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Phytochemical Analysis and Hepatoprotective Study of Leaves Extract of *Luffa Acutangula*

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Abstract: Liver health is crucial for various bodily functions, including nutrient metabolism, detoxification, and storage of vitamins and minerals. Liver diseases, commonly resulting from poor dietary habits, alcohol abuse, and drug misuse, can lead to severe conditions such as fibrosis, cirrhosis, and liver cancer. Oxidative stress and hepatotoxicity are key factors contributing to liver damage, prompting interest in natural alternatives for liver protection. Medicinal plants such as fenugreek, turmeric, onion, neem, Punarnava, and tulsi have demonstrated hepatoprotective properties, but their bioavailability and safety require further investigation. *Luffa Acutangula* (L.) Roxb, or ridge gourd, is a plant with diverse pharmacological properties, including antioxidant, anti-inflammatory, antimicrobial, and hepatoprotective effects. While traditional use supports its liver-protective potential, scientific evidence remains limited. This study investigates the hepatoprotective activity of *Luffa Acutangula* extract in an animal model, focusing on its ability to mitigate liver damage induced by hepatotoxic agents. Biochemical markers, such as liver enzyme levels, will be assessed to evaluate the effectiveness of the extract in preserving liver function, offering insight into its therapeutic potential for managing liver disorders.

Keywords: Hepatoprotective, Hepatotoxicity, *Luffa Acutangula*r, Wistar rats

1. Introduction

The liver, the largest solid organ and gland in the body, is essential for metabolizing nutrients, detoxifying harmful substances, and storing vitamins and minerals. It processes materials from the digestive system and ensures their safe distribution to the bloodstream, with its failure leading to life-threatening consequences (1). Key functions of the liver include bile production for fat digestion, filtering contaminants like bacteria and alcohol, and detoxifying harmful substances such as drugs and heavy metals. It also synthesizes critical proteins, such as enzymes and clotting factors, while storing glycogen to provide energy during low blood sugar. Liver diseases, prevalent in developing regions due to poor dietary habits, alcohol abuse, and drug misuse, are categorised into acute and chronic types, with chronic conditions often progressing to fibrosis, cirrhosis, or liver cancer. Oxidative stress, caused by reactive oxygen species (ROS), is a common driver of liver damage, leading to inflammation, cellular injury, and impaired function (2). Hepatotoxicity, or liver injury from drugs or chemicals, poses a significant global health burden, with synthetic drugs often associated with adverse effects. This has increased interest in medicinal plants as safer alternatives due to their efficacy and lower toxicity. Plants like fenugreek, onion, neem, turmeric, Punarnava, and tulsi have demonstrated hepatoprotective properties (3). Fenugreek and turmeric are known for their antioxidant

and detoxifying effects, while onion and neem help repair oxidative damage. Punarnava and tulsi provide anti-inflammatory and liver-supportive benefits. These herbal remedies promise to manage liver disorders, but their bioavailability, formulations, and safety profiles require further scientific validation to maximise therapeutic potential. By integrating medicinal plants with evidence-based approaches, liver health can be effectively maintained, offering a sustainable solution to liver diseases and reducing dependency on synthetic drugs (4).

Luffa Acutangula (L.) Roxb, commonly known as ridge gourd, is recognized for its diverse pharmacological properties, including antioxidant, antimicrobial, anticancer, hepatoprotective, antidiabetic, anti-inflammatory, and analgesic activities. The plant's various parts fruits, roots, leaves, flowers, and seeds possess significant medicinal benefits, with several extracts showing promising therapeutic effects. For instance, TiO2 nanoparticles synthesized from its leaf extract demonstrated strong antimicrobial properties against both bacterial and fungal strains (5). Additionally, antioxidant and anticancer effects were observed in animal models, where ethanolic and aqueous extracts significantly reduced tumour volumes. Methanolic extracts were particularly effective in managing diabetes and lipid imbalance, outperforming aqueous extracts in these areas (6). Despite its traditional use in medicine, scientific validation of *Luffa Acutangula*'s hepatoprotective properties remains limited. It has shown promise in protecting the liver from damage induced by toxins, drugs, and alcohol through mechanisms like free radical scavenging and immune modulation (7). This study aims to evaluate the in-vivo hepatoprotective activity of *Luffa Acutangula* extract using an animal model, focusing on its potential to mitigate liver damage caused by hepatotoxic agents. Biochemical parameters, including liver enzyme levels, will be measured to assess the extract's effectiveness in preserving liver function (8).

2. Materials and Methods

Materials

The chemicals used in this study were sourced from various suppliers in Mumbai and New Delhi. These include potassium mercuric iodide, iodine, potassium iodide, potassium bismuth iodide, picric acid, sodium nitropruside, sodium hydroxide, pyridine, ferric chloride, gelatin, lead acetate, nitric acid, copper acetate, aluminium chloride, methanol, ethanol, chloroform, folin-ciocalteu reagent, Fehling’s solution, quercetin, and gallic acid. Key suppliers include Thomas Baker, Loba ChemiePvt. Ltd., S.D. Fine Chem. Ltd., Qualigens Fine Chemicals, Hi Media, and Central Drug House Ltd.

Extraction Process

The maceration process was employed to extract active compounds from the shade-dried and coarsely powdered leaves of *Luffa Acutangula*. Initially, the plant material underwent defatting by extraction with petroleum ether. Subsequently, 60 g of the defatted powder was extracted using hydroalcoholic solvents (80% ethanol) through maceration for 48 hours (9). The extract was then filtered and concentrated using a vacuum evaporator at 40°C to obtain the final dried product. This traditional extraction method ensures the preservation of bioactive compounds (8).

Determination of percentage yield

The percentage yield of each extract was calculated by using following formula:

Phytochemical Screening

Phytochemical screening involves analyzing plant extracts to identify and quantify bioactive compounds that contribute to their potential health benefits and biological activities. These naturally occurring substances, while not vital for plant growth, protect plants against pathogens, pests, and environmental stress. They include alkaloids, flavonoids, terpenoids, phenolics, glycosides, and others, each offering specific health benefits such as antioxidant, antimicrobial, anti-inflammatory, or anticancer properties (10). Standardized chemical tests were performed on *Luffa Acutangula* extracts to detect these compounds. Alkaloids were identified using Mayer’s, Wagner’s, Dragendorff’s, and Hager’s tests through characteristic precipitate formations. Carbohydrates were confirmed via Molisch’s, Benedict’s, and Fehling’s tests, while glycosides were detected using Modified Borntrager’s and Legal’s tests. Saponins were verified through froth and foam tests, phenols by ferric chloride reaction, and tannins using the gelatin test. Flavonoids were identified through alkaline reagent and lead acetate tests, and proteins and amino acids

were confirmed by xanthoproteic and ninhydrin reactions. Finally, diterpenes were detected using the copper acetate test (11). These findings underline the rich phytochemical composition of the plant, suggesting its potential therapeutic applications.

Estimation of total phenolic content

The total phenolic content of the extract was assessed using the modified Folin-Ciocalteu method. For the standard, 50 mg of gallic acid was dissolved in 50 ml methanol, and aliquots ranging from 5 to 25 µg/ml were prepared. The extract was prepared by dissolving 10 mg of dried extract in 10 ml methanol, filtering it, and using 2 ml of the resulting solution (1 mg/ml) for analysis. In the procedure, 2 ml of each extract or standard was mixed with 1 ml of Folin-Ciocalteu reagent (diluted 1:10 with distilled water) and 1 ml of sodium carbonate solution (7.5 g/l)(12). The mixture was vortexed for 15 seconds, left to develop color for 15 minutes, and the absorbance was measured at 765 nm using a spectrophotometer.

Estimation of total flavonoid content

The total flavonoid content was determined using the aluminium chloride method. For the standard preparation, 50 mg of quercetin was dissolved in 50 ml methanol, and aliquots with concentrations ranging from 5 to 25 µg/ml were prepared. For the extract preparation, 10 mg of dried extract was dissolved in 10 ml of methanol, and filtered, and 3 ml of the resulting solution (1 mg/ml) was used for analysis. In the procedure, 1 ml of 2% aluminium chloride solution in methanol was mixed with 3 ml of either the extract or standard solution (13). The mixture was allowed to stand for 15 minutes at room temperature, after which the absorbance was recorded at 420 nm.

Isoniazid-induced hepatoprotective activity of leaves of *Luffa Acutangula*

Animals

Wistar rats (180-250 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55-65%). Rats received standard rodent chow and water ad libitum. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried out in a noise-free room between 08.00 to 15.00 h. A separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Drugs & Chemicals

INH (Isoniazid), and Silymarin (Micro labs, India) were used in present study. All other chemicals and other biochemicals used in the experiments were of analytical grade from different finns.

Acute toxicity studies

Acute oral toxicity was conducted according to the method of Organisation for Economic Co-operation and Development (OECD) (OECD, 2001). Animals were kept fasting providing only water, and leaves of *Luffa Acutangula* (50,100,150,200,300 mg/kg/day) were administered orally for 4 days of six groups of rats (n=6) and the animals were kept under observation for mortality as well as any behavioral changes for evaluation of a possible hepatoprotective effect.

Experimental Designs

The experimental design involved dividing animals into five groups of six each to evaluate the effects of **Luffa Acutangula** extract and silymarin against INH-induced toxicity. Group I served as the normal control, receiving sterile distilled water (1 ml/kg, p.o.). Group II received INH (100 mg/kg, p.o.). Groups III and IV were administered hydroalcoholic extract of *Luffa Acutangula* leaves at doses of 100 mg/kg and 200 mg/kg (p.o.), respectively, along with INH (100 mg/kg, p.o.). Group V received silymarin (2.5 mg/kg, p.o.) combined with INH (100 mg/kg, p.o.). The treatments were given once daily for 21 days. On the final day, animals were anaesthetized with ether for blood collection via retro-orbital plexus and subsequently sacrificed under ether anesthesia to harvest the liver for biochemical analysis.

Biochemical Evaluation in Serum

Serum glutamic pyruvate transaminase (SGPT), Serum glutamic oxaloacetic transaminase (SGOT), Alkaline phosphatase (ALP) and total bilirubin were estimated by using commercial kits as per the manufacturer's instructions (14).

3. Results and Discussion

Determination of Percentage Yield

The percentage yield of *Luffa Acutangula* leaf extracts were determined following the maceration extraction process, where the crude extracts were concentrated using a water bath to evaporate the solvents completely. The yields were calculated for extracts obtained using different solvents. The petroleum ether extract yielded 2.52% (w/w), while the hydroalcoholic extract showed a higher yield of 6.44% (w/w), as summarized in Table 1.

Table 1: % Yield of leaves extracts of *Luffa Acutangula*r

S. No.	Solvents	% Yield (WAV)
1.	Pet ether	2.52%
2.	Hydroalcoholic	6.44%

Phytochemical screening of extracts

A small portion of the dried extracts were subjected to the phytochemical test using Kokate (1994) methods to test for alkaloids, glycosides, saponins, flavonoids and phenol separately for extracts of all samples. A small amount of each extract is suitably resuspended into the sterile distilled water to make the concentration of 1 mg per ml. The outcomes of the results are discussed separately in Table 2.

Table 2: Phytochemical screening of extracts of *Luffa Acutangula*r

S. No.	Constituents	Hydroalcoholic Extract
1.	**Alkaloids**	
	Mayer's Test	-ve
	Wagner's Test	-ve
	Dragendroff's test	-ve
	Hager's test	-ve
2.	**Glycosides**	
	Modified Bomtrager's Test	-ve
	Legal's test	-ve
3.	**Flavonoids**	
	Lead acetate	+VC
	Alkaline test	+ve
4.	**Phenol**	
	Ferric Chloride Test	+VC
5.	**Proteins and Amino acids**	
	Xanthoproteic test	+ve
	Ninhydrin Test	-ve
6.	**Carbohydrates**	-VC
	Molisch's Test	-ve
	Benedict's Test	+ve
7.	**Saponins**	
	Froth Test	+ve
	Foam test	+VC
8.	**Diterpins**	

	Copper acetate test	-ve
9.	**Tannins**	
	Gelatin Test	+ve
[+ve=Positive; -ve=Negative]		

Total Phenolic content estimation (TPC)

Figure 1. The content of total phenolic compounds (TPC) content was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: Y = 0.042X+0.002, R2= 0.999, where X is the gallic acid equivalent (GAE) and Y is the absorbance (Figure 1).

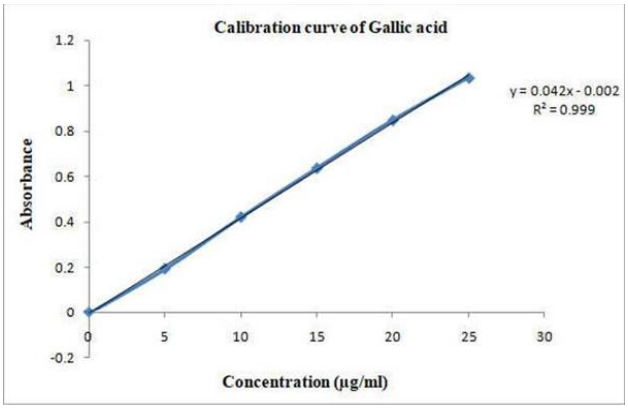

Figure 1: Total Phenolic content estimation (TPC)

Total flavonoid content estimation (TFC)

Figure 2. The content of total flavonoid compounds (TFC) content was expressed as mg/l00mg of quercetin equivalent of dry extract sample using the equation obtained from the calibration curve: Y = 0.06X+0.019, R2= 0.999, where X is the quercetin equivalent (QE) and Y is the absorbance (Figure 2).

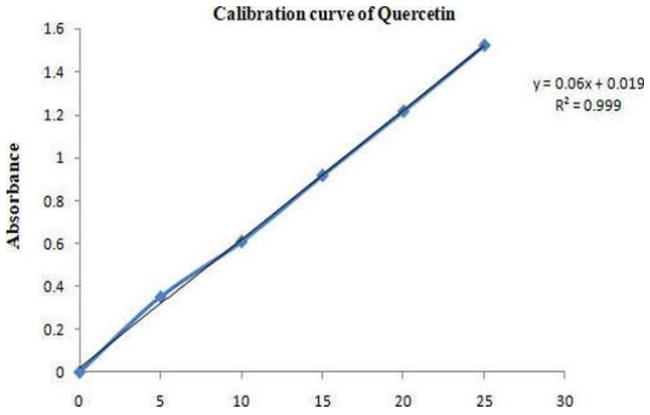

Figure 2: Total flavonoid content estimation (TFC)

Effect of Hydroalcoholic Extract of *Luffa Acutangula* and Silymarin on % SGOT Levels in Isoniazid hepatotoxicity in Rats

The study evaluates the hepatoprotective effects of hydroalcoholic extract of *Luffa Acutangula* and silymarin on SGOT levels in rats with isoniazid (INH)-induced liver damage. INH significantly increases SGOT levels, indicating liver damage. *Luffa Acutangula* extract and silymarin reduce SGOT levels, with *Luffa Acutangula* showing a dose-dependent effect. At 200 mg/kg, the extract reduces SGOT to 195.0 ± 2.9 U/L, which is less

effective than silymarin (165.0 ± 1.6 U/L) but still significant. These results suggest that *Luffa Acutangula* has hepatoprotective properties, though silymarin remains more effective. Further research is needed to understand *Luffa Acutangula*'s mechanisms and potential clinical applications fully.

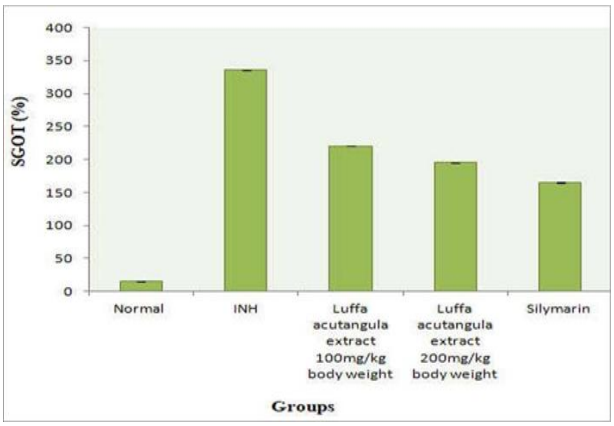

Figure 3: Effect of Luffa Acutangula and Silymarin on %SGOT levels in Isoniazid induced hepatotoxicity in rats

Effect of Hydroalcoholic Extract of *Luffa Acutangula* and Silymarin on %SGPT Levels in Isoniazid Induced Hepatotoxicity in Rats

The study examines the effects of hydroalcoholic extract of *Luffa Acutangula* and silymarin on SGPT levels in rats with isoniazid (INH)-induced liver damage. INH significantly increases SGPT levels, indicating liver injury. Both *Luffa Acutangula* extract and silymarin reduce SGPT levels, with the extract showing a dose-dependent effect: 100 mg/kg reduces SGPT to 200.0 ± 4.50 U/L and 200 mg/kg lowers it further to 191.0 ± 3.40 U/L. Silymarin is more effective, reducing SGPT to 145.0 ± 3.40 U/L. These results suggest that while *Luffa Acutangula* has notable hepatoprotective properties, silymarin remains more effective. Further research is needed to explore the mechanisms and therapeutic potential of *Luffa Acutangula*.

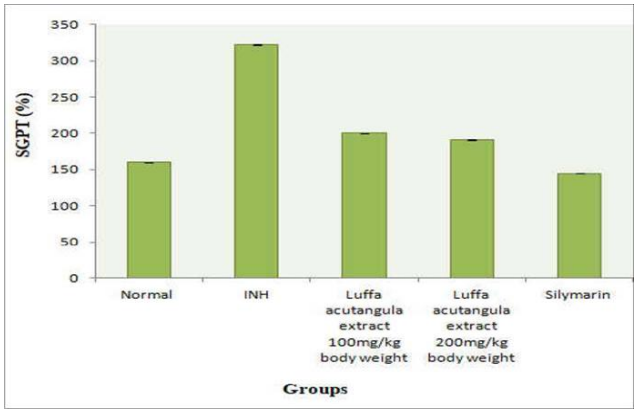

Figure 4: Effect of Luffa Acutangula and Silymarin on %SGPT levels in Isoniazid induced hepatotoxicity in rats

Effect of *Luffa Acutangula* and Silymarin on % serum bilirubin levels in Isoniazid induced hepatotoxicity in rats

The study explores the effects of *Luffa Acutangula* extract and silymarin on serum bilirubin levels in rats with isoniazid (INH)-induced hepatotoxicity. INH significantly elevates serum bilirubin to 275.0 ±2.15 pmol/L, indicating liver dysfunction. Treatment with *Luffa Acutangula* extract decreases bilirubin levels in a dose-dependent manner, with 100 mg/kg reducing it to 165.0 ± 4.65 pmol/L and 200 mg/kg further lowering it to 145.0 ± 1.50 pmol/L. Silymarin normalizes bilirubin levels to 125.0 ± 5.50 pmol/L, similar to the normal control group. These

results demonstrate that *Luffa Acutangula* extract has significant hcpatoprotcctivc effects, though silymarin is more effective in restoring bilirubin levels to normal.

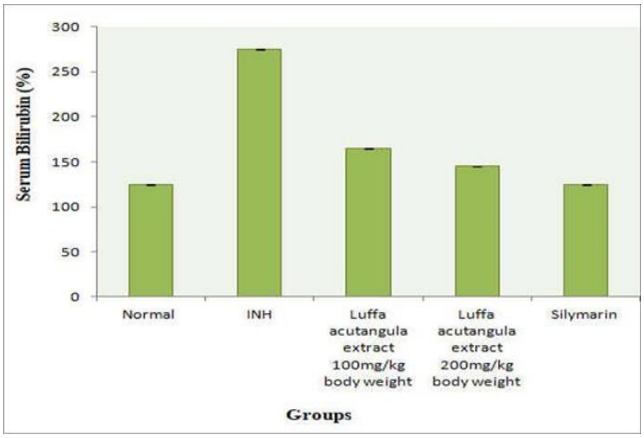

Figure 5: Effect of Luffa Acutangula and Silymarin on % serum bilirubin levels in Isoniazid induced hepatotoxicity in rats

Effect of *Luffa Acutangula* and Silymarin on % ALP levels in Isoniazid induced hepatotoxicity in rats

The study evaluates the effects of *Luffa Acutangula* extract and silymarin on alkaline phosphatase (ALP) levels in rats with isoniazid (INH)-induced hepatotoxicity. INH treatment significantly raises ALP levels to 325.0 ± 4.48 U/L, indicating liver damage. *Luffa Acutangula* extract shows a dose-dependent reduction in ALP levels: 100 mg/kg reduces ALP to 225.0 ± 3.50 U/L, and 200 mg/kg lowers it further to 195.0 ± 3.45 U/L. Silymarin is more effective, normalizing ALP levels to 136.0 ± 3.45 U/L. These findings suggest that while *Luffa Acutangula* extract provides significant hepatoprotection, silymarin is more effective in normalizing ALP levels.

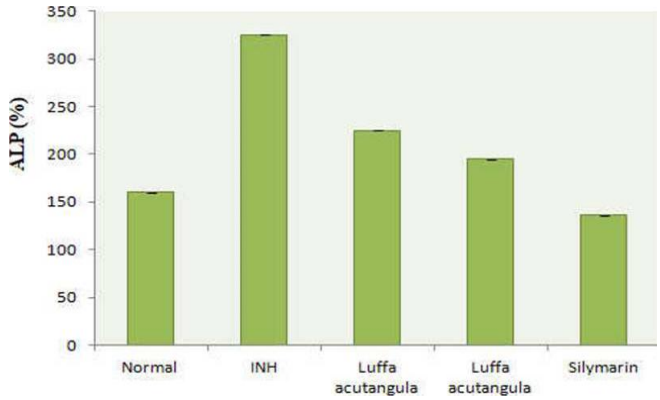

Figure 6: Effect of Luffa Acutangula and Silymarin on % ALP levels in Isoniazid induced hepatotoxicity in rats

4. Conclusion

The study assessed the phytochemical composition, antioxidant properties, and hepatoprotective potential of hydroalcoholic extract of *Luffa Acutangula*. Hydroalcoholic extraction yielded 6.44%, higher than petroleum ether (2.52%). Phytochemical analysis revealed the presence of flavonoids, phenols, carbohydrates, saponins, and tannins, while alkaloids, glycosides, and certain proteins were absent. Total phenolic content (TPC) and total flavonoid content (TFC) were measured as 0.655 mg and 0.521 mg per 100 mg of dry extract, respectively, suggesting a significant antioxidant capacity. The hepatoprotective effects were evaluated in rats with isoniazid (INH)-induced liver damage. INH caused notable increases in liver function markers, including SGOT, SGPT, serum bilirubin, and ALP levels, indicative of hepatotoxicity. *Luffa Acutangula* extract reduced these markers in a dose-dependent

manner. At 200 mg/kg, it lowered SGOT, SGPT, bilirubin, and ALP to 195.0 U/L, 191.0 U/L, 145.0 pmol/L, and 195.0 U/L, respectively. Silymarin, a standard hepatoprotective agent, demonstrated superior efficacy, normalizing these markers closer to control levels. These findings highlight the antioxidant and hepatoprotective potential of *Luffa Acutangula*, likely due to its bioactive phenolics and flavonoids. While silymarin outperformed the extract across all parameters, *Luffa Acutangula* showed significant protective effects, supporting its therapeutic potential against oxidative stress and liver damage. Further research is recommended to clarify its mechanisms and optimize its clinical use.

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