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Comparative Analysis of *Schisandra* Fruit Extracts and Polysaccharides from Different Origins: Chemical Composition and Prebiotic and Antimicrobial Activity

Lili Fu [1,2,*], Tomasz Ruman [3], Joanna Niziol [4], Zhuo Zhang [5], Hongfei Zhao [5], Bolin Zhang [5], Aleksandra Owczarek-Januszkiewicz [6], Monika A. Olszewska [6], Justyna Rosicka-Kaczmarek [7] and Adriana Nowak [1,*]

1 Department of Environmental Biotechnology, Faculty of Biotechnology and Food Sciences, Lodz University of Technology, Wolczanska 171/173, 90-530 Lodz, Poland
2 Interdisciplinary Doctoral School, Lodz University of Technology, Żeromskiego 116, 90-924 Lodz, Poland
3 Department of Inorganic and Analytical Chemistry, Faculty of Chemistry, Rzeszów University of Technology, Powstancow Warszawy 6, 35-959 Rzeszow, Poland; tomruman@prz.edu.pl
4 Department of Polymers and Biopolymers, Faculty of Chemistry, Rzeszów University of Technology, Powstancow Warszawy 6, 35-959 Rzeszow, Poland; jniziol@prz.edu.pl
5 Department of Food Science and Engineering, College of Biological Sciences and Technology, Beijing Forestry University, Beijing 100083, China; zhuo.zhang@uclouvain.be (Z.Z.); zhaohf518@163.com (H.Z.); zhangbolin888@bjfu.edu.cn (B.Z.)
6 Department of Pharmacognosy, Faculty of Pharmacy, Medical University of Lodz, 1 Muszynskiego St., 90-151 Lodz, Poland; aleksandra.owczarek@umed.lodz.pl (A.O.-J.); monika.olszewska@umed.lodz.pl (M.A.O.)
7 Institute of Food Technology and Analysis, Faculty of Biotechnology and Food Sciences, Lodz University of Technology, Stefanowskiego 2/22, 90-537 Lodz, Poland; justyna.rosicka-kaczmarek@p.lodz.pl
* Correspondence: lili.fu@dokt.p.lodz.pl (L.F.); adriana.nowak@p.lodz.pl (A.N.)

Abstract

Schisandra is a plant whose fruit possesses high biological potential and beneficial health effects. The pharmacological properties of *Schisandra* are attributed to its bioactive components, primarily polyphenols and polysaccharides. This study aimed to obtain *Schisandra* fruit extracts (SCE) from different locations in China and Poland, as well as *Schisandra* polysaccharides (SPO), and to compare their chemical composition and selected biological activities. The prebiotic and antibacterial effects of SCE and SPO on lactic acid bacteria (LAB), human and foodborne pathogens, and gut microbiota were investigated. The chemical composition of the three Chinese SCE was similar, whereas SCE from Poland (SCE-PL) differed. The main bioactive compounds differentiating the Chinese SCE were quercetin, isorhamnetin, and nicotiflorin, while gamma-tocopherol and mevalonic acid distinguished SCE-PL, as indicated through LC-QTOF-MS/MS metabolomic profiling. All SCE and SPO promoted the growth of LAB strains, confirming their prebiotic potential and ability to serve as effective carbon sources for LAB. Additionally, all SCE inhibited the growth of certain pathogens, with *S. chinensis* extract from China showing the strongest activity, whereas SPO did not exhibit such activity. Variations in chemical composition among SCE and SPO contribute to differences in their prebiotic and antimicrobial activity, highlighting the importance of species and geographical origin in determining their functional properties.

Keywords: *Schisandra* genus extracts; bioactive components; prebiotics; lactic acid bacteria; pathogens; simulated digestive tract; polysaccharides

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1. Introduction

The plants of the genus *Schisandra* Michx., a member of the Schisandraceae family, produce fruit with high biological potential and beneficial health effects [1]. The fruits of *Schisandra* are used to treat many diseases in Traditional Chinese Medicine [2]. There are approximately 20–30 species of *Schisandra*, widely distributed across China, eastern Russia, Korea, and Japan, and successfully cultivated in Europe and America [3–6]. The center of *Schisandra* species diversity lies in northeastern and south-central China, where 19 species are found. *S. chinensis* (Turcz.) Baill. is the dominant species, occurring naturally in the north-eastern provinces of China, as well as in eastern Russia, Korea, and Japan [7]. *S. sphenanthera* Rehder & E. H. Wilson is another popular species, distributed in the central and southern provinces of China [8]. In addition, many other *Schisandra* species have attracted the attention of researchers in recent years. For instance, *S. arisanensis* Hayata is a Schisandraceous plant from Taiwan [9], while *S. henryi* C.B. Clarke is endemic to Yunnan Province in China [10]. Some minor species have demonstrated high chemical and biological potential, such as *S. rubriflora* Rehder & E. H. Wilson or *S. viridis* A. C. Sm. [11,12]. In recent years, the *Schisandra* genus has become an important source of raw materials for the medicinal and health food industry in China and worldwide due to its high economic and pharmaceutical value [13,14]. In addition to providing effective agents for use in medicine, cosmetics, and health supplements, *Schisandra* is also a source of edible fruit. It is suitable for use in tea, beverages, jam, and seasonings as a functional food ingredient with a unique flavor [15–17].

The *Schisandra* genus is characterized by a rich chemical composition. Numerous reports have suggested that the major functional bioactive constituents in this genus are the lignans schisandrin A, schisandrin B, gomisin A, gomisin N, essential oils, and polysaccharides [1,18–20]. The chemical composition and resulting biological activity of *Schisandra* plants depend on humidity, light, soil type, latitude, season, maturity, harvest time, geographical location, temperature, and other factors [21]. Therefore, the majority of studies have mainly focused on the biologically active compounds of *Schisandra* extracts (SCE) and their specific chemical constituents [22,23]. Differences in chemical composition among various SCE are attributed to different plant species, geographical origins, extraction types, and characterization methods [24,25]. However, there have been few comparative studies on the phytochemical profiles of different *Schisandra* species to date, with most available data focusing only on *S. sphenanthera* and *S. chinensis* [26,27]. Moreover, most previous studies on the pharmacological activity of *Schisandra* plants have focused solely on lignans, while there has been little discussion about other SCE components, such as *Schisandra* polysaccharides (SPO) [28–30].

Prebiotics can positively impact well-being and health by selectively stimulating the growth of gut microbiota, thereby positively influencing it [31]. The main goal of supplementing the diet with probiotics and prebiotics is to increase the population of beneficial bacteria, eliminate pathogens, and induce lasting favorable changes in the composition of gut microbiota [32]. Recently, SCE and SPO have been found to possess noticeable prebiotic properties, as they exhibit strong antimicrobial, antioxidant, and anti-inflammatory effects, which are attributed to specific functional bioactive components [33,34]. Nevertheless, the prebiotic and antimicrobial potential of SCE and SPO from different species and geographical origins has not been assessed previously.

To date, the role of SCE and SPO in the modulation of the human gut microbiota, and thus their subsequent pro-health effects, has received considerable attention [35–37]. Recent antimicrobial studies have focused predominantly on determining their antibacterial effects on foodborne pathogens, resident skin microbiota, oral microorganisms, and others [38–40]. There is still little research on the influence of SCE and SPO on the growth of probiotic

strains, the modulation of gut microbiota, and the effects of co-administration of probiotics with SCE or SPO. It is imperative to ascertain the role of SCE and SPO in the alteration of the intestinal microbiota composition, a process that undergoes considerable changes and is related to the progression of disease [41]. Therefore, an assessment of the prebiotic activity of SCE and SPO is essential for evaluating their potential health effects.

The aim of the study was to prepare SCE from *Schisandra* fruits originating from different regions of China and Poland, and to isolate SPO from selected Chinese extracts using different extraction methods. The chemical compositions of both SCE and SPO were then compared, and their prebiotic potential was evaluated both directly and under simulated gastrointestinal conditions. In addition, the antimicrobial activity of SCE and SPO against selected pathogens was assessed.

2. Results

2.1. Fruit Extraction and Preparation of SCE and SPO

2.1.1. Preparation of SCE

This is the first study that has attempted to compare the extraction yields and phytochemical profiles of *Schisandra* fruit of certain species, collected from selected locations in China and Poland. The results showed that the extraction yields of *Schisandra* fruit of different origin (China and Poland) extracted under the same extraction conditions (reflux extraction with water at 90 °C) were similar. As shown in Table 1, they ranged from 6.30% to 10.17% for dried fruit of *Schisandra* plants. The highest extraction yield was obtained for SCE-1, originating from China (10.17%), and the lowest for SCE-PL, originating from Poland (6.30%).

Table 1. Extraction yield of SCE from different geographic locations in China and Poland. *S. chinensis* extracts from China were designated SCE-1, *S. sphenanthera* extracts from China were named SCE-2, *S. henryi* extracts from China were named SCE-3, and *S. chinensis* extracts from Poland were named SCE-PL.

Extracts	Origin	SCE/Dry Fruit	Extraction Yield (%)
SCE-1	*S. chinensis* (China)	0.1017 kg/1.00 kg	10.17
SCE-2	*S. sphenanthera* (China)	0.0904 kg/1.00 kg	9.04
SCE-3	*S. henryi* (China)	0.0842 kg/1.00 kg	8.42
SCE-PL	*S. chinensis* (Poland)	0.0630 kg/1.00 kg	6.30

2.1.2. Isolation of SPO

Ultrasonic-assisted extraction with water and subsequent precipitation with organic solvents provided a 13.12% yield of SPO-1 from *S. henryi* fruit extract (SCE-3), while *L. plantarum* CICC 23121 fermentation-assisted extraction followed by precipitation with the same solvents provided an 11.12% yield of SPO-2 from *S. sphenanthera* fruit extract (SCE-2) (Table 2).

Table 2. The isolation yield of SPO isolated from different SCE. *S. henryi* polysaccharides from China were designated SPO-1, and *S. sphenanthera* polysaccharides from China were designated SPO-2.

Polysaccharides	SCP/SCE (g/g)	Extraction Yield (%)
SPO-1	26.24 g/200 g	13.12
SPO-2	22.24 g/200 g	11.12

2.2. Comparison of the Chemical Composition of SCE and SPO

2.2.1. Chemical Composition of SCE from Different Locations

In the present work, the metabolite profiles of four types of SCE from different species and locations in China and Poland were determined using the LC–MS/MS method. In total, 937 different metabolites were detected. Significant differences in the metabolomes were observed between the SCE from China (SCE-1, SCE-2, and SCE-3) and those from Poland (SCE-PL) (Table S1). The division of all SCE sample metabolomes into two distinct groups was confirmed by multivariate analysis (Figures 1 and 2). The horizontal axis (PC1) represents the first principal component, explaining 63.8% of the variance, while the vertical axis (PC2) represents the second principal component, accounting for 7.6% of the variance. The data points for the SCE from China (SCE-1, SCE-2, and SCE-3) are more dispersed along both PC1 and PC2, enclosed by a red confidence ellipse, whereas the data points for the SCE from Poland (SCE-PL) are more tightly clustered (Figure 1a). The differences between the metabolomic profiles of the four types of SCE are also illustrated by the heatmap (Figure 1b), where the color scale represents the relative abundance levels—red indicates higher values, and blue indicates lower values. These results indicate that the metabolite profiles of SCE-2 and SCE-3 are more similar to each other than to other pairs. Additionally, the PCA plot shows different dispersion patterns: SCE-PL lies closest to its cluster centroid and SCE-2 clusters moderately tightly, while SCE-1 is positioned further from the China group centroid, indicating a greater deviation from the group average. The VIP score plot shows the most discriminating metabolites between the China and Poland groups (Figure 2a). The volcano plot depicts the differential accumulation of biological compounds between both groups (Figure 2b). There are several similarities among SCE-1, SCE-2, and SCE-3, whereas SCE-PL differed significantly from the China group. The results are consistent with the PCA and heatmap analysis.

A total of 937 compounds meeting the statistical criteria were identified by their VIP score. Table S1 lists the top 50 metabolites identified through the VIP scores. The dominant discriminating compounds in the China group included quercetin, angeloylgomisin, isorhamnetin, nicotiflorin, genistein, sophoricoside, peonidin-3-O-beta-galactoside, stearidonic acid, rutin, and 3-hydroxy-4-methoxycinnamic acid. Conversely, the abundant compounds in the Poland group were gamma-tocopherol and mevalonic acid (Figure 2a).

Significant differences in the metabolomes were observed between the Polish (SCE-PL) and Chinese (SCE-1, SCE-2, SCE-3) samples. However, certain differences in the metabolomes among SCE-1, SCE-2, and SCE-3, all of Chinese origin, were also revealed (Tables S2–S7). The numbers of compounds specific to SCE-1, SCE-2, and SCE-3 (each compared to SCE-PL) were 943, 966, and 977, respectively. There were 60 compounds specific to SCE-1 and SCE-2, 55 compounds differentiating SCE-1 and SCE-3, and 25 compounds specific to SCE-2 and SCE-3. Lists of all putatively detected compounds distinguishing the particular extracts can be found in Tables S2–S7. In comparison to SCE-PL, the more abundant differentiating compounds in SCE-1 were isoimperatorin and angeloylgomisin P, while angeloylgomisin P was the dominant specific compound in both SCE-2 and SCE-3.

2.2.2. Chemical Composition of SPO

The average molecular weight and monosaccharide composition were studied to chemically characterize the isolated polysaccharides. As shown in Table 3, the molecular weight of SPO-1 ranged from 0 to 3200 Da and the polysaccharide was composed of two neutral sugars, which are glucose and xylose. SPO-2 had a molecular weight in the range of 0 to 30,000 Da and was composed of eight neutral sugars and two uronic acids, which included glucose, mannose, galactose, rhamnose, xylose, arabinose, fructose, ribose, glucuronic acid, and galacturonic acid [42].

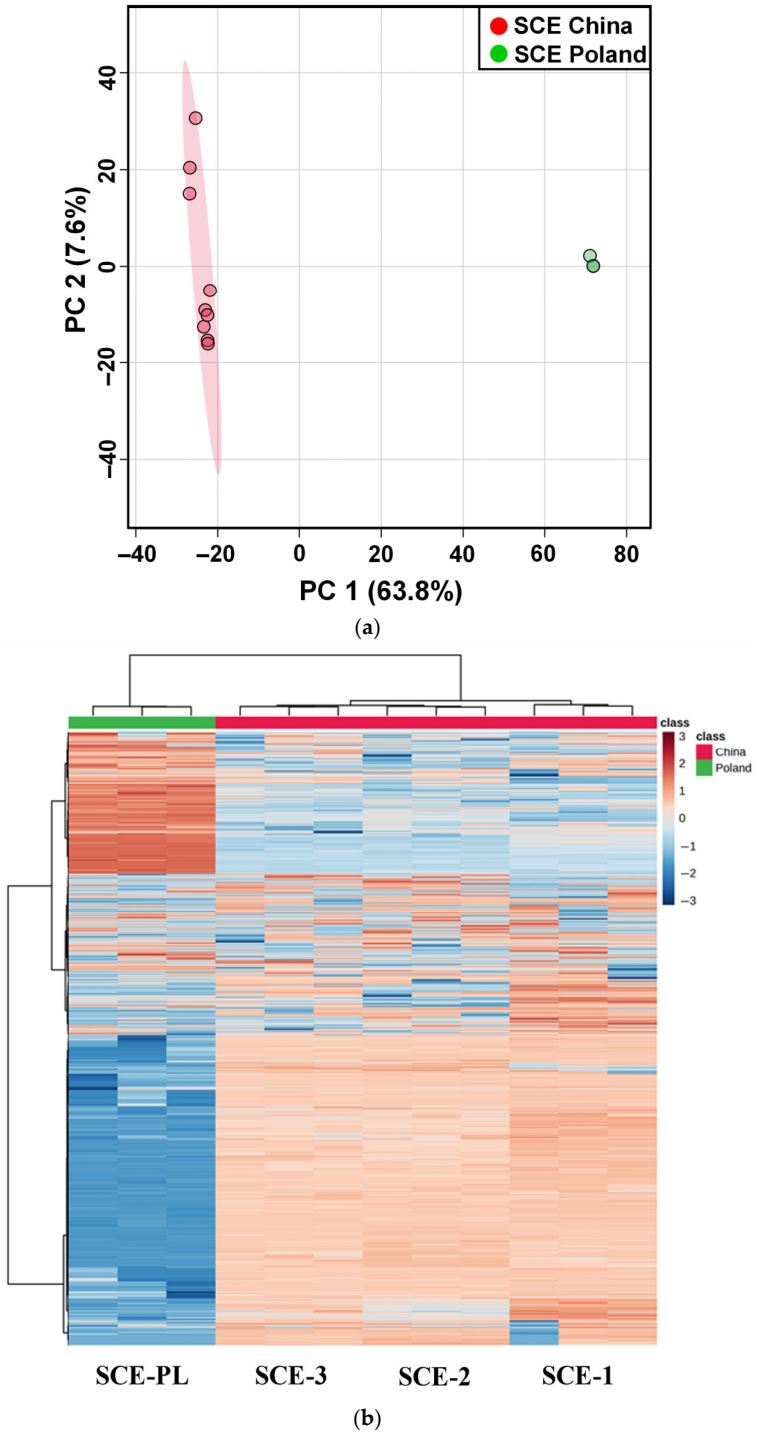


Figure 1. PCA analysis and heatmap of different SCE. (**a**) PCA analysis of different SCE. Red points represent SCE from China, green points represent SCE from Poland. The horizontal axis (PC1) represents the first principal component, while the vertical axis (PC2) represents the second principal component. The data points from China are more dispersed along PC1 and PC2, with a red confidence ellipse around them. (**b**) Heatmap shows statistically significant differences between metabolites detected in SCE samples. The heatmap indicates the relative abundance of each metabolite in China and Poland groups, with red representing high abundance and blue representing low abundance.

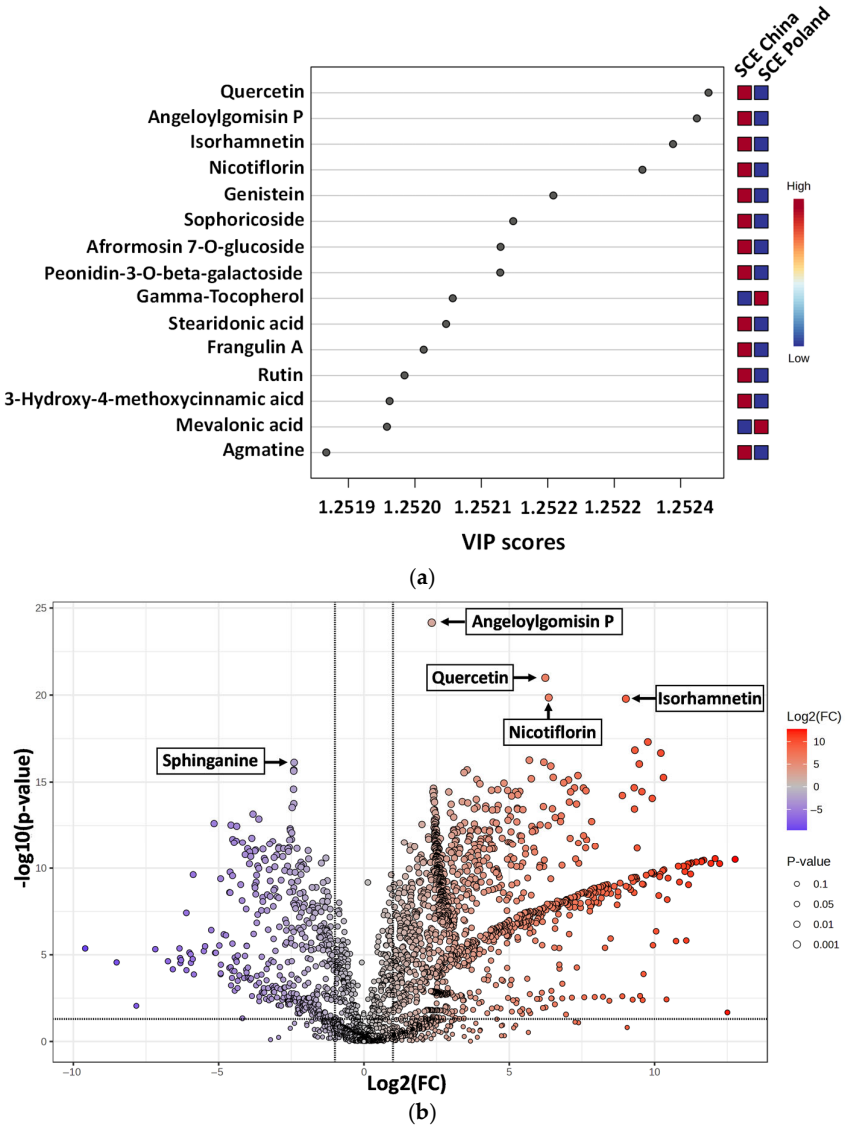


Figure 2. Multivariate analysis of different SCE. (**a**) VIP score plot showing the most discriminating metabolites between China and Poland groups. The metabolites on the Y-axis are ranked based on their VIP scores, as shown on the X-axis. Higher VIP scores indicate greater contribution to the separation between the two groups. (**b**) Volcano plot depicting the differential expression of biological compounds between China and Poland groups. The X-axis ($\log_2$FC) represents the log-transformed fold change in expression, while the Y-axis ($-\log_{10}(p)$) indicates the statistical significance (p-value) of each compound. Red points represent compounds that are significantly more abundant in SCE from China, while blue points represent compounds that are significantly more abundant in SCE from Poland. Gray points denote compounds that did not reach the significance threshold. Group China.

2.3. Prebiotic Effect of SCE and SPO

In the preliminary screening test, the agar-well diffusion method was performed to assess whether the tested SCE and SPO inhibited the growth of LAB and *Bifidobacterium* strains. Nine LAB strains and one *Bifidobacterium* strain were selected to determine the antimicrobial activity of SCE-(1–3). Due to the limited amount of SCE-PL and SPO, four strains (*Lacticaseibacillus rhamnosus* LOCK 0997, *Levilactobacillus brevis* OK-C, *Pediococcus acidilactici* 21/1, and *Bifidobacterium. breve animalis* ssp. *lactis* Bb-12) were selected to determine the antimicrobial activity of SCE-PL and SPO. There were no growth inhibition zones observed in any of the tested microorganisms and noticeable bacterial aggregation was observed around the wells where SCE and SPO were added (Figures 3 and S1). The tested

SCE and SPO displayed the ability to promote the growth of tested LAB and *Bifidobacterium* strains. These results confirmed the potential prebiotic properties of SCE and SPO and allowed for further experiments.

Table 3. The chemical composition of SPO isolated from different SCE. *S. sphenanthera* extracts from China were designated SCE-2, and *S. henryi* extracts from China were designated SCE-3. *S. henryi* polysaccharides from China were designated SPO-1, and *S. sphenanthera* polysaccharides from China were designated SPO-2.

Composition	SPO-1	SPO-2
Research Material	*S. henryi* (SCE-3)	*S. sphenanthera* (SCE-2)
Extraction method	Ultrasonic-assisted extraction	*L. plantarum* CICC 23121 fermentation-assisted extraction
Molecular weight	0 to 3200 Da	0 to 30,000 Da
Monosaccharide composition	Glucose and xylose	Glucose, xylose, galactose, rhamnose, mannose, arabinose, fructose, ribose, glucuronic acid, and galacturonic acid

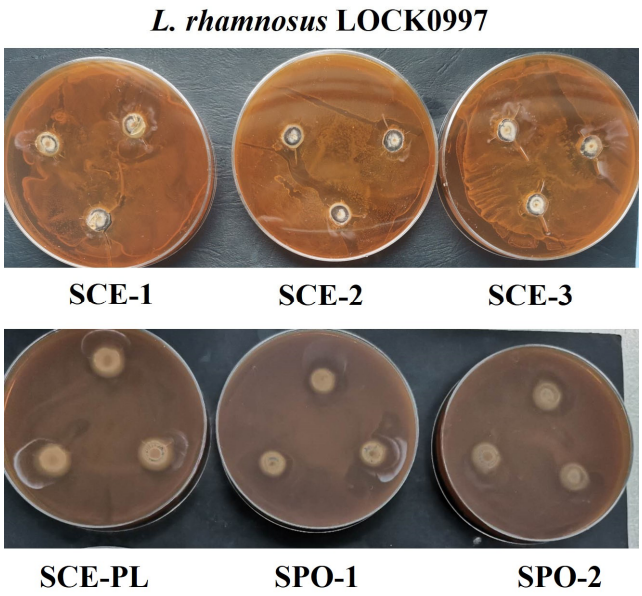


Figure 3. Exemplary photographs showing antimicrobial activity of SCE and SPO against *L. rhamnosus* LOCK 0997 as evaluated with the agar-well diffusion method.

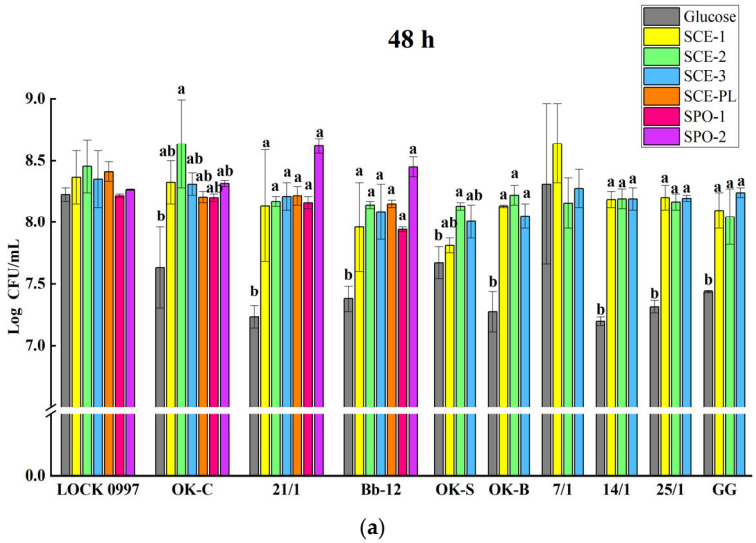
In the next stage of the study, it was tested whether SCE could serve as a carbon source instead of glucose, and whether the growth and viability of the strains were enhanced in the presence of SCE compared to glucose. For this purpose, nine LAB and one *Bifidobacterium* strains were used. It was observed that SCE-(1–3) showed growth stimulation of all LAB and *Bifidobacterium* strains after 48 h as compared to the glucose control. The effect (at the death stage of growth) was significantly weakened after 168 h of incubation, which was demonstrated by *L. rhamnosus* LOCK 0997 ($p = 0.021$); *Pediococcus pentosaceus* OK-S ($p = 0.021$); *L. brevis* OK-C ($p = 0.021$); *Lactiplantibacillus plantarum* OK-B ($p = 0.043$); *P. acidilactici* 7/1 ($p = 0.021$); and *B. breve* Bb-12 ($p = 0.043$), respectively (KWW test).

Due to the limited amount of SCE-PL and SPO, four strains (*L. rhamnosus* LOCK 0997, *L. brevis* OK-C, *P. acidilactici* 21/1, and *B. breve* Bb-12) were chosen for further research, selected on the basis of the previous experiments (the prebiotic effect of SCE-(1–3)), according to

their high growth ability and viability. Subsequently, the growth capacity of these four strains in the presence of SCE-PL and SPO was assessed. The growth capacity of the strains was weakened with extended incubation time, and a significant decline was observed after 168 h of incubation in comparison to 48 h with *L. rhamnosus* LOCK 0997 ($p = 0.021$), *L. brevis* OK-C ($p = 0.021$), and *B. breve* Bb-12 ($p = 0.043$) (respectively, KWW test).

In Figure 4 and Table S8, a comprehensive comparison of the growth of different LAB strains in the presence of SCE and SPO after 48 h and 168 h incubation is shown. After 48 h of incubation, the SCE and SPO demonstrated significant growth stimulation of all tested strains except for *L. rhamnosus* LOCK 0997 and *P. acidilactici* 7/1 ($p < 0.05$, LSD test). However, the SCE and SPO significantly stimulated the viability of *L. rhamnosus* LOCK 0997, *P. acidilactici* 7/1, *P. acidilactici* 21/1, and *B. breve* Bb-12 after 168 h of incubation ($p < 0.05$, LSD test). The growth capacity of LAB in the presence of SCE-2 was the highest after 48 h of incubation, as demonstrated by *L. brevis* OK-C, for which strong growth stimulation was observed (1.01 log CFU/mL). The growth capacity of LAB in the presence of SPO-2 was the strongest after 48 h of incubation, as demonstrated by *P. acidilactici* 21/1, for which strong growth stimulation was observed (1.39 log CFU/mL). After 168 h of incubation, *L. rhamnosus* LOCK 0997 (by 0.78 log CFU/mL), *L. brevis* OK-C (by 0.32 log CFU/mL), and especially *P. acidilactici* 21/1 (by 1.07 log CFU/mL) exhibited greater viability in the presence of SCE-PL.

Concerning the acid metabolites generated during the growth of selected LAB and *Bifidobacterium* strains in the presence of SCE and SPO, the pH after the incubation of strains in the culture medium for 168 h was measured (Figure 5 and Table S9). The initial pH of the media before inoculation was at about 6.0. For all of the tested strains, the pH of the culture medium after the cultivation of bacteria in the presence of SCE or SPO showed significant differences ($p < 0.05$, LSD test), and this was strain-specific. For most of the strains tested, the pH of the culture medium dropped to about 5.0, while for glucose, it dropped to about 4.5. In the presence of SPO, a slight change in the pH value of the culture medium for all tested strains was observed. In the presence of SCE-PL, the pH value of the culture medium for all tested strains displayed a significant decrease as compared to its initial pH (dropping to about 5.0). In the presence of SCE-(1–3), the pH value of the culture medium of *L. rhamnosus* LOCK 0997 and GG, *L. brevis* OK-C, *P. pentosaceus* OK-S, *L. plantarum* OK-B, and *P. acidilactici* 7/1 decreased to approximately 5.0.



(**a**)

Figure 4. *Cont.*

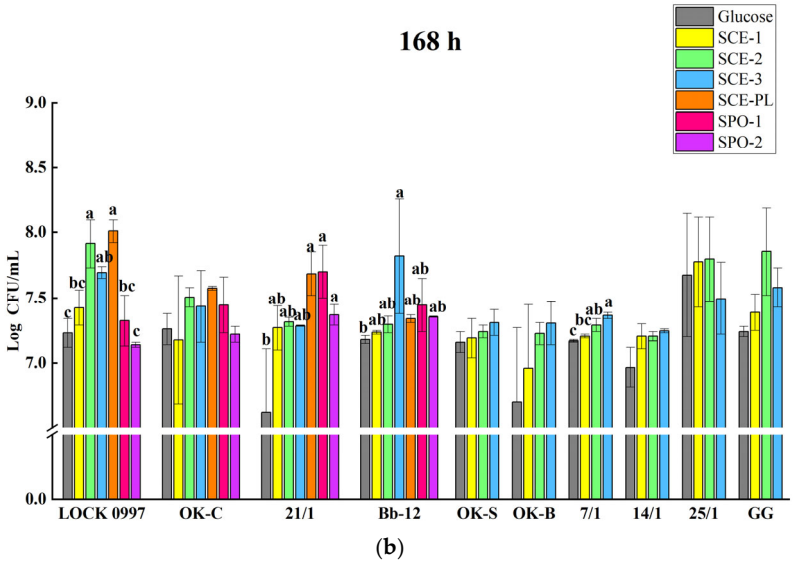


Figure 4. (**a**) The growth of LAB strains in the presence of 1% SCE or SPO after 48 h incubation. (**b**) The viability of bacterial strains in the presence of 1% SCE or SPO after 168 h incubation. Results are presented as mean ± SEM of two independent experiments (n = 2). Different letters above the bars indicate significant differences at ($p < 0.05$), revealed using a one-way ANOVA with an LSD multiple comparison test.

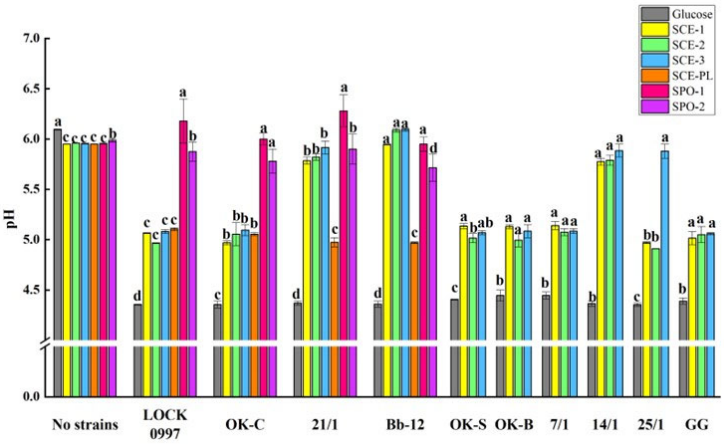


Figure 5. The pH of culture medium after 168 h incubation of bacterial strains in the presence of 1% SCE and SPO. Results are presented as mean ± SEM of two independent experiments (n = 2). Different letters above the bars indicate significant differences at ($p < 0.05$), revealed using a one-way ANOVA with an LSD multiple comparison test.

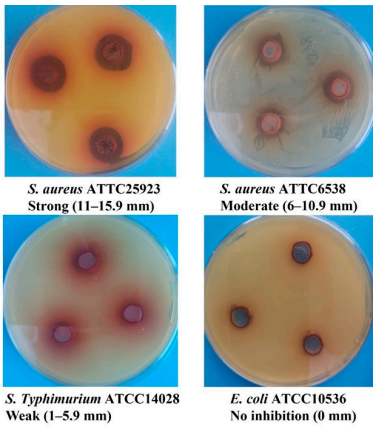
2.4. Antibacterial Activity of SCE and SPO Against Human and Food Spoilage Pathogens

The next experiment tested the antibacterial activity of SCE and SPO against selected human and food pathogens. As shown in Figure 6, all SCE were able to inhibit the growth of pathogens, whereas SPO showed no such activity. In the presence of diverse SCE, the antibacterial activity was shown to be significantly different, which depended on the pathogen. The significant differences between the tested SCE groups were as follows: for SCE-1 ($p < 0.001$), for SCE-2 ($p < 0.001$), for SCE-3 ($p = 0.016$), and for SCE-PL ($p < 0.001$) (KWW test). In the case of *L. monocytogenes* ATCC19115, *E. coli* ATCC25922, and *S. aureus* ATTC25923, all SCE demonstrated antibacterial activity. *S. aureus* ATTC25923 in the presence of SCE-1 turned out to be the most sensitive among the tested pathogens, where the growth inhibition diameter reached 13.24 ± 1.90 mm. *S. aureus* ATTC25923 also showed strong sensitivity to SCE-2 (11.58 ± 2.58 mm), and moderate sensitivity to SCE-3

(10.36 ± 2.42 mm) and SCE-PL (7.35 ± 3.43 mm). SCE-PL showed the weakest antibacterial activity towards *E. coli* ATCC10536, and the diameter of growth inhibition reached 2.25 ± 0.93 mm. SCE-PL also exhibited weak antagonism of *E. coli* ATCC10536 and *Citrobacter* sp. C1/1, and the growth inhibition diameters were 2.25 ± 0.93 mm (*E. coli* ATCC10536) and 2.95 ± 0.83 mm (*Citrobacter* sp. C1/1). SCE-1 displayed antibacterial activity towards *S.* Typhimurium ATCC14028 and *Staphylococcus epidermidis* S4/2, and the growth inhibition diameters were 2.27 ± 0.66 mm (*S.* Typhimurium ATCC14028) and 6.21 ± 1.79 mm (*S. epidermidis* S4/2). Out of all 14 tested pathogens, SCE-1 displayed antibacterial activity against 8, and the diameter of growth inhibition ranged from 2.27 ± 0.66 mm to 13.24 ± 1.90 mm. The SCE-2 showed antibacterial activity against five pathogens, and the diameter of growth inhibition ranged from 4.86 ± 0.89 mm to 11.58 ± 2.85 mm. The SCE-3 showed antibacterial activity towards three pathogens, and the diameter of growth inhibition ranged from 6.07 ± 1.11 mm to 10.36 ± 2.42 mm. SCE-PL demonstrated antibacterial activity against seven pathogens, and the diameter of growth inhibition ranged from 2.25 ± 0.93 mm to 12 ± 0.88 mm. Additionally, it was noticed that SCE displayed stronger antibacterial activity against Gram-positive than against Gram-negative bacteria.

2.5. Survival of LAB in the Simulated Gastrointestinal Tract in the Presence of SCE and SPO

Three strains (*L. rhamnosus* LOCK 0997 and GG, *P. acidilactici* 21/1) for which the greatest growth stimulation was observed in the previous experiment were selected for further study. The survival of the strains (in the presence of SCE and SPO) in the simulated digestive tract in vitro is presented in Figure 7 and Table S10. The negative control indicates pure LAB culture in 0.85% NaCl and the control indicates LAB incubated in gastric and intestinal juices without SCE and SPO. In fresh simulated saliva fluid for 10 min, the survival rate of LAB in the presence of SCE and SPO was similar to that for all controls; no conspicuous differences could be detected at this stage. After 2 h of incubation in the simulated gastric juice, the viability of LAB mildly dropped, but the obtained results were not statistically significant. At this stage, *L. rhamnosus* LOCK 0997 displayed the highest survival rate in the presence of SPO-2 (97.92%), while in the presence of SCE-PL, it showed the lowest survival rate (94.07%). *L. rhamnosus* GG exhibited the highest survival rate in the presence of SPO-2 (94.38%), whereas the lowest survival rate was observed in the control sample (89.36%). Similarly, *P. acidilactici* 21/1 showed the highest survival rate when supplemented with SPO-2 (94.78%), while the control group exhibited the lowest survival rate (92.47%). A significant decrease in LAB survival was observed after incubation in simulated intestinal juices for *L. rhamnosus* LOCK 0997 (p = 0.0009); *L. rhamnosus* GG (p = 0.0007), and *P. acidilactici* 21/1 (p = 0.0001) (respectively, KWW test). In intestinal juices, *L. rhamnosus* LOCK 0997 in the presence of SPO-2 demonstrated the highest survival rate (76.48%), while for the control, the lowest survival rate, at 69.86%, was observed. In the presence of SCE-1 and SCE-PL, *L. rhamnosus* LOCK 0997 also demonstrated a higher survival rate as compared to the control, with rates of 71.73% (SCE-1) and 72.52% (SCE-PL). The viability of *L. rhamnosus* GG in the presence of SCE and SPO was higher than that for the control (70.21%), ranging from 75.57% to 76.52%. Among these, *L. rhamnosus* GG in the presence of SCE-PL exhibited the highest survival rate (76.52%). In the presence of SPO-2, *P. acidilactici* 21/1 showed the highest survival rate (68.14%), while the control displayed the lowest survival rate (65.12%). *P. acidilactici* 21/1 in the presence of SCE-1 (66.43%) and SCE-PL (66.12%) also exhibited a higher survival rate than the control.

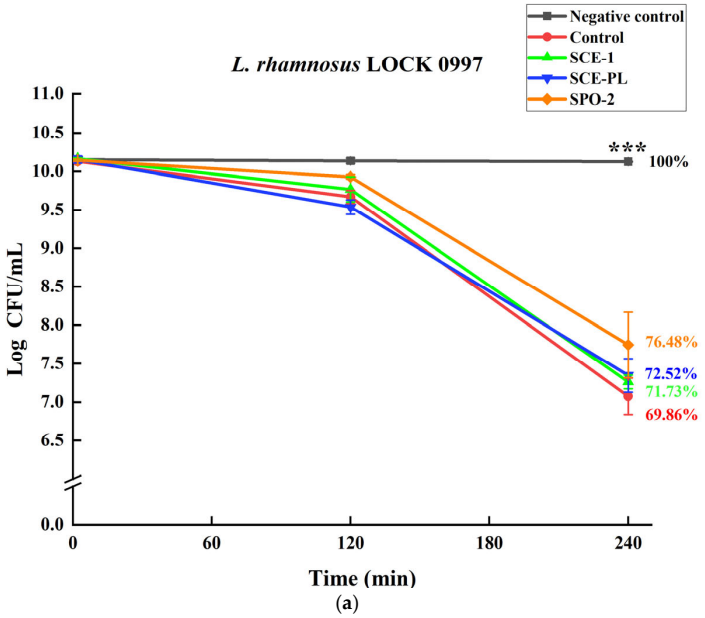


(**a**)

Pathogens \ SCE	SCE-1	SCE-2	SCE-3	SCE-PL	SPO-1	SPO-2
S. aureus ATTC6538	8.86±1.32 ^{b}	5.56±0.93 ^{b}	0	0	0	0
L. monocytogenes ATCC19115	6.92±0.81 ^{c}	4.86±0.89 ^{b}	6.41±1.15 ^{b}	8.48±1.63 ^{b}	0	0
E. coli ATCC10536	0	0	0	2.25±0.93 ^{d}	0	0
P. aeruginosa ATCC15442	5.28±0.79 ^{d}	6.77±0.77 ^{b}	0	12±0.88 ^{a}	0	0
S. Typhimurium ATCC14028	2.27±0.66 ^{e}	0	0	0	0	0
B. faecis DSM24798	0	0	0	0	0	0
Citrobacter sp. C1/1	0	0	0	2.95±0.83 ^{d}	0	0
P. stuartii P1/2	3.26±1.43 ^{e}	0	0	2.53±0.71 ^{d}	0	0
E. cloacae E3/1	0	0	0	0	0	0
S. epidermidis S4/2	6.21±1.79 ^{cd}	0	0	0	0	0
K. pneumoniae K4/3	0	0	0	0	0	0
E. coli ATCC25922	6.65±0.9 ^{cd}	4.93±0.67 ^{b}	6.07±1.11 ^{b}	5.45±0.76 ^{c}	0	0
S. aureus ATTC25923	13.24±1.9 ^{a}	11.58±2.85 ^{a}	10.36±2.42 ^{a}	7.35±3.43 ^{bc}	0	0
P. aeruginosa ATCC24755	0	0	0	0	0	0
Significant differences between the groups	***	***	**	***	-	-

(**b**)

Figure 6. (**a**) Exemplary photographs of antibacterial activity of SCE-1 against selected pathogens. (**b**) Antibacterial activity [mm] of different SCE against selected human and food pathogens. Type of inhibition: dark blue—strong (11–15.9 mm), medium dark blue—moderate (6–10.9 mm), light blue—weak (1–5.9 mm), white—no inhibition (0 mm). Results are presented as mean from three measurements ±SEM of two independent experiments (n = 2). Significant differences were analyzed using the KWW test, with ** $p \leq 0.01$, *** $p \leq 0.001$. Different letters indicate significant differences at ($p < 0.05$), revealed using a one-way ANOVA with an LSD multiple comparison test.



(**a**)

Figure 7. *Cont.*

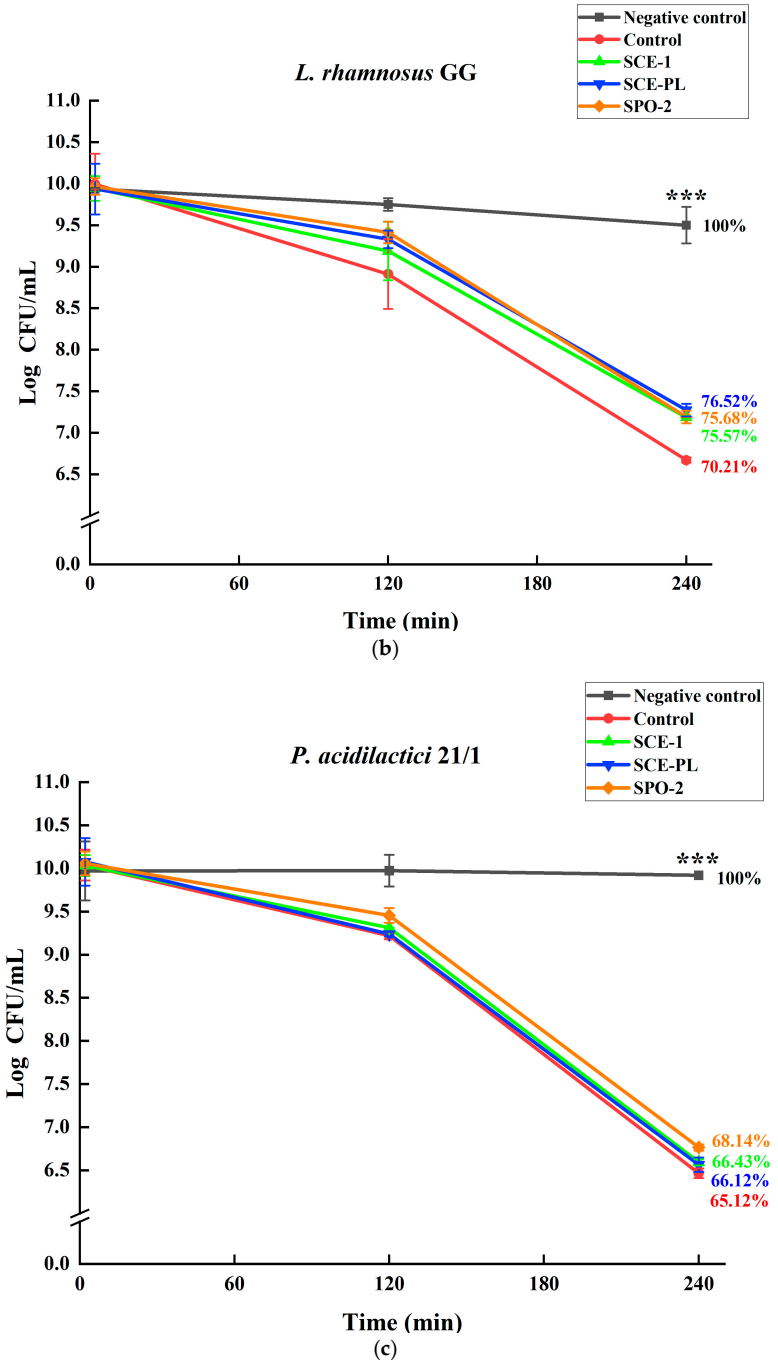


Figure 7. Survival of *L. rhamnosus* LOCK 0997 (**a**), *L. rhamnosus* GG (**b**), and *P. acidilactici* 21/1 (**c**) in the presence of SCE and SPO in gastric (t < 2 h) and intestinal (t > 2 h) conditions. The negative control indicates pure LAB culture in 0.85% NaCl and the control indicates LAB incubated in gastric and intestinal juices without SCE and SPO. Results are presented as mean ± SEM of two independent experiments (n = 2). Significant differences in the presence of SCE and SPO between the experimental groups of different gastric and intestinal conditions were analyzed using the KWW test. Statistically significant results were calculated against the initial concentration of LAB (t = 0 h) and marked with *** $p \leq 0.001$.

3. Discussion

The extraction methods used to obtain SCE often affect its extraction yield and bioactive properties [24,25,43]. In the present work, hot-water extraction was applied because it is a widely used, non-toxic, readily available, and environmentally friendly method that produces extracts with high antioxidant capacity and great cytoprotective effects [44,45].

Geographical origin, climate, cultivation year, harvest time, manufacturing procedures, and storage conditions may also be responsible for variations in extraction yields and bioactive marker content [21]. However, according to our findings, the extraction yield of *Schisandra* fruit is relatively stable independent of the species and origin of the plant (Table 1).

Several reports have suggested that the major bioactive constituents of *Schisandra* fruit are phenolics, primarily lignans and flavonoids, as well as essential oils and polysaccharides [1,18–20]. Our findings revealed that the typical compounds found in the group of Chinese samples were quercetin, rutin, and angeloylgomisin, which have been previously identified in *Schisandra* plants [20,46]. Quercetin is the most abundant aglycone flavonoid that forms schisandra flavonoid glycosides. It is also one of the most studied flavonoids generally due to its potent anti-inflammatory, antioxidant, antiviral, anticancer, and anti-obesity activities [47–49]. Rutin is also a well-known flavonoid with anti-inflammatory, antihypertensive, hypolipidemic, cytoprotective, anti-tumor, antibacterial, and antiviral activities [48,50,51]. It was revealed to dominate among flavonoids of the fruit of *S. chinensis*, followed by the free aglycone quercetin [20]. In 2001, quercetin, kaempferol, and (E)-cinnamic acid were analyzed from *S. chinensis* extracts using reversed-phase high-performance liquid chromatography (RP-HPLC) [52]. Angeloylgomisin has been isolated from the leaves and fruits of *S. sphenanthera* [46,53]. In contrast, in the Polish sample, the dominant discriminating compounds (gamma-tocopherol and mevalonic acid) that we found have not been previously reported in *Schisandra*. Gamma-tocopherol is of considerable interest due to its strong antioxidant and anticancer effects, as well as its other unique bioactivities [54,55]. Mevalonic acid is a key compound that contributes to the dynamic production of isoprenoid compounds [56,57]. Intriguingly, angeloylgomisin P emerged as the dominant compound in SCE-1, SCE-2, and SCE-3 compared to SCE-PL, which is particularly noteworthy given that both SCE-1 and SCE-PL are classified as *S. chinensis* extracts. This finding suggests that the geographical region in which *Schisandra* fruit is grown has a greater impact on its composition.

The findings of the present study evidenced marked discrepancies between SPO-1 and SPO-2 in terms of molecular weights and monosaccharide composition, which may be attributed to differences in both species and extraction methods. Polysaccharides isolated from *S. sphenanthera* and *S. chinensis* have been reported to differ in their sugar, protein, and uronic acid contents, resulting in distinct bioactivities [58,59]. Three purified jujube polysaccharides obtained using DEAE-Sepharose Fast Flow and Sephacryl S-300 columns with different adsorption capacities exhibited different antioxidant activities [60]. Moreover, different extraction and purification methods can significantly influence the molecular weights, monosaccharide compositions, and structural characteristics of polysaccharides derived from *Schisandra* [61]. According to literature reports, the carbohydrate composition, molecular weight, spatial configuration, and other structural features of polysaccharide molecules can affect their interactions with biomolecules, thereby regulating their biological activity [62]. Consequently, it is hypothesized that the two polysaccharides obtained in the present study also exhibit distinct bioactivities.

While the antimicrobial activity of *Schisandra* extracts against pathogenic microorganisms has been well-documented [40,63,64], little research has been conducted on their impact on the growth of probiotic strains. Only the studies mentioned indicated that *S. chinensis* polysaccharide could promote the proliferation of the beneficial intestinal bacterium *Limosilactobacillus reuteri* to repair the impaired intestinal barrier in alcohol-associated liver disease [65]. Bioactive extracts or compounds can enhance the survivability and colonization of probiotics [66,67].

One of the crucial potential biological features of SCE and SPO is their ability to affect microbial growth, either by inhibiting or stimulating it [68–70]. According to the

present results, the antimicrobial activity tests showed that SCE and SPO from various *Schisandra* species and sources promoted the growth of *Bifidobacterium* spp. and *Lactobacillus* spp. Based on the limited data available on their impact on the growth of probiotic strains, the prebiotic activity of 1% SCE and SPO after 48 h and 168 h of exposure was assessed. Generally, SCE and SPO increased the growth of almost all of the tested LAB and *Bifidobacterium* strains after 48 h of culturing compared with the control. Moreover, the viability of the strains increased with extended incubation to 168 h compared with the control. SCE and SPO supply carbohydrates as a carbon source during microbial growth, and their utilization rate as a carbon source is higher than that of the glucose control. It is inferred that this can be attributed to SCE and SPO providing a more nutritious and stimulating environment for lactic acid and probiotic bacteria, thereby promoting their growth, viability, and activity [71,72]. In our study, LAB and *Bifidobacterium* strains were able to metabolize SCE and SPO, using them as a carbon source to support growth and viability. Previous reports have demonstrated that the growth of *L. casei* was stimulated by aqueous extracts, and this effect could be related to the composition of the extracts, which are mainly composed of polyphenols and polysaccharides [68,73]. This is consistent with our observations. Specifically, SPO-2 demonstrated a stronger prebiotic effect than SPO-1, which we attribute to its higher molecular weight and more diverse monosaccharide profile—both factors known to influence the fermentability and selectivity of prebiotic carbohydrates [74–76]. It is also important to highlight that the effect of supplementation with these prebiotics (SCE and SPO) may differ among strains of the same LAB species (for example, *L. rhamnosus* LOCK 0997 and GG) owing to wide metabolic differences among the strains. Similarly, the prebiotic effect of plant extracts, such as green tea, is known to be dose- and compound-specific, with certain polyphenols enhancing probiotic growth at optimal levels while inhibiting it at higher concentrations [77–79]. Our findings with the *Schisandra* extracts align with this principle, where the extract composition dictates microbial response. Among the tested strains, *B. breve* Bb-12 and *P. acidilactici* 21/1 exhibited the strongest growth stimulation in the presence of SPO-2, whereas *L. rhamnosus* LOCK 0997 and *L. brevis* OK-C showed a less pronounced response. This suggests that SPO-2 is preferentially utilized by *Bifidobacterium* and *Pediococcus*, potentially due to their superior enzymatic capacity to hydrolyze complex polysaccharides into fermentable sugars [80,81]. The existing literature has shown that *Schisandra* extracts modulate the composition of intestinal microbiota to improve overall gut health [65,82]. However, it is imperative to focus on the compatibility of bioactive compounds (in terms of content and composition) in *Schisandra* extracts with LAB and *Bifidobacterium*. Overall, the main issue with using *Schisandra* extracts in prebiotic formulations is the variation in bioactive composition between different cultivars and extraction systems. Therefore, further research is necessary to elucidate the potential of *Schisandra* extracts and to minimize these constraints.

A key finding of our fermentation study was that SCE and SPO resulted in higher growth and viability than in the glucose control, while eliciting a more gradual and less pronounced decrease in culture pH. This contrasts with the rapid and sharp acidification triggered by the fast and complete fermentation of glucose [83–85]. We propose that this difference stems from the structural complexity of the polysaccharides and other bioactive compounds in SCE and SPO [86–89]. Their slower and potentially partial degradation leads to a steadier production of metabolites, including not only lactate but also acetate and butyrate via mixed-acid fermentation [90]. The resulting moderated acidification likely creates a more sustainable microenvironment, which can prolong bacterial growth and survival compared to the stressful, rapid pH drop caused by glucose [91–93]. Furthermore, the observation that SPO induced an even milder pH decline than SCE aligns with its

more complex structure, suggesting a direct relationship between substrate complexity and fermentation kinetics [94].

In vitro studies show that the viability of LAB at a low environmental pH and in the presence of bile salts, or under simulated gastrointestinal conditions, is a key characteristic to be considered when evaluating probiotics [95,96]. This approach is distinct from, and complementary to, assays measuring direct prebiotic activity (e.g., growth and viability) and those measuring direct probiotic metabolic activity (e.g., acid production) or adhesion. *L. rhamnosus* demonstrated high survivability under conditions of low pH and in the presence of bile salts, especially *L. rhamnosus* GG, which was patented as a probiotic [96,97]. *P. acidilactici* 21/1 also showed a high survival rate of above 90% in simulated GIT [98]. Therefore, the protective effect of SCE and SPO can be evaluated using the indicator strains *L. rhamnosus* LOCK 0997 and GG and *P. acidilactici* 21/1. Efficient prebiotics (SCE and SPO) must be resistant to GIT conditions and selectively promote the growth of probiotic bacteria (*Lactobacillus* spp. and *Bifidobacterium* spp.) [99,100]. Our findings suggested that SCE and SPO exhibited an excellent protective effect on the three indicator strains in simulated GIT conditions. SCE and SPO displayed stimulated viability in the simulated digestive tract in vitro; specifically, SPO-2 displayed the highest survival rate. In summary, all LAB strains, both in the presence and absence of SCE and SPO, exhibited a viable cell count above 6 log CFU/mL, well above the recognized detection limit for probiotic bile salt resistance of 3 log CFU/mL. This finding indicates the ability of the selected LAB strains to colonize environments resembling conditions prevailing in the digestive tract.

The potential use of SCE as an antimicrobial agent has already been widely described. The potential of the chemical constituents in *Schisandra perulata* essential oils from Vietnam as a source of antimicrobial agents has been reported [40]. The antimicrobial activity of *S. henryi* tested against strains of Gram-positive and Gram-negative bacteria and fungi showed the highest activity against *Helicobacter pylori* [63]. The results of the present study showed that SCE selectively inhibited the growth of different pathogens, and the antibacterial activity of different SCE samples varied significantly. SPO did not display antimicrobial activity against any pathogens. According to the results, it can be inferred that the inhibition of pathogens is more likely connected with other components of SCE than with polysaccharides. Existing research has reported that polyphenols from plant extracts exhibit high antibacterial activity and strong anti-oxidant effects, for instance, gallic acid and quercetin [101,102]. Notably, SCE-1 showed the strongest antibacterial activity towards the tested pathogens. This result may be explained by the different content and composition of diverse SCE samples. Previous reports have demonstrated that the ethanolic extract of grapefruit is active against all tested Gram-positive species but inactive against Gram-negative species, which is consistent with earlier observations [101,103].

This study provides some insights into the relationships between composition and prebiotic and antimicrobial activities of diverse SCE and SPO. However, its scope and conclusions are subject to certain limitations. While we demonstrated clear variations in bioactivity linked to geographical origin and extraction method, our analysis was confined to only one sample per geographic region (Poland and three from China). In the future, a larger, more diverse set of samples from each region should be incorporated, including multiple cultivars and harvest years, so that the observed trends can be confirmed and clearer correlations between geographical factors and chemical composition can be established. Our primary focus was on the general prebiotic and in vitro antioxidant properties of polysaccharide-rich fractions and crude extracts. Consequently, we did not perform detailed mechanistic studies to isolate and identify the specific antibacterial compounds within our extracts, nor did we evaluate their activity against a broad spectrum of pathogenic bacteria (e.g., oral pathogens like *Streptococcus mutans* or foodborne pathogens like *Listeria*

and *Salmonella*), despite the literature confirming such potential in *Schisandra* [38,39]. Future work should therefore aim to isolate, characterize, and validate the efficacy of these specific antimicrobial agents (e.g., phenolic acids like tartaric acid) in our extracts, bridging the gap between compositional analysis and targeted application development.

4. Materials and Methods

4.1. Research Material

In August and September 2022, *Schisandra* fruits were gathered from Liaoning, China (*S. chinensis* (Turcz.) Baill.), Shaanxi, China (*S. sphenanthera* Rehder & E. H. Wilson), and Yunnan, China (*S. henryi* C.B. Clarke) (Figure 8). In September 2022, *Schisandra* fruits (*S. chinensis* (Turcz.) Baill.) were also gathered from the Botanical Garden of Lodz, Poland. The *Schisandra* fruit samples were identified by Dr. Hongfei Zhao, a professor at the College of Biological Sciences and Technology, Beijing Forestry University, Beijing, China. The materials (designated as SCE-1, SCE-2, SCE-3, and SCE-PL) were deposited in the Department of Environmental Biotechnology, Faculty of Biotechnology and Food Sciences, Lodz University of Technology, Lodz, Poland.

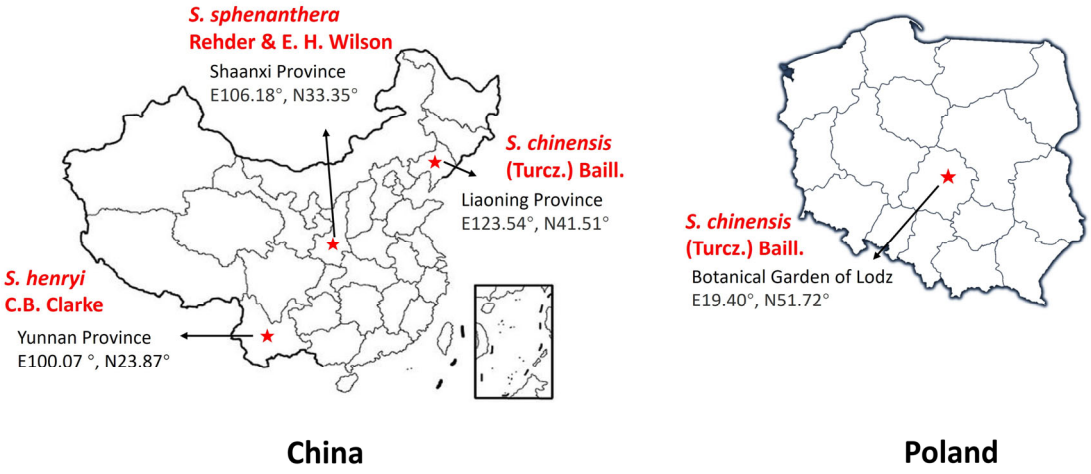


Figure 8. The origin of raw material of *Schisandra* genus fruits.

4.2. Chemicals and Reagents

In order to obtain SCE and SPO, the following chemicals and reagents were used: chloroform, n-butanol, and anhydrous ethanol. They were all purchased from Merck Life Science, Warsaw, Poland. For evaluating the interactions of SCE and SPO with LAB, pathogens, and gut microbiota, the following chemicals and reagents were used: glucose, MRS (de Man–Rogosa–Sharpe) broth and agar, TSA (Tryptic Soy Agar), TSB (Tryptic Soy Broth), BHI (Brain Heart Infusion) broth. All were purchased from Merck Life Science, Warsaw, Poland. Syringe filters (0.22 μm pore size) were purchased from Labindex S.A., Warsaw, Poland. AnaeroGen™ Atmosphere Generation Systems sachets were purchased from Thermo Fisher Scientific, Waltham, MA, USA. Cryobanks™ were from Copan Diagnostics Inc., Jefferson Avenue Murrieta, Murrieta, CA, USA.

4.3. Bacterial Strains and Growth Conditions

A total of nine strains of LAB were used for these studies. The strain *Lacticaseibacillus rhamnosus* LOCK 0997 originated from the Pure Culture Collection of the Institute of Fermentation Technology and Microbiology (LOCK 105), Lodz University of Technology. The strains *Lactiplantibacillus plantarum* OK-B, *Levilactobacillus brevis* OK-C, *Pediococcus pentosaceus* OK-S, 25/1, and 14/1, as well as *Pediococcus acidilactici* 7/1 and 21/1, were isolated

from environments such as human infant feces, fermented cucumbers, flowers, and honey, and were obtained from the collection of the Department of Environmental Biotechnology, Lodz University of Technology. Additionally, commercial probiotic strains—*L. rhamnosus* GG and *Bifidobacterium breve animalis* ssp. *lactis* Bb-12—commonly used in probiotic preparations were included in the study.

A total of 14 strains of human and food spoilage pathogens were used to study the potential of the analyzed extracts and polysaccharides. These included *Staphylococcus aureus* ATTC 6538 and ATTC 25923, *Listeria monocytogenes* ATCC 19115, *Escherichia coli* ATCC 10536 and ATCC 25922, *Pseudomonas aeruginosa* ATCC 15442 and ATCC 24755, *Salmonella enterica* subsp. *enterica* serovar Typhimurium ATCC 14028, *Citrobacter* sp. C1/1, *Providencia stuartii* P1/2, *Enterobacter cloacae* E3/1, *Staphylococcus epidermidis* S4/2, *Klebsiella pneumoniae* K4/3, and *Bacteroides faecis* DSM 24798. The strains were purchased from the American Type Culture Collection (labeled as ATCC) or the German Collection of Microorganisms and Cell Cultures GmbH (DSMZ, labeled as DSM). Strains with a alphanumeric symbols were obtained from the collection of the Department of Environmental Biotechnology, Lodz University of Technology. Microorganisms were cultured on the following media: nine LAB strains and *B. breve* Bb-12 on MRS; 13 pathogens on TSB; and *B. faecis* DSM 24798 on BHI. All microorganisms were stored in Cryobanks™ at −20 °C. Before conducting experiments, strains were activated, passaged three times (3% inoculum) and subsequently cultivated in the appropriate medium for 24 or 48 h under aerobic or anaerobic conditions (AnaeroGen™ Atmosphere Generation Systems sachet) at 37 °C.

4.4. Preparation of Schisandra Fruit Extracts (SCE)

Fresh fruits of three *Schisandra* species were washed and dried at 40 °C for 4 days to obtain dried fruit. The ground dried fruit powder was sieved with a 40 mesh (0.425 mm) sieve. Then, *Schisandra* powder and sterile water were added to round-bottom flasks at a ratio of 1:30 (*w*/*v*), which was followed by hot-water extraction. The extraction conditions were a temperature of 90 °C and duration of 5 h. A thermostat water bath was used as the experimental device. After extraction and centrifugation, the supernatant was rotary-evaporated at 70 °C to remove some water and then lyophilized to obtain *Schisandra* crude extract (SCE) powder. The SCE powder was collected and stored at −20 °C. The SCE powders were named as follows: SCE-1 (from *S. chinensis*), SCE-2 (from *S. sphenanthera*), and SCE-3 (from *S. henryi*). One of the SCE powders was obtained from *S. chinensis* fruit growing in Poland and it was named SCE-PL.

The extraction yield of SCE was calculated as the mass of the SCE powder divided by the mass of the dry *Schisandra* fruit [42].

The total lignans, total polysaccharides, total flavone, and total polyphenol content of SCE from China were determined using Ultraviolet–Visible (UV-Vis) spectrophotometry [42] (Table S11), which was carried out by Shanyang Lianfeng Biotech Co., Ltd. (Shangluo, Shanxi, China).

4.5. Isolation of Polysaccharides (SPO)

Preparation of the crude polysaccharide SPO-1 was carried out using an ultrasonic-assisted extraction method. The polysaccharide was isolated according to the method described earlier [42,104], with slight modifications. SPO-1 was isolated from SCE-3 powder, as it had the highest polysaccharide content (41.9%) among all of the received SCE samples. The SCE-3 powder was dissolved in distilled water (1:10, *w*/*v*) and placed in an ultrasonic bath for 40 min at 70 °C to obtain the crude polysaccharide. Chloroform and n-butanol were added to the crude polysaccharide solutions in the proportion of water solution: chloroform: n-butanol = 25:5:1 (*v*/*v*/*v*). The mixture was shaken for 30 min, then allowed

to stand and centrifuged to collect the aqueous phase. This process was repeated five times. After adding four volumes of anhydrous ethanol, the solution was kept overnight, centrifuged, and the precipitate was rotary-evaporated at 70 °C and lyophilized.

Preparation of the polysaccharide SPO-2 was carried out using *L. plantarum* CICC 23121 fermentation-assisted extraction, as has been described previously [103]. It was isolated from SCE-2 powder as the polysaccharide content was also very high (41.7%). In short, the SCE-2 powder was dissolved in distilled water (1:10, *w*/*v*), then was inoculated (3%) with *L. plantarum* CICC 23121 and fermented for 15.5 h. Further steps were carried out, as in the case of SPO-1.

The yield of extracted SPO (%) was calculated per the mass of dry SCE.

4.6. Compositional Analysis of SCE

4.6.1. LC-MS Sample Preparation

Water extracts (SCE-1, SCE-2, SCE-3, and SCE-PL) and methanol (at the ratio of 250:1, *w*/*v*) were transferred into Eppendorf tubes. The samples were placed in an ultrasonic bath for 1 h and then incubated at room temperature for an additional hour. After this time, the extracts were centrifuged at 9800× *g* for 10 min. The supernatants were transferred to new tubes, and the solvents were evaporated in a SpeedVac vacuum concentrator (Thermo Fisher Scientific, Waltham, MA, USA) (0.9 mbar vacuum) at room temperature. The dried materials were resuspended in 1 mL of methanol, placed in an ultrasonic bath for approximately 30 s, mixed on a vortex shaker (30 s), and then centrifuged at 9800× *g* for 5 min. Finally, the samples were diluted 10-fold and transferred to HPLC (high-performance liquid chromatography) vials.

4.6.2. LC-MS (Mass Spectrometry–Liquid Chromatography) Metabolomic Analysis

Mass spectrometry–liquid chromatography analyses were performed, consistent with our previous work [105], on a Bruker Elute UHPLC (Ultra-High-Pressure Liquid Chromatography) system operated with Hystar 3.3 software, coupled to an ultra-high-resolution (60,000+) mass spectrometer Bruker Impact II (Bruker Daltonik GmbH, Bremen, Germany) ESI QTOF-MS (Electrospray ionization Quadrupole Time-of-Flight Mass Spectrometry). The system was equipped with Data Analysis 4.2 (Bruker Daltonik GmbH), TASQ (2022b), and Metaboscape (2022b). The ion source used was a Bruker VIP-HESI (Vacuum Insulated Probe Heated ElectroSpray Ionization) with optimized flow rates and temperatures. The column used for Auto MS/MS (Auto Tandem Mass Spectrometry) measurements was a Bruker Intensity Solo C18, 2 µm particle size, with dimensions of 2.1 × 100 mm. Eluent A was water with 0.1% formic acid (HCOOH), and B was acetonitrile with 0.1% formic acid. For Auto MS/MS measurements, the gradient and flowrates were as follows: 0 and 2 min, 99% A; at 17 min, 1% A; 20 min, 1% A; at 20.1, 22 and 30 min, 99% A. The flow rate was 0.25 µL min^{-1} from 0 to 20 min and 0.35 µL min^{-1} from 20.1 to 30 min. The column temperature was maintained at 40 °C. The column exit was connected to ESI source. The injection volume was 5 µL. Analyses were performed in positive and negative Auto MS/MS mode with an m/z range of 50–1200. CID (Collision-Induced Dissociation) was applied with the following settings: absolute area threshold of 5000 counts; active exclusion after 2 spectra; release after 0.3 min; isolation mass: for m/z = 100, width was 4; for 300, width was 5; for 500, was 6; and for 1000, was 8; collision energy value was set at 30 eV. The internal calibration on 10 mM sodium formate (1:1, *v*/*v*, water: isopropanol) ions was performed automatically in Metaboscape with the use of a syringe pump at an infusion flow rate of 0.12 mLh^{-1}, using the high-precision calibration mode.

Untargeted annotations were performed in Metaboscape (ver. 2022b) with a criterion of mass deviation ($\Delta m/z$) under 2 ppm and mSigma value under 20 as the maximum

acceptable deviation of the mass of the compound and the isotopic pattern, respectively. All molecular formulas were generated using the Smart Formula tool and the C, H, N, O, P, S, Cl, Br, I and F elements. MS/MS spectra were automatically matched against MS/MS libraries: Bruker HMDB 2.0 library (spectral data with retention times), MoNA library (Mass Bank of North America, 2022), and NIST ver. 2020 MSMS library (Mass Spectrometry Data Center, 2022). The identification data obtained from the UHPLC-HRMS analysis are provided in Table S12 of the Supplementary Materials.

4.7. Compositional Analysis of SPO

4.7.1. Molecular Weight of SPO

SPO-1 (10 mg) was dissolved in 1 mL of deionized water and loaded into a 1 mL syringe. Spectra were recorded by direct injection using a syringe pump at a flow rate of 20 uL/min. Mass spectrometry analyses were performed in direct injection mode on an ultra-high-resolution (60,000+) mass spectrometer Bruker Impact II (Bruker Daltonik GmbH) ESI QTOF-MS, equipped with Data Analysis 4.2 (Bruker Daltonik GmbH), TASQ (2022b), and Metaboscape (2022b). The ion source used was a Bruker VIP-HESI with optimized flow rates and temperatures. Measurements were made in MS^1 mode at m/z 500–20,000 range; after observing no signals in the 3000–20,000 m/z range, it was adjusted to 500–3200 m/z. Molecular weight of SPO-2 was measured according to a method described previously [42].

4.7.2. Monosaccharide Composition of SPO

The carbohydrate profile of the SPO-1 sample was determined according to the method described by [106] with slight modifications. Firstly, 10 mg of the sample was mixed with 4 mL of 2 M trifluoroacetic acid (TFA) and incubated at 100 °C for 90 min. After hydrolysis, the samples were immediately cooled and centrifuged using a Centurion Scientific K3 Series centrifuge at 3852× *g* for 15 min at 25 °C. Then, 2 mL of the supernatant was transferred into 5 mL tubes and dried under a stream of nitrogen to evaporate the TFA. The dry residue was dissolved in 1 mL of HPLC-grade water. The solution was then filtered through nylon syringe filters with a pore size of 0.2 m into HPLC vials and subjected to HPLC-RI analysis. The chromatographic separation of sugars was carried out using a UHPLC+ Dionex UltiMate 3000 system (Thermo Fisher Scientific Inc., Waltham, MA, USA) equipped with a refractive index detector (Shimadzu, Kyoto, Japan) and a Rezex RPM Monosaccharide Pb^{2+} New Column (8.0 µm, 7.8 × 30 mm) stabilized at 80 °C with a thermostat. The injection volume was 10 µL for both samples and standards. Isocratic elution was performed using distilled water as the mobile phase at a flow rate of 0.6 mL min^{-1}. Detection was done with a Shodex R1–101 detector at 40 °C. Sugars in the samples were identified by comparing their retention times with those of standard compounds. The content of each carbohydrate was determined using the external standard method. All measurements were performed in triplicate. Monosaccharide analysis of SPO-2 was performed according to the method described earlier [42].

4.8. Preparation of SCE and SPO for Biological Tests

The SCE and SPO powders were not initially sterile. To minimize microbial contamination prior to conducting experiments involving biological tests, the SCE and SPO were subjected to tyndallization. Glucose, SCE, and SPO powder (0.1 g), along with sterile glucose-free MRS, TSA, or BHI broth, were added to 15 mL falcon tubes at a ratio of 1:10 (*w*/*v*), followed by tyndallization. The tyndallization conditions were temperature 80 °C and duration 15 min, for three consecutive days. A thermostat water bath was used as the experimental device. After tyndallization, samples were stored at 4 °C.

4.9. Antimicrobial Activity of SCE and SPO

The antimicrobial action of SCE against LAB and *Bifidobacterium* strains, as well as against selected human and food spoilage pathogens, were screened using the agar-well diffusion method. Before the experiments, the strains were activated, passaged threefold (3% inoculum), and cultured in MRS broth or TSB for 24 h at 37 °C. *B. breve* Bb-12 was cultured anaerobically in MRS broth. Activated strains at a density of 1.8×10^9 CFU/mL (6.0 according to the McFarland standard) were inoculated onto Petri dishes containing MRS agar or TSA. Subsequently, discs with a diameter of 10 mm were cut in triplicate from the inoculated agar medium using a sterile corkborer, and 200 µL of SCE or SPO solutions (SCE or SPO: sterile water, 1:10, *w*/*v*) were added to each well. After incubation for 24 h at 37 °C, zones of growth inhibition were measured, subtracting the diameter of the disc from the result. Each strain was tested in triplicate in two or three independent experiments.

To compare the antibacterial activity of SCE and SPO against the tested bacteria, the following criteria were adopted [107,108]: growth inhibition diameter above 16 mm—very strong inhibition; 11–15.9 mm—strong inhibition; 6–10.9 mm—moderate inhibition; 1–5.9 mm—weak inhibition; and 0 mm—no inhibition.

4.10. Effect of SCE and SPO on the Growth and Viability of LAB and Bifidobacterium Strains

To the 10 mL of glucose-free MRS broth, 1% SCE or SPO (SCE-1, SCE-2, SCE-3, SCE-PL, SPO-1, and SPO-2) was added. Liquid MRS broth containing 1% glucose with 3% inoculum of LAB or *Bifidobacterium* strains served as the control. MRS media supplemented with 1% SCE-1, SCE-2, or SCE-3 were inoculated with 3% inoculum of LAB or *Bifidobacterium* strains and cultured for 48 h and 168 h. Similarly, MRS media containing 1% SCE-PL, SPO-1, or SPO-2 were inoculated with 3% inoculum of four selected strains (*L. rhamnosus* LOCK 0997, *L. brevis* OK-C, *P. acidilactici* 21/1, and *B. breve* Bb-12) and cultured for the same time periods. The prebiotic effect of SCE and SPO on LAB growth and viability was evaluated using Koch's plate method. Each strain was tested in two independent experiments. pH measurements were taken throughout the experiment.

4.11. Growth Ability of LAB in the Presence of SCE and SPO in the Simulated Digestive Tract (INFOGEST)

The experiment was conducted with three selected strains: *L. rhamnosus* LOCK 0997 and GG and *P. acidilactici* 21/1. The growth ability of LAB in the presence of selected SCE and SPO was evaluated in vitro using a simulated digestive tract model. This experiment was based on existing studies and the INFOGEST simulated digestive tract protocol, with some modifications [109–112]. Overnight LAB cultures grown in MRS broth were centrifuged (10,733× *g*, 15 min), the supernatant was decanted, and the cells were suspended in sterile 0.85% NaCl. This washing step was repeated twice to completely remove the culture medium. The LAB biomass was then suspended in 0.85% NaCl (negative control) or in 1% solutions of selected SCE (SCE-1, SCE-PL) or SPO-2. Subsequently, all samples were suspended in fresh simulated saliva fluid (100 U/mL α-amylase, 0.85% NaCl, pH adjusted to 6.0, filtered through sterile 0.22 µm pore size syringe filters) and incubated for 10 min at 37 °C with shaking (80 rpm). Afterward, fresh simulated gastric juice (0.15% pepsin, 0.1% lipase, 0.85% NaCl, pH 3.0, filtered through sterile 0.22 µm pore size syringe filters) was added, and samples were incubated for 2 h at 37 °C with shaking (80 rpm). Finally, fresh simulated intestinal juice (1% bile salts, 0.1% pancreatin, 0.85% NaCl, pH 7.5, filtered through sterile 0.22 µm pore size syringe filters) was added, and the samples were incubated for 2 h at 37 °C with shaking (80 rpm). At each time point (initial 0 h, after 10 min incubation in simulated saliva fluid, after 2 h incubation in simulated gastric juice, and after a further 2 h incubation in simulated intestinal juice), 1 mL of each sample was transferred

to sterile 0.85% NaCl, mixed, diluted, and plated on MRS agar plates following Koch's plate method. After 48 h of incubation, the colonies growing on the agar were counted. Each strain was tested in two independent experiments.

4.12. Statistical Analysis

The statistical analysis, performed according to the methodology described in our previous work [113,114], was conducted using MetaboAnalyst 6.0 to assess the compositional differences between SCE and SPO. All metabolite datasets exported from Metaboscape v.2022 were analyzed using MetaboAnalyst 6.0 [115].

The results of prebiotic and antimicrobial effects are presented as the mean ± SEM of two or three independent experiments (n = 3). Normality of distribution was first assessed using the Shapiro–Wilk test. For datasets (e.g., differences between sample groups) that did not meet normality assumptions, the non-parametric Kruskal–Wallis test was employed, followed by Dunn's post hoc test for multiple comparisons between sample groups. For normally distributed data (e.g., differences between SCE and SPO samples), one-way analysis of variance (ANOVA) was performed, followed by Fisher's least significant difference (LSD) post hoc test for groupwise comparisons. All statistical analyses were conducted using Origin 2024 software (Northampton, MA, USA). A p-value < 0.05 was considered statistically significant.

5. Conclusions

Differences are observed in the bioactive components of the four SCE obtained from different geographic locations in China and Poland. The results highlight the relationship between the chemical composition of SCE and the geographical origin of the *Schisandra* species. Moreover, the extraction methods applied for SPO influence the molecular weight and monosaccharide composition of the isolated polysaccharides, indicating that both the origin and extraction procedure affect the physicochemical properties of the final product. SCE and SPO display the ability to stimulate the growth of tested LAB and *Bifidobacterium* strains, confirming their potential application as prebiotic supplements. The strongest growth stimulation is observed for *L. rhamnosus* LOCK 0997, *L. brevis* OK-C, and *P. acidilactici* 21/1 strains. All SCE display the ability to inhibit the growth of certain pathogens, whereas SPO do not exhibit such activity. Among the tested pathogens, *S. aureus* ATTC25923 is the most resistant. The tested LAB demonstrates the capacity to colonize environments by simulating the digestive tract. SCE and SPO enhance the viability of LAB under these conditions in vitro, with the highest survival observed in LAB treated with SPO-2, indicating their potential as prebiotic supplements in gastrointestinal applications. These findings indicate that SCE and SPO can be considered as functional ingredients in bioprocess design and formulation of food or feed products, and SCE also display potential as antimicrobial agents in food and industrial applications.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/molecules31040641/s1: Table S1: Characteristics of key metabolites discriminating between group China and group Poland; Table S2: Characteristics of key metabolites discriminating between SCE-1 and SCE-PL; Table S3: Characteristics of key metabolites discriminating between SCE-2 and SCE-PL; Table S4: Characteristics of key metabolites discriminating between SCE-3 and SCE-PL; Table S5: Characteristics of key metabolites discriminating between SCE-1 and SCE-2; Table S6: Characteristics of key metabolites discriminating between SCE-1 and SCE-3; Table S7: Characteristics of key metabolites discriminating between SCE-2 and SCE-3; Table S8: The growth of different LAB strains with 1% SCE and SPO after 48 h and 168 h incubation; Table S9: The pH of culture media of different LAB and *Bifidobacterium* strains with 1% SCE after 168 h of incubation; Table S10: Survival of selected LAB strains in the presence of SCE and SPO under the gastric (t < 2 h)

and intestinal (t > 2 h) conditions; Table S11: The total lignans, total polysaccharides, total flavone, and total polyphenol content of SCE from China; Table S12: Identification data of statistically and biologically significant metabolites analyzed by UHPLC-UHRMS; Figure S1: Examples of photographs showing antimicrobial activity of SCE and SPO against selected LAB and *Bifidobacterium* strains as evaluated with the agar-well diffusion method.

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