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Analysis of Charantin Steroid Glycoside from Momordica Charantia Extract and Investigation of Its Intranasal Therapeutic Potential in Type 2 Diabetic Rats

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Abstract This study aimed to identify the characteristic steroidal glycoside charantin in the fruit extract of Momordica charantia using UV spectrophotometric analysis. Absorbance values were recorded at different concentrations of the extract. The 10% solution exhibited a distinct absorption peak at 254 nm, whereas the 50% solution showed a peak around 266 nm. A consistent increment in absorbance with increasing concentration of extract was observed, indicating the presence and progressive accumulation of charantin in the extract. These findings confirm that UV spectrophotometry is an effective method for the detection of charantin and validate its characteristic spectral properties in ethanolic extracts of Momordica charantia. To investigate its biological activity, thirty rats with experimentally induced type 2 diabetes mellitus were used. The animals were maintained on a high-calorie diet containing 0.2% cholesterol and 2% margarine for 8 weeks, reaching a body weight of 470±20 g. Intranasal administration of liposome-encapsulated charantin for five consecutive days led to a marked decrease in blood glucose levels—from 7.57 mmol/L before treatment to 5.33 mmol/L after treatment.

Keywords Momordica Charantia, Charantin, Steroidal Glycoside, UV Spectrophotometry, Ethanolic Extract, Phytochemical Analysis, Model animals, Absorption Peak, Antidiabetic Compound

1. Introduction

It is well known that the fruits of the Momordica charantia plant, or bitter melon (sometimes called Indian pomegranate), have scientifically proven health benefits [1-3]. This plant has been used in traditional medicine since ancient times, especially for the treatment of diabetes mellitus. In recent years, with the identification of new phytochemicals in Momordica charantia, scientific research has intensified into the mechanisms of its antidiabetic, antioxidant, antitumor and antimicrobial action [1-3] (Table 1).

This plant contains bioactive phytochemicals such as polysaccharides, polypeptides, steroids, flavonoids, vitamins and minerals [1-3]. Of the bioactive substances in M. charantia, triterpenoids such as charantin, vicine and cucurbitan have been shown to lower blood glucose levels. These substances enhance insulin secretion, improve glucose uptake by cells and regulate the activity of important metabolic enzymes responsible for glucose metabolism [4]. Among the bioactive compounds present in the fruits, charantin—a steroidal saponin—plays a secondary yet supportive role in modulating these physiological processes. [1]. Charantin acts on the body like insulin and regulates blood glucose levels. This property is similar to the effect of modern hypoglycemic drugs such as metformin. Charantin is present in 13.85 ± 3.55 μg/g dry weight of Momordica charantia fruits [11]. In this regard, the identification of bioactive substances such as charantin in M. charantia, extract and the study of their therapeutic potential require careful scientific analysis and open the way to the development of new effective natural remedies for the treatment of common diseases such as diabetes mellitus.

Charantin exerts its antidiabetic effects through multiple physiological pathways, particularly demonstrating significant efficacy in type 2 diabetes models. Research by Wang et al. demonstrated that charantin-rich extract of Momordica charantia (CEMC) significantly decreased insulin levels while simultaneously promoting insulin sensitivity in type 2 diabetic models [22]. Specifically, charantin stimulates insulin release from pancreatic β-cells while also blocking glucose formation in the bloodstream, effectively addressing two critical aspects of type 2 diabetes pathophysiology [23]. Additionally, charantin increases the expression of glucose transporter 4 (GLUT4) and enhances glucose utilization in both liver and muscle tissues of diabetic rats, facilitating

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more efficient glucose uptake and metabolism [24]. The compound also appears to influence thyroid hormone stimulation and AMP-activated protein kinase (AMPK) activity, which further contributes to improved glucose homeostasis [25].

2. Materials and Methods

The object of the study was ripe parts of M. charantia fruits. For purification, they were washed in special solutions. Then, divided into small pieces, dried in a "TC-1/80" thermostat and ground to a homogeneous powder. Extraction processes was performed from the prepared dry raw materials using the alcohol extraction method. The resulting extract was concentrated on a "RE 100-Pro Dlab" vacuum rotary evaporator, obtaining a viscous extract. Extract solutions of various concentrations were prepared, filtered and solid residues were separated in a minicentrifuge. To determine the composition of the extract, a "Nano One Ultra Micro UV-Vis" spectrophotometer (in the range of 200-800 nm) was used and characteristic spectra were determined. Qualitative reactions of charantin identification were carried out using the Molisch reagent for carbohydrates, the Lieberman -Burkhardt method for steroids and the lead acetate method for tannins. Statistical analysis of the obtained results was performed using the "EpiCalc 2000" program.

30 adult rats (male, 10–12 weeks old) weighing 390 ± 20 g were used in this study. All animals were housed under standard laboratory conditions (22 ± 2 ℃, 12 h light/dark cycle) with ad libitum access to food and water. Type 2 diabetes mellitus was induced by maintaining the animals on a high-calorie diet containing 0.2% cholesterol and 2% margarine for 8 weeks, resulting in stable hyperglycemia and increased body weight. The study was conducted at the Metabolomics Laboratory of the Institute of Biophysics and Biochemistry under the National University of Uzbekistan in accordance with the Helsinki Declaration and international guidelines for laboratory animal care and use.

Liposomal formulations containing M. charantia extract were administered intranasally to diabetic rats at a standardized dose once daily for five consecutive days.

Blood glucose concentrations were measured using the Cypress Diagnostics Glucose Kit (Cypress Diagnostics, Belgium), an in vitro diagnostic medical device designed for the quantitative determination of glucose in citrate plasma free from hemolysis and turbidity. For analysis, blood samples were collected from the tail vein into citrate-containing microtubes. Plasma was separated by centrifugation at 3000 rpm for 10 minutes, and the assay was performed according to the manufacturer's protocol. Absorbance was measured spectrophotometrically at 510 nm, and glucose concentration was expressed in mmol/L.

Table 1. Biologically active substances in Momordica charantia fruits

№	Name of the substance	Chemical group	Main biological effect	Quantity (in dry weight)	Source
1	Charantin	Steroid glycoside	Hypoglycemic	13.85 ±3.55 μg/g	12
2	Polypeptide-P (plant insulin)	Protein (insulin-like)	Insulin-like effect	0.87 mg/g	13
3	Momordicin I, II, III	Triterpene glycoside	Antiviral, antioxidant	458.78 ±35.08 μg/g	14
4	Vicine	Glycoside	Hypoglycemic, antioxidant	19.6 μg/mL	15
5	Coumarins	Benzopyrone compounds	Anticoagulant, antibacterial	0.2–0.5%	16
6	Saponins	Glycosides	Stimulates immunity, has antibacterial effect	10-25 μg/mL	17
7	Alkaloids	Nitrogen-containing organic matter	Neurological effect, antibacterial	0.3–0.6%	16
8	Flavonoids (quercetin, kaempferol)	Polyphenolic compounds	Antioxidant, anti-inflammatory	16.55 ±0.47 μg/g	18
9	Carotenoids (β-carotene)	Terpenes	Provitamin A, antioxidant	5.3 μg/g	19
10	Luteolin and Apigenin	Flavonoid	Antioxidant, reduces inflammation	43.77 ±3.13 μg/g	18
11	Phenolic acids (gallic acid)	Phenolic compounds	Antioxidant, anticarcinogenic	146.2 ±1.95 μg/g	18
12	Omega-3 and omega-6 fatty acids	Fatty acids	Good for the cardiovascular system	0.5–1.0%	16

3. Results

In recent years, the methods for isolating charantin have been significantly improved. Among them, ultrasonic extraction (UE), supercritical fluid extraction (SFE) and microwave-assisted extraction (MAE) have shown high efficiency compared to traditional methods [7-9].

Charantin consists of two components: β-sitosterol-D-glycoside and stigmasterol-D-glycoside (Fig. 1).

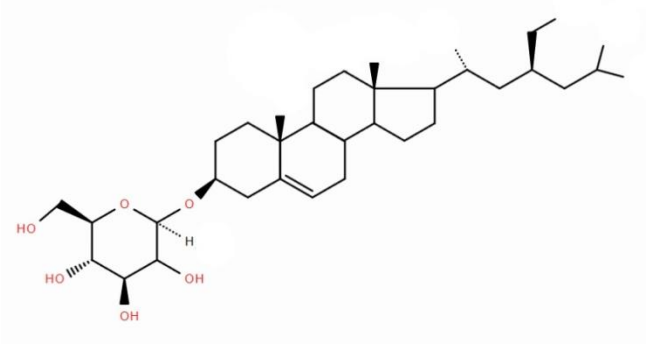


Figure 1. Chemical structure of the substance charantin

The presence of a steroid base in the form of an aglycone region increases its solubility in non-polar solvents such as chloroform and dichloromethane, and the presence of a glucose residue makes it partially soluble in polar solvents such as ethanol or methanol [6]. However, since the quantity and quality of charantin depend on the stage of plant maturation, source and extraction conditions, standardization of extraction products remains an urgent problem [10]. Therefore, modern scientific research is aimed at optimizing the parameters of charantin extraction and developing methods for its determination.

Qualitative reactions for the presence of the steroid glycoside charantin in the extract of Momordica charantia fruits:

Qualitative reaction for carbohydrates using Molisch reagent

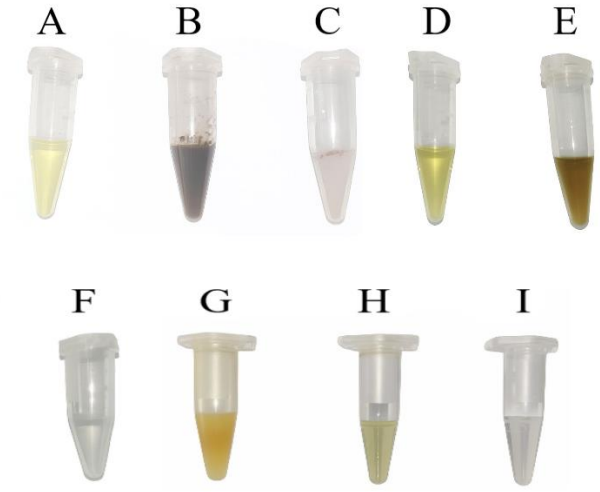
For this qualitative reaction, 1 drop of Molisch reagent (α-naphthol 5%, ethanol 95%) is added to 200 µl of the extract dissolved in 80% ethyl alcohol. Then 200 µl of concentrated H_2SO4 are added to the solution. If the color of the solution changes to reddish-violet (Figure 2), this confirms the presence of carbohydrates in the solution (part of the substance charantin is formed from O-glucose) [20].

Qualitative reaction for steroids using the Lieberman-Burchard method

First, 2 ml of acetic anhydride are mixed with 500 µl of the extract solution. Then 2 ml of concentrated H_2SO_2 are added to the solution. As a result, the color of the solution changes from yellow to blue-yellow or green (Figure 2). This indicates the presence of steroid fragments in the solution (charantin is a substance of steroid nature) [21].

Qualitative reaction for tannins using lead acetate

For this qualitative reaction, a few drops of 1% lead acetate solution are added to 500 µl of the extract dissolved in 80% ethyl alcohol. As a result, a yellow precipitate of lead-tannin complexes forms in the solution (Fig. 2).



Note. A – extract before qualitative reaction, B – extract after qualitative reaction, C – control reaction; Qualitative reaction for determination of steroids. D – extract before qualitative reaction, E – extract after qualitative reaction, F – control reaction; Qualitative reaction for determination of tannins. G – extract after qualitative reaction, H – extract before qualitative reaction, I – control reaction.

Figure 2. Qualitative reaction for determination of carbohydrates

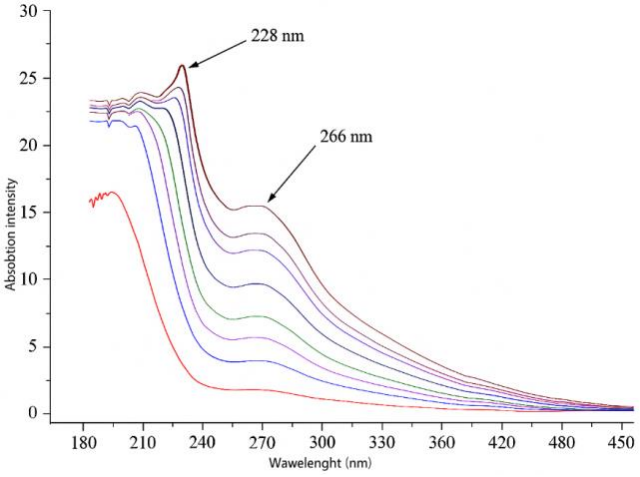


Figure 3. Spectrophotometric results of M. charantia extract

Before spectrophotometric analysis of M. charantia extract, 80% ethanol was used as a "blank" solution. Spectrophotometric analysis of substances in the M. charantia fruit extract showed a characteristic absorption wavelength at 266 nm. In 10% extract solution, characteristic absorbance maximum appeared at wavelengths of 220 and 254 nm, while in 50% solution, this maximum was recorded around 228 and 266 nm (Figure 3). The progressive redshift in absorption wavelength with increasing concentration, as observed during our analysis, provides strong evidence for the presence and accumulation of the compound charantin. Our results are consistent with the results of the scientific study "Evaluation of cytotoxic potential of Momordica charantia" conducted by researcher Rohan I. and his colleagues [6].

According to their data, it was proved that the absorption spectrum of charantin in a solution of Momordica charantia fruit extract in methanol is at a wavelength of 278 nm.

All animals maintained on a high-calorie diet containing 0.2% cholesterol and 2% margarine for 8 weeks developed signs of moderate obesity and hyperglycemia, consistent with type 2 diabetes mellitus. The average body weight of rats before treatment reached 390 ± 20 g, and fasting blood glucose levels averaged 7.57 ± 0.25 mmol/L, confirming successful model induction. No significant behavioral abnormalities or signs of acute toxicity were observed during the experimental period.

Intranasal administration of a 50% extract containing 50 μL of liposome-encapsulated charantin for five consecutive days resulted in a significant reduction in fasting blood glucose levels in diabetic rats. Post-treatment measurements indicated a mean glucose concentration of 5.33 ± 0.18 mmol/L, corresponding to an approximate 29.6% decrease compared with pre-treatment levels ($p < 0.05$). In contrast, control animals receiving physiological saline showed no significant change in glycemia during the same period.

The reduction in glucose levels suggests that intranasal delivery of liposomal charantin facilitates rapid systemic absorption and enhances its hypoglycemic efficacy. The observed glucose-lowering effect supports the potential of charantin as an active antidiabetic compound capable of modulating glycemic control through non-invasive administration routes.

4. Conclusions

In this study, qualitative assays for carbohydrates and steroids were conducted to identify the characteristic steroidal glycoside, charantin, in the extract of Momordica charantia fruits. The presence of steroidal glycosides was confirmed, and the detection of characteristic absorption peaks at 220 and 228 nm indicates the presence of polyphenolic compounds, particularly tannins, which was further corroborated by a qualitative reaction with lead acetate.

Beyond analytical characterization, the study demonstrated that intranasal administration of liposome-encapsulated charantin produced a marked hypoglycemic effect in rats with diet-induced type 2 diabetes mellitus, reducing blood glucose levels from 7.57 mmol/L to 5.33 mmol/L within five days of treatment. These findings indicate that charantin possesses potent antidiabetic activity and that its liposomal intranasal delivery may provide an effective and non-invasive route for therapeutic application. The integration of analytical detection and biological evaluation confirms charantin as a promising natural compound for further pharmacological development in the management of type 2 diabetes mellitus.

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Abbreviations

UV – Ultraviolet
nm – Nanometer
MAE – Microwave-Assisted Extraction
SFE – Supercritical Fluid Extraction
UE – Ultrasonic Extraction
HPLC – High-Performance Liquid Chromatography
EtOH – Ethanol
H_2SO_4 – Sulfuric Acid

Conflict of Interest

The authors declare no conflict of interest.

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