Potential of peel extracts of *Punica granatum* and *Citrus aurantifolia* on alloxan-induced diabetic rats

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**Abstract**

**Background:** Peel is one of the major by-products in fruit processing industry. Fruit products (non-edible parts) are also considered as waste products and often discarded in the environment. Fruit peels are now serving as one of the primary sources for isolation and extraction of secondary metabolites in pharmaceutical industry. The present investigation was carried out to screen the phytochemical constituents and HPTLC analysis of peel extracts of *Punica granatum* and *Citrus aurantifolia* and their antidiabetic potential in alloxan-induced diabetic rats.

**Results:** Among the different solvent extracts, methanol solvent extract was found to possess more amounts of secondary metabolites. In addition, HPTLC analysis of the plant samples revealed the presence of 13 peaks in both the plants by using gallic acid as marker. Different biochemical parameters such as blood glucose, cholesterol, protein, urea, creatinine, and triglycerides level were subjected for estimation by collecting the blood samples from the treated diabetic rats after 21 days. A sharp decline in blood glucose, cholesterol, triglycerides, creatinine, and urea level was noticed when methanolic extracts of *Punica granatum* and *Citrus aurantifolia* were given to experimental animals when compared with negative control. However, protein and weight of the animal were found to be enhanced when treated with methanolic extracts of both the plants.

**Conclusion:** It can be concluded that fruit peels of both the plants exhibited antidiabetic potential on alloxan-induced diabetic rats which can be attributed to wide range of active pool of secondary metabolites. Further, screening and isolation of secondary metabolites along with their mode of action is required for effective use of plant-based drugs as antihyperglycemic agent.

**Keywords:** Fruit Peel, *Punica granatum*, *Citrus aurantifolia*, Antidiabetic activity, Gallic acid, HPTLC analysis

**1 Background**

Diabetes mellitus (DM) is a major chronic metabolic disorder and an extremely serious condition from both clinical and public health standpoints. It is recorded that every 5th Indian have diabetes by 2025, it may be assumed 40 million diabetics in India expected to be 70 million by 2025 [1]. Diabetes mellitus can directly affect serum lipid levels causing diabetic dyslipidemia which is one of its complications [2]. Among the available therapeutic agents, insulin, metformin, sulfonylureas (SU), and thiazolidinediones (TZDs) are mostly used for the control of diabetes [3]. Plants are an excellent source of drugs, and many of the currently available drugs have been derived directly or indirectly from them [4]. It is obvious due to the richness and complexity of the compounds in plants. A multiple targeting is a double-edged sword in diabetes therapies. The multiple targets associated with antidiabetic herbal medicine could play a beneficial role in the control of diabetics.

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Fruit and vegetable wastes are used to extract and isolate potential bioactive compounds and used in the food, pharmaceutical, cosmetics, and textile industries. Studies have revealed that appreciable quantity of phytochemical compounds and other essential nutrients are present in the seeds, peels, and other components of fruits and vegetables [5]. Likewise, several works have confirmed that fruit peel parts possess phenolic compounds and exhibit higher antioxidant activity [6]. Even though several attempts are carried out on antidiabetic activities of medicinal plants and animal-based studies, studies regarding fruit peels are scanty. Therefore, the present study was made to evaluate the antidiabetic potential of fruit peel of Punica granatum and Citrus aurantifolia on alloxan-induced diabetic rats and identify the bioactive compounds using HPTLC analysis.

2 Methods

2.1 Collection of plant materials
The fruits of Citrus aurantifolia and Punica granatum which were procured from Tamil Nadu Agricultural College and Research Institute (TNAU), Madurai, Tamil Nadu was identified and authenticated by an botanist (also an expert in plant taxonomist) from our institute by referring standard taxonomic characteristic features (keys) according to Flora of Madras Presidency [7] and the Flora of Tamil Nadu Carnatic [8]. The voucher samples (SN-9718) and photographs were deposited in the institute for future reference.

2.2 Selection and maintenance of experimental animals
Albino Wister strain rats of both sex, 6 months old that weighed 180-220 g with a mean weight of 200 g were used in this study. The animals were allowed to acclimatize for 2 weeks in the animal house at the Department of Pharmacy in Ultra College of Pharmacy, Madurai. The rats were housed in polypropylene cages, maintained under standard laboratory conditions. Animals were fed with standard laboratory pellets diet and water. Animal studies were done at Department of Pharmacy in Ultra College of Pharmacy, Madurai and experimental protocols and procedures were approved by Institute of Animal Ethics Committee of Ultra College of Pharmacy Institute (approval number: MKU/IAEC/KMCP/88/P9718 Ph.D./2013).

2.3 Extraction of Punica granatum and Citrus aurantifolia
The fruit peel of Punica granatum and Citrus aurantifolia were washed thoroughly with running tap water to remove dust particles, adhering epiphytes, etc. They were air-dried and crushed into powder in a grinding machine. The powder (0.5 kg) was extracted in an Erlenmeyer flasks with different solvents, viz., 90% petroleum ether, ethyl acetate chloroform and methanol, and aqueous at room temperature. The whole extract was combined, filtered (Whatman filter paper no.1), and concentrated at 40 °C in vacuum and finally, the extract was freeze-dried to get 50 g of crude extract.

2.4 Preparation of aqueous extract
Fresh plant materials (0.5 kg) were harvested and were surface sterilized with 0.1% (w/v) HgCl2 solution for 5 min. The plant material was grounded with mortar and pestle and the tissue was centrifuged at 3500 rpm for 20 min. The supernatant alone was taken as aqueous extract. All the extracts were concentrated by distillation and evaporated to dryness using a flash evaporator. After evaporation, each of the solvent extracts was weighed and preserved at 5 °C in airtight bottles.

2.5 Phytochemical analyses of the fruit peel of Citrus aurantifolia and Punica granatum
Phytochemical screening of crude solvent extracts were performed using the following reagents and chemicals: alkaloids with Wagner reagent, tannins with 5% ferric chloride, saponins with ability to produce foam by adding water and olive oil, carbohydrates with Molish reagents and concentrated sulfuric acid, glycosides with glacial acetic acid, ferric chloride and concentrated sulfuric acid, steroids with chloroform, acetic anhydride and concentrated sulfuric acid, terpenoids with chloroform and concentrated sulfuric acid, and fixed oil using spot and oil staining methods [9, 10].

2.6 HPTLC analysis
2.6.1 Development of chromatogram
Sample solutions were applied onto the plates with an automated Camag HPTLC system comprising of Linomat V as sample applicator (Camag, Muttenz, Switzerland) and TLC Scanner III controlled by win CATS software 1.4.3 was used for quantitative evaluation [11]. A TLC scanner III with win CATS software was used for scanning the TLC plates, and pre-coated silica gel aluminum plates 60 F254 20 × 10 cm with 0.2 mm-μm thickness (Merck, Darmstadt, Germany) were used for all determinations. The plates were pre-washed with methanol and activated at 60 °C for 5 min, prior to chromatography. Five different aliquots (2, 4, 8, 12, and 16μl) of standard solutions were applied in triplicates on 20 × 10 cm TLC plates for the preparation of the calibration curve. Six such plates were prepared. A constant application rate of 0.1 μl s−1 was employed with a bandwidth of 6 mm. Bandwidth was set at 20 nm. The mobile phase (10 ml) consisted of toluene, ethyl acetate, formic acid, and methanol (3:6: 1:6: 0.4) (v/v). The chamber saturation time for mobile phase was 15 min (optimum) at relative humidity of 60% ± 5. The chromatogram run length was 8.0 cm. After development, chromatographic
plates were dipped into derivatization reagent, i.e., modified Dragendorff reagent and again dried for 10 min using hair drier on hot mode. After drying, the plates were heated at 70°C for 15 min in a pre-heated oven. The formation of orange-colored spots corresponding to various phytocompounds of plant (fruit peel of *Citrus aurantifolia* and *Punica granatum*) extracts was observed. The plates were scanned within 10 min using a densitometric scanner III in the remission mode at 254 nm. The spots and or peaks were detected and their Rf values, and peak areas were calculated.

### 2.7 Identification of the optimum alloxan-monohydrate dose to induce diabetes

Intraperitoneal optimum dose of alloxan to induce diabetes was done by using a logarithmic scale with 5 dose levels (50, 100, 150, 200, and 250 mg/kg body weight) [12]. The doses were intraperitoneally administered in 0.1 ml physiological saline once for each level to five albino Wistar rats. The animals were frequently monitored for changes in blood sugar within 24 to 48 h. Any mortality, body weakness, or abnormality observed at any dose level was recorded. After 48 h, the diabetic animals were examined for suitability in the bioassays by measuring blood glucose levels after every 2 h consistently for 24 h. The observations were compared, and the optimum dose was selected for further studies.

#### 2.7.1 Induction of hyperglycemia

Hyperglycemia was induced in albino Wister rats from both sexes aged 6 months (180-220 g body weight) experimentally by intraperitoneal administration of a single dose of 150 mg/kg body weight (identified as optimum dose) of a freshly prepared 10% alloxan monohydrate (2,4,5,6 tetraoxypyrimidine; 5-6-dioxypyrimidin), obtained from Sigma (Steinhein, Switzerland). Blood glucose level was measured using a glucometer after 48 h. Rats with fasting blood glucose ≥ 200 mg/dl were considered as diabetic. Before initiation of this experiment, the animals were fasted for 8-12 h but providing water until the end of this experiment and maintained at room temperature in plastic cages [13].

#### 2.7.2 Acute toxicity study

Acute toxicity was calculated and determined according to the method of Lorke [14]. Wistar albino rats weighing between 150 and 220 g were used for the study. In the first phase, rats were distributed to four groups of four animals each with four groups as control (1 ml saline). Methanolic extracts of *Citrus aurantifolia* and *Punica granatum* (250, 500, 1000, 1500, 2000 mg/kg) were administered. In the second phase, three higher doses of 1000, 1500, and 2000 mg/kg of the extract were administered to each rat from each group. The animals were monitored for 24 h for behavior and mortality. Further, they were all placed for more observation for 72 h (3 days) for the signs of physical changes, lethality, toxic symptoms, behavioral changes, or deaths.

#### 2.7.3 Experimental design

Among the different solvents used, only methanolic extracts were taken and used to determine the hypoglycemic activity of *Punica granatum* and *Citrus aurantifolia*. After observing the acute toxicity study, 500 mg of methanol extract of *Citrus aurantifolia* and *Punica granatum* were selected as dosage for this study. Doses were prepared in distilled water by dissolving 500 mg of methanol extract of *Punica granatum* and *Citrus aurantifolia* with 1% Tween-80 as a surfactant. The experimental animals with an average weight of 180-220 g were randomly divided into five groups of five animals each. The extracts were administered orally for 28 days.

- **Group I** consisted of normal rats orally administered with 1.0 ml physiological saline and fed with normal pellet diet as positive control.
- **Group II** consisted of alloxan-induced diabetic rats orally administered with 1.0 ml physiological saline, as diabetic control (negative control).
- **Group III** consisted of alloxan-induced diabetic rats orally administered with 1.8-2.2 mg of glibenclamide, standard commercial drug (10 mg/kg body weight) in 1.0 ml physiological saline.
- **Group IV** consisted of alloxan-induced diabetic rats orally administered with peel extract of *Citrus aurantifolia* (500 mg/kg body weight) in 1.0 ml physiological saline.
- **Group V** consisted of alloxan-induced diabetic rats orally administered with peel extract of *Punica granatum* (500 mg/kg body weight) in 1.0 ml physiological saline respectively.

#### 2.7.4 Duration of treatment

The extracts/drug treatment was given orally for 28 days. Fasting blood glucose levels were estimated on 0, 7, 14, 21, and 28 days. Similarly, the blood samples were withdrawn from all animals at 7 days interval up to 28 days by retro-orbital plexus. Serum glucose [15], serum protein [16], serum cholesterol [17], serum triglycerides [18], serum creatinine [19], and serum urea [20] were estimated using standard procedures. The initial body weight and final body weight were also measured. Finally, euthanasia was performed by cervical dislocation under deep anesthesia with 5% isoflurane.

#### 2.8 Statistical analysis

The data were collected from five replicates of each experimental study. Values were expressed as mean of total number of replicates ± SE and compared with diabetic control.
3 Results and discussions

3.1 Acute toxicity study

During the acute toxicity study, the extracts did not produce any drug-induced harmful physical signs and no mortality was detected. There was no lethality and no toxic reaction was observed at any of the doses selected till the end of the treatment. This designates the two extracts as safety product.

3.2 Phytochemical screening

Differential occurrence of phytocompounds was observed when different solvent extracts of *Punica granatum* and *Citrus aurantifolia* were subjected to phytochemical screening. Among various solvents used, methanolic extract was found to show positive results for the majority of compounds when compared to aqueous, ethyl acetate, petroleum ether, and chloroform solvent. Methanolic peel extracts of *Punica granatum* showed positive response for alkaloids, carbohydrates, saponins, phenols, tannins, flavanoids, gums, and mucilages (Table 1). Similarly, methanolic extracts of *Citrus aurantifolia* peel exhibited the presence of phenolic compounds, tannins, flavonoids and glycosides, alkaloids and carbohydrates (Table 1).

3.3 HPTLC analysis

HPTLC chromatograms of methanolic solvent extracts were scanned under UV 254 nm. The densitometric scanning at 254 nm revealed that the methanol extract exhibited 12 peaks in *Citrus aurantifolia* in 4.0 μl concentration in the chromatogram with start Rf value at 0.46 and end Rf value at 0.55. The chromatogram starts at the height of 6.7 and ends at 0.4. The highest peak (8th peak) in the chromatogram was appeared at Rf value 0.50 as for the respective marker gallic acid and it showed peak area of 7873.1. Extracts of *Punica granatum* fruit peel exhibited 13 peaks with different Rf values in the chromatogram. The peaks appeared to show start Rf at 0.45 and end Rf at 0.54 in 4.0 μl concentration. The chromatogram showed start height at 0.5 and end height at 3.3. The 10th peak in the chromatogram of *Punica granatum* peels appeared at the same Rf value (0.50) as that of the respective marker (standard gallic acid) with peak area 9919.2 (Fig. 1).

3.4 Antidiabetic potential of fruit peel of *Punica granatum* on alloxan-induced diabetic rats

3.4.1 a) Biochemical parameters

As expected, the blood glucose level of untreated diabetic rats (negative control) continued to increase significantly from day 1 to day 28. Methanolic peel extracts of *Punica granatum* exhibited 39% reduction in fasting blood glucose level at the end of day 28 whereas standard drug reduced the blood sugar level by 44%. In the case of blood glucose level, the diabetic animals treated with standard drug glibenclamide recorded a reduction of 59% in blood glucose level when compared to individual plant extracts of *Punica granatum* (47%). Treatment of diabetic rats with methanolic peel extracts of *P. granatum* and glibenclamide for 28 days exhibited the same level of reduction in serum cholesterol level (58%). But in the case of serum triglycerides level, diabetic rats, when administered with standard drug glibenclamide, brought down the level triglycerides by 56%. But, *P. granatum* reduced the triglyceride level by 64% which was found to be higher than standard drug. Serum creatinine level was found to be reduced by 59% in those rats

| Table 1 Phytochemical screening of extracts of fruit peel of *Citrus aurantifolia* and *Punica granatum* |
|---|---|---|---|---|---|---|---|---|---|
| Extracts | *Citrus aurantifolia* | | | | | | | | |
| | Petroleum ether | Chloroform | Ethyl acetate | Methanol | Water | Petroleum ether | Chloroform | Ethyl acetate | Methanol | Water |
| Alkaloid | - | - | + | + | - | - | - | + | - |
| Carbohydrates | - | - | + | + | - | - | - | + | - |
| Saponin | - | - | - | - | - | - | - | + | - |
| Triterpenoids | - | - | - | - | - | + | - | - | - |
| Phenolic compounds and tannins | - | - | ++ | ++ | - | - | - | ++ | - |
| Proteins and amino acids | - | - | - | - | - | - | - | - | + |
| Steroids and sterols | + | + | + | + | - | + | - | - | - |
| Fixed oil and fat | - | - | - | - | - | - | - | - | - |
| Flavones and flavonoids | - | - | ++ | ++ | - | - | - | ++ | - |
| Glycosides | - | - | + | + | - | + | + | + | + |
| Gums and mucilages | - | - | - | - | - | - | - | + | - |

+++Maximum
++Moderate
+Minimum, not detected
a) HPTLC chromatogram of methanolic extract of *C. aurantifolia*

![HPTLC chromatogram of methanolic extract of *C. aurantifolia*](image)

| Peak | Start Rf | Start Height | Max Rf | Max Height | % Max | End Rf | End Height | Area | % Area | Assigned Substance |
|------|----------|-------------|--------|------------|-------|--------|-----------|------|--------|-------------------|
| 1    | 0.46     | 6.7         | 0.50   | 308.7      | 100.00| 0.55   | 0.4       | 7873.1| 100.00 | gallic acid       |

b) HPTLC chromatogram of methanolic extract of *P. granatum*

![HPTLC chromatogram of methanolic extract of *P. granatum*](image)

| Peak | Start Rf | Start Height | Max Rf | Max Height | % Max | End Rf | End Height | Area | % Area | Assigned Substance |
|------|----------|-------------|--------|------------|-------|--------|-----------|------|--------|-------------------|
| 1    | 0.45     | 0.5         | 0.50   | 395.3      | 100.00| 0.54   | 3.3       | 9919.2| 100.00 | gallic acid       |

**Fig. 1 a** HPTLC chromatogram of methanolic extract of *C. aurantifolia*. **b** HPTLC chromatogram of methanolic extract of *P. granatum*
which were fed with P.granatum peel extract. But, 69% of reduction in serum creatinine was observed when diabetic rats were fed with standard drug glibenclamide. It was clear that the plant extract exhibited 10% less reduction efficiency as compared to glibenclamide. At the end of day 28, diabetic rats that received peel extract of P.granatum brought down the level of serum urea by 32%. But, in glibenclamide treated animals, serum urea level was found to be reduced by 41% which is found to be maximum when compared to individual plant extract. Protein content was found to be enhanced in the diabetic rats, which were supplemented with Punica grana-
tum fruit peel and glibenclamide. In the untreated group, there was a gradual reduction in serum protein level from day 1 to day 28 over control. Intraperitoneal administration of Punica grana-
tum peel extracts to alloxan-induced diabetic rats enhanced the protein level in serum by about 38% over negative control at day 28. Administration of standard drug glibenclamide also increased the protein level to about 53% which was comparatively higher when compared to P.granatum treatment (Table 2).

3.4.2 b) Body weight
A gradual increase in the body weight of both the nor-
mal and control rats and treated diabetic rats was no-
ticed throughout the study. Continuous oral treatment of diabetic rats increased the body weight by 12%. Fur-
ther, body weight of the diabetic rats was found to be increased by 14% when rats were fed with glibenclamide drug (Table 2).

3.5 Antidiabetic potential of fruit peel of Citrus auranti-
folia on alloxan-induced diabetic rats
3.5.1 a) Biochemical parameters
Alloxan induced diabetic rats when treated with gliben-
clamide and methanolic extracts of Citrus aurantifolia fruit peel showed a gradual decrease in fasting blood glucose level. As expected, the blood glucose level of un-
treated diabetic rats (diabetic control) continued to in-
crease significantly on the following days. Peel extracts of C. aurantifolia exhibited 41% reduction in the fasting blood glucose level whereas glibenclamide reduced the fasting blood glucose level by 42%. In the case of blood glucose level, diabetic animals treated with standard drug glibenclamide recorded a reduction of 59% whereas Citrus aurantifolia fruit peel reduced the blood glucose level by 54%. As the days were progressing, on 28th day, cholesterol level was found to be decreased in alloxan-
induced diabetic rats by 56% and 57% when treated with methanolic peel extracts of C. aurantifolia and glibenclam-
vide respectively. Diabetic rats that received C. auranti-
folia peel extract reduced the serum triglycerides level by 65% which was found to be higher than glibenclamide treatment (56%). Moreover, at the end of 28 days treat-
ment, a decrease in serum creatinine by 56% and serum urea level by 37% was observed in those diabetic rats which were supplemented with C. aurantifolia peel ex-
tract. But, glibenclamide treated animals exhibited 69% of reduction in serum creatinine and 41% of reduction in serum urea which was found to be more when com-
pared to individual plant extracts. However, the level of protein was influenced considerably by the standard drug and the plant extract. An increase in protein content by 43% was observed in those rats which were ad-
ministered with C. aurantifolia peel extracts whereas standard drug glibenclamide also increased the protein level to about 53% which was comparatively higher than C. aurantifolia extracts (Table 3).

3.5.2 b) Body weight
In case of body weight, a gradual increase in body weight was noticed as the days of treatment was progressed. At the end of 28th day, 12% and 14% increase in body weight was recorded in diabetic rats which were fed with C. aurantifolia peel extracts and glibenclamide drug treatment (Table 3).

In general, P.granatum and C. aurantifolia peel ex-
tracts exhibited antidiabetic potential which was found to be equal to the performance of standard drug gliben-
clamide. Both P.granatum and C. aurantifolia extracts exhibited a positive change in all biochemical constitu-
ents when compared to diabetic control. The antidia-
betic potential of peel extracts of P.granatum and C. aurantifolia may be attributed to the qualitative and quantitative occurrence of phytochemical compounds that could have regulated the carbohydrate metabolism and increasing the secretion of β cells in islets of the pancreas and thus exhibiting hypoglycemic potential.

4 Discussion
Several authors have reported that the red color of peel may be attributed to anthocyanidins, flavanols, flavones and flavanones, punicalin and punicalagin and these compounds exhibit antidiabetic potential [21, 22]. The inedible Punica grana-
tum peel contains as much as three times the total amount of polyphenols, including condensed tannins and catechins, gallo catechins, and prodelphinidins [23]. The antidiabetic activity of medici-
nal plants may be due to the presence of phenolic compounds, alkaloids, terpenoids, and flavonoids [24–26]. Preliminary screening of C. aurantifolia fruit and other parts showed the presence of alkaloids, caroten-
oids, coumarins, essential oils, flavanoids, tannins, sapo-
nins, steroids, cardiac glycosides, carbohydrates, phenols, and reducing sugars [27–30] Besides citrus pulps, citrus peels are also a promising source of bioactive com-
ounds, which are rich in carotenoids, phenolic acids,
Table 2 Effect of methanolic peel extracts of *Punica granatum* on biochemical parameters of alloxan-induced diabetic rats

| Biochemical parameters | Diabetic control | Glibenclamide | Punica granatum |
|------------------------|------------------|---------------|-----------------|
|                        | Day 1 | Day 7 | Day 14 | Day 21 | Day 28 | Day 1 | Day 7 | Day 14 | Day 21 | Day 28 |
| Fasting Blood Glucose (mg/dl) | 91    | 105   | 111    | 116   | 121   | 73   | 71    | 68    | 67    | 67    | 79   | 73   | 71    | 68    | 67    | 67    | 74   | 74    | 74    | 74    | 74    |
| Blood Glucose (mg/dl)    | 210.3 | 215.4 | 236.5  | 278.1 | 295.7 | 205.2| 189.2 | 154.6 | 130.3 | 126   | 208.3| 179.6| 163.4 | 150.3 | 156.7 | 156.7 |
| Triglycerides (mg/dl)    | 178.2 | 186.5 | 192    | 201.3 | 210.4 | 130.1| 92.4  | 85.3  | 78.6  | 72.1  | 140.5| 110.2| 97.73 | 84.6  | 74.9  | 74.9  |
| Cholesterol (mg/dl)      | 178.2 | 180.1 | 188.5  | 198.6 | 218.6 | 182.8| 163.6 | 154.3 | 111.6 | 94.6  | 190.2| 165.2| 143.2 | 102.6 | 95.4  | 95.4  |
| Urea (mg/dl)             | 45.37 | 48.9  | 54.76  | 62.18 | 67.2  | 48.1 | 47.3  | 47.2  | 41.3  | 40.1  | 54.6 | 52.2 | 51.7  | 49.8  | 41.9  | 41.9  |
| Creatinine (mg/dl)       | 1.21  | 1.32  | 1.41   | 1.47  | 1.52  | 1.17 | 0.97  | 0.67  | 0.59  | 0.47  | 1.16 | 1.02 | 0.97  | 0.84  | 0.63  | 0.63  |
| Protein (g/dl)           | 5.22  | 5.01  | 4.62   | 4.07  | 3.98  | 4.92 | 5.42  | 5.72  | 5.99  | 6.1   | 4.63 | 4.92 | 5.05  | 5.39  | 5.22  | 5.22  |
| Bodyweight (g/individual)| 210   | 208   | 206    | 202   | 198   | 208 | 208   | 222   | 225   | 206   | 210 | 210 | 215   | 222   | 222   | 222   |

Values given inside the bracket are percent decrease over diabetic control
| Biochemical parameters | Diabetic control | Glibenclamide | Citrus aurantifolia |
|------------------------|-----------------|--------------|-------------------|
|                        | Day 1 | Day 7 | Day 14 | Day 21 | Day 28 | Day 1 | Day 7 | Day 14 | Day 21 | Day 28 | Day 1 | Day 7 | Day 14 | Day 21 | Day 28 |
| Fasting Blood Glucose (mg/dl) | 91 | 105 | 111 | 116 | 121 | 73 (−19%) | 71 (33%) | 68 (−38%) | 67 (−42%) | 67 (−42%) | 77 (−15%) | 73 (−30%) | 74 (−33%) | 72 (−37%) | 71 (−41%) |
| Blood Glucose (mg/dl) | 2103 | 215.4 | 236.5 | 278.1 | 295.7 | 205.2 (−4%) | 189.2 (−13%) | 154.6 (−36%) | 130.3 (−55%) | 126 (−59%) | 209.24 (−1%) | 156.5 (−27%) | 155.2 (−34%) | 149.3 (−46%) | 1484 (−54%) |
| Triglycerides (mg/dl) | 1782 | 186.5 | 192 | 201.3 | 210.4 | 130 (−27%) | 92.4 (−50%) | 85.3 (−56%) | 78.6 (−615) | 72.1 (−56%) | 132.2 (−26%) | 105.3 (−45%) | 920 (−53%) | 84.6 (−59%) | 75.2 (−65%) |
| Cholesterol (mg/dl) | 1782 | 180.11 | 188.5 | 198.6 | 218.6 | 182.8 (−3%) | 163.6 (−9%) | 154.3 (−18%) | 111.6 (−44%) | 94.6 (−57%) | 192.3 (−7%) | 178.3 (−2%) | 151.2 (−20%) | 109.3 (−46%) | 978 (−56%) |
| Urea (mg/dl) | 4537 | 48.9 | 54.76 | 62.18 | 67.2 | 48.1 (5%) | 47.3 (−4%) | 47.2 (−14%) | 41.3 (−34%) | 40.1 (−41%) | 53.1 (16%) | 50.92 (3%) | 49.78 (−10%) | 47.82 (−24%) | 43.1 (−37%) |
| Creatinine (mg/dl) | 1.21 | 1.32 | 1.41 | 1.47 | 1.52 | 1.17 (−3%) | 0.97 (−27%) | 0.67 (−52%) | 0.59 (−60%) | 0.47 (−69%) | 1.14 (−6%) | 1.09 (−18%) | 1.02 (−28%) | 0.99 (−37%) | 0.67 (−56%) |
| Protein (g/dl) | 5.22 | 5.01 | 4.62 | 4.07 | 3.98 | 4.92 (−6%) | 5.42 (8%) | 5.72 (23%) | 5.99 (47%) | 6.1 (53%) | 4.31 (−18%) | 4.56 (−9%) | 5.16 (11%) | 5.5 (36%) | 5.81 (43%) |
| Body weight (g/individual) | 210 | 208 | 206 | 202 | 198 | 208 (1%) | 211 (2%) | 220 (7%) | 222 (10%) | 225 (14%) | 200 (−5%) | 204 (−1%) | 217 (6%) | 219 (8%) | 222 (12%) |

Values given inside the bracket are percent decrease over diabetic control
flavonoids, terpenoids, and vitamin C [31]. In citrus fruits, peels are reported to possess the highest amounts of polymethoxylated flavones compared to other edible parts of the fruit [32]. In our study, methanolic extract of Punica granatum and Citrus aurantifolia peel possessed phytochemical compounds such as phenols, flavonoids, alkaloids, etc., which might have regulated the key metabolic pathways such as carbohydrate metabolism, triglycerides, and other blood biochemical parameters. The beneficial effects of peel extract may be attributed to its wide range of active bioactive compounds, and also opens up a new avenue for food and pharmaceutical industry. The beneficial effects of peel extract may be attributed to its wide range of active bioactive compounds present in Citrus aurantifolia and Punica granatum peels which might have played a vital role in controlling the diabetic condition by increasing the insulin secretion, reducing hepatic glucose output, regulating certain enzymes that are involved in carbohydrate metabolism. Therefore, it is important to characterize the secondary plant metabolites (phytochemicals) and also to unveil their mode of action in controlling this multifactorial endocrine disorder.

**5 Conclusion**

Fruit peels are commonly discarded in both domestic and industrial processing. It serves as an important source for the isolation and extraction of bioactive compounds and also opens up a new avenue for food and pharmaceutical industry. The beneficial effects of peel extract may be attributed to its wide range of active bioactive compounds present in Citrus aurantifolia and Punica granatum peels which might have played a vital role in controlling the diabetic condition by increasing the insulin secretion, reducing hepatic glucose output, regulating certain enzymes that are involved in carbohydrate metabolism. Therefore, it is important to characterize the secondary plant metabolites (phytochemicals) and also to unveil their mode of action in controlling this multifactorial endocrine disorder.

**Abbreviations**

HIPTLC: High Performance Thin Layer Chromatography; GLUT4: Glucose transporter type 4; TLC: Thin layer chromatography; RF: Retardation factor; UV: Ultraviolet; HDL: High density lipoprotein; LDL: Low density lipoprotein; MKU: Madurai Kamaraj University; IAEC: Institutional Animal Ethical Committee; KMCP: K.M.College of Pharmacy; TNAU: Tamil Nadu Agricultural University

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following their guidelines regarding protocols and procedures after getting
obtained from the institution to use the animals for this study. Animal
Ethics approval and consent to participate

We would like to state that a written consent and approval has been
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Competing interests

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