

## Pharmacodynamics evaluation of Eggplant wastes formulated as lyophilized tablets in an attempt for preparation of a bioactive natural antidiabetic dosage form

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### ABSTRACT

**Introduction:** Eggplant fruits (*Solanum melongena* L.) are widely consumed worldwide due to their generous composition of nutraceuticals. They are enriched with different classes of phenolic phytochemicals. Among the polyphenolic constituents, the fruit peels show high content of quercetin-3-rutinoside, chlorogenic acid and delphinidine-3-rutinoside. The plant possesses various biological activities such as antioxidant and antidiabetic activities. It can manage type 2 diabetes by controlling glucose absorption. **Aim of study:** The objective of the present study was to standardize delphinidine-3-rutinoside, chlorogenic acid, and quercetin-3-rutinoside, present in the eggplant peels extract, then formulate the extract as a lyophilized tablet, in order to evaluate its effect on glucose levels in an attempt for preparation of a bioactive natural antidiabetic dosage form. **Materials and Methods:** Various extracts of Eggplant peels were prepared using different extraction solvents. Pharmacodynamics studies were carried out on formulated lyophilized tablets containing the most promising extract. Pharmacodynamics study was conducted to assess the effect of the fruit peels extract on the management of blood glucose level in diabetic rats. **Results:** The results revealed that the Eggplant fruit peels extract in the form of lyophilized tablets could be promising in controlling the high blood glucose level in type 2 diabetic patients, as the freeze-dried tablets of the Eggplant peels extract resulted in lowering the blood glucose level to ~148 mg/dl after 10 days of twice daily administrations to diabetic rats. **Conclusion:** this report, addresses the important approach of finding and exploiting the significance of using food wastes in drug discovery.

**Keywords:** Eggplant peels – anthocyanins - pharmacodynamics- antidiabetic.

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## INTRODUCTION

Eggplant fruits (*Solanum melongena L.*) are widely consumed worldwide due to their generous composition of nutraceuticals.<sup>1</sup> They are enriched with different classes of bioactive compounds namely flavonoids, phenolic acids, and anthocyanins. Eggplant peels are highly enriched with high content of anthocyanins, thus possessing potent antioxidant,<sup>2</sup> anti-diabetic,<sup>3</sup> anti-cancer,<sup>4</sup> anti-inflammatory<sup>5</sup> and anti-microbial activities.<sup>6</sup> Among the anthocyanins which are particularly present in high concentrations in Eggplants are delphinidin derivatives such as delphinidin-3-rutinoside.<sup>7</sup> The fruit peels showed high content of delphinidine-3-rutinoside, chlorogenic acid, the most abundant phenolic acid ester in eggplant, and quercetin-3-rutinoside (**Figure 1**).

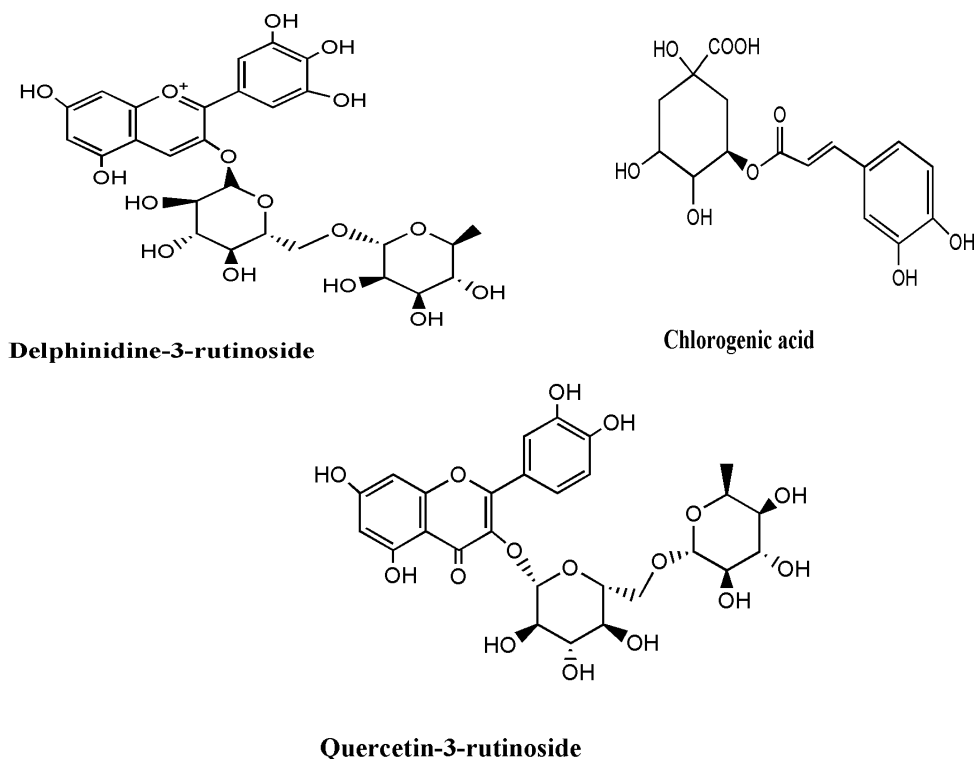
Chlorogenic acid is formed as a result of esterification between cinnamic acid and quinic acid.<sup>8</sup> It is well known that it could effectively decrease the blood glucose level, and could have a beneficial effect on human health in management of type 2 diabetes mellitus by controlling glucose absorption.<sup>9</sup> Diabetes mellitus (DM) is a well-known metabolic disorder. Its progression could be either due to deficiency in insulin secretion or resistance to its action. Insulin resistance and

the beta cell dysfunction are caused by the imbalance between reactive oxidative stress (ROS) and antioxidants. The previously mentioned polyphenolic compounds proved to have antidiabetic effects.<sup>10</sup> Freeze-drying (lyophilization) is a dehydration procedure used to improve the physical and chemical stability of the extractives.<sup>11,12</sup> The freeze-dried powders are distinguished by their simple preparation, low residual moisture content, elegant appearance and long-term stability. To overcome the difficulties of physical instability that may develop during lyophilization, cryoprotectants as PVA [poly (vinyl alcohol)] can be used.<sup>11,12</sup> The goal of this study was to formulate the eggplant peels extract as a lyophilized tablet and evaluate their effect on the blood glucose levels in experimental animals to develop bioactive natural antidiabetic dosage form.

## MATERIAL AND METHODS

### Materials

The fresh fruits of *Solanum melongena L.* (Eggplant) were bought from the Egyptian market during November 2021. Poly vinyl alcohol (PVA) was purchased from Sigma–Aldrich, Inc. (St. Louis, MO). Delphinidin-3-rutinoside was purchased from Phytolab, Germany, while chlorogenic acid and quercetin-3- rutinoside were purchased from



**Figure 1:** Structures of delphinidine-3-rutinoside, chlorogenic acid, Quercetin-3-rutinoside.

Nawah scientific center, Egypt. Sodium dihydrogen phosphate, TEA, and orthophosphoric acid were supplied by Adwic; (Egypt). Acetonitrile (HPLC grade) was purchased from Sigma-Aldrich (Germany).

### Methods

The fruits were washed, and peels were removed with a knife in thin uniform strips, subsequently the peels were left for air drying on paper towels and ground to powder. The powder was stored in an airtight glass container and kept in the refrigerator until extracted and analyzed.

Small scale extraction was done at Misr International University using three solvent

systems, ethanol 70% (Extract A),<sup>13</sup> ethanol 70% with 2g citric acid (Extract B),<sup>14</sup> and ethanol 70% with 0.2% HCl (Extract C). 1 g of air-dried sample was immersed in 20 ml of each solvent system separately for 3 times under sonication for 30 mins in the dark. Each time the temperature and frequency were set to 40° C and 40 KHz, respectively. pH value was adjusted, as any pH changes could affect the extraction yield<sup>15,16</sup> Each extract was filtered through a cellulose filter paper. The filtrate was centrifuged separately at 14,000 rpm for about 10 min at 4 °C (13), to remove the fine suspended particles and the supernatant was obtained, concentrated at 45 °C under reduced pressure using

Rotatory evaporator, then stored in a freezer for further analysis.

The use of Ultrasound-assisted technology (UAE) was selected rather than classical solid-liquid extraction (SLE) methods, as UAE offers greater extraction yield of polyphenolic compounds, lower consumption of organic solvents, less operation time, in comparison to SLE methods.<sup>17</sup> High-Performance Liquid Chromatography (HPLC) analysis of the three extracts was done against the standards, so as to detect which solvent system could give the highest yield of delphinidin-3-rutinoside, chlorogenic acid and quercetin-3-rutinoside.

#### **Conditions for HPLC method:**

Chromatographic analyses were performed using an Agilent 1100 HPLC system with a UV detector. Samples were loaded into a manual Rheodyne injector (model G1328B, USA) equipped with a gradient pump and a ZORBAX ODS Column (250 mm×4.6 mm×5 µm). Mobile phase: Solvent A (0.01 M phosphate buffer pH 2.5), Solvent B (Acetonitrile), Flow rate: 1mL/min. The mobile phase was filtered through 0.45-µm membrane filters (Millipore, USA). The UV detector was set at 230 nm. All determinations were made at room temperature.

#### **Construction of calibration curve:**

Stock solution of 10 mg/mL of delphinidin, chlorogenic acid and quercetin were dissolved in ethanol. All standards were stored away from light in low temperature (-4 °C). Serial dilutions of each standard were prepared from the stock solution having the final volume of (0.5, 0.75, 1, 1.25 and 1.5 µg/mL for delphinidin), (1, 5, 10, 25, 50 µg/mL for chlorogenic acid) and (10, 50, 100, 150 and 250 µg/mL for quercetin).

#### **Formulation of lyophilized tablet of Eggplant peels extract**

Lyophilized tablets of Eggplant peel extract were prepared by adding the alcoholic extract dropwise to 6% polyvinyl alcohol solution at 5 °C under magnetic stirring at 1500 rpm for 15 min. The resulting mixture was poured into the pockets of a PVC blister pack to obtain an extract dose of 60 mg per tablet. The tablet blister pack was then transferred to a freezer at -20 °C for 24h followed by freeze drying in a Cryodos-50 lyophilizer (Telstar Cryodos, Terrassa, Spain). Freeze drying was performed at a pressure of 0.2 mbar, at -48 °C for 48h, then the lyophilized tablets were kept in tightly closed containers at room temperature until further use.<sup>12</sup>

#### **Pharmacodynamics Study**

The *in vivo* evaluation of the extract in experimental animals was all approved and carried out in accordance with Guide for Care and Use of Laboratory Animals of Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt. All experiments were carried out in an environmentally controlled breeding room under the institutional guidelines for the human treatment of laboratory animals. Animals were received from the animal house of the National Research Center (Dokki, Giza, Egypt).

Pharmacodynamics study was carried out using healthy male albino Sprague-Dawley rats (~275 g). Rats were acclimatized

in the animal house for one week before the study. Rats were fed on a standard laboratory rat diet with a free access to tap water and kept under constant environmental conditions (22 ±3 °C; 50 ±5% relative humidity and 12 hr. of light/dark cycle). Type II diabetes mellitus (non-insulin-dependent) was developed in rats *via* single intraperitoneal injection (140 mg/kg) of freshly dissolved alloxan monohydrate (diabetogenic agents) in normal saline.<sup>18</sup> On the third day of injection, rats were considered diabetic, and were isolated for the next day's experiment when blood glucose

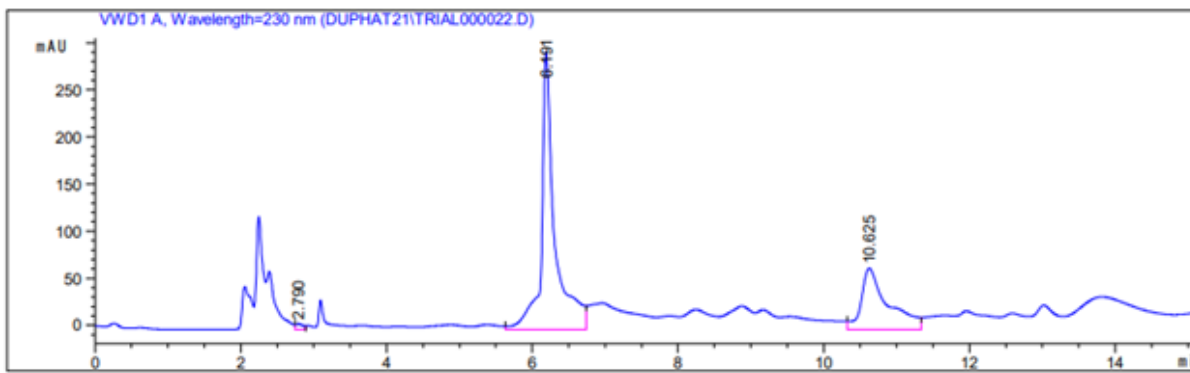
levels were determined to be more than 250 mg/dl.

Diabetic rats on the fourth day were randomized into two groups (n= 5). The first group acted as the positive control group, while the second group administrated orally the lyophilized tablets containing 60 mg of the extract twice daily for ten days. Blood glucose levels of rats were monitored daily using one-touch blood glucose monitoring system (LifeScan Inc, USA) by collecting blood from the tail vein. The hypoglycemic activity was determined as the percentage reduction in blood glucose level and was plotted against time.

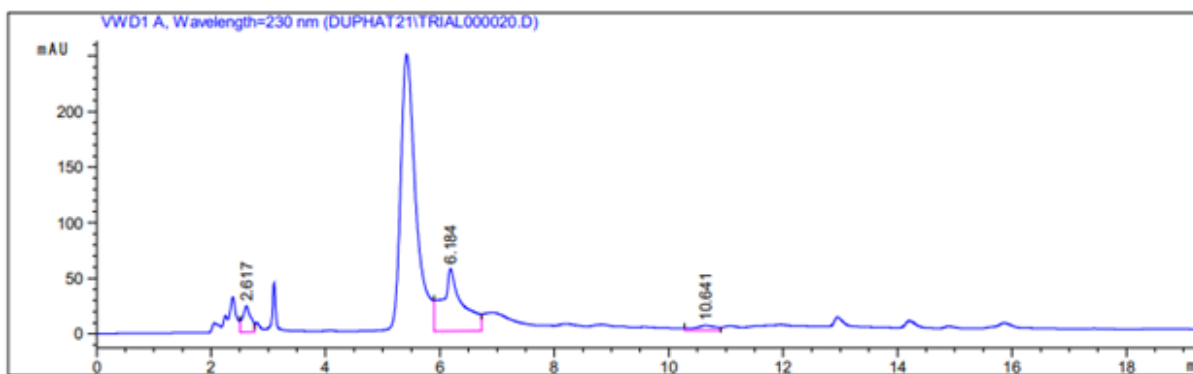
$$\text{Percentage reduction in blood glucose level} = \frac{\text{Blood glucose level at } t=0 - \text{Blood glucose level at } t=t}{\text{Blood glucose level at } t=0} \times 100$$

## RESULTS

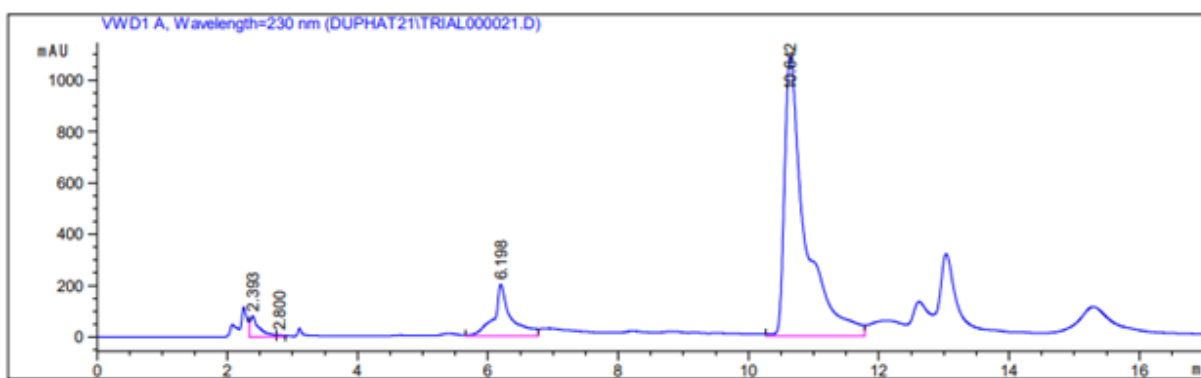
The results of the HPLC analysis (**Figures 2-4**) showed that extract **C** (ethanol 70% with 0.2% HCL) showed relatively higher intensities of peaks of the three compounds than extracts **A** and **B**. Consequently, the percentages of these three main compounds were found to be higher in the sample of extract **C**. Consequently, successive extraction was performed, where 100g of Eggplant peels were dissolved in 2L of solvent system (1400 mL ethanol/600 mL distilled water/12 mL HCl) for three consecutive times. The extract was filtered,



**Figure 2:** HPLC chromatogram showing compounds extracted using ethanol 70%.



**Figure 3:** HPLC chromatogram showing compounds extracted using ethanol 70% with 2g citric acid.



**Figure 4:** HPLC chromatogram showing compounds extracted using ethanol with 0.2% HCL.

centrifuged, and dried, adopting the previously mentioned methods, to give 37 g

dried residue, with percentage yield of 37% (Table 1).

**Table 1:** Results of the HPLC analysis of the three extracts.

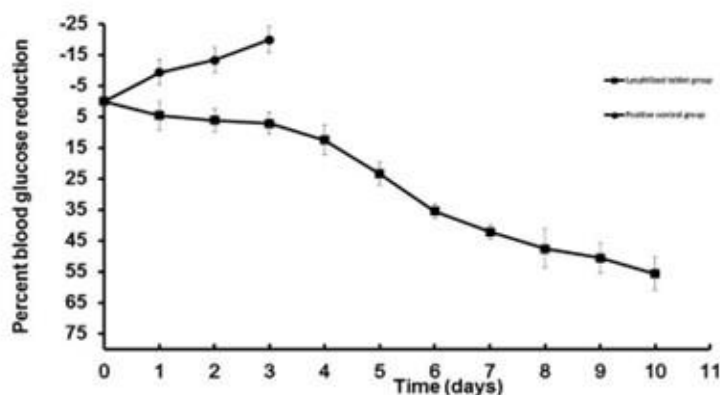
Sample	Delphinidin-3-rutinoside ( $\mu\text{g/ml}$ )	Chlorogenic acid ( $\mu\text{g/mL}$ )	Quercetin-3-rutinoside ( $\mu\text{g/ml}$ )
Extract using ethanol 70% (Extract A)	0.5	21.5	15.3
Extract using ethanol 70% with 2g citric acid (Extract B)	0.74	6.07	0.7
Extract using ethanol with 0.2% HCL (Extract C)	1.37	21.47	225.8

## DISCUSSION

### Pharmacodynamics

Lyophilized tablets were prepared using the hydrophilic polymer polyvinyl alcohol, that aimed to increase the wettability of tablets, and act as binder and cryoprotectant. It has been reported that medications that produce 25% drop in blood glucose level have a significant antidiabetic effect.<sup>19</sup> **Figure 5** shows the percentage of reduction in the blood glucose level of diabetic rats versus time, where the lyophilized tablets of

the Eggplant peels extract resulted in reduction of the blood glucose level to ~ 55% (~148 mg/dl) after 10 days of twice daily administrations, which could be attributed to the high content of delphinidine-3-rutinoside, chlorogenic acid, and quercetin-3-rutinoside. These compounds play a key role in the reduction of blood glucose levels by inhibiting the absorption of glucose from the intestines, in addition to improving glucose utilization and uptake by peripheral tissues.<sup>10</sup> Furthermore, they could inhibit  $\alpha$ -amylase

**Figure 5:** Percentage of reduction in the blood glucose level of diabetic rats versus time.

and  $\alpha$ -glucosidase enzymes, which will lead to reduction in the blood glucose levels.<sup>20</sup> Many animals in the positive control group died after 3 days of diabetes induction as a result of the continuous increase in the blood glucose level.

## CONCLUSION

Results of pharmacodynamics study suggested the antidiabetic activity of lyophilized tablets containing Eggplant peel extracts is safe, cheap, and effective, and could be a promising natural alternative to control the high blood glucose levels in type 2 diabetic patients.

## RECOMMENDATION

Based on the obtained results, it is deemed of interest to carry out pharmacokinetic studies, which are currently under process.

## CONFLICT OF INTEREST

The authors report no conflicts of interest.

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