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Green Waste from Cucumber (*Cucumis sativus* L.) Cultivation as a Source of Bioactive Flavonoids with Hypolipidemic Potential

Daniil N. Olennikov * and Nina I. Kashchenko

Laboratory of Biomedical Research, Institute of General and Experimental Biology, Siberian Division, Russian Academy of Science, 6 Sakhyanovoy Street, Ulan-Ude 670047, Russia; ninkk@mail.ru
* Correspondence: olennikovdn@mail.ru

Abstract: Cucumber is an important agricultural crop that is cultivated under greenhouse conditions. Cucumber cultivation generates substantial quantities of green waste that require proper disposal. The lack of data on the chemical composition of cucumber greens and their biological activity emphasizes the necessity for research on useful products resulting from this widely available waste. Our examination of the flavonoid contents in the leaves and stems of 30 cucumber cultivars revealed 6 cultivars with high flavonoid contents. In addition, the cutting time did not cause negative effects, and we observed a positive effect with 0.05–0.10% organomineral fertilizer application on the flavonoid levels in the greens. Liquid chromatography–mass spectrometry detected 38 apigenin derivatives, including acylated and non-acylated cucumerins and *C*-, *O*-, and *C*,*O*-glycosides. Among these, 12 known flavonoids and 18 novel compounds were identified. The concentrations of these compounds in the six flavonoid extracts varied at 39.85–181.53 mg/g for the non-acylated flavones, 14.67–293.31 mg/g for the cucumerins, and 401.73–892.17 mg/g for the acylated flavones. Oral administration of the total flavonoid extracts (at a dosage of 100 g/kg/day) resulted in a hypolipidemic effect in hyperlipidemic hamsters, with subsequent normalization of their serum lipid profiles, malondialdehyde levels, and liver antioxidative enzyme activities. These results substantiate the lipid-lowering potential of cucumber waste extracts. Our investigation of the selected flavonoid activity showed that isovitexin-2′′-*O*-glucoside-6′′-*O*-*p*-coumarate (administered at 50 g/kg/day) had the highest hypolipidemic potency. These results can contribute to the practical use of cucumber green waste and the development of novel supplements for diseases linked to high-fat consumption.

Keywords: cucumber; liquid chromatography–mass spectrometry; metabolite profiling; total flavonoid extract; industrial food waste; bioactive ingredients; hyperlipidemia; isovitexin

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1. Introduction

In recent years, the world has witnessed a surge in closed agronomic initiatives. According to the World Health Organization (WHO), the total area occupied by greenhouses is approximately 10 million hectares [1]. These increasing initiatives have led to a substantial increase in plant waste volume, which includes non-fruit secondary materials (such as stems, leaves, and roots). These materials are plant byproducts and are not used for commercial purposes [2]. The main approach for recycling green waste is composting; however, plant biomass can be used as a source to obtain economically valuable products [3]. Contemporary research on the problem of greenhouse waste disposal is limited. Therefore, in national long-term development plans, the rational use of raw materials is important. Thus, the development of a set of measures for the creation and implementation of competitive domestic technologies based on the latest scientific achievements is necessary [4].

Cucumber (*Cucumis sativus* L., Cucurbitaceae) is the main crop in greenhouse crop rotations, with a global production of approximately 10 million tons in 2021 [5] (Figure 1). A distinctive biological feature of this culture is the formation of elongated shoots (of stems and leaves); their mass may account for up to 50–60% of that of the fruit. Preliminary

estimates suggest that the volume of this waste may reach 20–30 million tons per year [6]. Despite the considerable utilization of cucumber fruits as a food product [7], scientific studies on the green shoots of this species have been limited to providing general information on their chemical composition. There is a lack of detailed information on the composition of their metabolites and their potential biological activities. Some studies have reported the presence of cucurbitacins [8], flavone *C*-glycosides [9,10], *C*-glycosyl flavonoid phytoalexins [11,12], and acylated flavones [13] in cucumber leaves, as well as flavonoids in the flowers [14]. Recently, it has been determined that specific cucumber flavonoids possess the ability to inhibit pancreatic lipase, indicating their possible hypolipidemic potential [12–14].

Considering the production volume of cucumber shoots, they could become a promising raw material for the pharmaceutical industry, in particular, for the creation of phytopreparations with lipid-lowering activity. Cucumber greens have a high flavonoid content, which inhibits the activity of food lipases and can absorb and remove lipids [15].

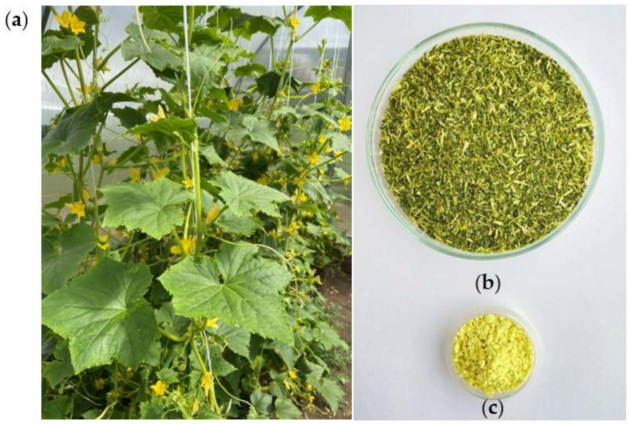


Figure 1. Cucumber plants (*Cucumis sativus* L.) under greenhouse conditions (cv. Zasolochnii) (**a**), dried cucumber waste green biomass (**b**), and flavonoid extract from cucumber waste green biomass (**c**).

A scientometric analysis of scientific databases revealed that the keywords "Cucumis sativus" and "cucumber" are found in over 14,000 articles. Of these, approximately 900 studies are devoted to the problems of the disposal of waste from the production of cucumber fruits, including greenhouse waste. Despite the many publications detailing advancements in successful cucumber cultivation and increasing productivity, there is a lack of information on waste disposal from this production. Currently, the problem of processing green biomass, a byproduct of seasonal and year-round cucumber production, into a valuable and economically significant product, remains unsolved [16]. In this regard, the study of cucumber waste disposal is an urgent objective for regional and global agricultural science. One proposal is to use plant residues to create new products with different biological activities, thus expanding the assortment of pharmaceuticals [17].

The creation of new approaches for collecting, transporting, processing, reusing, or disposing of greenhouse green waste is currently one of the most rapidly developing branches of agricultural science. One of the objectives is to understand the potential of this raw material, which is produced in large quantities around the world and, in most cases, remains unused, and to obtain economically valuable products [18]. Research on greenhouse green waste disposal has been performed from several directions, including the development of various methods for composting organic residues, the use of dried plants as a substrate for growing mushrooms, and the development of methods for processing organic waste into carbohydrate-containing products and/or their derivatives [19]. The main composting processes are labor-intensive and inefficient, while the cultivation of mushrooms and deep processing leads to the underutilization of the plant metabolome, which may have potential biological activity [20]. The latter two processing methods can

be applied to green waste after the removal of valuable extractive substances because the methods are based on the utilization of the carbohydrate components in plants [21].

Successful examples of this strategy have reduced the environmental burden and increased the profitability of existing agribusinesses engaged in food production. However, such examples are extremely rare, and there are no solutions for the non-compostable utilization of cucumber biomass. This issue likely exists because specialists in the field of phytochemistry and bioorganic chemistry generally lack sufficient interest in this area of agricultural science. Thus, in this study, a comprehensive chemical and biological investigation of the unused cucumber waste green biomass was performed for the first time. Specifically, metabolite profiling and quantification of the cucumber waste green biomass extracts was performed using liquid chromatography–mass spectrometry, followed by a study of the hypolipidemic potential of the extracts and selected compounds using the experimental model of alimentary hyperlipidemia in hamsters.

2. Materials and Methods

2.1. Plant Materials

For the screening study, seeds of 30 cucumber cultivars (Alfavit, Altai, Amur, April, Arcadia, Crystall Apple, Dachnyi, Dinamit, Holland Yellow, Kartoshka, Konkurent, Kurazh, Lemon, Madhur, Masha, Nezhinskii, Parisian Gherkin, Parizhskii Kornishon, Perseus, Secret, Shruti, Sibirskaya Girlyanda, Sibirskii Express, Sikkim, Strelets, Titus, Tornado, Tulsi, Zasolochnii, and Zozula) were cultivated in grow box conditions (Secret Jardin Hydro Shoot HS480W System, Secret Jardin Agomoon SRL, Manage, Belgium) using Plagron Soil Promix (Plagron, Weert, Netherlands) as an artificial ground. The leaves and stems were collected 1 month after germination of the plants. One sample included the stems and leaves from one plant, and the analyte included five samples. Fresh biomass was dried in an IPLS-131 convection drying oven (Besteq Engineering, Inc., Rostov-On-Don, Russia) at 45 °C to a moisture content of 10% and stored in a D-450A Edry auto dry cabinet (Edry Co., Ltd., Taichung, Taiwan; humidity 2%) before extraction and analysis. The green biomass of six high-flavonoid cucumber cultivars was cultivated by greenhouse farming within 4 months, and the remaining fruits, roots, and rotting leaves were cleared off before extraction and analysis (Table S1). Fertilizer experiments were performed using Lignogumat BM (RET Ltd., Saint Petersburg, Russia) organomineral fertilizer (composition: salts of humic substances, 16.2%; humic acids, 10%; fulvic acids, 5%; potassium (K), 1.8%; sulfur (S), 0.6%; iron (Fe), 0.036%; manganese (Mn), 0.022%; copper (Cu), 0.022%; zinc (Zn), 0.022%; molybdenum (Mo), 0.0027%; selenium (Se), 0.0009%; boron (B), 0.027%; and cobalt (Co), 0.022%), which was added to the tank mix for plant watering.

2.2. Chemicals

The reference standards were purchased from ChemFaces (Wuhan, Hubei, PRC). These included isovitexin (Cat. No. CFN98620; purity 98%), isovitexin-2″-*O*-rhamnoside (Cat. No. 72036-50-1; purity 98%), and isovitexin-4′,2″-*O*-glucoside (Cat. No. 63316-27-8; purity 98%). From MCE Med Chem Express (Monmouth, NJ, USA), we obtained isovitexin-2″-*O*-glucoside (meloside A; Cat. No. HY-N5124; purity 98.55%) and isovitexin-7-*O*-glucoside (saponarin; Cat. No. HY-N5083; purity 98.84%). Selected flavonoids were plant-derived metabolites isolated in our laboratory, including cucumerins C and D, isovitexin-7,4′-*O*-glucoside (saponarin-4′-*O*-glucoside) [12], isovitexin-4′-*O*-glucoside-2″-*O*-(6‴-*O*-*p*-coumaroyl)-glucoside, isovitexin-4′-*O*-glucoside-2″-*O*-(6‴-*O*-feruloyl)-glucoside, isovitexin-2″-*O*-(6‴-*O*-*p*-coumaroyl)-glucoside, isovitexin-2″-*O*-glucoside-6″-*O*-*p*-coumarate, isovitexin-2″-*O*-glucoside-6″-*O*-ferulate, isovitexin-2″-*O*-(6‴-*O*-feruloyl)-glucoside, isovitexin-2″-*O*-*p*-coumarate, isovitexin-2″-*O*-(6‴-*O*-feruloyl)-glucoside-6″-*O*-ferulate, isovitexin-6″-*O*-*p*-coumarate [13], isovitexin-7-*O*-(6″-*O*-glucosyl)-glucoside, apigenin-7-*O*-(6″-*O*-feruloyl)-glucoside (saponarin-6″-*O*-ferulate) [22], and isovitexin-4′-*O*-glucoside [23].

2.3. Total Flavonoid Spectrophotometric Assay

Powdered plant material (100 mg) was mixed with 70% ethanol (10 mL) in a plastic tube (15 mL) and sonicated for 30 min at 40 °C. The extract was centrifuged (15 min, 6000× *g*), the supernatant was transferred into a volumetric flask (25 mL), and the same conditions were used for the second extraction. The final volume in the volumetric flask reached 25 mL using 70% ethanol (solution A). Solution A (50 µL) was mixed with 70% ethanol (250 µL) in a 96-well plate (solution B), and the optical density of solution B was measured at 271 nm using 70% ethanol as the blank solution. Isovitexin was used as the reference compound. To prepare the stock solutions, isovitexin (5 mg) was dissolved in 10 µL of DMSO in a volumetric flask (10 mL), and the final volume in the volumetric flask reached 10 mL using 70% ethanol. The standard calibration curve was generated using 2.5, 5.0, 10.0, 20.0, 40.0, and 80.0 µg/mL concentrations. The calibration curves were created by plotting the optical density vs. the concentrations. All the analyses were carried out in triplicate, and the data are expressed as the means ± standard deviation (S.D.).

2.4. Flavonoid Extract Isolation

Our previous method of flavonoid isolation [14] was used to obtain the flavonoid extract of cucumber green waste. In brief, the dry cucumber biomass (1 kg) was ground and extracted using MeOH (15 L) in an ultrasonic bath (×3, 60 min, 40 °C, ultrasound power of 100 W, frequency of 35 kHz). After centrifuging (6000× *g* rpm, 20 min), the supernatant was concentrated under vacuum. The dry residue was treated with hexane, $CHCl_3$, and n-BuOH to produce the n-butanol fraction separated by polyamide solid-phase extraction (2 kg) after elution with water and 0.5% NH_3 in ethanol. The alkaline eluate was concentrated and dissolved in 40% ethanol (5 mg/mL). It was separated using the macroporous resin Amberlite XAD-2, equilibrated for 6 h, and eluted using 40% ethanol at a flow rate of 2-bed volumes/h, with 5-bed volumes eluted. The resulting eluate was vacuum-concentrated and dried (Figure 1c). The yields and total flavonoid contents are shown in Table 1.

Table 1. Yields and flavonoid contents in the flavonoid extracts of green waste biomass from six cucumber cultivars.

Cultivar	Extract Yield, % of Dry Plant Weight	Flavonoid Content, % of Dry Plant Weight
Altai	1.2 ± 0.0	90.83 ± 1.84
Konkurent	1.6 ± 0.0	90.65 ± 1.86
Masha	3.4 ± 0.1	92.61 ± 2.11
Parizhskii Kornishon	2.8 ± 0.1	90.33 ± 2.01
Zasolochnii	1.2 ± 0.0	91.51 ± 1.93
Zozula	2.0 ± 0.1	91.04 ± 2.08

2.5. Liquid Chromatography–Mass Spectrometry Profiling and Quantification

High-performance liquid chromatography with photodiode array detection and electrospray ionization triple quadrupole mass spectrometric detection (HPLC–PDA–ESI–tQ-MS) was applied for the flavonoid profiling and quantification of flavonoid extracts of the cucumber waste green biomass from six cultivars, as described previously [14]. An LC-20 Prominence liquid chromatograph (Shimadzu, Columbia, MD, USA) coupled with an SPD-M30A photodiode array detector (wavelength range 200–600 nm) and LCMS 8050 triple quadrupole mass spectrometer (all Shimadzu, Columbia, MD, USA) were used for the separation. A GLC Mastro column (2.1 × 150 mm, 3 µm; Shimadzu, Kyoto, Japan) was eluted with a gradient binary eluent system of 0.5% formic acid in water (eluent A) and 0.5% formic acid in acetonitrile (eluent B) using the following gradient program: 0–2 min 5–8% B, 2–5 min 8–9% B, 5–12 min 9–36% B, 12–13 min 36–59% B, 13–15 min 59–78% B, 15–20 min 78–90% B, and 20–25 min 90–5% B. The flow rate, column temperature, and injection volume were set as 100 µL/min, 30 °C, and 1 µL, respectively. Mass spectrometric studies were performed in negative electrospray ionization mode (source voltage

3 kV, collision energy −35 eV, scanning range m/z 80–2000), various temperatures for the ESI interface (300 °C), desolvation line (250 °C), and heat block (400 °C), and specific flow rates of the nebulizing gas (N_2; 3 L/min), heating gas (air; 10 L/min), and collision-induced dissociation gas (Ar; 0.3 mL/min). To identify metabolites, information about the retention time, ultraviolet spectra, and mass spectra in comparison with reference standards and literature was used, managed via LabSolution (Shimadzu) workstation software (https://www.tecan.com/fluent-automated-workstation?utm_term=laboratory%20automation%20software&utm_campaign=SO-Liquid+Handling&utm_source=adwords&utm_medium=ppc&hsa_net=adwords&hsa_tgt=kwd-1357969953253&hsa_ad=646626667096&hsa_acc=9279258943&hsa_grp=145508071093&hsa_mt=p&hsa_cam=19637594876&hsa_kw=laboratory%20automation%20software&hsa_ver=3&hsa_src=g&gad=1&gclid=CjwKCAjw6p-oBhAYEiwAgg2PgnIuzmWBLKDO5llBJSXRoByXZ2k1EXBZU41-NC06zOngBwPm_ZIprhoCdLkQAvD_BwE, accessed on 20 August 2023) loaded with an internal LC–MS library.

2.6. Hypolipidemic Activity

Our previous method [24] for the experimental study of hyperlipidemia in Golden Syrian hamsters was adopted to assess the hypolipidemic potential of cucumber extracts and pure flavonoids. The animal study was conducted according to the guidelines of the Declaration of Helsinki, and was approved by the Russian Health Ministry (protocol code 708H, 23 August 2010) and the Ethics Committee of the Institute of General and Experimental Biology (protocol code LM-0324, 28 January 2014). Male Golden Syrian hamsters (weight of 71–73 g; BioNursery Stezar, Vladimir, Russia) were housed one per cage with a 12 h light/dark cycle (humidity of 50–55%) and had free access to regular rodent cholesterol-free chow (Assortiment-AGRO Company, Sergiev Posad, Russia) and water. After two weeks of adaptation, the animals were weighed, divided into fifteen groups (n = 10), fed the regular rodent cholesterol-free chow for three months, and switched to a high-fat–1%-cholesterol diet (10% lard, 10% yoke powder, 1% cholesterol, 79% regular rodent cholesterol-free chow) for the subsequent six months. The groups were as follows: (1) a normal-diet group; (2) a group with a high-fat–1%-cholesterol diet; (3) a group with a high-fat–1%-cholesterol diet + simvastatin (10 mg/kg/day); (4) a group with a high-fat–1%-cholesterol diet + flavonoid extract cv. Konkurent (100 mg/kg/day); (5) a group with a high-fat–1%-cholesterol diet + flavonoid extract cv. Masha (100 mg/kg/day); (6) a group with a high-fat–1%-cholesterol diet + flavonoid extract cv. Zozula (100 mg/kg/day); (7) a group with a high-fat–1%-cholesterol diet + flavonoid extract cv. Parizhskii Kornishon (100 mg/kg/day); (8) a group with a high-fat–1%-cholesterol diet + flavonoid extract cv. Altai (100 mg/kg/day); (9) a group with a high-fat–1%-cholesterol diet + flavonoid extract cv. Zasolochnii (100 mg/kg/day); (10) a group with a high-fat–1%-cholesterol diet + isovitexin-2″-*O*-glucoside-6″-*O*-*p*-coumarate (20 mg/kg/day); (11) a group with a high-fat–1%-cholesterol diet + isovitexin-2″-*O*-glucoside-6″-*O*-*p*-coumarate (50 mg/kg/day); (12) a group with a high-fat–1%-cholesterol diet + isovitexin-2″-*O*-glucoside (20 mg/kg/day); (13) a group with a high-fat–1%-cholesterol diet + isovitexin-2″-*O*-glucoside (50 mg/kg/day); (14) a group with a high-fat–1%-cholesterol diet + isovitexin (20 mg/kg/day); and (15) a group with a high-fat–1%-cholesterol diet + isovitexin (50 mg/kg/day). Ready-to-use Sigma-Aldrich assay kits were used to measure serum cholesterol (No. MAK436), serum triglycerides (determination kit No. TR0100), serum high-density lipoprotein–cholesterol, low-density lipoprotein–cholesterol (No. MAK045), serum malondialdehyde (Elabscience Biotechnology, Inc., Houston, TX, USA; No E-BC-K025-S), liver superoxide dismutase (No. 19160), glutathione peroxidase (No. MAK437), and catalase (No. MAK100). All analyses were performed five times, and the data are expressed as means ± S.D.

2.7. Statistical Analysis

Statistical analyses were performed through one-way analysis of variance, and the significance of the mean difference was determined using Duncan's multiple range test.

Differences at $p < 0.05$ were considered statistically significant. The results are presented as the means ± S.D. The linear regression analysis and generation of calibration graphs were conducted using Advanced Grapher 2.2 (Alentum Software, Inc., Ramat-Gan, Israel).

3. Results

3.1. Selection of Cucumber Cultivars Based on the Total Flavonoid Content

The total number of cucumber varieties grown in the world is not definitively known. However, the Russian State Register of Breeding Achievements approved approximately 1700 zoned cucumber cultivars for use in 2023 [25]. With this diversity, it was reasonable to conduct a preliminary screening to select the target cucumber cultivars with the highest flavonoid contents. In this study, we assessed the green tissues (leaves and stems) of 30 cultivars that are commonly used for greenhouse cultivation in the Baikal region of Siberia.

Due to the lack of a standardized method for analyzing flavonoids in cucumber greens, we explored new method that would enable the rapid screening of tissues to determine the flavonoid presence. Spectrophotometry, which is traditionally used for the quantification of flavonoids in plants, was chosen as the analysis method. Examining the absorption spectra of ethanol extracts derived from 30 cucumber varieties, we observed similar spectral patterns characterized by two distinct bands (Figure 2). The variance in the position of band II was very narrow and did not exceed 2 nm (272–274 nm), whereas the absorption maximum of band I was in a wide wavelength range (315–325 nm).

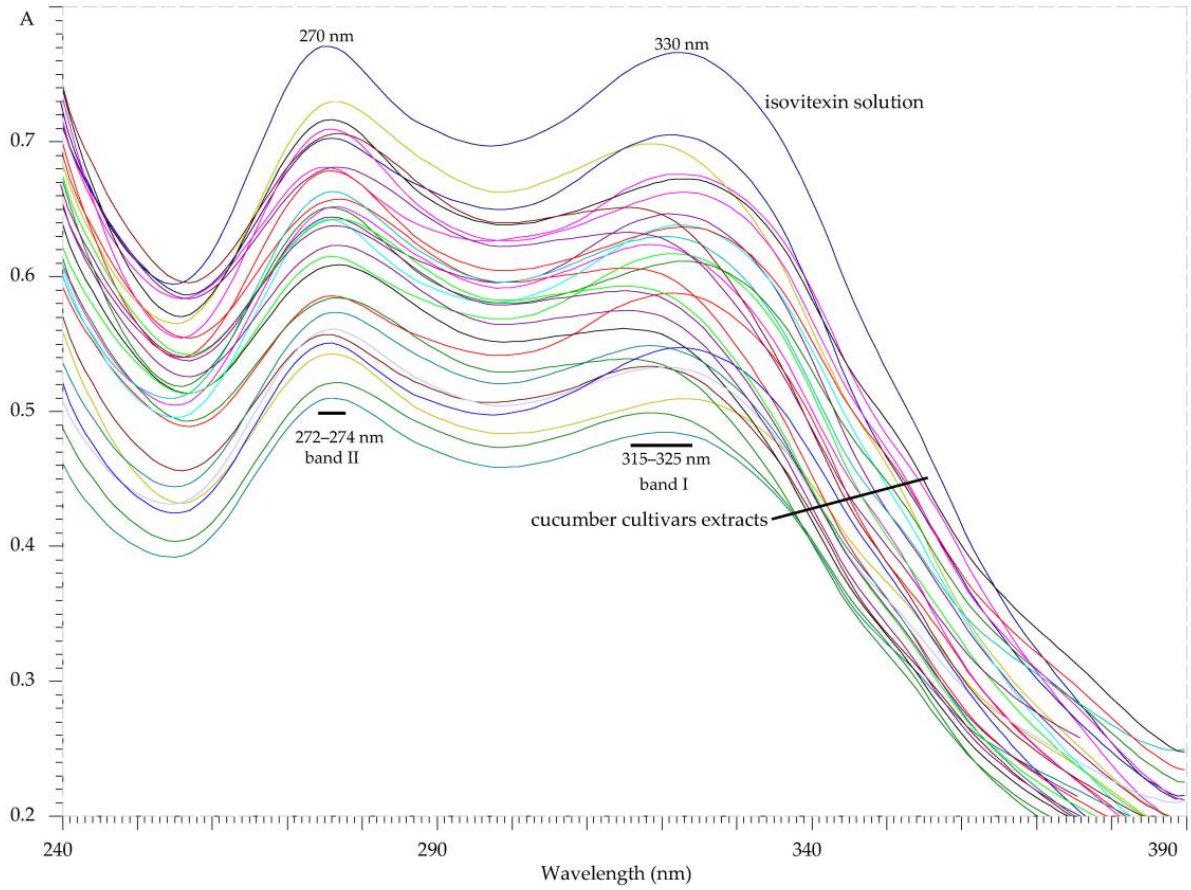


Figure 2. Ultraviolet spectra of the ethanolic extracts from the herbs of 30 cucumber cultivars and isovitexin (reference standard).

The absorption spectra were characteristic of flavones belonging to the apigenin group, which was confirmed by previous data on various apigenin derivatives in cucumber greens [9–14]. A comparative analysis of the spectra of various commercially available

apigenins showed that isovitexin has a similar spectrum (Figure 2). Moreover, isovitexin derivatives are most frequently identified in cucumbers. The 271 nm wavelength was chosen as the working wavelength because it is located centrally between the extremes of the isovitexin and cucumber extracts. Choosing a long wavelength band as the analytical wavelength is less successful because the maximum absorption of isovitexin is at 330 nm, which is 5 nm farther from the extreme position of band II for cucumber extracts. Additional studies have shown that 70% ethanol is the best flavonoid extractant for cucumber greens, and extraction should be performed using double sonication at 50 °C for 30 min (Table S2) [14]. The principal validation criteria, including correlation coefficients, standard deviation, limits of detection, limits of quantification, and linear ranges, were suitable for subsequent quantitative analysis (Table S3).

According to the analysis, the flavonoid content in the cucumber leaves varied from 1.14 to 52.11 mg/g of dry plant weight. Meanwhile, in the stems, the flavonoid content ranged from 0 to 2.17 mg/g of dry plant weight (Table 2). Despite the absence of an official method for categorizing plant tissues based on flavonoid content, we established specific thresholds for flavonoid content segregation. These thresholds were chosen only for interpretation of the results.

Table 2. Total flavonoid contents (TFCs) in the leaves and stems of 30 cucumber cultivars (±S.D.)

Cultivar	TFC in Leaves, mg/g DPW	TFC in Stems, mg/g DPW	Cultivar Group
Masha	52.11 ± 1.04 ^{a}	2.17 ± 0.04 ^{a}	Cultivars with high flavonoid content: >40 mg/g in leaves, >1 mg/g in stems
Parizhskii Kornishon	48.63 ± 0.97 ^{b}	2.02 ± 0.04 ^{ab}	
Zozula	46.82 ± 0.93 ^{bc}	1.92 ± 0.04 ^{b}	
Zasolochnii	45.79 ± 0.91 ^{c}	1.27 ± 0.03 ^{c}	
Altai	40.26 ± 0.80 ^{d}	1.63 ± 0.03 ^{b}	
Konkurent	40.01 ± 0.81 ^{d}	1.52 ± 0.03 ^{b}	
Alfavit	32.67 ± 0.65 ^{e}	0.80 ± 0.02 ^{d}	Cultivars with medium flavonoid content: 10–40 mg/g in leaves, 0.1–1 mg/g in stems
Nezhinskii	30.09 ± 0.60 ^{e}	0.83 ± 0.02 ^{d}	
Arcadia	25.11 ± 0.50 ^{f}	0.64 ± 0.02 ^{e}	
Titus	23.67 ± 0.45 ^{f}	0.30 ± 0.01 ^{g}	
Dachnyi	20.82 ± 0.41 ^{g}	0.43 ± 0.02 ^{f}	
Perseus	18.63 ± 0.37 ^{h}	0.35 ± 0.01 ^{fg}	
Sibirskaya Girlyanda	17.14 ± 0.34 ^{h}	0.15 ± 0.00 ^{h}	
Madhur	15.22 ± 0.30 ^{i}	0.27 ± 0.01 ^{g}	
Amur	15.08 ± 0.30 ^{i}	0.25 ± 0.00 ^{g}	
April	14.29 ± 0.26 ^{i}	0.61 ± 0.02 ^{e}	
Tulsi	11.27 ± 0.20 ^{j}	0.14 ± 0.00 ^{h}	
Sibirskii Express	8.62 ± 0.17 ^{k}	ND	Cultivars with low flavonoid content: <10 mg/g in leaves, <0.1 mg/g in stems
Dinamit	7.29 ± 0.14 ^{kl}	0.08 ± 0.00 ^{i}	
Strelets	7.04 ± 0.12 ^{l}	ND	
Kurazh	6.33 ± 0.12 ^{lm}	ND	
Tornado	6.27 ± 0.11 ^{m}	ND	
Shruti	6.02 ± 0.11 ^{mn}	0.05 ± 0.00 ^{j}	
Parisian Gherkin	5.71 ± 0.14 ^{n}	ND	
Secret	3.75 ± 0.07 ^{o}	0.06 ± 0.00 ^{ij}	
Holland Yellow	3.56 ± 0.06 ^{o}	ND	
Kartoshka	2.83 ± 0.05 ^{p}	ND	
Sikkim	1.69 ± 0.03 ^{q}	ND	
Lemon	1.54 ± 0.03 ^{q}	ND	
Crystall Apple	1.14 ± 0.02 ^{r}	ND	

DPW, dry plant weight. ND, not detected. Values in the same column followed by different superscript letters are significantly different from each other ($p < 0.05$).

Cultivars with a flavonoid content >40 mg/g in leaves and 1 mg/g in stems were classified as high-flavonoid-content cultivars. This group includes six cultivars: Masha, Parizhskii Kornishon, Zozula, Zasolochnii, Altai, and Konkurent. Eleven cultivars had

medium flavonoid levels, with flavonoid concentrations in the range of 10–40 mg/g in the leaves and 0.1–1 mg/g in the stems. Thirteen cultivars had low flavonoid contents (<10 mg/g in the leaves and <0.1 mg/g in the stems). Previous studies have indicated that the total concentration of flavonoids in various cultivars grown in other countries is up to 1 mg/g in China [26], up to 1.46 mg/g in Indonesia [27], and up to 2 mg/g in the USA [28].

This means that different cucumber cultivars are used in industrial greenhouses, and their greens accumulate different levels of flavonoids ranging from negligible to high. Utilizing cultivars having a low flavonoid content would inevitably result in a product with a low concentration of desired metabolites. This, in turn, can adversely affect bioactivity and reduce drug quality. Therefore, we chose to focus our further research on six varieties with high flavonoid contents.

3.2. Variations in the Total Flavonoid Content in Cucumber Greens Based on Harvest Time and Organic Fertilization

The content of secondary metabolites in the green parts of plants is inherently influenced by their age. When cultivating cucumbers under greenhouse conditions, plants are traditionally grown "in one stalk". This practice involves the removal of lateral shoots, which are then collected to form a sample of greens. This collected material is the cucumber waste green biomass. The lateral shoots are removed continuously throughout the entire cucumber growth season. Determining the optimal timing for collection of this green waste is important and needs to be studied. Furthermore, in greenhouse farming, various types of fertilizers are often utilized. Specifically, organic and organomineral fertilizers based on humic acids have emerged as particularly promising and nontoxic fertilizers. In our study, we employed the organomineral fertilizer Lignogumat BM, which is a humic–fulvic preparation containing 16.2% humic acids and 5% fulvic acids. This fertilizer was added to the watering tank mix at levels of 0.01–0.1% and administered every two weeks during the cucumber growth season. The plants were harvested at intervals of 1–4 months after seed sowing, and the total flavonoid content in the entire biomass was analyzed (Table 3).

In the samples collected from the six cucumber varieties, the total flavonoid content fluctuated minimally from the first to the fourth month of growth. Despite a slight average increase observed from the first to the third month of cultivation, these variations were not statistically significant ($p < 0.05$). This suggests that plant age does not significantly affect the flavonoid content. Therefore, the removal of lateral shoots can be performed throughout the cultivation period without compromising the plant material quality.

The use of fertilizer led to an increase in flavonoid content during all harvesting periods. The minimum concentration of the organomineral fertilizer (0.01%) had a negligible effect on the chemical composition of the green biomass. Although the mean values increased, this increase was not statistically significant ($p < 0.05$). At concentrations of 0.05% and 0.1%, significant increases in flavonoid content were observed in all studied varieties. Moreover, a significant increase in flavonoid content was observed in samples obtained during the third and fourth months of harvesting compared with those from the first month of harvesting. This significant difference was not observed in samples that did not receive fertilizer or that were subjected to 0.05% fertilizer. This is compelling evidence that the use of organomineral fertilizers based on humic acids affects the metabolism of green parts of vegetable crops grown under greenhouse conditions and increases the flavonoid content.

It has previously been shown that the use of organomineral fertilizers increases crop productivity and changes the biochemical parameters of the target product and green biomass, which can be used as an additional source of bioactive metabolites. Increases in the contents of flavonoid glycosides and caffeic acid derivatives were observed when organomineral fertilizers were used on a culture of red lettuce (*Lactuca sativa* L.) grown in open ground [29]. The same effect was also observed in grapes [30]: anthocyanin and flavonoid concentrations increased when organomineral fertilizers were used. A positive effect on the chemical composition of fruits and leaves in Golden Delicious apples has been noted with the regular use of organomineral fertilizers [31]. Previous studies have

indicated the effectiveness of using organomineral fertilizers in increasing the productivity of *C. sativus* and improving the nutrient composition of its fruits [32]. However, our study shows, for the first time, that the use of organomineral fertilizers increases flavonoid contents in cucumber greens.

Table 3. Total flavonoid content in the green biomass from six cucumber cultivars cut after 1–4 months of sowing with and without fertilizer use.

Fertilizer Concentration	Total Flavonoid Content, mg/g Dry Plant Weight (±S.D.)			
	1st Month	2nd Month	3rd Month	4th Month
	cv. Masha			
0.00%	28.11 ± 2.52	29.52 ± 2.30	31.28 ± 2.53	30.63 ± 2.48
0.01%	31.27 ± 2.84	31.67 ± 2.51	32.84 ± 2.69	31.62 ± 2.94
0.05%	35.69 ± 3.21 *	36.84 ± 3.31 *	41.81 ± 3.26 *,**	40.83 ± 3.14 *,**
0.10%	36.07 ± 3.54 *	36.96 ± 3.39 *	42.67 ± 3.49 *,**	41.85 ± 3.30 *,**
	cv. Parizhskii Kornishon			
0.00%	21.67 ± 1.73	22.12 ± 1.83	22.54 ± 1.78	20.81 ± 1.65
0.01%	22.16 ± 1.77	23.93 ± 1.63	24.08 ± 1.92	22.08 ± 1.67
0.05%	25.18 ± 2.01 *	27.04 ± 2.14 *	32.69 ± 2.61 *,**	30.82 ± 2.40 *,**
0.10%	25.96 ± 2.11 *	27.96 ± 2.21 *	32.93 ± 2.69 *,**	31.07 ± 2.35 *,**
	cv. Zozula			
0.00%	17.80 ± 1.42	19.63 ± 1.50	20.08 ± 1.60	20.31 ± 1.60
0.01%	18.22 ± 1.47	20.11 ± 1.59	21.86 ± 1.66	21.59 ± 1.75
0.05%	22.09 ± 1.79 *	24.83 ± 1.89 *	25.69 ± 2.05 *,**	25.06 ± 2.01 *,**
0.10%	22.53 ± 1.83 *	25.69 ± 1.93 *	26.37 ± 2.12 *,**	25.93 ± 2.11 *,**
	cv. Konkurent			
0.00%	14.02 ± 1.12	14.02 ± 1.14	14.27 ± 1.16	14.63 ± 1.20
0.01%	14.93 ± 1.22	14.69 ± 1.21	15.89 ± 1.29	15.92 ± 1.29
0.05%	17.03 ± 1.40 *	17.27 ± 1.42 *	20.93 ± 1.52 *,**	20.53 ± 1.51 *,**
0.10%	17.43 ± 1.48 *	17.53 ± 1.40 *	21.22 ± 1.60 *,**	20.99 ± 1.53 *,**
	cv. Zasolochnii			
0.00%	10.42 ± 0.83	10.83 ± 0.80	11.93 ± 0.97	11.47 ± 0.95
0.01%	10.93 ± 0.89	11.27 ± 0.92	12.89 ± 1.05	12.93 ± 1.01
0.05%	14.22 ± 1.14 *	14.97 ± 1.25 *	16.83 ± 1.45 *,**	16.29 ± 1.42 *,**
0.10%	14.59 ± 1.21 *	15.18 ± 1.26 *	17.22 ± 1.49 *,**	16.97 ± 1.53 *,**
	cv. Altai			
0.00%	8.97 ± 0.75	9.04 ± 0.70	9.18 ± 0.75	9.02 ± 0.72
0.01%	10.22 ± 0.85	10.54 ± 0.82	10.69 ± 0.85	10.75 ± 0.82
0.05%	11.83 ± 0.95 *	11.93 ± 0.99 *	15.22 ± 1.22 *,**	15.02 ± 1.20 *,**
0.10%	12.69 ± 1.04 *	12.90 ± 1.07 *	15.89 ± 0.27 *,**	15.93 ± 1.22 *,**

*, $p < 0.05$ vs. 0.00% fertilizer concentration group. **, $p < 0.05$ vs. 1st greens cutting time group

To evaluate the chromatographic profile of flavonoids, we collected green waste from the cultivation of six cucumber varieties after applying an organomineral fertilizer at a concentration of 0.5% in the third month after sowing.

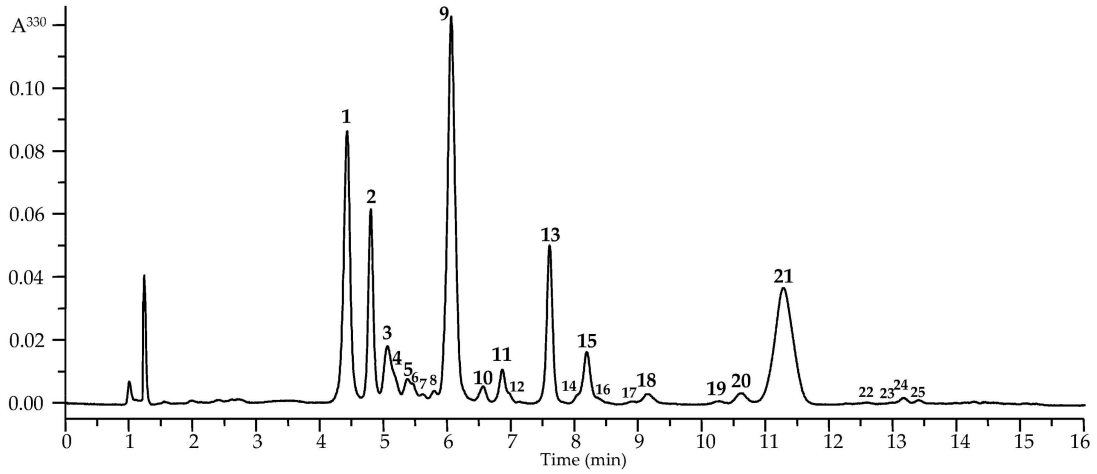
3.3. LC–MS Profiling of Flavonoids in Cucumber Waste Green Biomass Extracts

The analysis of flavonoid extracts isolated from the cucumber waste green biomass of six *C. sativus* cultivars with the highest flavonoid contents was performed using HPLC–PDA–ESI–tQMS. This approach has previously been employed for the analysis of cucumber flower flavonoids [14]. The separation of these extracts provided the identification of 38 flavonoids based on their retention times, ultraviolet and mass spectrometric spectra, and a comparison with the reference standards and literature data [9–14,33–36] (Figure 3, Table 4 and Table S4). Twenty compounds were thoroughly characterized (the first-level metabolite identification according to their structures is shown in Figure 4) [37]. Eighteen compounds were putatively annotated (second-level metabolite identification).

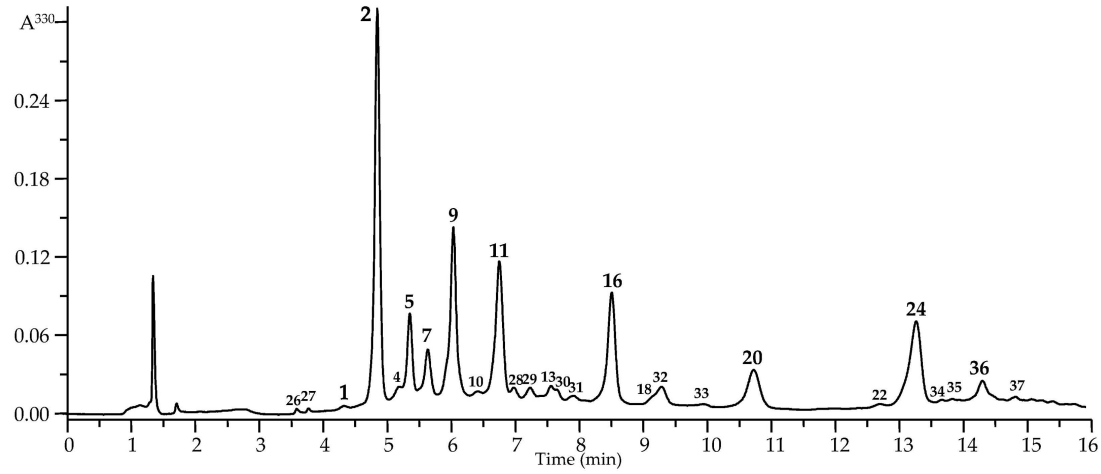
Table 4. Chromatographic (t), ultraviolet (UV), and mass-spectrometric (ESI-MS) data of compounds **1–38** found in the flavonoid extracts of the cucumber waste green biomass.

No.	t, min	UV Pattern [a]	ESI–MS, $[M + H]^-$, m/z	Compound [Ref.]	IL [b]	Found in Cultivar [c]						Early Data [d]
						I	II	III	IV	V	VI	
1	4.45	A	715	Cucumerin C [12]	1	+	+	+	+	+	+	L [12], F [14]
2	4.83	A	715	Cucumerin D [12]	1	+	+	+	+	+	+	L [12], F [14]
3	5.11	A	685	Cucumerin A/B *O*-pentoside [11,12]	2	+	–	+	+	+	+	
4	5.18	A	919	Apigenin-*C*-hexoside-tri-*O*-hexoside [9,13,33]	2	+	+	–	–	–	–	
5	5.42	A	757	Isovitexin-7,4′-*O*-glucoside (saponarin-4′-*O*-glucoside) [33,34]	1	+	+	+	+	+	+	L [12], L* [9]
6	5.47	A	699	Cucumerin A/B *O*-desoxy hexoside [11,12]	2	+	–	–	–	–	–	
7	5.63	A	757	Isovitexin-4′,2″-*O*-glucoside [33,34]	1	+	+	–	–	–	–	L [12]
8	5.80	A	699	Cucumerin A/B *O*-desoxyhexoside [11,12]	2	+	–	+	+	–	–	
9	6.02	B	903	Apigenin-*C*-hexoside-di-*O*-hexoside-*O*-*p*-coumarate [9,13,33]	2	+	+	+	+	+	+	
10	6.53	A	757	Isovitexin-7-*O*-(6″-*O*-glucosyl)-glucoside [35]	1	+	+	+	+	+	+	
11	6.90	B	903	Isovitexin-4′-*O*-glucoside-2″-*O*-(6‴-*O*-*p*-coumaroyl)-glucoside [9,13]	1	+	+	+	+	+	+	L [13], L* [9]
12	7.09	B	933	Apigenin-*C*-hexoside-di-*O*-hexoside-*O*-ferulate [9,13,33]	2	+	–	–	+	+	+	
13	7.53	C	933	Isovitexin-4′-*O*-glucoside-2″-*O*-(6‴-*O*-feruloyl)-glucoside [9,13]	1	+	+	+	+	+	+	L [13], L* [9]
14	8.03	B	903	Apigenin-*C*-hexoside-di-*O*-hexoside-*O*-*p*-coumarate [9,13,33]	2	+	–	–	+	+	+	
15	8.17	A	595	Isovitexin-2″-*O*-glucoside (meloside A) [34,36]	1	+	–	+	+	+	+	L [10,12]
16	8.46	A	595	Isovitexin-4′-*O*-glucoside [34,36]	1	+	+	–	–	–	–	
17	8.82	C	933	Apigenin-*C*-hexoside-di-*O*-hexoside-*O*-ferulate [3,9,13]	2	+	–	–	+	+	+	
18	9.20	B	903	Apigenin-*C*-hexoside-di-*O*-hexoside-*O*-*p*-coumarate [9,13,33]	2	+	+	+	+	+	+	
19	10.24	C	609	Apigenin-7-*O*-(6″-*O*-feruloyl)-glucoside (saponarin-6″-*O*-ferulate) [35]	1	+	–	+	+	+	+	F [14]
20	10.63	B	741	Isovitexin-2″-*O*-(6‴-*O*-*p*-coumaroyl)-glucoside [9,13]	1	+	+	+	+	+	+	L [13], L* [9]
21	11.27	B	741	Isovitexin-2″-*O*-glucoside-6″-*O*-*p*-coumarate [13]	1	+	–	+	+	+	+	L [13]
22	12.61	C	771	Isovitexin-2″-*O*-glucoside-6″-*O*-ferulate [13]	1	+	+	–	–	–	–	L [13]
23	12.94	B	741	Apigenin-*C*-hexoside-*O*-hexoside-*O*-*p*-coumarate [9,13,33]	2	+	–	–	–	–	–	
24	13.18	C	771	Isovitexin-2″-*O*-(6‴-*O*-feruloyl)-glucoside [9,13]	1	+	+	+	+	+	+	L [13], L* [9]
25	13.47	B	579	Isovitexin-2″-*O*-*p*-coumarate [33]	1	+	–	+	+	+	+	
26	3.62	A	877	Cucumerin C/D *O*-hexoside [11,12]	2	–	+	–	–	–	–	
27	3.71	A	877	Cucumerin C/D *O*-hexoside [11,12]	2	–	+	–	–	–	–	
28	6.98	A	595	Isovitexin-7-*O*-glucoside (saponarin) [35]	1	–	+	–	–	–	–	L [12], L* [9], F [14]
29	7.24	B	741	Apigenin-*C*-hexoside-*O*-hexoside-*O*-*p*-coumarate [9,13,33]	2	–	+	–	–	–	–	
30	7.71	B	741	Apigenin-*C*-hexoside-*O*-hexoside-*O*-*p*-coumarate [9,13,33]	2	–	+	–	–	–	–	
31	7.92	B	741	Apigenin-*C*-hexoside-*O*-hexoside-*O*-*p*-coumarate [9,13,33]	2	–	+	–	–	–	–	
32	9.31	A	579	Isovitexin-2″-*O*-rhamnoside [34,36]	1	–	+	–	–	–	–	L [12]
33	9.94	A	433	Isovitexin [36]	1	–	+	–	–	–	–	L [10,12], L* [9,11], F [14]
34	13.54	C	947	Isovitexin-2″-*O*-(6‴-*O*-feruloyl)-glucoside-6″-*O*-ferulate [13]	1	–	+	–	–	–	–	L [13]
35	13.81	B	579	Isovitexin-6″-*O*-*p*-coumarate [34,36]	1	–	+	–	–	–	–	
36	14.27	B	725	Apigenin-*C*-hexoside-di-*O*-*p*-coumarate [9,13,33]	2	–	+	–	–	–	–	
37	14.81	C	755	Apigenin-*C*-hexoside-*O*-*p*-coumarate-*O*-ferulate [9,13,33]	2	–	+	–	–	–	–	
38	15.21	C	785	Apigenin-*C*-hexoside-di-*O*-ferulate [9,13,33]	2	–	–	–	–	+	+	

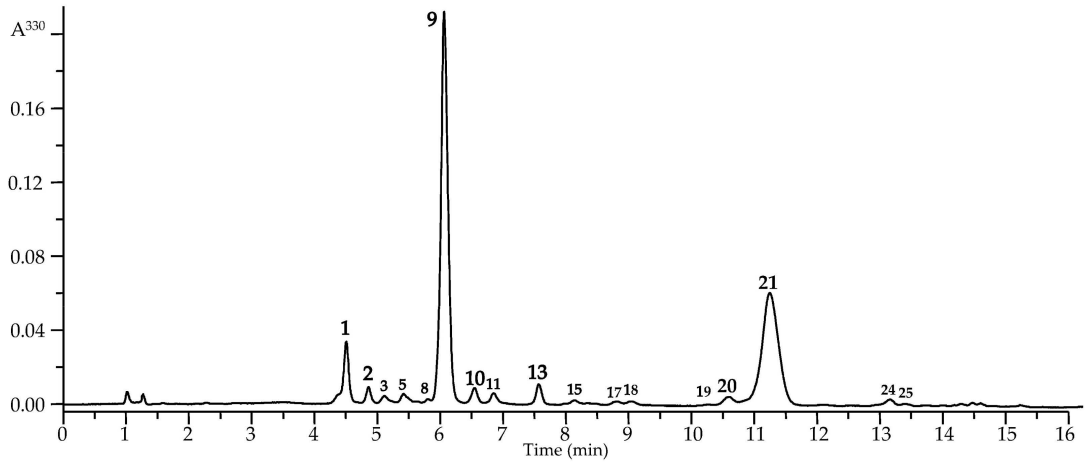
[a] UV patterns: A, 269 ± 3, 332 ± 2 nm; B, 270 ± 1, 290 ± 1, 319 ± 3 nm; C, 272 ± 1, 292 ± 1, 321 ± 3 nm. [b] Identification levels: (1) putatively annotated compounds after comparison of UV and MS data with literature data; (2) identified compounds after comparison of UV and MS data and retention times with reference standards. [c] Cultivars: I, Konkurent; II, Masha; III, Zozula; IV, Parizhskii Kornishon; V, Altai; VI, Zasolochnii. "+", found in the cultivar, "–", not found in the cultivar. [d] Known information about the presence of compound in *C. sativus* parts (L, healthy leaves; L*, leaves infected with *Sphaerotheca fuliginea*; F, flowers).



(**a**)

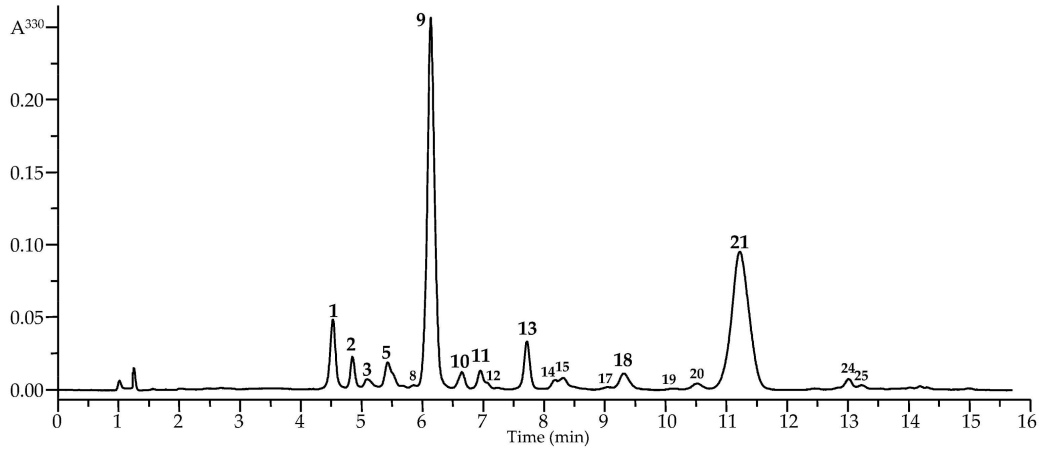


(**b**)

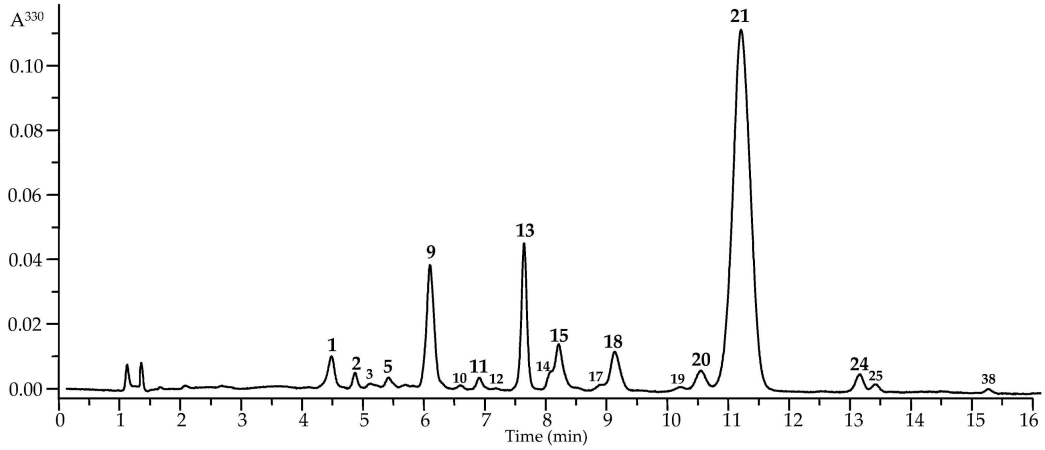


(**c**)

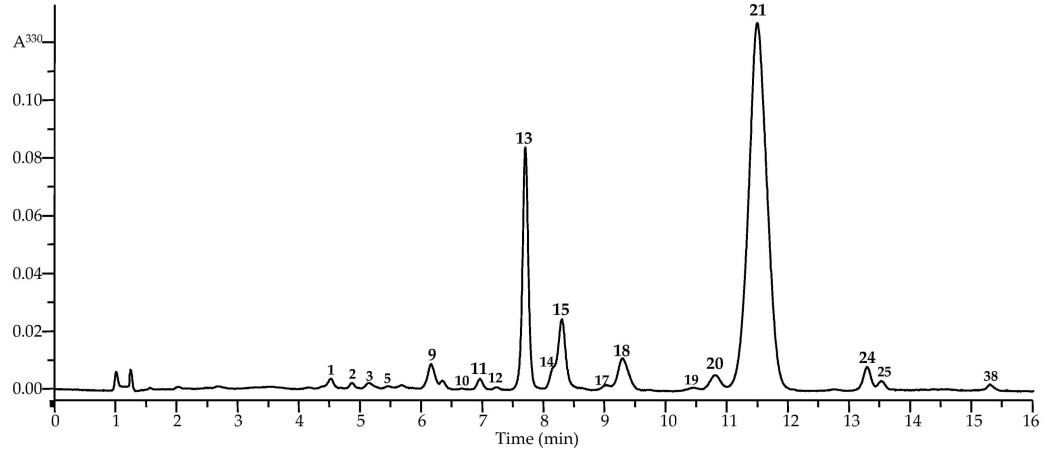
Figure 3. *Cont.*



(**d**)



(**e**)



(**f**)

Figure 3. High-performance liquid chromatography data of the Amberlite XAD-2 fraction of cucumber waste green biomass with photodiode array detection (λ 330 nm) for cv. Konkurent (**a**), Masha (**b**), Zozula (**c**), Parizhskii Kornishon (**d**), Altai (**e**), and Zasolochnii (**f**). The compounds are numbered as listed in Table 5.

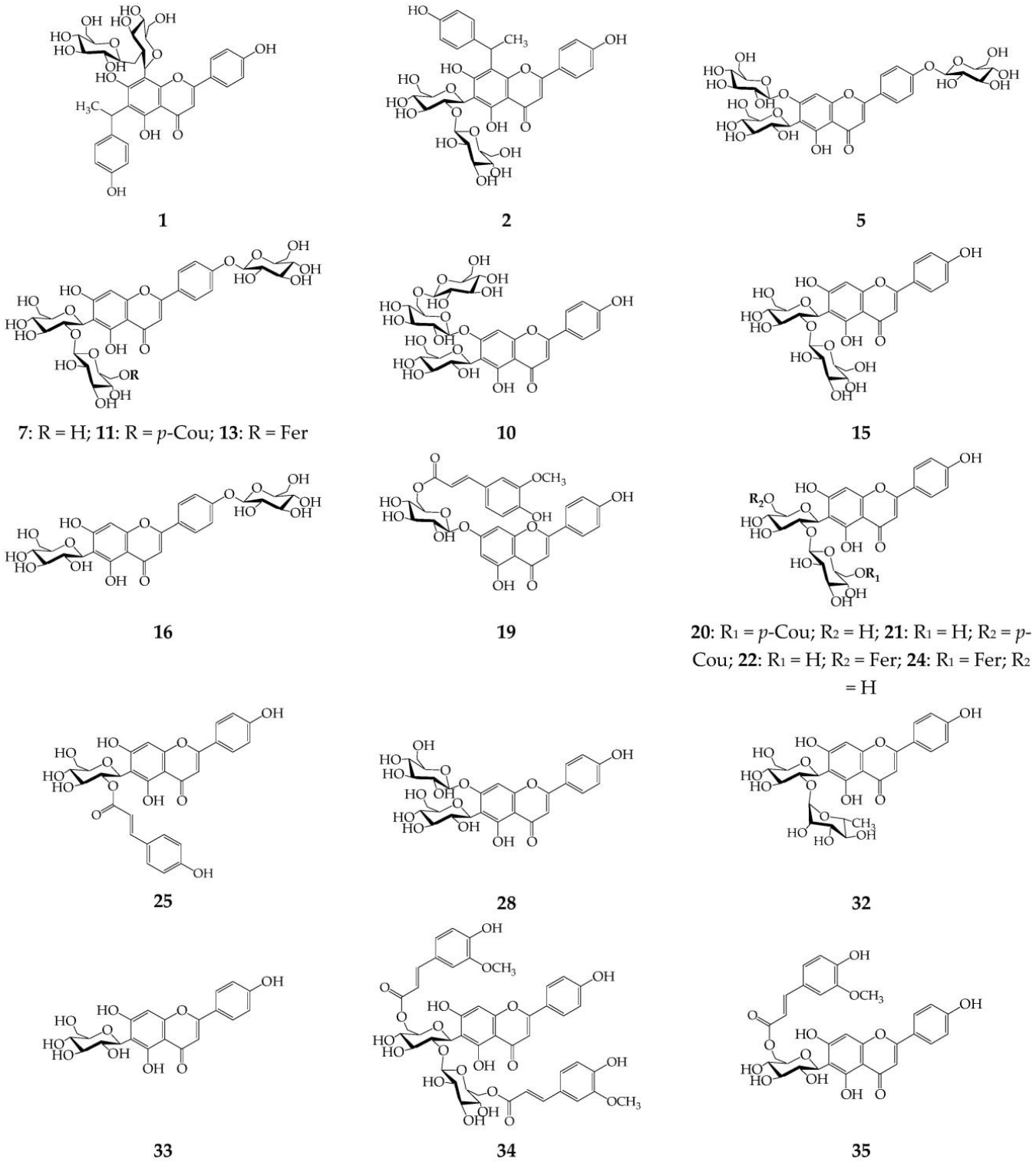


Figure 4. Structures of known flavonoids **1**, **2**, **5**, **7**, **10**, **11**, **13**, **15**, **16**, **19**–**22**, **24**, **25**, **28**, and **32**–**35** found in the cucumber waste green biomass. Abbreviations: *p*-Cou, *p*-coumaroyl; Fer, feruloyl.

Table 5. Compound contents in flavonoid extracts of the cucumber waste green biomass.

Compound	Content in Flavonoid Extract from the Cultivar [a], mg/g ± S.D.					
	I	II	III	IV	V	VI
Non-acylated flavone *C*- and *C*,*O*-glycosides						
Isovitexin	-	<1.00	-	-	-	-
Isovitexin-7-*O*-glucoside	-	6.03 ± 0.14 [b]	-	-	-	4.71 ± 0.09 [d]
Isovitexin-4′-*O*-glucoside	<1.00	59.73 ± 1.14 [i]	-	-	-	-
Isovitexin-2′′-*O*-rhamnoside	-	21.44 ± 0.24 [e]	-	-	-	-
Isovitexin-2′′-*O*-glucoside	22.08 ± 0.41 [e]	-	5.72 ± 0.12 [c]	15.62 ± 0.32 [g]	38.06 ± 0.77 [j]	54.73 ± 1.02 [k]
Isovitexin-7,4′-*O*-glucoside	20.06 ± 0.40 [e]	47.21 ± 0.95 [g]	15.83 ± 0.32 [g]	33.41 ± 0.69 [i]	6.14 ± 0.12 [e]	1.42 ± 0.03 [a]
Isovitexin-4′,2′′-*O*-glucoside	4.21 ± 0.08 [b]	33.18 ± 0.69 [f]	-	-	-	-
Isovitexin-7-*O*-(6′′-*O*-glucosyl)-glucoside	10.32 ± 0.22 [d]	2.86 ± 0.06 [a]	18.30 ± 0.37 [h]	15.04 ± 0.32 [g]	2.18 ± 0.05 [b]	<1.00
Apigenin-*C*-hexoside-tri-*O*-hexoside 4	<1.00	11.08 ± 0.23 [c]	-	-	-	-
Acylated flavone *C*- and *C*,*O*-glycosides						
Isovitexin-2′′-*O*-*p*-coumarate	<1.00	-	2.63 ± 0.04 [a]	4.37 ± 0.09 [c]	6.02 ± 0.11 [f]	6.73 ± 0.12 [e]
Isovitexin-6′′-*O*-*p*-coumarate	-	<1.00	-	-	-	-
Isovitexin-2′′-*O*-(6′′′′-*O*-*p*-coumaroyl)-glucoside	11.02 ± 0.23 [d]	51.26 ± 1.01 [h]	15.64 ± 0.34 [g]	9.43 ± 0.19 [e]	22.18 ± 0.45 [i]	17.41 ± 0.35 [i]
Isovitexin-2′′-*O*-(6′′′′-*O*-feruloyl)-glucoside	4.04 ± 0.09 [b]	115.03 ± 2.34 [k]	9.97 ± 0.18 [e]	11.86 ± 0.23 [f]	14.35 ± 0.29 [h]	15.24 ± 0.33 [h]
Isovitexin-2′′-*O*-glucoside-6′′-*O*-*p*-coumarate	183.39 ± 3.69 [h]	-	309.27 ± 6.22 [k]	329.02 ± 6.71 [l]	613.09 ± 12.26 [m]	643.34 ± 12.89 [m]
Isovitexin-2′′-*O*-glucoside-6′′-*O*-ferulate	<1.00	<1.00	-	-	-	-
Isovitexin-4′-*O*-glucoside-2′′-*O*-(6′′′′-*O*-*p*-coumaroyl)-glucoside	22.07 ± 0.45 [e]	104.82 ± 2.14 [j]	15.24 ± 0.29 [g]	12.93 ± 0.14 [f]	7.25 ± 0.15 [f]	6.08 ± 0.12 [e]
Isovitexin-4′-*O*-glucoside-2′′-*O*-(6′′′′-*O*-feruloyl)-glucoside	81.50 ± 1.63	<1.00	20.35 ± 0.42 [i]	39.29 ± 0.78 [j]	81.83 ± 1.65 [k]	137.10 ± 2.75 [l]
Isovitexin-2′′-*O*-(6′′′′-*O*-feruloyl)-glucoside-6′′-*O*-ferulate	-	<1.00	-	-	-	-
Apigenin-*C*-hexoside-*O*-hexoside-*O*-*p*-coumarate 23	<1.00	-	-	-	-	-
Apigenin-*C*-hexoside-*O*-hexoside-*O*-*p*-coumarate 29	-	7.11 ± 0.15 [b]	-	-	-	-
Apigenin-*C*-hexoside-*O*-hexoside-*O*-*p*-coumarate 30	-	<1.00	-	-	-	-
Apigenin-*C*-hexoside-*O*-hexoside-*O*-*p*-coumarate 31	-	<1.00	-	-	-	-
Apigenin-*C*-hexoside-di-*O*-hexoside-*O*-*p*-coumarate 9	222.61 ± 4.48 [i]	105.39 ± 2.16 [j]	453.18 ± 9.11 [l]	371.53 ± 7.49 [l]	91.03 ± 1.85 [l]	17.24 ± 0.33 [i]
Apigenin-*C*-hexoside-di-*O*-hexoside-*O*-*p*-coumarate 14	<1.00	-	-	7.26 ± 0.15 [e]	9.43 ± 0.19 [g]	11.18 ± 0.21 [g]
Apigenin-*C*-hexoside-di-*O*-hexoside-*O*-*p*-coumarate 18	2.34 ± 0.05 [a]	<1.00	6.08 ± 0.12 [d]	22.46 ± 0.43 [h]	34.62 ± 0.69 [j]	32.92 ± 0.66 [j]
Apigenin-*C*-hexoside-di-*O*-hexoside-*O*-ferulate 12	<1.00	-	-	5.62 ± 0.12 [d]	1.01 ± 0.02 [a]	1.52 ± 0.03 [a]
Apigenin-*C*-hexoside-di-*O*-hexoside-*O*-ferulate 17	<1.00	-	5.14 ± 0.10 [c]	2.31 ± 0.05 [b]	4.14 ± 0.08 [d]	3.41 ± 0.07 [c]
Apigenin-*C*-hexoside-di-*O*-*p*-coumarate 36	-	18.12 ± 0.34 [d]	-	-	-	-
Apigenin-*C*-hexoside-*O*-*p*-coumarate-*O*-ferulate 37	-	<1.00	-	-	-	-
Apigenin-*C*-hexoside-di-*O*-ferulate 38	-	-	-	-	-	3.84 ± 0.07 [c]
Flavone *O*-glycosides						
Apigenin-7-*O*-(6′′-*O*-feruloyl)-glucoside	<1.00	-	<1.00	1.52 ± 0.03 [a]	4.03 ± 0.08 [d]	2.81 ± 0.05 [b]
Cucumerins						
Cucumerin C	154.12 ± 3.08 [h]	<1.00	60.83 ± 1.22 [j]	53.92 ± 1.09 [k]	22.71 ± 0.46 [i]	7.43 ± 0.15 [f]
Cucumerin D	86.71 ± 1.73 [g]	210.44 ± 4.22 [l]	14.06 ± 0.29 [f]	21.18 ± 0.42 [h]	7.16 ± 0.15 [f]	3.02 ± 0.06 [b]
Cucumerin C/D *O*-hexoside 26	-	<1.00	-	-	-	-
Cucumerin C/D *O*-hexoside 27	-	<1.00	-	-	-	-
Cucumerin A/B *O*-pentoside 3	46.21 ± 0.92 [f]	-	10.63 ± 0.21 [e]	11.73 ± 0.23 [f]	3.42 ± 0.06 [c]	4.22 ± 0.09 [c]
Cucumerin A/B *O*-desoxyhexosides 6	6.27 ± 0.12 [c]	-	4.24 ± 0.08 [b]	-	-	-
Cucumerin A/B *O*-desoxyhexosides 8	<1.00	-	<1.00	3.25 ± 0.07 [c]	-	-
Subtotal non-acylated *C*- and *C*,*O*-glycosides	56.67	181.53	39.85	64.07	46.38	60.86
Subtotal acylated *C*- and *C*,*O*-glycosides	526.97	401.73	837.50	816.08	884.95	892.17
Subtotal *O*-glycosides	<1.00	-	<1.00	1.52	4.03	2.81
Subtotal cucumerins	293.31	210.44	89.76	90.08	33.29	14.67
Total flavonoids	876.95	793.70	967.11	971.75	968.65	970.51

[a] Cultivars: I, Konkurent; II, Masha; III, Zozula; IV, Parizhskii Kornishon; V, Altai; VI, Zasolochnii. Values in the same column followed by different superscript letters are significantly different from each other ($p < 0.05$).

3.3.1. Non-Acylated Flavonoids

Cucumerins. Cucumerins are a small group of natural apigenin derivatives with an unusual structure consisting of a basic flavone skeleton to which the *C*-fused fragment of 4-hydroxy-1-ethylbenzene is attached at C-6 or C-8. The first identified cucumerins, A and B, were found in the hydrolysate of *C. sativus* leaves infected with the pathogenic fungus *Podosphaera xanthii* [11] and subsequently detected in healthy cucumber greens [12]. Struc-

turally, cucumerin A is a derivative of vitexin (6-*C*-(4-hydroxy-1-ethylbenzene)-apigenin 8-*C*-glucoside 6-(4-hydroxy-1-ethylbenzene), and cucumerin B is derived from isovitexin (8-*C*-(4-hydroxy-1-ethylbenzene)-apigenin 6-*C*-glucoside).

Two isomeric glycosides of cucumerins A and B [**1** (cucumerin C) and **2** (cucumerin D), respectively] yielded protonated ions with *m*/*z* 715. These compounds, which were initially identified in cucumber leaves of cv. Masha [12] and in cucumber flowers [14], were detected in the cucumber waste green biomass of six cultivars (Figure 5a). Compound **3**, with a protonated ion *m*/*z* 685, exhibited a mass spectrum that is similar to those of **1** and **2**, but without a fragment with *m*/*z* 132, which is characteristic of pentose [38] (Figure 5b). The most likely structure of **3** is *O*-pentoside of cucumerins A or B; however, the exact structure is still unknown. Isomers **6** and **8** with protonated ions at *m*/*z* 699 were identified as *O*-desoxyhexosides of cucumerins A or B; however, the exact structures are also unknown (Figure 5c). Compounds **26** and **27** yielded protonated ions with *m*/*z* 877, which is 162 a.m.u. heavier than those of **1** and **2**, indicating that the possible structures of cucumerins C and/or D are *O*-hexosides (Figure 5d). Unknown compound **6** was detected in all cultivars, with **3** detected in five cultivars, **8** in cv. Konkurent and Masha, and **26** and **27** only in cv. Masha.

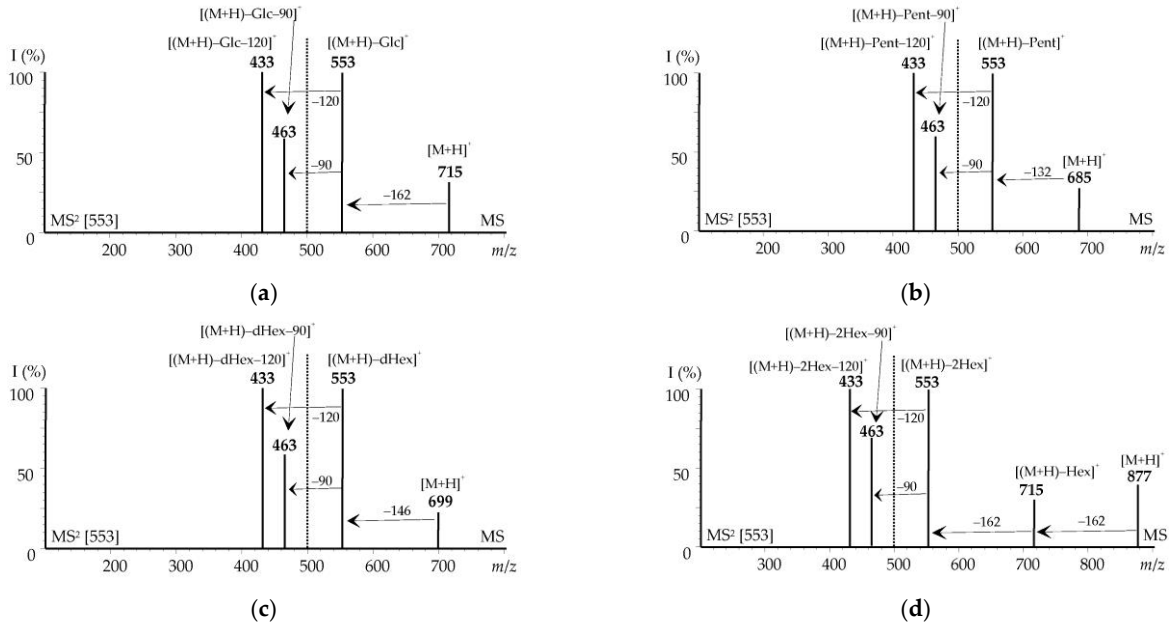


Figure 5. Mass spectra (MS: right; MS^2: left) of compounds **1** (**a**), **3** (**b**), **6** (**c**), and **26** (**d**). dHex, desoxyhexose; Glc, glucose; Hex, hexose; Pent, pentose.

Apigenin *C*- and *C*,*O*-glycosides. Nine non-acylated glycosides of apigenin were found in the studied cultivars, including the known compounds of isovitexin (**33**), isovitexin-7-*O*-glucoside (**28**), isovitexin-4′-*O*-glucoside (**16**), isovitexin-2″-*O*-glucoside (**15**), isovitexin-2″-*O*-rhamnoside (**32**), isovitexin-7-*O*-(6″-*O*-glucosyl)-glucoside (**10**), isovitexin-7,4′-*O*-glucoside (**5**), and isovitexin-4′,2″-*O*-glucoside (**7**). These were identified by comparing spectral data with reference standards. Isovitexin has previously been identified in cucumber leaves of cv. Corona [11], Cezar, Delicius [10], and Mustang [9]. Isovitexin-2″-*O*-glucoside has been identified in the leaves of six Polish cultivars [10], and isovitexin-7-*O*-glucoside has been found in Mustang [9]. Compounds **5**, **7**, **10**, **16**, and **32** were identified in *C. sativus* for the first time.

Compound **4** with a protonated ion at *m*/*z* 919 showed a consecutive loss of three hexose fragments (*m*/*z* 919→757, 595, 433), followed by a typical vitexin/isovitexin cleavage (*m*/*z* 433→343, 313) [39] (Figure 6). This suggests that compound **4** is a tri-*O*-hexoside of apigenin-*C*-hexoside; however, the exact structure is unknown. Only isovitexin-7-*O*-(6″-*O*-

glucosyl)-glucoside was found in all cultivars, whereas the remaining compounds were found in one to four cultivars.

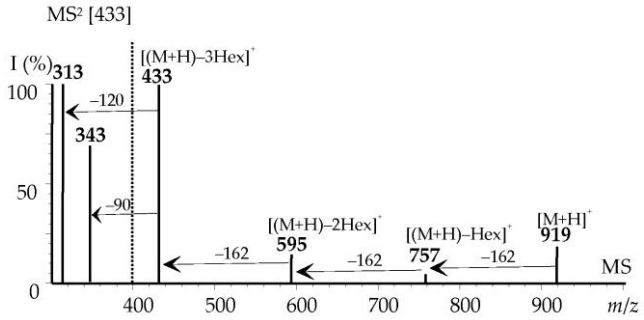


Figure 6. Mass spectrum (MS: right; MS^2: left) of compound **4**. Hex, hexose.

3.3.2. Acylated Flavonoids

Apigenin *O*-glycoside. Apigenin-7-*O*-(6″-*O*-feruloyl)-glucoside (saponarin-6″-*O*-ferulate; **19**) was identified in the leaves of five cucumber cultivars after comparison with reference standards.

Apigenin *C*- and *C*,*O*-glycosides. The presence of apigenin *C*- and *C*,*O*-glycosides acylated with fragments of coumaric and ferulic acids has previously been reported in cucumber leaves [9,13]. The cucumber waste green biomass of commercially grown cucumbers also contains these compounds. Finally, 21 compounds were identified. Among these, nine flavones, isovitexin-4′-*O*-glucoside-2″-*O*-(6‴-*O*-*p*-coumaroyl)-glucoside (**11**), isovitexin-4′-*O*-glucoside-2″-*O*-(6‴-*O*-feruloyl)-glucoside (**13**), isovitexin-2″-*O*-(6‴-*O*-*p*-coumaroyl)-glucoside (**20**), isovitexin-2″-*O*-glucoside-6″-*O*-*p*-coumarate (**21**), isovitexin-2″-*O*-glucoside-6″-*O*-ferulate (**22**), isovitexin-2″-*O*-(6‴-*O*-feruloyl)-glucoside (**24**), isovitexin-2″-*O*-*p*-coumarate (**25**), isovitexin-2″-*O*-(6‴-*O*-feruloyl)-glucoside-6″-*O*-ferulate (**34**), and isovitexin-6″-*O*-*p*-coumarate (**35**), have previously been identified by us in cucumber leaves [13], and were found for the first time in the cucumber waste green biomass.

Twenty compounds with tentative structures were characterized as derivatives of apigenin *C*-hexoside due to the presence of the principal fragments in the MS^2 spectra at *m*/*z* 343 and 313 [40]. Three compounds, **9**, **14**, and **18**, yielded protonated ions with *m*/*z* 903 and, in combination with their UV patterns (λ_{max} 270 ± 1, 290 ± 1, 319 ± 3 nm), indicated the loss of the coumarate fragment (*m*/*z* 903→757), followed by the loss of two hexose moieties (*m*/*z* 757→595, 433) (Figure 7a).

Structures **9**, **14**, and **18** are possibly apigenin-*C*-hexoside-di-*O*-hexoside-*O*-*p*-coumarates; these compounds do not have known analogues from natural sources. Flavonoids **23**, **29**, **30**, and **31** were relatively light (162 a.m.u. heavier than **9**), which suggests that they are apigenin-*C*-hexoside-*O*-hexoside-*O*-*p*-coumarates (Figure 7b). Two flavonoids, **12** and **17**, with UV patterns at 272 ± 1, 292 ± 1, and 321 ± 3 nm, demonstrated the loss of a fragment at *m*/*z* 176, typical for ferulic acid [41], resulting in the presence of basic ions in the MS spectrum at *m*/*z* 757, 595, and 433 in the spectra of **9** (Figure 7c). These data suggest that **12** and **17** are possibly unknown apigenin-*C*-hexoside-di-*O*-hexoside-*O*-ferulates.

The mass spectrum of compound **36** indicated the presence of two *p*-coumaroyl fragments coupled with apigenin-*C*-hexoside (*m*/*z* 725→579, 433). This was confirmed by the longer retention time of **36** compared with that of isovitexin-6″-*O*-*p*-coumarate (**35**) at 14.27 min vs. 13.81 min, respectively (Figure 8a). The most likely structure of **36** is vitexin/isovitexin di-*O*-*p*-coumarate, which is not found naturally. Similar considerations were applied to compounds **37** and **38**, which yielded protonated ions at *m*/*z* 755 (Figure 8b) and 785 (Figure 8c), respectively, and were identified as apigenin-*C*-hexoside-*O*-coumarate-*O*-ferulate and apigenin-*C*-hexoside-di-*O*-ferulate, respectively.

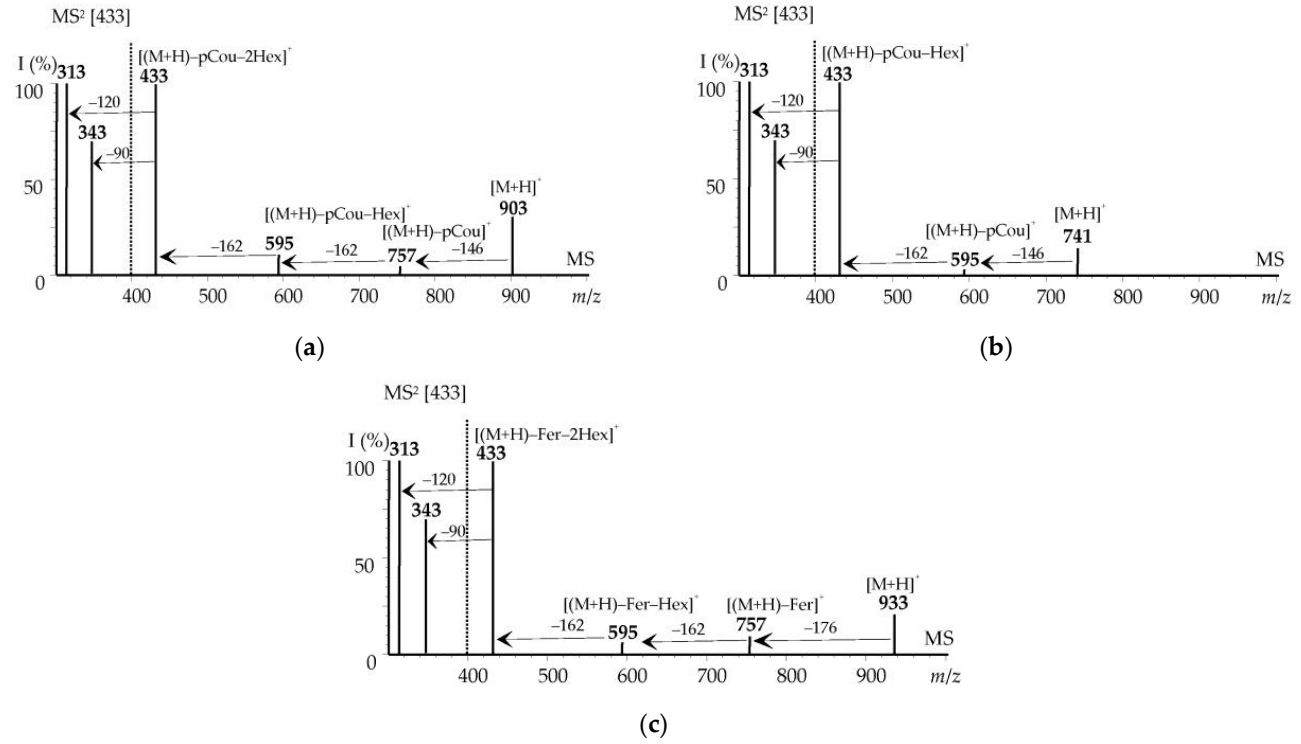


Figure 7. Mass spectra (MS: right; MS^2: left) of compounds **9** (**a**), **23** (**b**), and **12** (**c**). Fer, ferulate; Hex, hexose; pCou, *p*-coumarate.

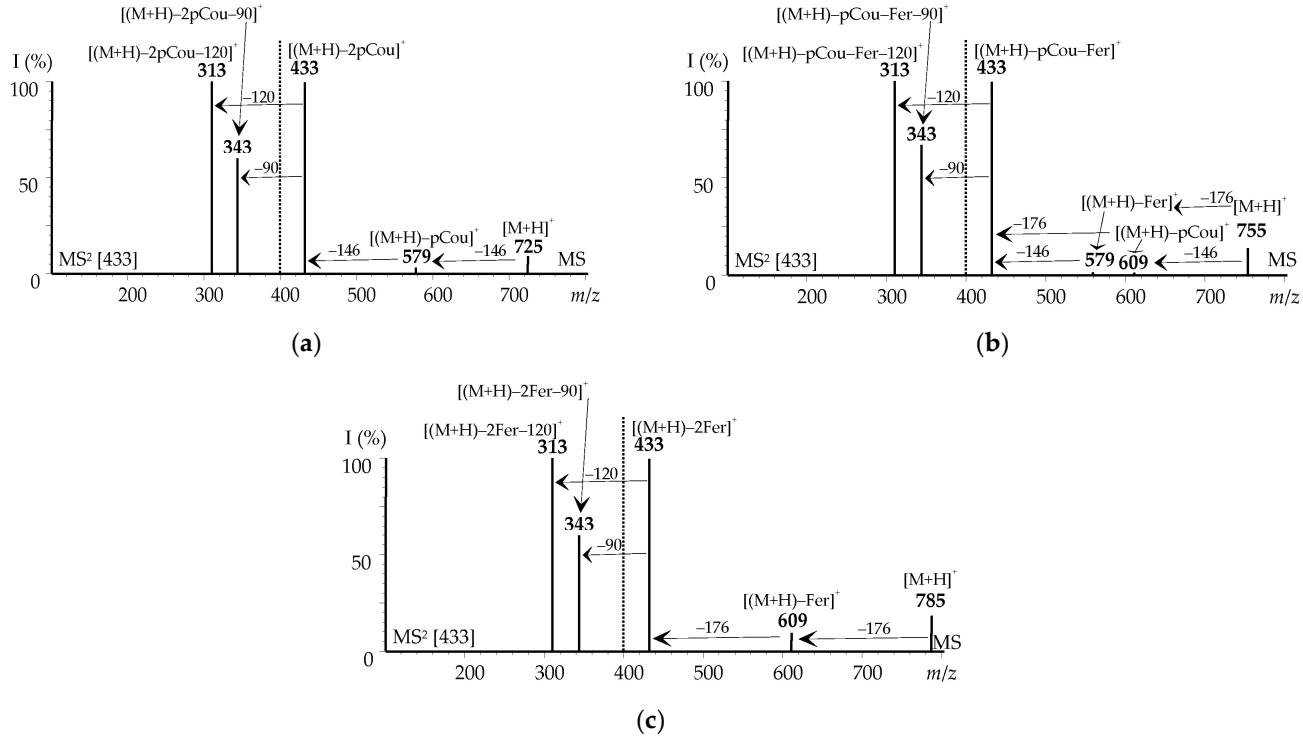


Figure 8. Mass spectra (MS: right; MS^2: left) of compounds **36** (**a**), **37** (**b**), and **38** (**c**). Fer, ferulate; pCou, *p*-coumarate.

Brown et al. [42] investigated foliar cucumber flavonoids and revealed the presence of nine unidentified compounds. In a later study of six Poland cultivar leaves, three flavonoids were identified as isovitexin, isoorientin, and isovitexin-2″-*O*-glucoside in com-

bination with two unknown di-*O*-glucosides of isovitexin and swertiajaponin [10]. Infection of the plants with *Sphaerotheca fuliginea* led to the accumulation of acylated derivatives isovitexin-2″-*O*-(6‴-*p*-coumaroyl)-glucoside, isovitexin-2″-*O*-(6‴-*p*-coumaroyl)-glucoside-4′-*O*-glucoside, isovitexin 2″-*O*-(6‴-feruloyl)-glucoside-4′-*O*-glucoside, isoscoparin-2″-*O*-(6‴-*p*-coumaroyl)-glucoside, isoscoparin-2″-*O*-(6‴-feruloyl)-glucoside-4-*O*-glucoside, apigenin-7-*O*-(6″-*O*-*p*-coumaroyl)-glucoside, isovitexin-2″-*O*-(6‴-feruloyl)-glucoside, and isoscoparin-2″-*O*-(6‴-feruloyl)-glucoside, as well as the non-acylated flavonoids isovitexin, saponarin, saponarin-4′-*O*-glucoside, and vicenin-2 [9]. Subsequent studies on the cucumber leaf tissue resistance against powdery mildew fungi led to the discovery of new flavonoids fused with the 4-hydroxy-1-ethylbenzene fragments, specifically vitexin-6-*C*-(4-hydroxy-1-ethylbenzene) (cucumerin A) and isovitexin-8-*C*-(4-hydroxy-1-ethylbenzene) (cucumerin B), as well as the known phenolics vitexin, isovitexin, orientin, isoorientin, and *p*-coumaric acid [11]. Cucumerin glucosides, cucumerins C and D, were later found in the foliar tissue of healthy cucumbers in addition to isovitexin, isovitexin-2″-*O*-rhamnoside, isovitexin-2″-*O*-glucoside, isovitexin-7-*O*-glucoside, isovitexin-7,2″-di-*O*-glucoside, isovitexin-7,4′-di-*O*-glucoside, and isovitexin-4′,2″-di-*O*-glucoside [12]. Seven acylated isovitexins were isolated from non-damaged cucumber leaves as isovitexin-2″-*O*-glucoside-6″-*O*-ferulate, isovitexin-2″-*O*-glucoside-6″-*O*-*p*-coumarate, isovitexin-2″-*O*-(6‴-*O*-feruloyl)-glucoside-6″-*O*-ferulate, isovitexin-4′-*O*-glucoside-2″-*O*-(6⁗-*O*-feruloyl)-glucoside, isovitexin-4′-*O*-glucoside-2″-*O*-(6⁗-*O*-*p*-coumaroyl)-glucoside, isovitexin-2″-*O*-(6⁗-*O*-feruloyl)-glucoside, and isovitexin-2″-*O*-(6⁗-*O*-*p*-coumaroyl)-glucoside [13]. Thus, during an almost 50-year history of studying cucumbers, 26 flavonoids have been accurately identified in *C. sativus* leaves. Despite the differences between various cucumber cultivars, apigenin-*C*-glucosides (isovitexins and vitexin) are the primary components of the flavonoid complex in contrast to the rarely occurring derivatives of luteolin (orientin and isoorientin) and chrysoeryol (isoscoparins). This study found only apigenin-*C*-glycosides in the cucumber waste green biomass, which agrees with prior estimates of cucumber foliar phenolics [9–14,42]. Of the 38 identified compounds, only 17 were previously described for the cucumber, and the remaining 22 flavonoids were found in this species for the first time. The data indicate that the study of foliar flavonoids can be further expanded to identify new metabolites.

3.4. Selected Compounds in the Flavonoid Extracts of Cucumber Waste Green Biomass

Quantitative analysis revealed distinct variations in the composition of the examined extracts among the different cucumber cultivars. The total flavonoid content in the extracts ranged from 793.70 mg/g (cv. Masha) to 971.75 mg/g (cv. Parizhskii Kornishon) (Table 5).

The primary group of compounds included acylated flavone *C*- and *C*,*O*-glycosides at 401.73 mg/g in cv. Masha (50.6% of the total flavonoid content, TFC) and 526.97 mg/g in cv. Konkurent (60.1% TFC) on the lower end, and 884.95 mg/g in cv. Altai (91.4% TFC) and 892.17 mg/g in cv. Zasolochnii (91.9% TFC) on the higher end. In cv. Konkurent, Zozula, Parizhskii Kornishon, Altai, and Zasolochnii, the major compounds were determined to be isovitexin-2″-*O*-glucoside-6″-*O*-*p*-coumarate flavones with concentrations in the range of 183.39–643.34 mg/g. In cv. Masha, isovitexin-2″-*O*-(6⁗-*O*-feruloyl)-glucoside (115.03 mg/g) and isovitexin-4′-*O*-glucoside-2″-*O*-(6⁗-*O*-*p*-coumaroyl)-glucoside (104.82 mg/g) were the major compounds, and in cv. Zasolochnii, isovitexin-4′-*O*-glucoside-2″-*O*-(6⁗-*O*-feruloyl)-glucoside (137.10 mg/g) was the major compound. Notably, compound **9**, tentatively identified as an apigenin-*C*-hexoside-di-*O*-hexoside-*O*-*p*-coumarate, exhibited concentrations in cv. Masha (105.39 mg/g), Konkurent (222.61 mg/g), Parizhskii Kornishon (371.53 mg/g), and Zozula (453.18 mg/g), warranting further structural elucidation. The contents of non-acylated flavone *C*- and *C*,*O*-glycosides varied from 39.85 mg/g (cv. Zozula) to 181.53 mg/g (cv. Masha). Within this category, isovitexin-2″-*O*-glucoside (5.72–54.73 mg/g) and isovitexin-7,4′-*O*-glucoside (1.42–47.21 mg/g) were the major compounds in four cultivars, while isovitexin-4′-*O*-glucoside (59.73 mg/g) and isovitexin-7-*O*-(6″-*O*-glucosyl)-glucoside (18.30 mg/g) were the major compounds in cv. Masha and Zozula, respectively.

The contents of cucumerins, a group of compounds found only in cucumbers, varied from 14.67 mg/g (cv. Zasolochnii) to 293.31 mg/g (cv. Konkurent). The contents of cucumerins C and D were the highest, with concentrations of 7.43–154.12 mg/g and 3.02–210.44 mg/g, respectively. The minor group of flavonoids, flavone *O*-glycosides, accounted for zero (cv. Masha), a trace amount (cv. Konkurent and Zozula), and 1.52–4.03 mg/g (cv. Parizhskii Kornishon, Altai, and Zasolochnii). Our study shows that the quantitative parameters of cucumber waste green biomass may vary across different cultivars used for flavonoid extract production. Changes in chemical composition among the different cultivars or varieties of cultivated plants are common. Previously, variations in the contents of specific phenolic compounds were observed in different vegetables and fruits [43–47]. Such variations originate from the peculiarities of parental varieties when genetic information is mixed, which contributes to the development of varieties with diverse chemical profiles. Thus, the aim of this study was to identify cultivars with characteristics that are most suitable for obtaining high-quality target products. Next, a cultivar (cultivars) was chosen that resulted in extracts with distinct biological activities.

3.5. Hypolipidemic and Antioxidant Potential of the Flavonoid Extract and Selected Compounds in Cucumber Waste Green Biomass on the Hyperlipidemia of Hamsters

Despite their diverse bioactivities, flavonoids have recently emerged as potential agents for lowering lipid levels [48]. Studies in animals and humans have demonstrated that diets rich in flavonoids lead to improved blood lipid profiles and reduced risks of cardiovascular diseases and contribute to the prevention of atherosclerosis. Of particular interest as sources of flavonoids are the widely available plant products or food waste, which can be used for producing flavonoid-rich extracts. For example, eggplant peel flavonoids were shown to have a pronounced hypoglycemic effect on cholesterol-fed rats [49]. Total flavonoid extracts of tea plant shiso or Korean perilla (*Perilla frutescens*) herb were highly effective in reducing total serum cholesterol, triacylglycerols, low-density lipoprotein–cholesterol, and adipose tissue lipid accumulation in hyperlipidemic rats [50]. Sea buckthorn (*Hippophae rhamnoides*) flavonoids normalized the lipid blood profile of mice while improving antioxidant blood protection indicators [51]. A hypolipidemic effect was found for avocado (*Persea americana*) peel flavonoid extract [52], chrysanthemum flavones [53], citrus polymethoxylated flavones [54], and hawthorn leaf flavonoids [55]. Therefore, flavonoid extracts from cucumber leaves are promising lipid-lowering agents, especially because certain compounds can inhibit pancreatic lipase activity [12–14].

The main groups of flavonoids from cucumber leaves are apigenin derivatives, which have a hypolipidemic effect [55]. Apigenin has been shown to improve hyperlipidemia in rats that are fed high-fat diets [56] and reduce lipid accumulation induced by palmitic acid by activating the AMPK/SREBP pathway in HepG2 cells [57]. Apigenin ameliorates lipid metabolism disorders by downregulating sterol regulatory element-binding protein 1c (SREBP-1c), sterol regulatory element-binding protein 2 (SREBP-2), and some downstream genes [58]. Building on these insights, we studied the hypolipidemic effect of cucumber leaf extracts isolated from six different cultivars using an experimental hyperlipidemia hamster model.

Following a six-month diet that included 1% cholesterol, significant increases ($p < 0.05$) were observed in the serum levels of total cholesterol (TC; 1.57 mmol/L→6.32 mmol/L), total triglycerides (TTG; 0.75 mmol/L→3.02 mmol/L), and low-density lipoprotein–cholesterol (LDLC; 0.81 mmol/L→3.25 mmol/L). In contrast, there was a reduction in the level of high-density lipoprotein–cholesterol (HDLC; 0.51 mmol/L→0.24 mmol/L). These changes indicated the progression of hyperlipidemia compared with that in normal-diet animals (Figure 9). Simvastatin, a known lipid-lowering agent [59], resulted in the lowering of TC (2.48 mmol/L), TTG (1.50 mmol/L), and LDLC (1.11 mmol/L), along with an elevation in HDLC levels (0.54 mmol/L) at a dosage of 10 mg/kg/day. However, the high-cholesterol diet negatively affected liver function, as evidenced by the high malondialdehyde level (MDA; 3.0 mmol/L→29.1 mmol/L) and low levels of superoxide

dismutase (SOD; 79.1→42.1 U/mg), glutathione peroxidase (GPx; 9.7→3.7 U/mg), and catalase (CAT; 122.7→41.6 U/mg). The administration of simvastatin did not lead to a noticeable improvement in the indicators of antioxidant protection in the liver.

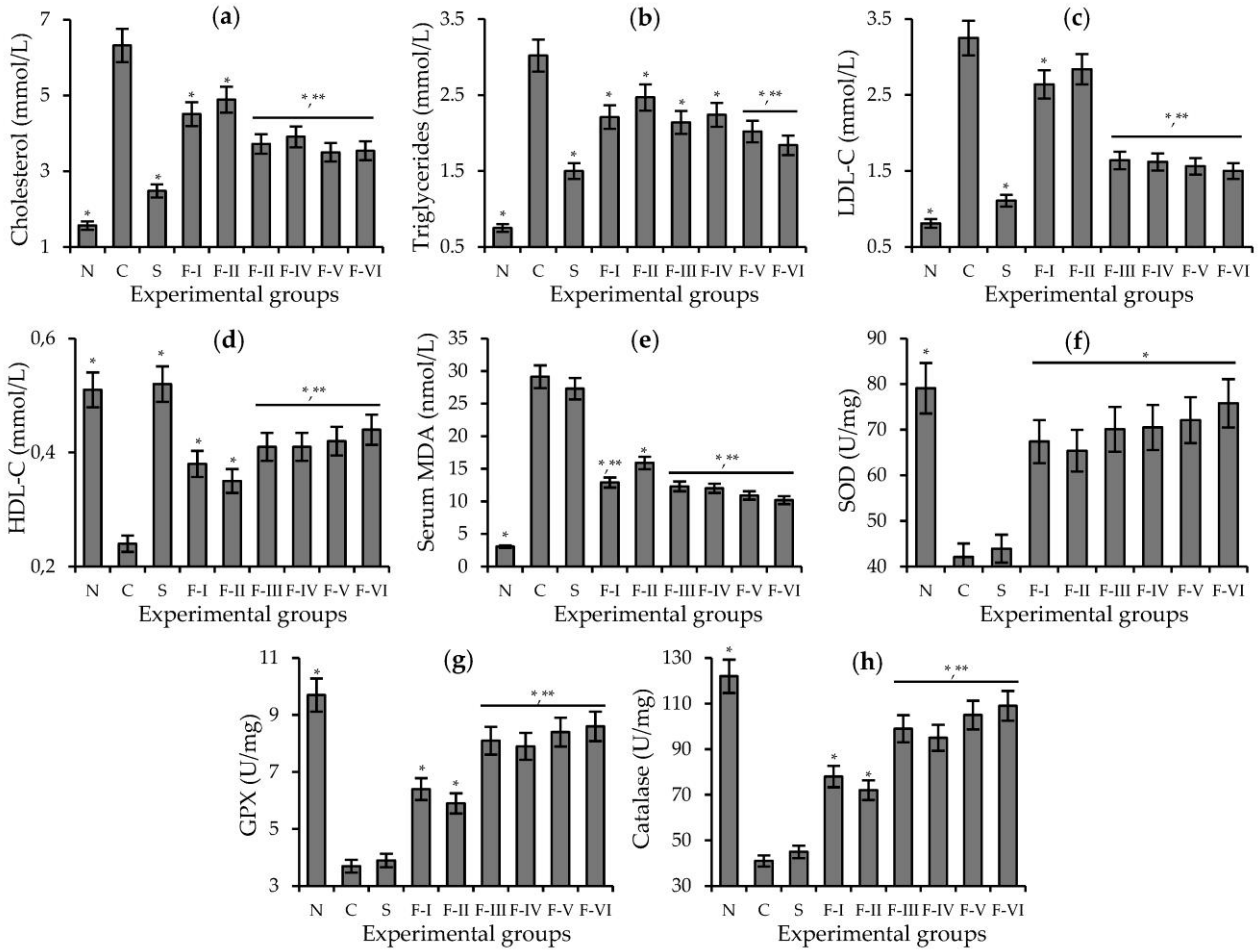


Figure 9. Changes in serum total cholesterol (**a**), serum total triglycerides (**b**), serum low-density lipoprotein–cholesterol (**c**), serum high-density lipoprotein–cholesterol (**d**), serum malondialdehyde (MDA; (**e**), liver superoxide dismutase (SOD; (**f**), liver glutathione peroxidase (GPX; (**g**) and liver catalase (**h**) for hamsters that were fed a normal diet (N) compared with those fed the 1% cholesterol diet (C), 1% cholesterol diet + simvastatin (10 mg/kg/day; S), with 1% cholesterol diet + flavonoid extract cv. Konkurent (100 mg/kg/day; F-I), 1% cholesterol diet + flavonoid extract cv. Masha (100 mg/kg/day; F-II), 1% cholesterol diet + flavonoid extract cv. Zozula (100 mg/kg/day; F-III), 1% cholesterol diet + flavonoid extract cv. Parizhskii Kornishon (100 mg/kg/day; F-IV), 1% cholesterol diet + flavonoid extract cv. Altai (100 mg/kg/day; F-V), and 1% cholesterol diet + flavonoid extract cv. Zasolochnii (100 mg/kg/day; F-VI). The asterisk (*) indicates a significant difference ($p < 0.05$) vs. 1% cholesterol diet group (C group); the double asterisk (**) indicates a significant difference ($p < 0.05$) vs. 1% cholesterol diet + flavonoid extract cv. Masha group (F-II group).

Administering flavonoid extracts from cucumber leaves to hamsters at a dose of 100 mg/kg/day resulted in a significant decrease ($p < 0.05$) in the serum levels of TC (3.50–4.89 mmol/L), TTG (1.84–2.47 mmol/L), and LDLC (1.50–2.84 mmol/L), with an increase in HDLC (0.35–0.44 mmol/L). The flavonoid extract from cv. Zasolochnii exhibited the highest efficacy, demonstrating maximal changes in TC (−44%), TTG (−39%), LDLC (−54%), and HDLC (+83%) compared with the 1% cholesterol diet group. Regression analysis revealed specific correlations between serum lipid parameters and the contents of different flavonoids in the cucumber leaf extracts. This suggests that extracts with higher

levels of acylated flavonoids exhibited the highest hypolipidemic activity (Figure S1). Prior research has identified acylation as a key factor contributing to the lipid-lowering properties of flavonoids [14] and triterpene saponins [60]. Therefore, this phenomenon likely also occurs in cucumber flavonoids. All extract groups showed a decrease in MDA values (10.2–15.9 nmol/L) and increases in SOD (65.4–75.8 U/mg), GPx (5.9–8.6 U/mg), and CAT (72.3–109.1 U/mg) levels, suggesting their prominent antioxidant properties. Prior studies have shown that there is direct correlation between the antioxidant potential and the hypolipidemic activity of plant extracts [51,53–55], including certain compounds, such as apigenin [56].

The bioactivity assessment of cucumber leaf extracts identified cv. Zasolochnii as a prime source for a product with the highest hypolipidemic and antioxidant activity. The essential chemical characteristic of this extract is the prevalence of acylated flavone *C*- and *C*,*O*-glycosides (892.17 mg/g) rather than non-acylated compounds. The main flavonoid in this extract is isovitexin-2″-*O*-glucoside-6″-*O*-*p*-coumarate (compound **21**) at 643.34 mg/g (Table 6). Therefore, we studied its lipid-lowering potential. To understand the possible structure–activity relationship, we selected a deacylated analog of **21**, isovitexin-2″-*O*-glucoside, in addition to the parent compound, isovitexin, for further investigation.

Administering two doses of isovitexin-2″-*O*-glucoside-6″-*O*-*p*-coumarate, 20 and 50 mg/kg/day for 6 months, significantly ($p < 0.05$) decreased the serum TC levels by 42% and 53%, respectively, compared with the 1% cholesterol diet group levels (Table 6). TTG decreased by 35% (20 mg/kg/day) and 43% (50 mg/kg/day), respectively; LDLC also decreased (by 44% and 57%, respectively). However, HDLC increased by 67% and 104%, respectively. The efficacy of isovitexin-2″-*O*-glucoside-6″-*O*-*p*-coumarate was significantly enhanced compared with that of the parent flavonoid extract of cv. Zasolochnii. Removing the acyl fragment from compound **21** resulted in the formation of isovitexin-2″-*O*-glucoside, which was inactive, similar to isovitexin. This highlights the crucial role of the *p*-coumaroyl moiety in inducing hypolipidemic effects.

Table 6. Levels of serum total cholesterol (TC), serum total triglycerides (TTG), serum low-density lipoprotein–cholesterol (LDLC), serum high-density lipoprotein–cholesterol (HDLC) after the application of simvastatin, isovitexin-2″-*O*-glucoside-6″-*O*-*p*-coumarate (IVitGC), isovitexin-2″-*O*-glucoside (IVitG), and isovitexin (IVit).

Experimental Group	Dose, mg/kg/day	TC, mmol/L	TTG, mmol/L	LDLC, mmol/L	HDLC, mmol/L
Normal Diet	-	1.57 ± 0.08 *	0.75 ± 0.06 *	0.81 ± 0.06 *	0.51 ± 0.04 *
1% Cholesterol Diet	-	6.32 ± 0.53	3.02 ± 0.21	3.25 ± 0.26	0.24 ± 0.02
1% Cholesterol Diet + Simvastatin	10	2.48 ± 0.18 *	1.50 ± 0.11 *	1.11 ± 0.09 *	0.54 ± 0.05 *
1% Cholesterol Diet + IVitGC	20	3.69 ± 0.32 *	1.96 ± 0.14 *	1.83 ± 0.14 *	0.40 ± 0.04 *
	50	2.95 ± 0.23 *,**	1.71 ± 0.12 *,**	1.40 ± 0.11 *,**	0.49 ± 0.04 *,**
1% Cholesterol Diet + IVitG	20	6.20 ± 0.57	3.05 ± 0.24	3.20 ± 0.25	0.25 ± 0.25
	50	5.72 ± 0.45	2.91 ± 0.22	3.06 ± 0.21	0.30 ± 0.02
1% Cholesterol Diet + IVit	20	6.27 ± 0.52	2.96 ± 0.21	3.18 ± 0.26	0.22 ± 0.02
	50	5.68 ± 0.47	2.78 ± 0.20	2.93 ± 0.23	0.32 ± 0.02

The asterisk indicates a significant difference ($p < 0.05$) vs. the 1% cholesterol diet group; the double asterisk (**) indicates a significant difference ($p < 0.05$) vs. the same group with a dose of 20 mg/kg/day.

All three flavonoids exhibited antioxidant effects in response to the high-cholesterol diet. Specifically, they successfully reduced the MDA level from 29.1 nmol/L in the 1% cholesterol diet group to 9.5–12.9 nmol/L for isovitexin-2″-*O*-glucoside-6″-*O*-*p*-coumarate, 15.9–26.4 nmol/L for isovitexin, and 17.8–27.1 nmol/L for isovitexin-2″-*O*-glucoside (Table 7). Furthermore, the levels of SOD, GPx, and CAT were elevated in all flavonoid-treated groups. This was expected, considering the well-documented antioxidative potential of apigenin derivatives [61]. However, this effect was observed for isovitexin and its derivatives for the first time in this experimental hyperlipidemia model. The potency of isovitexin-2″-*O*-glucoside-6″-*O*-*p*-coumarate was significantly ($p < 0.05$) higher than

that of non-acylated compounds, which is likely due to the presence of the *p*-coumarate fragment [62]. Thus, the flavonoid extract derived from cv. Zasolochnii is a promising source of the bioactive compound isovitexin-2″-*O*-glucoside-6″-*O*-*p*-coumarate, which has been shown to exhibit hypolipidemic potential.

Table 7. Levels of serum malondialdehyde (MDA), liver superoxide dismutase (SOD), liver glutathione peroxidase (GPx), and liver catalase (CAT) after the application of simvastatin, isovitexin-2″-*O*-glucoside-6″-*O*-*p*-coumarate (IVitGC), isovitexin-2″-*O*-glucoside (IVitG), and isovitexin (IVit).

Experimental Group	Dose, mg/kg/day	MDA, nmol/L	SOD, U/mg	GPx, U/mg	CAT, U/mg
Normal Diet	-	3.0 ± 0.18 *	79.1 ± 5.2 *	9.7 ± 0.6 *	122.7 ± 9.8 *
1% Cholesterol Diet	-	29.1 ± 1.8	42.1 ± 2.8	3.7 ± 0.2	41.6 ± 3.3
1% Cholesterol Diet + Simvastatin	10	27.3 ± 1.7	43.9 ± 3.5	3.9 ± 0.2	45.9 ± 3.4
1% Cholesterol Diet + IViGC	20	12.9 ± 0.8 *	60.7 ± 4.1 *	7.8 ± 0.5 *	86.1 ± 6.9 *
	50	9.5 ± 5.6 *,**	75.9 ± 5.0 *,**	9.2 ± 0.7 *,**	115.3 ± 9.2 *,**
1% Cholesterol Diet + IVitG	20	27.1 ± 1.6	54.7 ± 3.5 *	4.5 ± 0.3 *	62.7 ± 5.0 *
	50	17.8 ± 1.1 *,**	61.8 ± 4.1 *,**	5.8 ± 0.3 *,**	73.7 ± 5.9 *,**
1% Cholesterol Diet + IVit	20	26.4 ± 1.6	57.8 ± 4.6 *	5.0 ± 0.3 *	69.3 ± 5.6 *
	50	15.9 ± 0.9 *,**	69.7 ± 5.6 *,**	6.1 ± 0.4 *,**	81.2 ± 6.9 *,**

The asterisk indicates a significant difference ($p < 0.05$) vs. the 1% cholesterol diet group; the double asterisk (**) indicates a significant difference ($p < 0.05$) vs. the same group with a dose of 20 mg/kg/day.

The hypolipidemic activity of plant flavonoids has been the subject of many studies, which confirmed the high lipid-lowering effect of total flavonoid extracts from *Actinidia kolomikta* leaves [63], *Chrysanthemum morifolium* flowers [53], *Citrus aurantium* pericarp [64], *Crataegus pinnatifida* leaves [55], *Polygonum capitatum* herb [65], *Polygonum perfoliatum* tubers [66], *Solanum melongena* fruits [48], and *Trichosanthes kirilowii* seeds [67] (Table S5). In most animal hyperlipidemia models, administering flavonoids in the diet for 3–6 weeks has resulted in a decrease in serum total cholesterol, total triglycerides, and low-density lipoprotein–cholesterol, coupled with an increase in high-density lipoprotein–cholesterol. Concurrently, these studies have reported gradual improvements in liver antioxidant parameters, indicating the positive effects of plant flavonoids on hepatic functions. The total flavonoid fraction extracted from *Cucumis sativus* leaves achieved comparable results, suggesting a similar effect and effectiveness.

Pure flavonoids, as components of plant total flavonoid extracts, have also shown a significant hypolipidemic effect through inhibition of the Niemann–Pick C1-like 1 protein, a decrease in high blood cholesterol levels [68], inhibition of cholesterol synthesis [69], and an improvement of hepatic steatosis [53]. This evidence supports the concept of the "second strike theory", which suggests that hyperlipidemia contributes to impaired liver function [70] upon antioxidant intake. Previous studies on natural flavonoids [71] and this study on three derivatives of isovitexin confirm this correlation. However, for cucumber flavonoids, one important structural characteristic influencing both hypolipidemic and antioxidant activities is the presence of a phenylpropanoid-type acyl group (*p*-coumarate). The lipid-lowering effect of acylated flavonoids has been shown for the first time in our animal hyperlipidemia model. Our study demonstrates the potential of cucumber leaf extracts and isovitexin-2″-*O*-glucoside-6″-*O*-*p*-coumarate as agents to normalize lipid metabolism in hyperlipidemic disorders.

4. Conclusions

The findings of this study emphasize the significant potential of cucumber (*Cucumis sativus* L.) cultivation waste as a valuable source of bioactive extracts. Pure flavonoids can improve liver function through antioxidant effects in animals with hyperlipidemia. Further research is needed to enhance the chemodiversity of cucumber flavonoids, which may serve as effective hypolipidemic agents. Additionally, comprehensive investigation of the precise mechanisms underlying the lipid-lowering effects of these compounds

is warranted. Overall, this study challenges the view towards agricultural waste as only being potentially useful as compost, biofuel, or non-pharmaceutical substances. Our results suggest that there is a more rational use for secondary metabolites formed from the unused green parts of greenhouse plants.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13092410/s1, Table S1: Basic characteristics of six flavonoid-rich cucumber cultivars; Table S2: Total flavonoid yield from cucumber leaves and stems under various extraction conditions; Table S3: Regression equation, correlation coefficient, standard deviation, limit of detection, limit of quantification, intra- and interday precision, repeatability, stability, and the recovery linear range for isovitexin in the spectrophotometric assay; Table S4: Mass spectrometric data of compounds 1–38 found in the flavonoid extracts of cucumber waste green biomass; Table S5: Hypolipidemic and antioxidant activities of plant total flavonoid extracts; Figure S1: Correlation graphs between flavonoid contents in extracts of cucumber waste green biomass and serum total cholesterol, serum total triglycerides, serum low-density lipoprotein–cholesterol, and serum high-density lipoprotein–cholesterol.

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