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EVALUATION OF ANTI-DIABETIC ACTIVITY OF FLAVONOID RICH FRACTION FROM METHANOLIC EXTRACT OF *COCCINIA GRANDIS*

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Abstract

The present investigation aimed to evaluate the extraction efficiency, phytochemical profile, chromatographic separation, toxicity, and antidiabetic potential of the methanolic extract and its isolated fractions from *Coccinia grandis* fruit. The methanolic extract exhibited a significantly higher yield (18.16%) compared to the petroleum ether extract (1.56%), indicating superior extraction of polar bioactive constituents. Phytochemical screening revealed the presence of alkaloids, phenolics, flavonoids, tannins, steroids, triterpenoids, and saponins, which was supported by high total phenolic content (88.03 mg GAE/g) and total flavonoid content (71.31 mg QE/g). Column chromatography yielded eight fractions, among which Fraction F2 demonstrated the highest flavonoid concentration (138.37 mg QE/g). Acute toxicity studies confirmed that Fraction F2 was safe up to 2000 mg/kg. In vivo antidiabetic evaluation in alloxan-induced diabetic rats showed that F2 significantly improved body weight, reduced fasting blood glucose levels, and restored lipid profiles in a dose-dependent manner. Treatment with F2 also normalized elevated biochemical markers such as SGOT, SGPT, creatinine, and urea, indicating hepatoprotective and nephroprotective effects. These therapeutic responses are attributed to the flavonoid-rich nature of F2, which may enhance insulin sensitivity, modulate glucose metabolism, and attenuate oxidative stress. The findings demonstrate that the flavonoid-rich Fraction F2 of *Coccinia grandis* exhibits potent antihyperglycemic, antihyperlipidemic, hepatoprotective, and nephroprotective activities. The study scientifically supports the traditional use of *Coccinia grandis* in diabetes management and highlights F2 as a promising natural therapeutic candidate for metabolic disorders.

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Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia caused by impaired insulin secretion or action. It is one of the most significant global health concerns, associated with long-term complications affecting the kidneys, eyes, nerves, and cardiovascular system. According to the International Diabetes Federation (IDF), the number of individuals living with diabetes continues to rise worldwide, indicating an urgent need for safer and more effective therapeutic approaches.[1]

Conventional antidiabetic drugs, including sulfonylureas, biguanides, and

thiazolidinediones, though effective, often produce adverse effects such as hypoglycemia, weight gain, and gastrointestinal disturbances.[2] These limitations have increased scientific interest in medicinal plants rich in bioactive phytochemicals. Among these, flavonoids have drawn considerable attention due to their antioxidant, insulin-mimetic, and glucose-lowering activities.[3] They can influence key biochemical pathways involved in glucose metabolism, such as modulation of glucose transporters, inhibition of carbohydrate-digesting enzymes, and enhancement of insulin sensitivity.

Coccinia grandis (Ivy gourd), belonging to the family Cucurbitaceae, is traditionally used across India and Southeast Asia for its antidiabetic, anti-inflammatory, and antioxidant properties.[4] The plant contains various phytoconstituents, including alkaloids, terpenoids, glycosides, and notably flavonoids, which are believed to be the primary contributors to its antidiabetic effects.[5] Studies have shown that extracts of *Coccinia grandis* exhibit significant hypoglycemic activity in diabetic animal models, supporting its traditional use.[6]

However, limited studies have focused specifically on the flavonoid-rich fraction of its methanolic extract. Isolation and evaluation of this fraction can provide deeper insight into its mechanism of action and therapeutic potential. Therefore, the present investigation aims to evaluate the anti-diabetic activity of the flavonoid-rich fraction from the methanolic extract of *Coccinia grandis* using appropriate experimental models.

Material and Methods

Material

The materials used for this investigation included fresh fruits of *Coccinia grandis*, which were collected, cleaned, shade-dried, and powdered for extraction. Methanol and petroleum ether (analytical grade) were used as extraction solvents. Gallic acid and quercetin served as standard compounds for determining total phenolic and flavonoid contents, respectively. Reagents such as Folin–Ciocalteu reagent, aluminum chloride, and other analytical chemicals were employed for phytochemical and spectrophotometric analyses. Silica gel (60–120 mesh) was used for column chromatography to isolate fractions from the methanolic extract. For in vivo antidiabetic studies, healthy Wistar albino rats, alloxan monohydrate (for diabetes induction), and standard laboratory feed and reagents were utilized. All chemicals and solvents used were of analytical grade to ensure accuracy and reproducibility of results.

Methods

Collection and Identification of the Plant

Fresh fruits of *Coccinia grandis* were procured from local roadside vendors in Gwalior. The plant material was taxonomically identified and authenticated by a qualified botanist at RB Science, Bhopal, and a voucher specimen was retained for future reference.

Preparation of Plant Material for Extraction

The collected fruits were thoroughly washed with tap water to remove adhering dust and impurities. They were then shade-dried at room temperature, pulverized into coarse powder, and stored in airtight containers until further use.**Extraction of the Fruit Peel**

A quantity of 65 g of the powdered fruit peel was first defatted using hexane at room temperature for 24 hours. After defatting, the dried marc was transferred to a Soxhlet apparatus and subjected to hot continuous extraction with methanol for about 7.5 hours. The obtained extract was filtered, and the solvent was evaporated using a water bath. The concentrated extract was then placed in a desiccator to remove residual moisture. The dried methanolic extract was stored in a desiccator for subsequent phytochemical screening and pharmacological evaluation.[7]

Phytochemical screening of plant material

The methanolic extract was subjected to preliminary qualitative phytochemical analysis to determine the presence or absence of major secondary metabolites. Standard chemical tests were performed for triterpenes/steroids, alkaloids, glycosides, flavonoids, saponins, tannins, and phenolic compounds. The development of characteristic color changes or precipitate formation was considered as a positive indication for each class of phytoconstituents.[8]

Quantitative Estimation of Total Phenolics

The total phenolic content of the extract was determined using the Folin–Ciocalteu colorimetric method, with gallic acid employed as the reference standard. Briefly, 200 µL of the extract was mixed with 1.4 mL of purified water followed by 100 µL of Folin–Ciocalteu reagent. After incubation at room temperature for 15 minutes, 300 µL of 20% Na_2CO_3 solution was added, and the mixture was incubated for an additional 2 hours. The absorbance was recorded at 760 nm using a UV–Visible spectrophotometer. Standard gallic acid solutions (10–100 ppm) were similarly processed to construct a calibration curve. A reagent blank containing methanol and all reagents was prepared under identical

conditions. Results were expressed as milligrams of gallic acid equivalents (GAE) per 100 g of dry sample.[9]

Quantitative Estimation of Total Flavonoids

Total flavonoid content was estimated using the aluminum chloride ($AlCl_3$) colorimetric method. Quercetin (50 mg) was dissolved in 50 mL of methanol, and aliquots ranging from 25–150 µg/mL were prepared to generate a standard curve. A total of 0.1 g of dried extract was extracted with 10 mL methanol, filtered, and the final volume adjusted to 100 mL. From this, 1 mL of extract (1 mg/mL) was mixed with 1 mL of 2% $AlCl_3$ solution and allowed to stand for 60 minutes at room temperature. Absorbance was measured at 420 nm.[10]

Thin Layer Chromatography (TLC) of Extract

The methanolic extract was dissolved in methanol and applied to precoated silica gel GF_{254} TLC plates. The chromatograms were developed using a mobile phase consisting of Toluene:Ethyl Acetate:Formic Acid (5:4:0.2). Developed plates were visualized under UV light at 254 nm and 365 nm to detect phytoconstituents.

Chromatographic Fractionation of the Extract

Column Packing

Column chromatography was performed using the wet packing method. Activated neutral silica gel was prepared as a slurry in the selected solvent system and slowly poured into the column. Continuous tapping was carried out to prevent the formation of air bubbles and to ensure uniform packing. The solvent level was maintained at 2–3 cm above the silica bed.

Preparation of Sample

Approximately 10 g of the crude extract was triturated with 50 g of silica gel (60–120 mesh) and a small volume of solvent until a free-flowing homogeneous mixture was obtained.

Loading of Sample

The prepared sample mixture was carefully introduced into the column using a hollow glass cylinder to avoid disturbing the stationary phase. The mobile phase was then added for elution.

Collection of Fractions

The solvent mixture Toluene:Ethyl Acetate:Formic Acid (5:4:0.2) was used for elution. Fractions of approximately 10 mL each were collected and analyzed by TLC on silica gel plates. Fractions exhibiting identical Rf values were pooled and concentrated for further analysis.[11]

Determination of Total Flavonoid Content of Fractions

The pooled chromatographic fractions were subjected to quantitative flavonoid estimation using the $AlCl_3$ colorimetric

method described.

Pharmacological Evaluation

Animals

Healthy male Wistar rats weighing 180–250 g were selected for the study. Animals were housed under standard laboratory conditions (12-hour light/dark cycle, temperature 17–26 °C) with free access to standard pellet diet and water. They were fasted for 12 hours prior to experimentation but had unrestricted access to water.

Acute Toxicity Study[12]

Acute toxicity was evaluated following OECD guidelines. Three rats received a single oral dose of the flavonoid-rich fraction (2000 mg/kg). Animals were observed individually during the first 30 minutes, periodically for the next 24 hours, and daily for 14 days to monitor changes in skin, fur, eyes, mucous membranes, respiration, autonomic responses (salivation, perspiration, urination, defecation), central nervous system activity (tremors, convulsions, drowsiness), cardiovascular signs, and mortality.

Induction of Diabetes

After overnight fasting (12 hours), diabetes was induced by intraperitoneal administration of freshly prepared streptozotocin (STZ) at a dose of 55 mg/kg body weight in cold 0.1 M citrate buffer (pH 4.5). To prevent acute hypoglycemia, rats were provided with 5% glucose solution overnight. Animals showing persistent hyperglycemia with fasting blood glucose levels >250 mg/dL on the third day post-STZ injection were considered diabetic and included in the study.[13]

Experimental Design

Animals were divided into five groups (n=6 per group):

Group I (Normal control): received distilled water.

Group II (Diabetic control): received STZ (55 mg/kg, i.p.).

Group III: diabetic rats treated with Glibenclamide (5 mg/kg, p.o.).

Group IV: diabetic rats treated with flavonoid-rich fraction (200 mg/kg, p.o.).

Group V: diabetic rats treated with flavonoid-rich fraction (400 mg/kg, p.o.).

Treatment continued for 21 days. Fasting blood glucose was recorded on days 0, 7, 14, and 21 using glucose oxidase–peroxidase strips and a glucometer. Blood samples were obtained from the tail vein.

Biochemical Analysis

At the last day animal was sacrificed by decapitation, blood samples were collected and serum was separated using centrifuge to study the biochemical parameters. The estimation of protein was carried out using the method of Lowry [14-15]. The extraction of serum

lipids were carried out by the method of Folch[63] and the serum cholesterol estimation was carried out by the method of Zlatkis[64] Serum triglycerides were estimated by the method of Foster and Dunn and HDL cholesterol was estimated by the method of Burstein[16]. The VLDL cholesterol was evaluated using the formula, TG/5 mg/dl. The serum LDL cholesterol was estimated by the method of Friedwald[17] SGOT and SGPT were measured by the method of Reitman and Frankel (Colorimetric method)[18] the plasma creatinine was measured by Jaffe's method[19] and serum urea was measured by the diacetyl monoxime method[20-22].

Results and Discussion

The present study focused on the extraction, phytochemical profiling, chromatographic separation, toxicity evaluation, and antidiabetic assessment of the methanolic extract and its isolated fractions of *Coccinia grandis* fruit. As shown in Table 1, the methanolic extract provided a substantially higher extraction yield (18.16%) compared to the petroleum ether extract (1.56%). This significant difference highlights the ability of polar solvents to extract bioactive compounds more efficiently. The dark brown, sticky nature of the methanolic extract also suggests the presence of polyphenolic and flavonoid-rich constituents.

Phytochemical screening results presented in Table 2 further support this observation, revealing that the methanolic extract contains a broad spectrum of secondary metabolites including alkaloids, phenolics, flavonoids, tannins, steroids, triterpenoids, and saponins. In contrast, the petroleum ether extract contained limited phytochemicals. Such findings are in agreement with previous reports that describe *Coccinia grandis* as a phytochemically rich plant traditionally used in metabolic and inflammatory disorders. In addition, quantitative estimation of the methanolic extract demonstrated high total phenolic content (88.03 mg GAE/g) and total flavonoid content (71.31 mg QE/g), as shown in Table 3, confirming its strong antioxidant potential.

Further chromatographic analysis yielded eight distinct fractions from the methanolic extract (Table 4). TLC analysis visualized under 365 nm and 254 nm (Figure 1) confirmed the presence of several major and minor phytoconstituents. Among the isolated fractions, Fraction F2 showed the highest flavonoid concentration (138.37 mg QE/g), followed by F3 (105.43 mg QE/g), as shown in Table 5. These quantitative data justified the selection of F2 for

subsequent pharmacological evaluation, as flavonoids play a major role in regulating glucose metabolism, lipid homeostasis, and oxidative stress.

Acute toxicity evaluation of Fraction F2 (Table 6) demonstrated that it was safe up to 2000 mg/kg, with no observed mortality or behavioral abnormalities. This confirms that the fraction is non-toxic and suitable for in vivo antidiabetic studies.

The experimental results of body weight variation (Table 7) revealed that diabetic control animals exhibited significant weight loss throughout the study period, a typical symptom of uncontrolled diabetes. In contrast, animals treated with Fraction F2 showed a gradual improvement in body weight from Day 7 onward, indicating enhanced carbohydrate utilization and restoration of metabolic balance. This improvement suggests that F2 may have protective effects against diabetes-induced muscle wasting.

One of the most significant findings of this study is the strong antihyperglycemic effect of Fraction F2. As shown in Table 8, diabetic control animals exhibited a progressive rise in blood glucose levels from Day 0 to Day 21. However, treatment with F2 led to a dose-dependent reduction in blood glucose levels, with the highest dose (Group V) showing near-normalization by Day 21. These improvements may be attributed to enhanced insulin sensitivity, improved pancreatic β-cell activity, or inhibition of carbohydrate-metabolizing enzymes—mechanisms commonly associated with flavonoid-rich extracts.

Fraction F2 also demonstrated potent antihyperlipidemic activity. The lipid profile data summarized in Table 9 show that diabetic animals displayed elevated levels of total cholesterol, triglycerides, LDL, and VLDL, along with reduced HDL. Treatment with F2 significantly improved all lipid parameters, particularly by decreasing LDL and VLDL while enhancing HDL levels. These properties indicate strong cardioprotective effects, likely due to the antioxidant and lipid-regulating potential of flavonoids.

Similarly, the biochemical parameters listed in Table 10 indicate that diabetes induced significant hepatic and renal disturbances, as reflected by elevated SGOT, SGPT, creatinine, and urea levels in the diabetic control group. Treatment with Fraction F2 resulted in a marked reduction of these elevated markers, suggesting hepatoprotective and nephroprotective activities. These protective effects are likely associated with the antioxidant and membrane-stabilizing properties of phenolic and flavonoid compounds.

The findings from all tables collectively

demonstrate that the flavonoid-rich Fraction F2 of *Coccinia grandis* exhibits significant antihyperglycemic, antihyperlipidemic, hepatoprotective, and nephroprotective activities. The phytochemical richness of the extract, high antioxidant content, and strong in vivo efficacy validate the traditional use of *Coccinia grandis* in the management of diabetes and associated metabolic disorders. Fraction F2 thus emerges as a promising natural therapeutic agent capable of offering broad-spectrum metabolic protection.

Table 1: Extraction yield and physical characteristics of extracts

Solvent	**Yield (%)**	**Appearance**	**Texture**
Petroleum Ether	1.56	Yellow	Powder
Methanol	18.16	Brown	Sticky

Table 2: Phytochemical screening of *Coccinia grandis* extract

Phytochemical Test	**Observation**	**Pet. Ether Extract**	**Methanolic Extract**
Alkaloids			
Mayer's Test	Cream-colored precipitate	–	+
Hager's Test	Yellow precipitate	–	+
Wagner's Test	Reddish-brown precipitate	–	+
Dragendorff's Test	Reddish-brown precipitate	–	+
Glycosides			
Legal Test	Pink/red coloration	–	–
Baljet Test	Orange/yellow coloration	–	–
Keller–Kiliani Test	Reddish-brown color in acid layer	–	–
Phenolics			
Ferric Chloride Test	Blue/green coloration	–	+
Lead Acetate Test	Yellow precipitate	–	+
Flavonoids			
Shinoda Test	Red coloration	–	+
Alkaline Reagent Test	Yellow color turning red on acidification	–	+
Proteins			

Biuret Test	Violet color	–	–
Ninhydrin Test	Purple/blue color	–	–
Triterpenoids			
Libermann–Burchard Test	Deep red color	+	+
Salkowski Test	Yellow color in lower layer	+	–
Carbohydrates			
Molisch's Test	Violet ring	+	+
Steroids			
Libermann–Burchard Test	Brown ring; upper layer turns green	+	+
Salkowski Test	Red color in chloroform layer	+	–
Tannins			
Lead Acetate Test	Yellow/red coloration	–	+
Saponins			
Foam Test	Persistent foam (1 cm)	–	+

Table 3: Total phenolic and total flavonoid content of methanolic extract of *Coccinia grandis*

Parameter	**Value (mg/g extract)**	**Method Used**
Total Phenolic Content (TPC)	88.03 mg GAE/g	Folin–Ciocalteu Method
Total Flavonoid Content (TFC)	71.31 mg QE/g	Aluminum Chloride Colorimetric Method

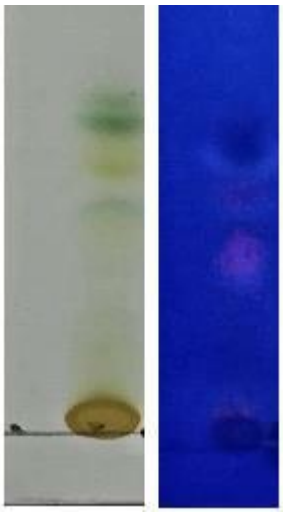

Figure 1: TLC of extract (A) 365 nm (B) 254 nm

Table 4: Fractions obtained from column chromatography of methanolic extract of *Coccinia grandis*

Fraction Collected	Rf Value
Fraction 1	0.68
Fraction 2	0.68
Fraction 3	0.68
Fraction 4	0.47
Fraction 5	0.47
Fraction 6	0.34
Fraction 7	0.34
Fraction 8	0.29

Table 5: Total flavonoid content in chromatographic fractions of *Coccinia grandis*

Fraction	Absorbance at 420 nm	Total Flavonoids (QE mg/g)
F1	0.398	79.35
F2	0.699	138.37
F3	0.531	105.43
F4	0.158	32.29

Table 6: Results of Acute Toxicity Study of Fraction F2

Group	Fraction	Number of Mice (n)	Dose (mg/kg)	Deaths (n)	Survival (n)	Mortality Rate (%)
1	F2	3	2000	0	3	0

Table 7: Effect of Fraction F2 on body weight in experimental animals

Group	Body Weight (g)			
	Day 0	Day 7	Day 14	Day 21
I	198.3 ± 1.910	197.5 ± 2.257	208.1 ± 1.033	216.4 ± 1.161
II	200.4 ± 2.010	160.1 ± 1.042	144.3 ± 1.266	135.2 ± 0.964
III	195.3 ± 1.930	184.2 ± 2.010	185.4 ± 1.033	193.6 ± 1.780
IV	194.4 ± 3.165	174.3 ± 2.026	175.5 ± 0.933	180.1 ± 1.033
V	197.6 ± 2.186	180.4 ± 1.865	182.3 ± 1.166	186.8 ± 1.303

Table 8: Effect of fraction F2 on blood glucose levels

	Blood Glucose (mg/dl)			
Group	Day 0	Day 7	Day 14	Day 21
I	94.56 ± 3.030	96.73 ± 3.636	98.97 ± 1.650	94.42 ± 2.066
II	101.43 ± 3.365	245.12 ± 1.822	278.25 ± 2.010	287.71 ± 1.495
III	105.51 ± 4.875	168.72 ± 1.529	140.95 ± 1.541	127.18 ± 1.757
IV	103.26 ± 2.257	226.57 ± 1.487	208.53 ± 2.088	168.13 ± 1.533
V	98.61 ± 3.010	187.68 ± 1.445	169.74 ± 1.341	130.28 ± 1.468

Table 9: Effect of Fraction F2 on Lipid Profile

Group	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
I	105.2 ± 0.357	85.7 ± 0.325	60.27 ± 0.338	40.4 ± 0.443	18.96 ± 0.46
II	122.36 ± 0.319	169.2 ± 0.440	33.65 ± 0.560	138.43 ± 0.710	40.71 ± 0.640
III	119.71 ± 0.567	95.16 ± 0.510	55.71 ± 0.960	85.95 ± 0.421	25.63 ± 0.382
IV	163.38 ± 0.921	133.42 ± 0.287	46.36 ± 0.733	118.12 ± 0.490	35.64 ± 0.295
V	142.73 ± 0.931	105.3 ± 0.817	58.12 ± 0.355	97.48 ± 0.157	29.48 ± 0.428

Table 10: Effect of Fraction F2 on Biochemical Parameters (SGOT, SGPT, Creatinine, Urea)

Group	Serum Urea (mg/dl)	Creatinine (mg/dl)	SGOT (U/L)	SGPT (U/L)
I	6.38 ± 0.167	0.54 ± 0.018	46.78 ± 0.21	22.80 ± 0.21
II	5.24 ± 0.143	1.46 ± 0.037	113.03 ± 0.46	47.81 ± 0.45
III	6.48 ± 0.182	0.53 ± 0.015	44.86 ± 0.85	25.86 ± 0.54
IV	6.23 ± 0.217	1.13 ± 0.026	64.83 ± 0.91	38.13 ± 0.69
V	6.82 ± 0.138	0.68 ± 0.018	51.86 ± 0.63	30.46 ± 0.33

Conclusion

The study confirms that the methanolic extract of *Coccinia grandis*, especially the flavonoid-rich Fraction F2, exhibits strong antidiabetic, antihyperlipidemic, hepatoprotective, and nephroprotective effects in alloxan-induced diabetic rats. Fraction F2 was found to be safe up to 2000 mg/kg and significantly improved glucose levels, lipid profile, and biochemical markers. These findings validate the traditional use of *Coccinia grandis* in diabetes management and highlight Fraction F2 as a promising natural candidate for treating metabolic disorders.

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