EXTRACTION AND STRUCTURAL CHARACTERIZATION OF GLUCOMANNAN FROM AMORPHOPHALLUS TONKINENSIS AND ITS EFFECT ON BLOOD GLUCOSE IN RATS

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ABSTRACT

Objective: To isolate glucomannan from the tuber of *Amorphophallus tonkinensis* (GMT) and investigate its chemical structure and effect on postprandial blood glucose in rats.

Methods: GMT was isolated and characterized using Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR) spectroscopy. Its biological effects were evaluated by administering GMT to rats and measuring postprandial blood glucose levels.

Results: GMT was a glucomannan composed of a backbone of -linked β-D-mannopyranosyl and β-D-glucopyranosyl residues, with a mannose/glucose molar ratio of 1.0:0.11. In vivo experiments showed that GMT intake could reduce the rate of glucose absorption, thereby decreasing postprandial blood glucose levels in rats.

Conclusion: Glucomannan was successfully isolated and structurally characterized from the tubers of *Amorphophallus tonkinensis*. In vivo studies in mice demonstrated that the GMT might modulate blood glucose levels, suggesting its potential as a functional food ingredient for glycemic control.

Keywords: Glucomannan, Cholesterol, Amorphophallus tonkinensis

I. INTRODUCTION

Glucomannan is a polysaccharide composed of D-glucose and D-mannose units linked by β-1,4-glycosidic bonds, found in plants of the *Amorphophallus* species (Araceae family, known as konjac or “khoai nưa” in Vietnam) [1], Aloe vera [2] and certain seaweeds [3]. Konjac glucomannan is the primary component of konjac flour extracted from the corm of *Amorphophallus* konjac, a plant mainly grown in East and Southeast Asia (Japan, China, Thailand, Vietnam). The term also applies to glucomannan from other *Amorphophallus* species [4]. Glucomannan exhibits valuable properties such as high viscosity in solution, stable gel formation, biocompatibility, and non-toxicity, enabling applications across industries. In food, amorphophallus has long been cultivated for diets as it provides no calories. Konjac glucomannan serves as a thickener (due to high water absorption and viscosity) and stabilizer (as a non-ionic compound, unaffected by ions unlike other thickeners, preventing precipitation). In medicine, it aids weight loss (being non-caloric), lowers blood cholesterol and lipids, regulates blood glucose, with minimal side effects [5], [6]. The FDA recognizes glucomannan as safe for food and pharmaceutical use. European Directive 95/2 permits konjac flour in foods, and the EU has funded biological and medical studies on it. Konjac flour is key in low-energy diets. In pharmaceuticals, it acts as an excipient for drug stability and coating [4].

Due to its superior properties and broad applications, global research especially in China and Japan has surged recently [4]. Vietnam hosts over 20 *Amorphophallus* species, however, to date, no published studies exist on glucomannan extraction from *Amorphophallus tonkinensis* and its ability to modulate glucose absorption for blood glucose regulation. Therefore, this paper reports work aimed at the isolation and structural characterization of glucomannan from *amorphophallus tonkinensis* tuber, followed by preliminary evaluation of its effects on blood glucose in Wistar rats.

II. SUBJECTS AND METHODS

2.1. Materials

Amorphophallus tonkinensis Engl. & Gehrm tubers (Araceae family) were collected from several northern provinces (classified by Dr. Nguyen Van Du, Vietnam Institute of Ecology and Biological Resources).

The main chemicals used in the study were analytical or pure grade, including ethanol (C_2H_5OH); sodium hydroxide (NaOH); hydrochloric

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Received date: 06/3/2026

Revised date: 05/5/2026

Accepted date: 11/5/2026

acid (HCl), carboxymethyl cellulose (CMC), glucose, gliclazide,… and other chemicals.

Wistar male and female rats, healthy, weighing 160-180 g, supplied by the Military Medical Academy. The experimental animals were acclimatized for 5 days post-purchase, fed standard pellets provided by the Hanoi Institute of Hygiene and Epidemiology, with free access to water.

2.2. Research Instruments and Equipment

The instruments and equipment used in the study included: magnetic stirrer with heating, drying oven, analytical balance, IMPACT-410 FTIR spectrometer, 500 MHz Bruker Avance NMR spectrometer, Ubbelohde viscometer, and other laboratory equipment.

2.3. Method for isolation and structure characterization of GMT

The process of isolating GMT from the tuber of *amorphophallus tonkinensis* was carried out as follows: the tubers were washed clean, peeled, cut into small pieces, and ground/crushed in water (tubers/water ratio of 100/200 g/ml). Filtration was performed to remove the residue, followed by settling and a second filtration to obtain a filtrate containing GMT in suspension. GMT was precipitated by adding the filtrate to 500 mL of 90% (v/v) ethanol. The resulting precipitate was collected and washed with ethanol, then dried at 60°C and used for further characterization.

The GMT flour content (GF) was calculated using the following formula: $GF\% = (m_1/m_2)\times 100\%$ where m_1 and m_2 were the weights of final white powder and original *amorphophallus tonkinensis* tubers, respectively.

The intrinsic viscosity of GMT was measured using an Ubbelohde viscometer according to method of Li and Wanchun. Mark-Houwink parameters were determined according to $\eta = 5.96\times 10^{-2} M_w^{0.7317}$ [7], [8].

The infrared spectrum (IR) of GMT was recorded in the 4000–400 cm^{-1} region, with the dried sample pelletized with KBr.

The nuclear magnetic resonance (NMR) spectra of GMT (^{1}H-NMR and ^{13}C-NMR) were acquired at 80°C (353K), using sample concentrations of 10 g/L in D_2O.

2.4. Effects of GMT administration on blood glucose levels in rats

Wistar rats, after being stably maintained in laboratory conditions, were divided into batches (7 rats per batch). Negative control group (NG): rats administered vehicle (CMC 1%); Positive control group (PG): rats administered gliclazide at 20 mg/kg body weight; Test group 1 (T1): rats administered GMT at 3 g/kg body weight; Test group 2 (T2): rats administered GMT at 9 g/kg body weight.

Rats received oral administration daily for 15 days, with glucose tolerance tests conducted twice. Test 1: 1 hour after the first drug administration on day 1; Test 2: 1 hour after drug administration on day 14.

Glucose tolerance test procedure was performed as follows: 1 hour after drug administration, baseline blood glucose was measured, followed by oral glucose administration at 3 g/kg body weight (rats fasted for 15 hours beforehand). Blood glucose was then measured at 30, 60, and 120 minutes post-glucose administration.

For glucose tolerance testing, blood glucose values and the degree of blood glucose increase at study time points were compared between the test groups and control groups at the same time points (relative to pre-glucose baseline) to assess the drug’s inhibitory effect on postprandial hyperglycemia.

The degree of blood glucose increase at time points after glucose administration (compared to immediately before glucose administration) was calculated using the following formula: $X = [(C_t - C_o)/C_o]\times 100\%$, where X is the degree of blood glucose increase (%); C_o is the blood glucose concentration immediately before glucose administration; C_t is the blood glucose concentration after glucose administration.

III. RESULTS

3.1. Isolation and structural characterization of GMT

The content of GMT in fresh tubers reached 7% (w/w), in white powder form, insoluble in water. The molecular weight of GMT isolated from *Amorphophallus tonkinensis was about* 1.043×10^6 Da.

The infrared (FTIR) spectrum and nuclear magnetic resonance (NMR) spectra of GMT are presented in Figures 1, 2 and 3, respectively.

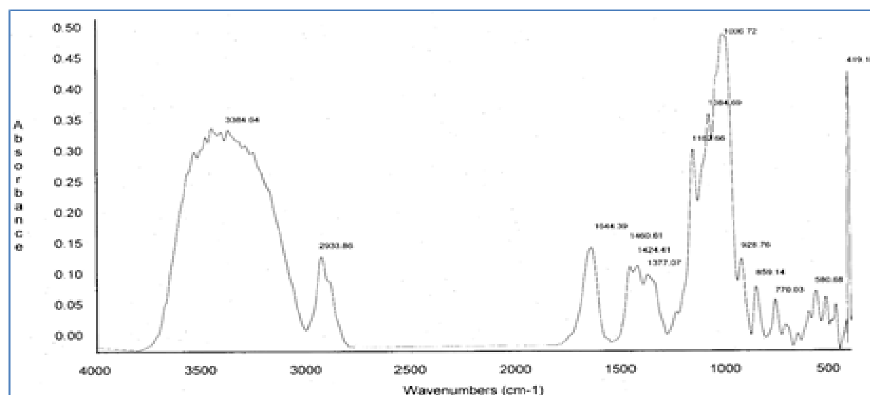

Fig. 1: Infrared (FTIR) spectrum of GMT

The FTIR spectrum (Fig. 1) exhibited characteristic absorption bands at 3000–3600 cm^{-1}, assigned to ν(OH); 2933.86 cm^{-1}, corresponding to ν(C–H); 1644.39 cm^{-1}, attributed to adsorbed H_2O; 1460.61, 1424.41, and 1377.07 cm^{-1}, assigned to δ(C–H); 1162.66 cm^{-1}, corresponding to ν(C–O–C) of inter-sugar ether linkages; 1084.69 and 1006.72 cm^{-1}, attributed to ν(C–O) of C–OH groups; and 900–800 cm^{-1}, corresponding to β-pyranose C–H vibrations characteristic of glucomannan anomeric configurations.

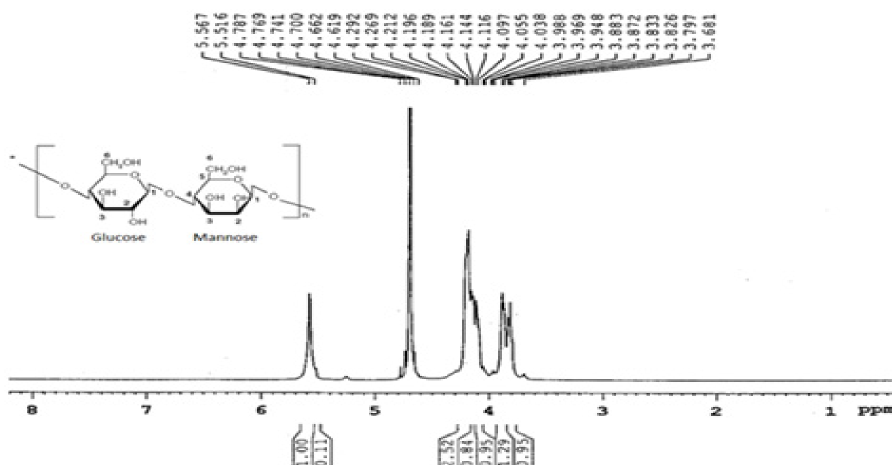

Fig.2: ^{1}H-NMR spectrum of GMT

The signals in the ^{1}H-NMR spectrum (Fig. 2) could be assigned as follows: H1 of mannose (δ 5.567 ppm); H1 of glucose (δ 5.516 ppm); H2 of mannose and glucose (δ 4.116–4.161 ppm, with overlapping signals); H3 of mannose and glucose (δ 3.797–3.833 ppm); H4 of mannose (δ 3.883 ppm) and H4 of glucose (δ 3.872 ppm); H5 of mannose (δ 4.292 ppm) and H5 of glucose (δ 4.269 ppm); H6a and H6b of mannose and glucose appearing at δ 4.212 ppm and 4.116 ppm, respectively.

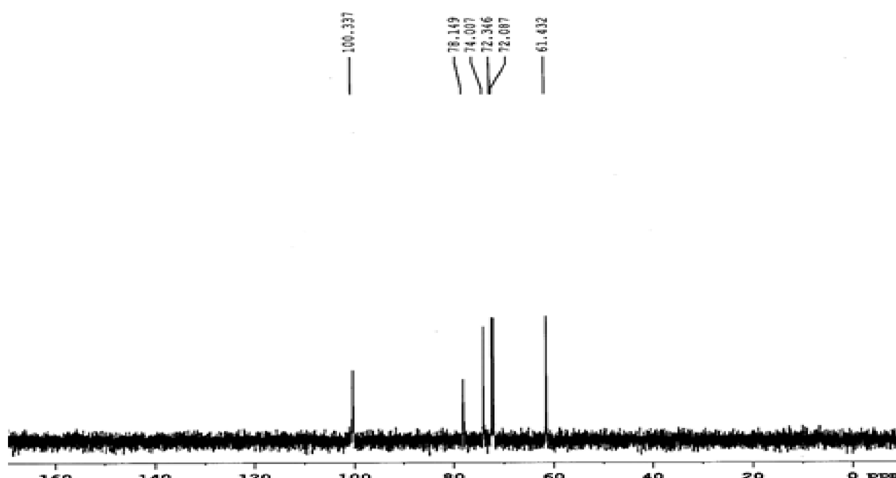

Fig. 3: ^{13}C-NMR spectrum of GMT

The ^{13}C-NMR spectrum of GMT (Fig. 3) showed an overlap between the signals characteristic of the carbons in the β-D-glucopyranose ring (glucose and mannose). Specifically, the signal characteristic of C1 appeared at 100.337 ppm, C2 at 72.087 ppm, C3 at 72.346 ppm, C4 at 78.149 ppm, C5 at 74.007 ppm, and C6 at 61.432 ppm.

3.2. Effects of GMT on glucose tolerance in white Wistar rats

The effect of GMT on glucose tolerance in Wistar rats was evaluated after 1 and 14 days of administration. Mean blood glucose concentrations were measured at defined time points before and after oral glucose loading, and the results are presented in Figures 4 and 5 for the respective treatment durations.

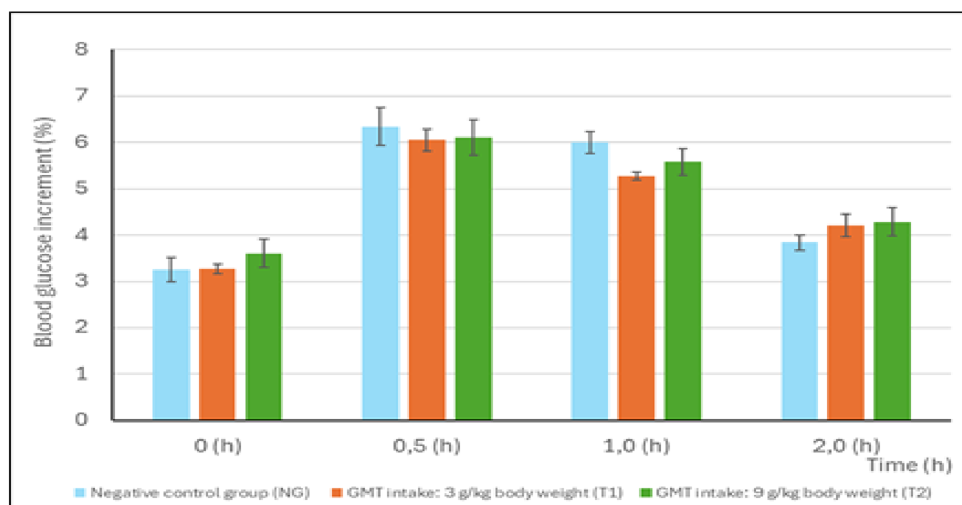

Fig. 4: Blood glucose increment (%) before and after glucose administration (rats administered GMT 1 day prior to testing)

The results (Fig. 4) showed no significant difference in blood glucose levels between the GMT-administered rat groups (3 g/kg body weight, T1; 9 g/kg body weight, T2) and the control group (NG) at all studied time points. GMT did not induce an increase in blood glucose levels in the studied rats. In contrast, blood glucose levels in the control group (without GMT) rose more rapidly during the observation period than in the GMT-treated groups after receiving the same glucose load.

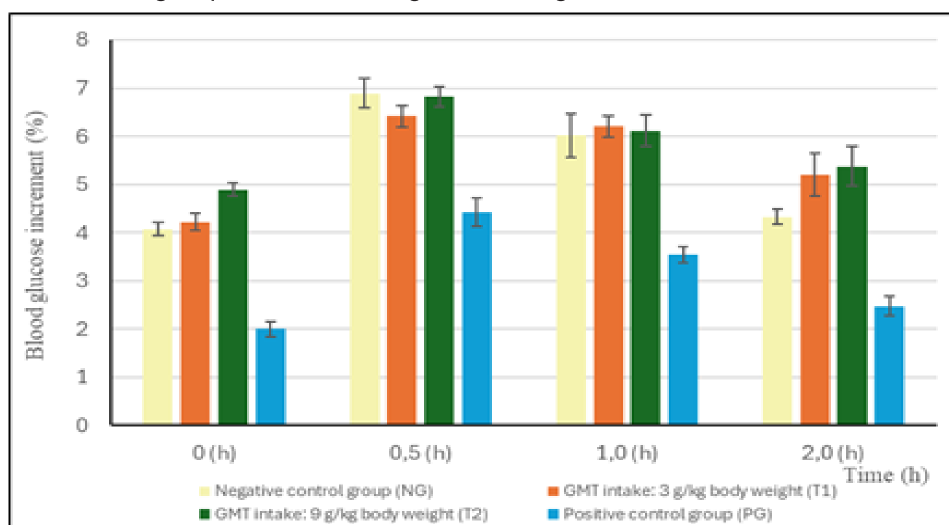

Fig. 5: Blood glucose increment (%) before and after glucose administration (rats administered GMT 14 days prior to testing)

The results (Fig. 5) showed that blood glucose levels in the gliclazide-treated group (PG; 20 mg/kg body weight) were lower than those in the control group (NG) at all evaluated time points: immediately before and 60, 90, and 120 min after glucose administration. In contrast, no significant differences were observed between the GMT-treated groups (3 g/kg, T1; 9 g/kg, T2) and the control group. These findings suggest that GMT did not increase blood glucose levels in Wistar rats.

IV. DISCUSSION

4.1. Isolation and structural characterizaation of GMT

Isolation of glucomannan from *Amorphophallus tonkinensis* tubers was optimized based on its poor water solubility. Traditional methods require drying tubers before extraction with chemicals, suitable for water-soluble polysaccharides [9]. However, since glucomannan from *Amorphophallus tonkinensis* was water-insoluble and denser than other tuber components, it was directly separated from crude flour by water dispersion, simultaneously removing water-soluble fats and proteins. This green method avoids toxic chemicals, making the product safer for food and medicine applications. The molecular weight of glucomannan isolated from *Amorphophallus tonkinensis* was 1.043×10^6 Da, which was comparable to commercial *Amorphophallus konjac* ($0.5 \div 2.0 \times 10^6$ Da) and indicated high viscosity potential essential for effective glucose absorption modulation and postprandial blood glucose control. This high molecular weight confirmed that glucomannan isolated from *Amorphophallus tonkinensis* possessed physicochemical properties suitable for pharmaceutical and functional food applications [10].

The FTIR spectrum GMT (Fig.1) revealed characteristic absorption bands typical of polysaccharide structures, confirming its identity as a β-1,4-linked glucomannan. The broad v_{OH} band at 3000–3600 cm^{-1} arose from hydrogen-bonded hydroxyl groups, the peak at 2933.86 cm^{-1} corresponded to C–H stretching in the aliphatic backbone; these aligned closely with reports for glucomannans from related *Amorphophallus* species, such as *Amorphophallus panomensis* (3000–3700 cm^{-1} for O–H and 2926 cm^{-1} for C–H) [10]. Bands at 1644.39 cm^{-1} (v_{H_2O}), 1460.61/1424.41/1377.07 cm^{-1} (δ_{CH}), 1162.66 cm^{-1} (v_{C-O-C}), and 1084.69/1006.72 cm^{-1} (v_{C-O}) indicated glycosidic linkages, adsorbed water, and ether/alcohol functionalities, respectively. The region at 900–800 cm^{-1} (δ_{CH}) specifically signified β-pyranose ring conformations of mannose and glucose units. These peaks matched published data for glucomannan from the tuber of *Amorphophallus panomensis* (e.g., 1645 cm^{-1} H_2O, 1159 cm^{-1} C–O–C, 1082 and 1014 cm^{-1} C–O, and 768–927 cm^{-1} β-pyranose). Minor variations in exact wavenumbers (e.g., 2926 vs. 2933.86 cm^{-1}) likely resulted from sample hydration, extraction methods, or instrumental resolution, but the overall profile validated the glucomannan purity without protein/lipid contaminants [11].

The peaks in the ^{1}H-NMR spectrum of glucomannan (GMT) from *Amorphophallus tonkinensis* were assigned by comparison with literature chemical shift data, revealing well-resolved anomeric proton signals at δ 5.567 ppm (H-1 of mannose) and δ 5.516 ppm (H-1 of glucose), alongside ring protons (H-2 to H-6) between δ 4.116–4.292 ppm; these patterns aligned with reported assignments for β-(1→4)-linked glucomannans from *Amorphophallus* species , where H-1 resonances shifted downfield due to deshielding by adjacent glycosidic oxygen (e.g., H-1 mannose δ 5.047–5.567 ppm; H-1 glucose δ 4.993–5.516 ppm) [12]. The mannose/glucose molar ratio was determined as 1.00:0.11 from H-1 integrals, matching *Amorphophallus panomensis* and *Amorphophallus konjac* data. Notably, the absence of acetyl signals (~δ 1.8–2.2 ppm) confirmed a fully deacetylated structure, unlike that of acetylated konjac, explaining its poor water solubility due to reduced hydrophilicity. These characteristic shifts verified that GMT shared the canonical backbone architecture dominated by β-D-mannopyranosyl and β-D-glucopyranosyl residues with β-(1→4) linkages, in agreement with structural models for konjac and related glucomannans [13].

The ^{13}C-NMR spectrum (Fig. 3) of glucomannan (GMT) isolated from *Amorphophallus tonkinensis* exhibited characteristic signals corresponding to β-linked hexopyranosyl units, confirming its deacetylated linear structure and showing good agreement with previous studies on *Amorphophallus* glucomannans. The anomeric C1 resonance at δ 100.337 ppm fell within the established range (98–103 ppm) for β-D-mannopyranosyl and β-D-glucopyranosyl residues, indicating a predominant β-anomeric configuration. The secondary carbons C2 (δ 72.087 ppm) and C3 (δ 72.346 ppm) were consistent with unsubstituted hexopyranose rings, while the C4 signal at δ 78.149 ppm showed the characteristic downfield shift associated with β-(1→4) glycosidic linkages due to deshielding effects. In addition, C5 (δ 74.007 ppm) and C6 (–CH_2OH, δ 61.432 ppm; within the typical 60–63 ppm

range) supported the presence of pyranose chair conformations and primary hydroxymethyl groups in hexose units. Importantly, the absence of acetyl methyl signals in the δ 20–23 ppm region confirmed a fully deacetylated backbone, distinguishing GMT from native konjac glucomannan (derived from *Amorphophallus konjac*), in which acetyl groups (typically accounting for 4–8% of hexose residues and commonly located at C3 and C6) enhance water solubility by disrupting intra- and intermolecular hydrogen bonding and promoting chain hydration [15]. Overall, the spectral profile was in good agreement with published ^{13}C-NMR data for glucomannans from *Amorphophallus konjac* and *Amorphophallus muelleri*, confirming that the isolated polysaccharide consists predominantly of a linear β-(1→4)-linked glucomannan backbone, with overlapping glucose and mannose carbon signals arising from their similar chemical environments.

4.2. Effects of GMT on glucose tolerance in Wistar rats

The blood glucose levels measured before and after oral glucose administration in Wistar rats following one day of glucomannan (GMT) treatment showed no statistically significant differences between the GMT-treated groups (3 g/kg and 9 g/kg body weight) and the control group at any time point (baseline, 30 min, 60 min, 120 min post-glucose; $p > 0.05$). In the control group, peak blood glucose reached 6.34 ± 0.41 mmol/L at 30 minutes, returning toward baseline by 120 minutes, consistent with a standard oral glucose tolerance test response in rodents, whereas GMT groups exhibited slightly blunted peaks (6.05-6.11 mmol/L) and slower declines (4.21-4.29 mmol/L at 120 min), indicating GMT did not induce hyperglycemia but modestly attenuated the postprandial glucose excursion without statistical significance at the tested doses. This attenuated response likely stems from GMT's properties as a soluble, viscous dietary fiber that forms a gel-like matrix in the gastrointestinal tract, delaying gastric emptying, impeding carbohydrate digestion, and slowing glucose diffusion to the intestinal mucosa for absorption, unlike the control, where glucose absorption proceeds unimpeded, resulting in a reduced rate of systemic glucose entry.

Blood glucose responses to oral glucose loading in Wistar rats following 14-day glucomannan (GMT) supplementation are presented in Figure 5. The gliclazide-treated positive control group (20 mg/kg) exhibited significantly lower blood glucose levels than the control group at baseline, 60 min, and 120 min after glucose loading ($p < 0.01–0.05$). In contrast, GMT supplementation at doses of 3 and 9 g/kg did not produce significant differences in blood glucose levels compared with the control group at any investigated time point ($p > 0.05$), suggesting that dietary GMT did not adversely affect glycemic regulation. These findings were consistent with previous reports indicating that glucomannan and other soluble dietary fibers modulated, rather than increased, postprandial glycemic responses [16]. The slightly attenuated increase in blood glucose levels during the initial phase (0–30 min) in GMT-treated groups might have been attributed to hydrogen bonding interactions between hydroxyl groups in the GMT structure and glucose molecules, thereby delaying intestinal glucose absorption. At later time points (60–120 min), blood glucose levels in GMT-treated groups remained comparable to those of the control group, indicating no evidence of abnormal glucose accumulation or hyperglycemic effects [17]. Overall, the results supported the glycemic safety of GMT under the investigated experimental conditions and suggested its potential as a functional dietary fiber for glycemic modulation.

V. CONCLUSION

This study represented the first report of glucomannan extraction from *Amorphophallus tonkinensis* tubers using a novel water-dispersion method that exploited its water-insolubility. The isolated glucomannan exhibited 7.0% content (fresh weight basis), $M_W = 1.043 \times 10^6$ Da, and a β-1,4-linked mannose:glucose (1.0:0.11) backbone. Importantly, in vivo administration in rats significantly attenuated postprandial glucose excursions, which confirmed its capacity to modulate glucose absorption via high-viscosity gel formation. These findings established *Amorphophallus tonkinensis* as a promising local source of glucomannan for functional foods and antidiabetic applications, warranting further clinical investigation.

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