



EVALUATION OF ANTIDIABETIC POTENTIAL *MURRAYA KOENIGII* & *ABELMORACHUS ESCULENTUS* EXTRACT IN DIABETIC RAT

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Article History

Volume 6, Issue 12, 2024

Received: 15 June 2024

Accepted: 10 July 2024

Doi:

[10.48047/AFJBS.6.12.2024.5474-5488](https://doi.org/10.48047/AFJBS.6.12.2024.5474-5488)

Abstract:

Diabetes is a metabolic illness that has spread throughout the world in the 20 century. The effect of *lady finger and curry leaves* ethanolic extract on blood sugar levels was investigated in acute and prolonged study models in ethanol -induced diabetic rats. In the acute trial, the following medications were given orally: control, standard, combined extract of formulations, individual curry leaves and lady fingers administered to them. Blood sugar levels were measured before and after dose in the strategic response at 1, 7, and 14 hours, correspondingly. The 300 and 500 mg/kg concentrations of ethanolic extract were discovered after hyperglycemic testing. Significantly lower serum sugar concentrations was obtained.

Keywords: *Murraya koenigii*, *Abelmoschus esculentus*, Diabetes, Blood sugar, Polyherbal

1. Introduction

Diabetes Mellitus is a long-term metabolic disorder marked by chronically high blood sugar levels. To distinguish the illness from diabetes insipidus, which is also associated with excessive urination, British physician John Rolle introduced the term "mellitus" in the late 1700s. It's a rapidly increasing global problem with serious social, medical, and economic consequences [1-3].

In the lack of clear hyperglycemia, a good outcome should be validated by repeating any of the following methods on another day. Because the ease of implementation and the significant time investment of official glucose sensitivity testing, that requires 2 hours to perform and has no predictive benefit over the overnight test, it is preferred to assess a fasting blood glucose. Two overnight glucose levels above 126 mg/dl (7.0 mmol/l) are deemed definitive for diabetes mellitus, per the modern interpretation.

Diabetes insipidus is a rare condition that has signs similar to insulin resistance but does not cause sugar metabolism problems (insipidus meaning "without taste" in Latin) but does not include the same disease processes [4].

A diet rich in whole grain and fibre, as well as selecting beneficial fats like vegetable oils found in nuts, trans fats, or fish, have been shown to be helpful in preventing diabetes. Reducing sugary drinks & consuming less red meat as well as other saturated fat sources also can aid in diabetes prevention. Continuous smoking has also been linked to an

elevated risk of diabetic, therefore quitting can be a valuable preventive approach [5]. Herbs, or plants with medical properties, are the source of herbal remedies. There are various groupings within the plant kingdoms; however, botanical classification is not provided in this section. Conversely, there are four types of herbal plants: climbers, woody perennials, perennials and biennials, trees, and shrubs. This page only discusses blooming plants; fungus, fern, moss, and algae are discussed in passing [6-8]. Medicinal herbalism, or the use of herbs, is the term for the practice of treating plants. Western modern medicine is not the same as medicinal Herbalism, but ultimately the two come together. For instance, using cascara or senna to relieve diarrhoea, sunburn and bruises with aloe Vera gel, and colds with friar's balsam or benzoin tincture [9]. Okra or lady finger is enriched in flavonoids, which are phenolic chemicals that are found naturally in a wide variety of foods, such as fruits, vegetables, and seeds. Flavonoids have been implicated as important potential chemopreventive agents due to mounting evidence of their preventative function against obesity and type 2 diabetes. We reviewed research on the impact of flavonoids and foods high in flavonoids on the modulation of the insulin-signaling pathway during type 2 diabetes and obesity, both *in vitro* and *in vivo*. It's interesting that not many studies involving humans have evaluated the modulatory impact of these phenolic chemicals at the molecular degree of insulin control [10-12]

Diabetes can be managed with a variety of medications and therapies, most of which have negative side effects and are costly (such as insulin and thiazolidinediones) for developing nations like India. Impacts (such as low blood sugar). India has a long history of using traditional medicine in addition to having an enormous stock of natural resources. These systems have listed several spices that are frequently used in Indian food as having anti-diabetic qualities. Aim of the study is to investigate and develop a novel plant-based anti-diabetic drug that was tested *in vitro* and *in vivo*.

2. Methodology:

Sample collection and preparation: pharmacology department certified the leaves of plant name were gathered. The plant samples were collected from the local area and then allowed to be washed with distilled water. The washed samples were kept for air dry to remove water traces. For 15 days, the samples were kept at room temperature. Then, using a motorized grinder, it was ground into dust. This extract was obtained using Ethyl acetate, Ethanol, and Ethanol in order of rising polarity. In a Soxhlet device, 300g of the crushed leaves was evenly put into a thimble & recovered with 1000ml Organic solvent [13].

Assay for the inhibition of alpha-amylase: 1000 µl of test materials with comparison medicine (100-1000 g.ml⁻¹) liquids are combined with 0.5 ml buffer solution (pH 6.9 and 0.2mM) and -amylase (0.50 mg/L) solution in 0.5 ml dilution method (pH 6.9 and 0.2mM) solutions and stored at 25.00°C for 20 seconds. Each tube was then given 500.00 µl of 1% starch solutions in phosphate buffer (pH 6.9 and 0.2mM). The resulting mixture was then maintained at 25°C for 10 minutes.

The reaction was terminated with 1 mL of DNS colour solution. The tubes were chilled to normal body temperature after five min in a water bath. The absorbance was measured at 540 nm after diluting the solution mixture using 5 mL D.W. Standards reflected 100 percent enzymatic activities and was performed out in the same way as extracts but with a different vehicle [13]

TLC slides preparation & activation: Mixture is composed of a mix of immobile & liquid phases. TLC plates are commonly prepared by pouring, dipping, sprinkling, or spraying. In the dropping technique, slurry is prepared and poured onto the glass. The slurry was spread out evenly across the glass plate. Dishes are cleaned in the oven. During the dipping procedure, two plates are dipped in slurry, separated, and then dried. The massive amount of slurry required is a disadvantage. TLC spreader is utilised in this approach. The glass plates are layered on top of each other on the base plate.

Slurry was pumped into the TLC spreader's reservoir. The spreader's thickness can be modified by turning a knob. For analytical applications, a thickness of 0.25 mm is commonly utilised. The plates was spreader rolled on them and then air dried. Plates are activated by exposing them to temperatures ranging from 100 to 120 degrees Celsius for one hour. For later usage, activated plates can be stored in a thermostatically controlled oven.

For excellent spotting the quantity of the solid material should be kept to a minimum. 2- Employing measuring cylinder, spot 4ug of a 1% mixture of reference or specimen. Spots ought to be 3 cm above the plate's bottom and must not be submerged in the solvent system. On a 1/4 plate, at least four locations can be found.

Industrial sheets with silica gel pre-applied are accessible. Choose a solvents by experimenting with different solvent on the sample. Dissolve a tiny amount of EtOH extract of unknown leaves in various flasks containing various polar solvent. Place the TLC plates in the chambers with the spotted flat side to decrease the pencil line around the solvents [14]. Allow this plates to dry after removing it from the potential depends.

Screening of Phytoconstituents: Preliminary phytochemical screening of ethanolic leaves extract of curry leaves & lady finger. The ethanolic leaves extract of was used for testing preliminary phytochemical screening in order to detect major chemical groups [15].

Pharmacological study: Healthy adult wistar rats strain between age 3week 1 month and weight approx. 200 gram Diabetes induce rat -Alloxan and Drug *murrya koenijii*, *Abelmoschus esculentus*.

Experimental design: There was two groups formed from the animals. There was six animals each group. The dosage schedule and group size details are as follows[16].

Group	Details of Group	Animal	Treatment and Dosing schedule	Duration of Treatment
1	<i>Normal control group</i>	6	<i>Vehicle treated(0.1% normal saline)</i>	<i>14 days</i>
2	<i>Disease control</i>	6	<i>Alloxan 130mg/kg I.P</i>	<i>14 days</i>
3	<i>Treatment control (1st dose level)</i>	6	<i>Curry leaves 200,400 mg/kg</i>	<i>14 days</i>
4	<i>Treatment control (2nd Dose level)</i>	6	<i>Lady finger 200,400 mg/kg</i>	<i>14 days</i>
5	<i>Only drug treated 2nd dose level</i>	6	<i>Curry leaves And lady finger dose</i>	<i>14 days</i>
6	<i>Standard drug</i>	6	<i>Metformin dose depend the animal weight</i>	<i>14 days</i>

Estimation of biochemical parameters

Collection of blood samples: Regular charting 14 days tail vein. Overnight blood specimens was taken, and blood sugar levels was measured after 1, 7, and 14 days of fasting.

Blood glucose level: The quantity of sugar in the blood specimen received from the mice is measured by the blood glucose level test. The testing is frequently done to look for high blood glucose concentrations which can indicate diabetic or insulin resistance [17].

Statistical analysis: Statistics research was performed by utilizing PRISM 5.0. All the data of Biochemical variables and body mass was represented as SEM. The values was examined for statistically relevance utilizing (ANOVA), comparing was performed by employing Dunnett’s t test. P values less than 0.05 was deemed significant P values ≤0.01 was deemed very substantial, P ≤0.001 was deemed extremely substantial, and was not substantial [18].

3.Results and Discussions:

Sample collection:

The samples were collected from the tree located in the Noida. Initially, the collected samples were washed with distilled water and then crushed into powder. The fine powdered samples were dipped into polar and nonpolar solvents. Further, the samples were filtered and the extracts were collected into marked tubes after evaporating the solvents; as explained in figure.

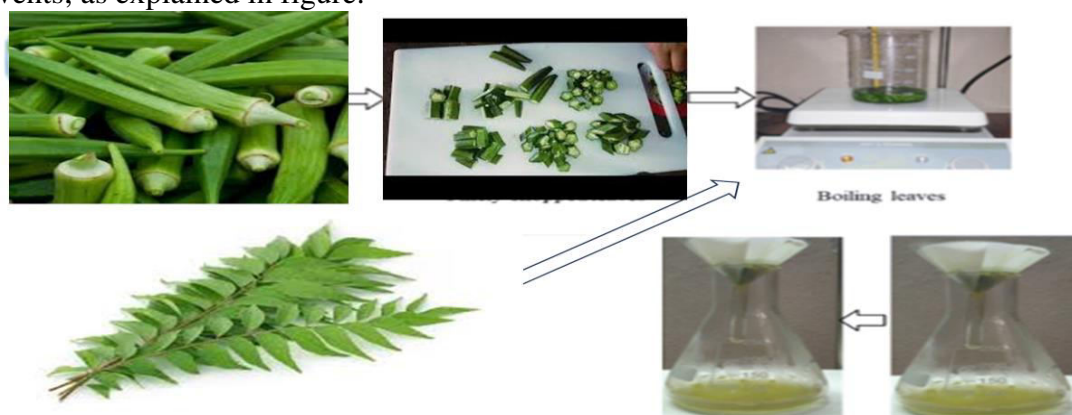


Figure 1: Extraction of bioactive compounds from lady finger and curry leaves.

Phytochemical screening:

The extracted compounds were analysed for the presence and absence of phytochemicals and found that the terpenoids are present in all of the extracts, while flavonoids are absent in 70% ethanolic extract. The results were illustrated in table

Table 1:Phytochemical Test Results for

Secondary metabolite	Lady finger			Curry leaves		
	Ethanol	Chloroform	Acetic acid	Ethanol	Chloroform	Acetic acid
Terpenoids	+	+	+	+	+	-
Flavonoids	+	+		+	+	-
Phlobatanins	+		+	+	-	+
Tanins	+	+	+	+	+	-
Courmarin	+		+	+	-	+
Leucoanthocyanin	-	-		-		-

Steroids	-	-	-	+	-	
Fatty acids	+	+	+	-	+	+

Table 2:Standard λmaxrange

S.No	Standard λmaxrange (innm)	λmaxobtained(innm) Lady finger	λmaxobtained(innm) curry leaves
1.	Tannins(300 nm–365 nm)	300	299
2.	Saponins(230 nm-355 nm)	244	254
3.	Steroids(310 nm – 340 nm)	310	254
4.	Terpenoids (330 nm – 385 nm) &(255 nm – 340 nm)	314	351

Animal study for diabetes:

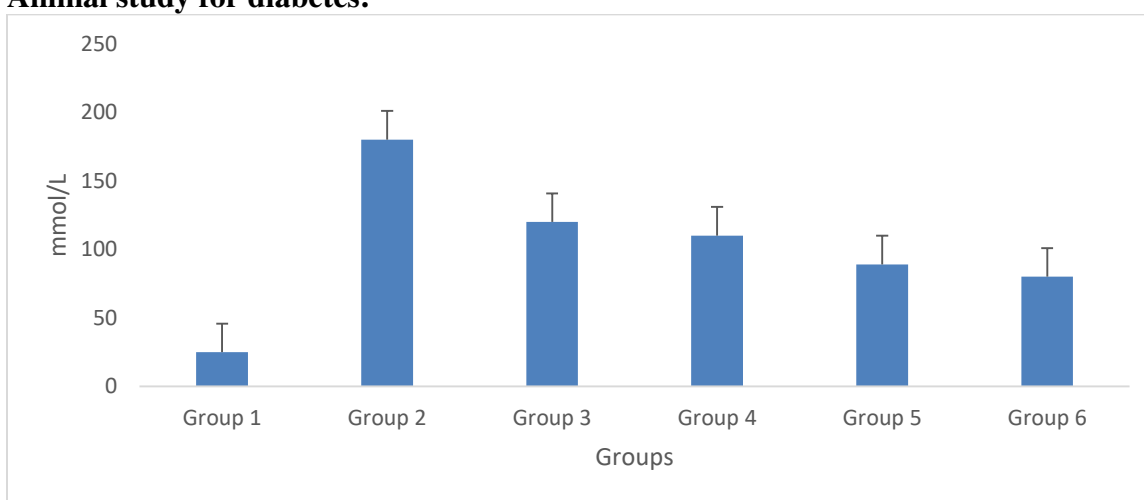


Table 3:Showingdifferentialcountsofleucocytesfoundindifferentgroups

Different Groups	Erythrocytes (NR: 7.0-10.1x10 ⁶ Cells/cmm)	WBC (NR:-3.8-12.7x10 ³ Cells/cmm)	Hemoglobin(NR: 14-18 gm/dl)	Platelets (NR:766–1677 x10 ³ Cells/cmm)
I	10.0	12.5	14.2	940.5
II	9.8	11.9	13.4	935.6
III	5.5	3.4	8.0	800.4
IV	9.3	11.0	13.0	856.5
V	9.8	11.6	14.0	840.0
VI	10.0	12.5	14.2	940.5

Groups	Neutrophil(NR:50-81%)	Lymphocyte (NR:14-44%)	Basophile(NR:0-1%)	Monocytes (NR:2-6%)	Eosinophil(NR:1-2%)
I	65.5±3	26.4±2	1.2±1	5.4±2	1.5±0.5
II	70.7±4.0	34.8±3	1.4±1	5.5±2	1.6±0.6
III	40.6±2.0	40.9±4	4.0±2	10.20±3	4.03±0.5
IV	58.4±3.0	32.9±3	1.30±0.3	5.4±0.5	2.15±0.2

V	63.5±3.0	27.8±2	1.5±1	5.6±2	2.12±0.2
VI	65.5±3	26.4±2	1.2±1	5.4±2	1.5±0.5

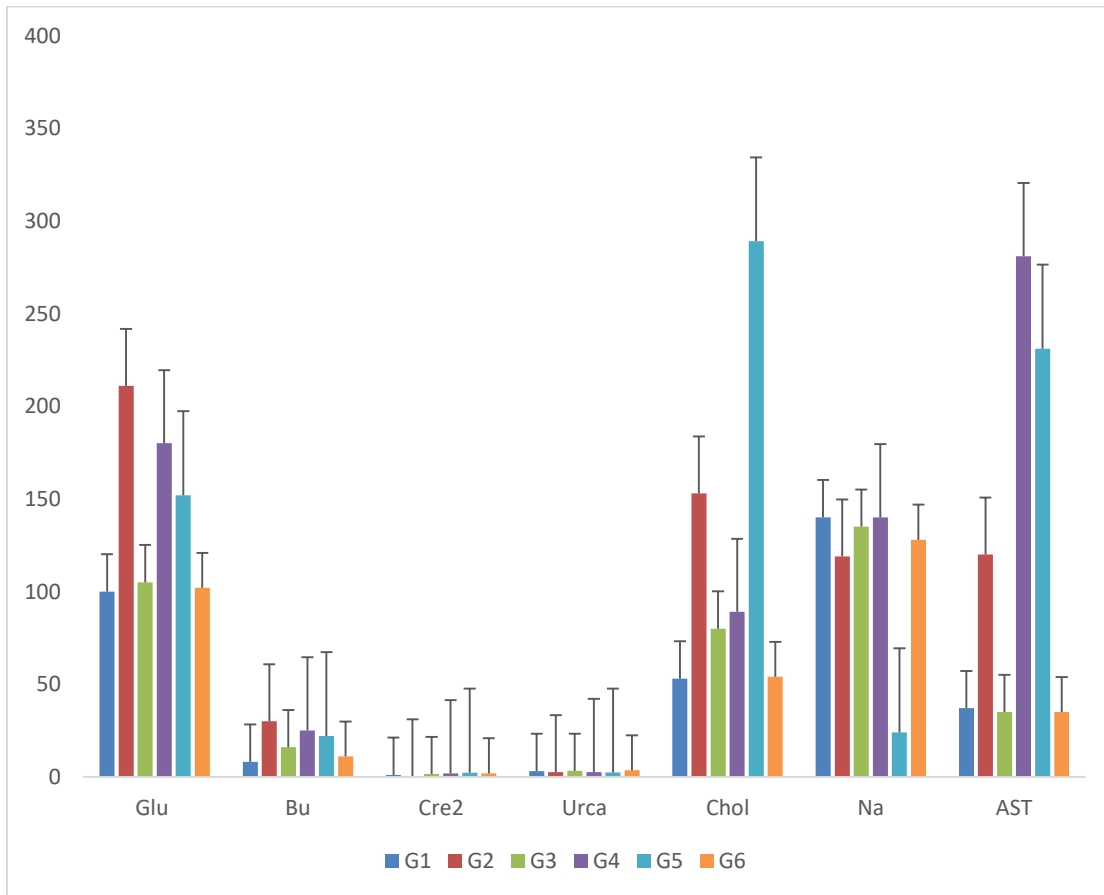
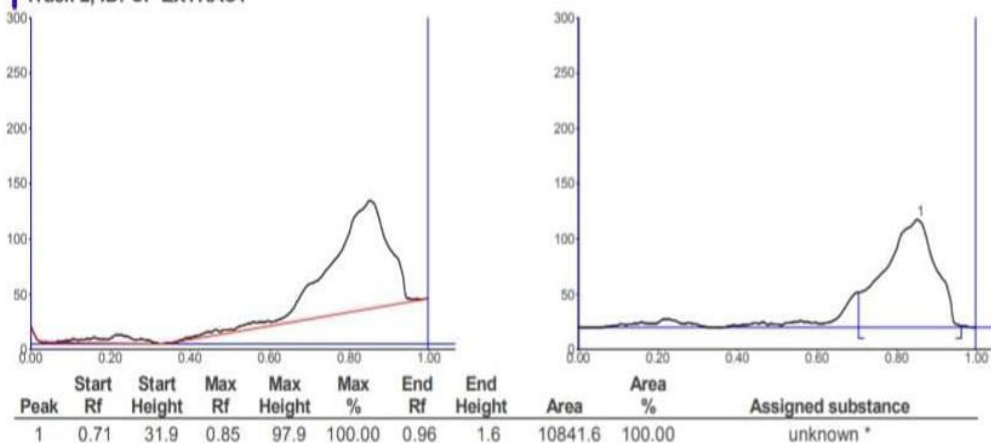


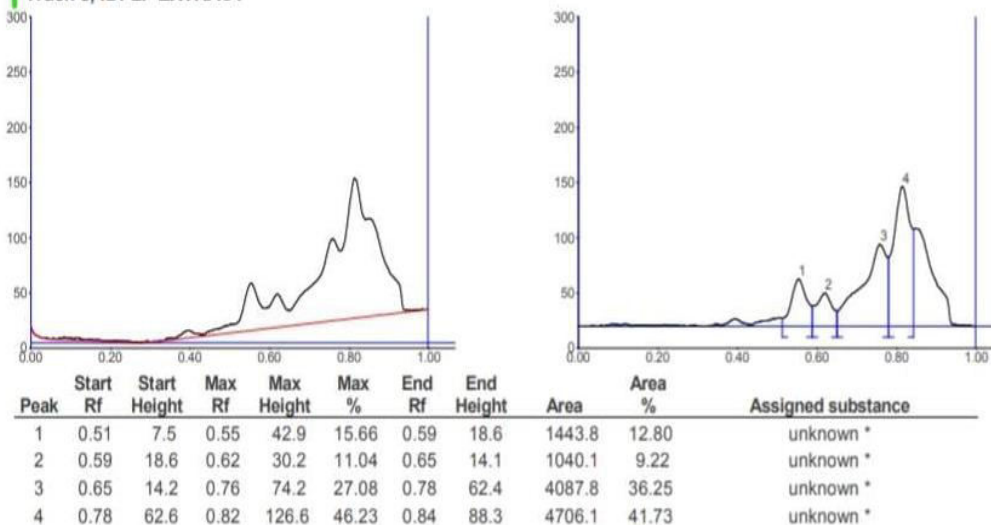
Figure 2: Serum routine test

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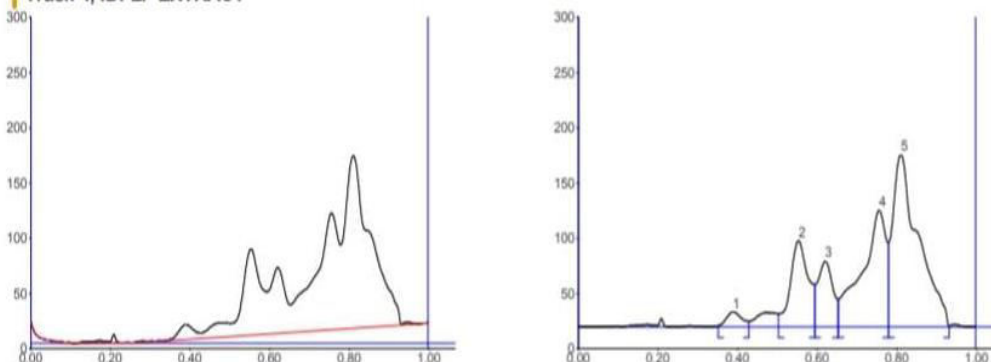
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Track 3, ID: LF EXTRACT

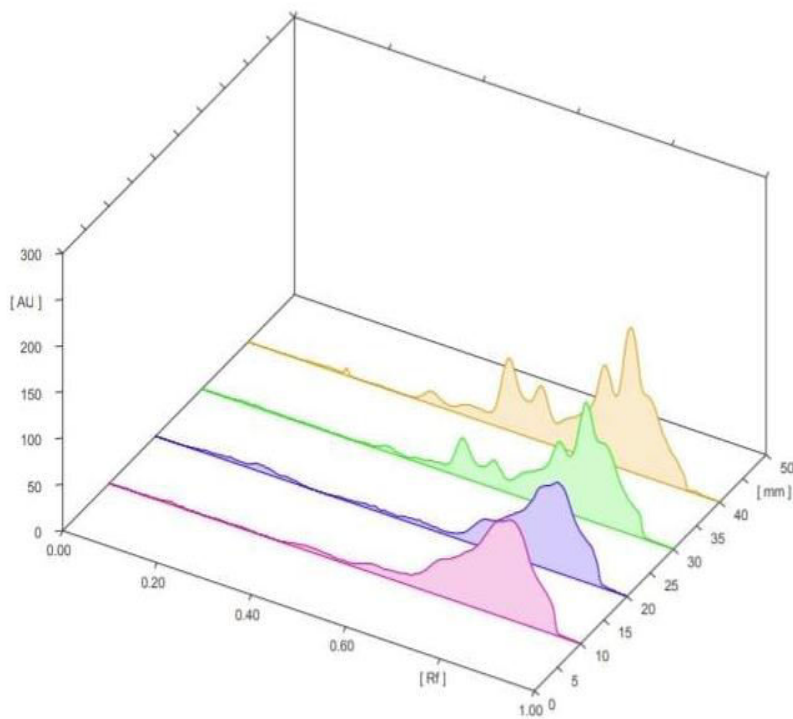


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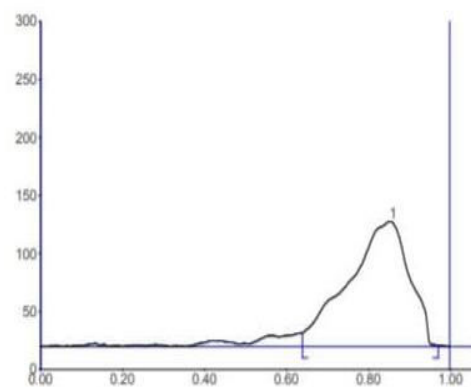
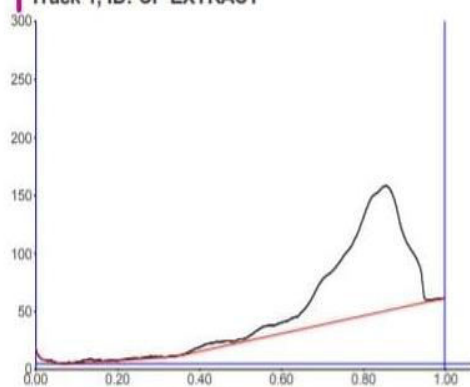


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All tracks at Wavelength



Track 1, ID: CP EXTRACT



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.64	11.5	0.85	107.8	100.00	0.97	1.0	13966.6	100.00	unknown *

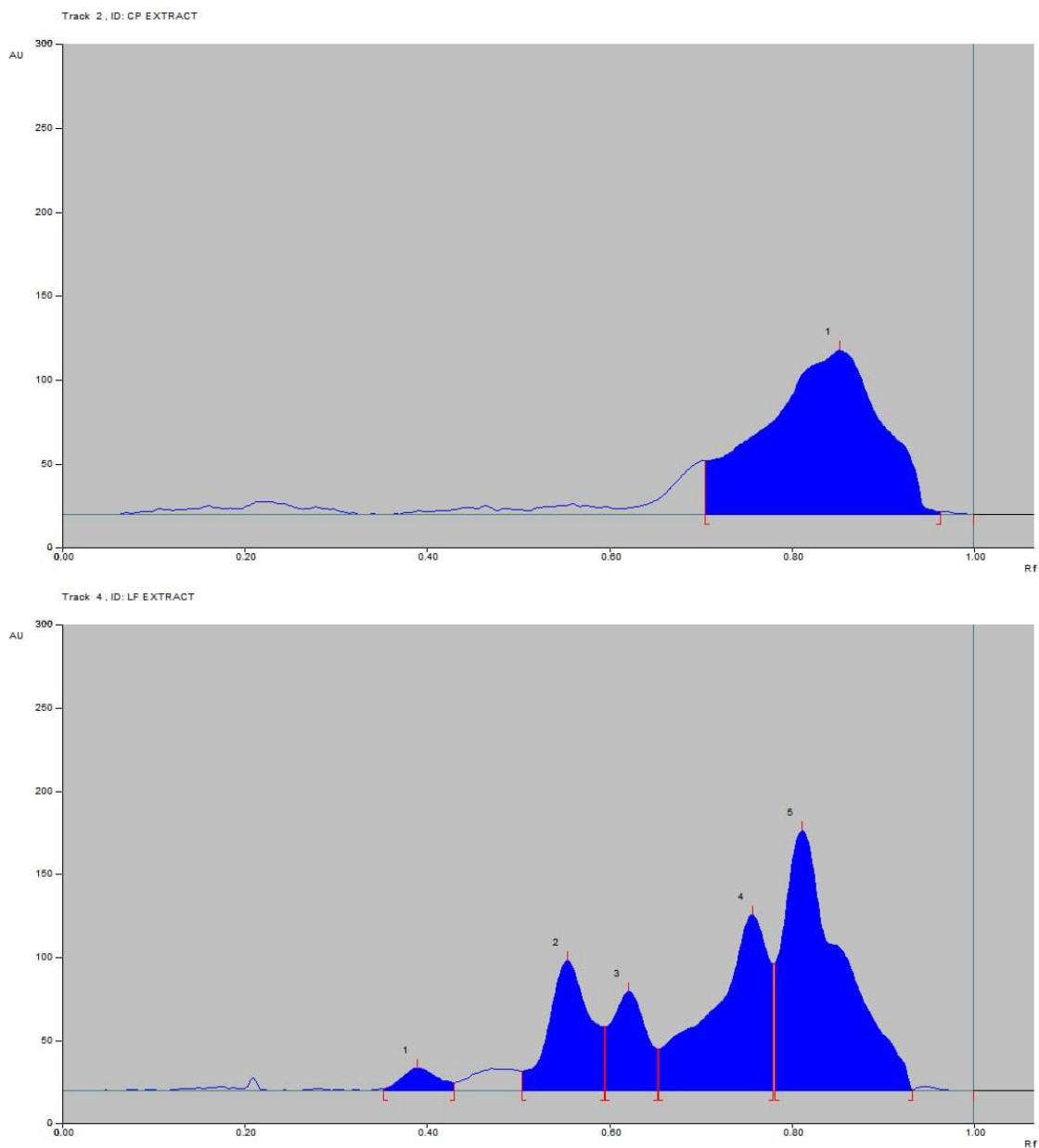
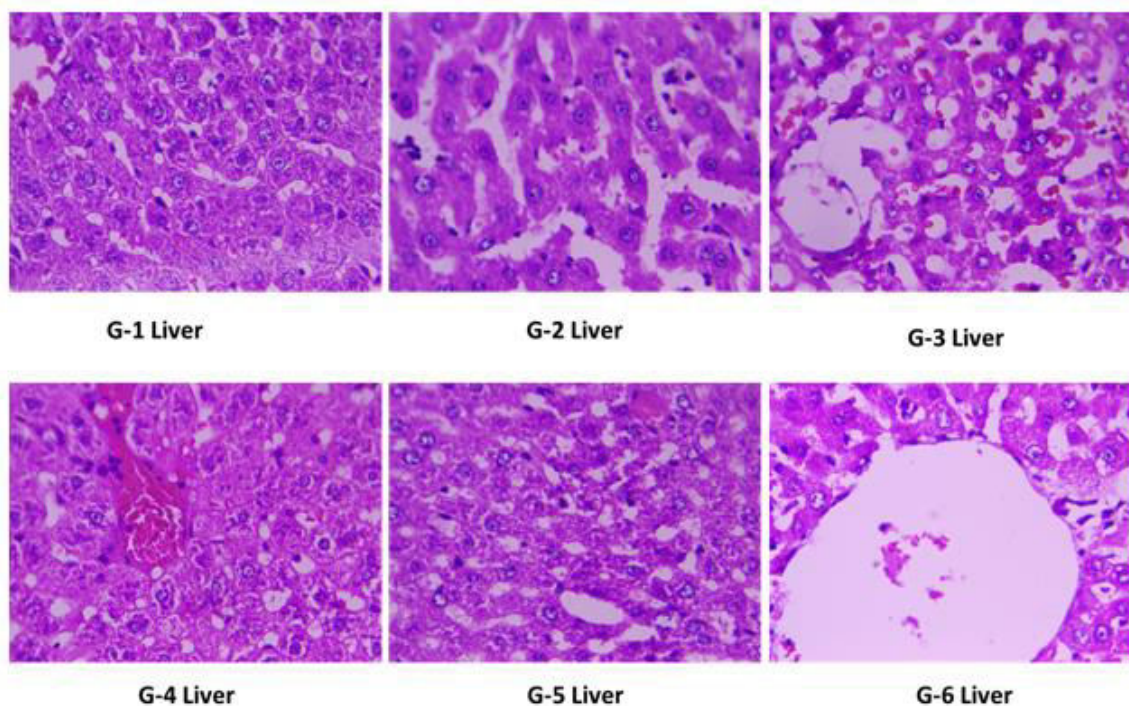


Figure 3: HPTLC analysis of the compounds

Peak	Rf	Area
1	0.35	466
2	0.50	3055
3	0.60	1959.6
4	0.65	5578.8
5	0.78	8823.3

Histopathology studies:**Figure 4: Histopathological studies on liver**

The histopathological examination of the liver across different experimental groups reveals distinct variations attributable to the specific treatments administered. In the G1 (Control) group, the liver tissue is expected to display normal histological architecture, characterized by well-preserved hepatocytes, clear central veins, and minimal signs of inflammation or necrosis, indicative of a healthy liver. In contrast, the G2 (Standard) group, which might be subjected to a known standard treatment, should exhibit liver histology close to normal, potentially with slight improvements or minor deviations depending on the nature of the standard treatment used.

In the G3 (Curry leaves + Lady finger) group, the combined treatment of curry leaves and lady finger is anticipated to demonstrate protective or restorative effects on the liver. Histologically, this group should show reduced signs of liver damage, with a significant decrease in inflammatory cell infiltration, necrosis, and fatty degeneration, indicating the hepatoprotective synergy of the combined extracts. The G4 (Curry leaves) group is expected to show similar hepatoprotective effects, potentially manifesting as improved liver architecture, reduced inflammation, and lesser necrosis, reflective of the beneficial properties of curry leaves alone.

For the G5 (Lady finger extract) group, the liver histopathology should reveal beneficial effects of the lady finger extract, with improved hepatocyte integrity, reduced signs of oxidative stress, and lesser inflammation, highlighting its protective role against liver damage. Lastly, the G6 (Alloxan) group, which serves as a model for induced liver damage, is anticipated to display significant pathological changes. These changes might include extensive hepatocyte necrosis, pronounced inflammatory infiltration, and possible fatty changes, indicative of alloxan-induced hepatotoxicity and oxidative stress.

Overall, these expected histopathological findings reflect the varying impacts of different treatments on liver health, providing insights into their potential therapeutic or toxicological effects.

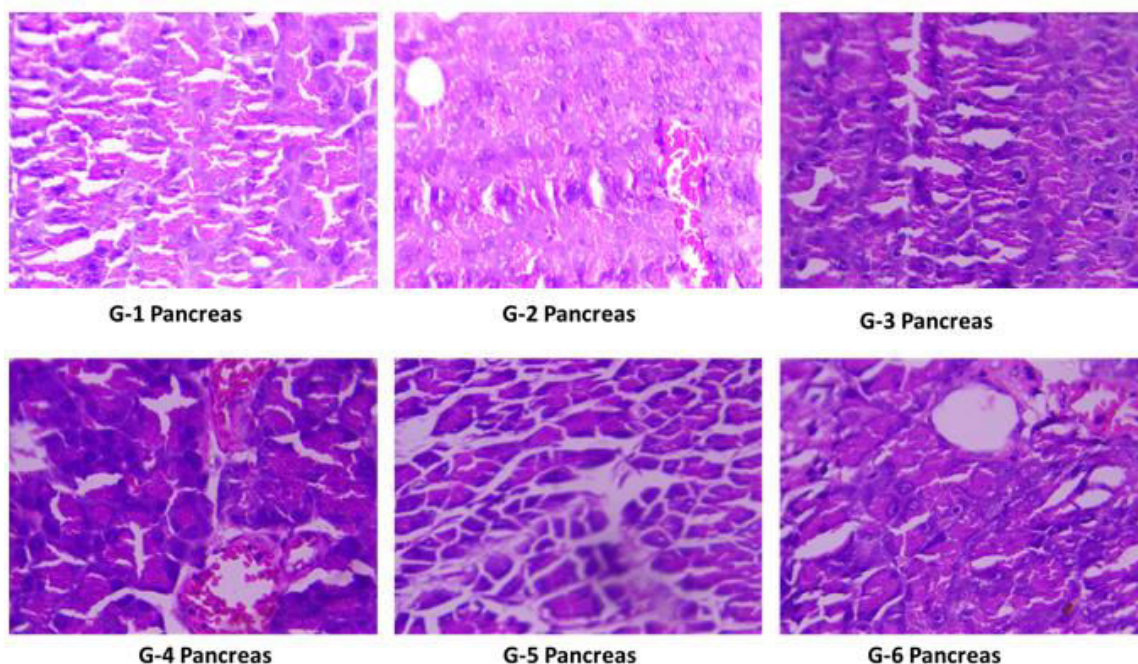


Figure 5: Histopathological studies on Pancreas

The histopathological examination of the pancreas across different experimental groups reveals distinct variations attributable to the specific treatments administered. In the G1 (Control) group, the pancreatic tissue is expected to display normal histological architecture, characterized by well-preserved acinar cells and intact islets of Langerhans, with minimal signs of inflammation or necrosis, indicative of a healthy pancreas. In contrast, the G2 (Standard) group, which might be subjected to a known standard treatment, should exhibit pancreatic histology close to normal, potentially with slight improvements or minor deviations depending on the nature of the standard treatment used.

In the G3 (Curry leaves + Lady finger) group, the combined treatment of curry leaves and lady finger is anticipated to demonstrate protective or restorative effects on the pancreas. Histologically, this group should show reduced signs of pancreatic damage, with a significant decrease in inflammatory cell infiltration, necrosis, and degeneration, indicating the protective synergy of the combined extracts. The G4 (Curry leaves) group is expected to show similar protective effects, potentially manifesting as improved pancreatic architecture, reduced inflammation, and lesser necrosis, reflective of the beneficial properties of curry leaves alone.

For the G5 (Lady finger extract) group, the pancreatic histopathology should reveal beneficial effects of the lady finger extract, with improved acinar cell integrity, reduced signs of oxidative stress, and lesser inflammation, highlighting its protective role against pancreatic damage. Lastly, the G6 (Alloxan) group, which serves as a model for induced pancreatic damage, is anticipated to display significant pathological changes. These changes might include extensive necrosis of the acinar cells, pronounced inflammatory

infiltration, and possible degeneration, indicative of alloxan-induced pancreatic toxicity and oxidative stress.

Overall, these expected histopathological findings reflect the varying impacts of different treatments on pancreatic health, providing insights into their potential therapeutic or toxicological effects.

Conclusion

- **Curry Leaves and Lady Finger Combination (Group III):** The combination treatment significantly disrupts blood parameters and induces a strong inflammatory response in the pancreas, suggesting potential adverse effects on health.
- **Curry Leaves (Group IV) and Lady Finger (Group V) Extracts:** Both individual treatments show mild protective effects on pancreatic tissue with slight impacts on blood health. These treatments may be beneficial in moderate doses but require careful monitoring of blood parameters, especially hemoglobin and platelet levels.
- **Control (Group I), Standard Treatment (Group II), and Alloxan (Group VI):** These groups maintain relatively normal blood and tissue health, indicating stability and minimal adverse effects from the treatments.

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