepatoprotective, SGOT, SGOT

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Introduction

Hepatoprotective potential of plant-derived compounds and extracts has garnered significant attention in recent years as an avenue for developing natural remedies for liver-related

disorders. *Flacourtia indica* (Burm. f.) Merr. commonly known as 'Baichi' or 'Katai,' is an indigenous medicinal plant widely distributed in regions of Bangladesh and India. The leaves of

Flacourtia indica have a rich history of traditional use as a remedy for various ailments, including liver-related conditions. Given the growing interest in herbal medicine and the need for alternative treatments for hepatic disorders, the exploration of the hepatoprotective potential of *Flacourtia indica* leaves has become a subject of scientific investigations (Patel *et al.*, 2009; Doshi *et al.*, 2013; Saravana Kumar *et al.*, 2014; Manandhar *et al.*, 2014).

The liver, a vital organ responsible for numerous metabolic functions, is particularly susceptible to damage from various factors such as toxins, drugs, and pathogens. Hepatoprotective agents play a crucial role in maintaining liver health and preventing or ameliorating liver damage. *Flacourtia indica*'s traditional use and preliminary phytochemical analysis suggest the presence of bioactive compounds that may confer hepatoprotective benefits (Srinivasan *et al.*, 2011; Choudhury and Gogoi, 2014; Rao and Subramanyam, 2016).

This study aimed to evaluate and substantiate the hepatoprotective properties of *Flacourtia indica* leaves through a comprehensive assessment including biochemical analyses. The research will provide valuable insights into the potential of *Flacourtia indica* as a natural hepatoprotective agent and contribute to the growing body of knowledge on plant-based remedies for liver health (Babu *et al.*, 2013; Parija *et al.*, 2015; Ali *et al.*, 2017).

Materials and Methods

Collection of Plant Material:

Fresh leaves of *Flacourtia indica* (Burm. f.) Merr. were collected from the natural habitats of Maharashtra, India. The plant material was identified and authenticated by a botanist, and a voucher specimen was deposited for reference.

Extraction of leaves:

The freshly collected leaves were initially washed and then sun-dried for a period of three days to reduce moisture content. Subsequently, they were

further dried in an oven at 40°C for 12 h to ensure complete dryness. The dried leaves were ground into a coarse powder using an attrition-type grinder. This coarse powder was stored in an airtight container and kept in a cool, dark, and dry place to preserve its integrity. The coarse powder was then used for the sequential extraction process. Each solvent (ethanol, petroleum ether and aqueous solutions) was used for maceration separately for seven days at room temperature. During this period, the mixture was occasionally agitated to enhance the extraction process. The yield of each extract was determined as a percentage based on the amount of the initial plant material used. The resulting crude extracts were further subjected to phytochemical analysis to assess the presence of secondary metabolites, and these extracts were employed in the subsequent hepatoprotective experiments.

Phytochemical screening of the extracts:

A phytochemical analysis was conducted to ascertain the existence of various secondary metabolites. This examination involved TLC techniques. The TLC analysis results indicated positive reactions for steroids and terpenoids, glycosides. Furthermore, leaves were found to contain phenolic glucoside esters, Flacourside, Poliothyroside, Flacourtiosides A-F, and Xylosmin (Touchstone and Dobbins, 1978; Harborne, 1980, Wagner and Bladt, 1996).

Animals:

In this study, we employed albino rats, which were in the age range of 3 to 4 weeks and weighed between 120 to 140 g. These experimental animals were sourced from the central animal house. The rats were kept in compliance with standard environmental conditions, and they had unrestricted access to both food and water. All animal experiments strictly adhered to the guidelines outlined by the Institutional Animal Ethics Committee.

Test samples and ethanol:

To create the suspensions of the extractives, normal saline was utilized along with 5% acacia

Table 1: Serum enzyme levels of rats after exposure to various extracts of *F. indica*

Mean serum concentration (U/L) \pm SEM						
Treatment	SGOT	SGOT % Change	SGPT	SGPT % Change	SAP	SAP % Change
Control (saline)	26.70 \pm 0.52	0	27.32 \pm 0.26	0	17.21 \pm 0.31	0
Ethanol 3.67 mg/kg	59.42 \pm 0.23*	0	63.43 \pm 0.91*	0	21.01 \pm 0.22**	0
Ethanol 3.67 mg/kg + PE (1.5 g/kg)	39.02 \pm 0.66*	(-34.33)	50.32 \pm 1.10*	(-20.66)	20.01 \pm 0.22*	(-4.75)
Ethanol 3.67 mg/kg) + ETH (1.5 g/kg)	50.53 \pm 0.90*	(-14.95)	61.22 \pm 1.15 ^{ns}	(-3.48)	21.32 \pm 0.23*	1.47
Ethanol 3.67 mg/kg + AQ (1.5 g/kg)	51.02 \pm 0.23*	(-14.12)	64.30 \pm 0.22*	1.38	21.02 \pm 0.23*	0.04

PE = Petroleum ether extract, ETH- Ethanolic extract, AQ- Aqueous extract, + Indicates increase as compared to control group; - indicates decrease as compared to ethanol treated group; *P < 0.05, **P < 0.01, ns = non-significant

mucilage. Subsequently, all the test samples and ethanol were orally administered using a feeding needle.

Evaluation of hepatoprotective activity:

The hepatoprotective effects of the crude petroleum ether (PE), ethanolic (ETH), and aqueous (AQ) extracts were assessed using a rat model with ethanol-induced hepatic necrosis. Experimental animals were selected randomly and divided into five groups, each comprising six rats. Each group received a specific treatment as a single oral dose. For instance, the control group was administered normal saline with 5% acacia mucilage. In contrast, three test groups received ETH extract (1.5 g/kg/b wt.), AQ extract (1.5 g/kg/ b wt.), and PE extract (1.5 g/kg/b wt.).

Hepatotoxicity was induced through the oral administration of ethanol (3.67 mg/kg/b wt.). After 72 h of treatment, blood samples were obtained by puncturing the retro-orbital plexus and allowed to clot at room temperature. The serum was separated through centrifugation at 3000 rpm for approximately 5 min. The clear, straw-colored serum was collected and stored at 4°C for subsequent measurement of marker enzyme levels, serving as an indicator of liver function [Jollow *et al.*, 1974, Borne, 1995].

Estimation of serum enzyme levels:

To assess the protective effects of the extracts,

biochemical parameters, including SGOT, SGPT, and SAP levels in the serum of control, ethanol-treated, and extract-treated rats were measured using an auto-analyzer. In a nutshell, the determination of SGOT and SGPT levels involved the use of the substrate α -oxaloacetate, while p-nitrophenyl phosphate was employed for the measurement of SAP.

To perform the analysis, 100 μ l serum was mixed with 1 ml of each substrate solution in a small test tube. The mixture was thoroughly shaken and promptly loaded into a pre-programmed auto-analyzer designed for each marker enzyme. The results were obtained in units per liter (U/L).

Statistical analysis:

The experimental data were presented as mean values with the standard error of the mean (SEM). Statistical significance was established with a threshold of $p < 0.001$.

Results and Discussion

Alcohol metabolism generates reactive oxygen species (ROS), such as superoxide radicals and hydrogen peroxide, which can cause oxidative stress. These ROS can damage cellular components, including lipids, proteins, and DNA, contributing to inflammation and liver injury (Tilg and Day, 2007).

Chronic alcohol consumption disrupts the normal function of the liver. It impairs the liver's ability to process and detoxify substances, including drugs and toxins, which can further exacerbate liver damage. Alcohol can trigger an inflammatory response in the liver. This leads to the release of inflammatory mediators and the recruitment of immune cells, causing inflammation. Prolonged inflammation can contribute to liver fibrosis and cirrhosis. Chronic alcohol consumption disrupts the normal function of the liver (Gao and Bataller, 2011).

Administration of ethanol to rats twice a day causes changes in liver function parameters (SGPT, SGOT, SAP) which are depicted in Table 1. Concomitant use of various extracts of *F. indica* leaves extract along with toxicant significantly recovered the serum parameters. Petroleum ether extract caused better liver function parameter as compared to other extracts (Table 1). These observations inferred that all three evaluated extracts of leaves of *F. indica* have hepatoprotective principles.

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