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Mixtures of *Scutellaria baicalensis* and *Glycyrrhiza* L. Extracts as Antibacterial and Antiviral Agents in Active Coatings

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Abstract: The aim of this study was to develop active packaging materials covered in active coatings (offering antibacterial and antiviral properties) that contain selected plant extracts. In addition, the synergistic effect of the active substances in these extracts was also analysed. The results of the study demonstrated that *Scutellaria baicalensis* and *Glycyrrhiza* L. extracts (two of six analysed plant extracts) were the most active agents against selected Gram-positive and Gram-negative bacterial strains. Additionally, the synergistic effect of *S. baicalensis* and *Glycyrrhiza* L. extracts was noted, meaning that the effect of these two plant extract mixtures on *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas syringae* growth was higher than the activity of individual pure extracts. Mixtures of the extracts were introduced into the coating carrier. A polyethylene (PE) foil was then coated with active layers containing mixtures of *S. baicalensis* and *Glycyrrhiza* L. extracts as antimicrobial agents. The results of this research showed that all of the active coatings had a bacteriolytic effect on *B. subtilis* and a bacteriostatic effect on *S. aureus* cells. The coatings were found to be inactive against *E. coli* and *P. syringae* cells. This means that the coatings could be used as internal coatings to preserve food products against Gram-positive bacteria that may be responsible for food spoilage. The results of this study also demonstrated that the coatings were highly active against phage phi 6 phage particles, used as SARS-CoV-2 surrogate. This means that the coatings could be used as external coatings to limit the spread of SARS-CoV-2 and pathogenic Gram-positive bacteria via human hands.

Keywords: SARS-CoV-2; phi 6 phage; antiviral properties; antibacterial properties; active coatings; active packaging; *Scutellaria baicalensis*; *Glycyrrhiza* L. extracts

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1. Introduction

Hippocrates taught the art of healing through the use of plant-based medicines. He used plants and their extracts to treat diseases around 460–370 BC. A return to utilising Nature's potential for the discovery and selection of new active compounds from renewable resources has become ever more popular. The main reason may be that natural derivatives have specific properties, high efficacy and offer a wide spectrum of activity [1,2]. It is important to be aware that on the whole pharmaceutical/chemical medicines usually only contain just one synthetic active substance, whereas plant extracts are simply a synergistic combination of dozens, if not hundreds of natural active compounds and vitamins [1]. The COVID-19 pandemic has affected many aspects of human life, including the use of herbal medicines. It is well known that the development of new synthetic drugs require many years of experimentation, animal and human trials, as well as lengthy legislation processes. This is why herbs/plants may play an important role to put this outbreak behind us. As already stated [2,3], herbal compounds/plant extracts may not only be used to

enhance the human immune system response against the SARS-CoV-2 virus, but also to prevent and effectively treat coronavirus-related diseases. Moreover, herb/plant extracts can be ideal solutions due to their synergistic effect on SARS-related and bacterial diseases, such as upper respiratory tract or lung infections [2–4]. Due to their synergistic effect, mixtures of many herb /plant extracts can be used to ensure microbial food safety and extend shelf life through the inhibition of microorganism growth, which is responsible for food product spoilage [2]. Active compounds extracted from plants/herbs can be added onto the surface of food products directly (as additives), or alternatively they can be introduced into a polymer matrix or into a coating carrier to cover a polymer foil. The created coatings or active films can be used to preserve food products and increase their shelf life as active packaging [2,4,5]. These active coatings are typically obtained using biopolymers derived from many natural plants, such as polysaccharides with the addition of active compounds, such as plant/herb extracts. Many of these extracts are dark brown or dark green in colour. They also have an intense smell, characteristic of the plant from which the extract was obtained [6–9]. The addition of plant/herb extract into a coating carrier may thus lead to the creation of active, non-transparent coatings with an intense smell. From the consumer's point of view, the coating cannot simply be active against selected microorganisms responsible for food spoilage, but also be transparent and odourless. An excellent example of an transparent, odourless coating (with antibacterial properties) containing plant extracts as active substances is the coatings offered in a previous study carried out by the authors [2]. These coatings were highly active against selected bacteria leading to them being an ideal internal layer for active packaging. Alternatively, these coatings can also be used as an external active packaging layer due to their antiviral properties. In earlier research carried out by the authors, it was demonstrated that these coatings had a marked effect on phi 6 bacteriophage particles, used as airborne virus surrogates, such as influenza and corona viruses, including SARS-Co-V-2 [2,4,10]. It should be mentioned that the external, active coatings of the packaging may be useful during the pandemic, not only because they may limit the spread of coronavirus particles, but also because they may protect consumers against bacterial infection. Regular hand disinfection in public places could lead to the spread of resistant bacteria via human hands. External active coatings might be a solution to this problem. Supermarkets could develop additional health safety measures, e.g., cardboard/ paper bags or foil bags coated with an antiviral and antibacterial external layer [2].

Due to their biological activities, such as antioxidative, antibacterial, hepatoprotective, antidiabetic, antiperspirant, antihypertensive, diuretic and tonic effects the roots of *Astragalus* species are well-known in folk medicine, most notably in China. *Astragalus* species extracts were found to contain cycloartane and triterpenoid saponins [11,12]. These substances are responsible for the observed biological activity, e.g., antiviral, immunostimulant, anti-protozoal and cardiovascular properties. El-Sebakhy et al. [12] indicated that *Astragalus* species extracts also contain isoflavones. In addition, the phenolic compounds of these extracts include chlorogenic acid, catechin hydrate, rutin and epicatechin, kaempferol, syringic acid, as well as quercetin, ferulic acid and cinnamic acid [11,12].

Uncaria tomentosa, also known as cat's claw because of the small curved thorns in its leaf axil, is a medicinal plant used over the centuries by the indigenous civilizations of the Amazon rainforest as an alternative treatment for different diseases [13,14]. Studies performed over a number of years have shown its antiviral, antibacterial, antioxidant, anti-inflammatory [13], antineoplastic, anticonceptive and immunostimulant activity [14], as well as its lack of toxicity [13].

Verbascum thapsus L. (commonly known as great mullein or common mullein) is a biennial herbaceous plant belonging to the *Scrophulariaceae* family [15]. This extract was found to contain boric acid and phenol as major compounds. Both of these compounds have antibacterial activity [16]. These extracts also contain iso-verbascoside, verbascoside, martynoside and leucosceptoside, as well as samioside, leucosceptoside B and alyssonoside [15].

Fomitopsis betulina (birch polypore) is a plant belonging to the *Fomitopsidaceae*, formerly known as *Piptoporus betulinus*. This plant is a member of the wood-rotting basidiomycete genus *Fomitopsis.* It is a medicinal and (when young) edible mushroom grow mainly in North America, Northern regions of Europe and Asia. The activity of *F. betulina* originate mostly from the triterpenoids, especially lanostane derivatives (e.g., 24-methylene-lanostane triterpenes) present in the fungus [17].

The most well-known species of the *Scutellaria* is *Scutellaria baicalensis*. The *S. baicalensis* genus (Baikal skullcap) is a perennial herb which is native to east Asia. Its root is commonly used in traditional Chinese Medicine and in other East Asian countries [18–20]. *S. baicalensis* contains a wide variety of polyphenols, especially flavonoids, which the main active compounds in the extracts of this plant [21]. More than 40 different polyphenols have been isolated and identified from *S. baicalensis*, including flavonoids and their flavonols, dihydroflavones and their dihydroflavonols, chalcones, tenaxin, viscudulin and biflavonoids. Among these, the most representative ingredients are baicalein, baicalin, wogonoside, wogonin, and norwogonin [18–22]. *S. baicalensis* extracts also contain small amounts of essential oils, lignan sterols, glycosides and amino acids [18]. The volatile oils in *S. baicalensis* have an aromatic smell and a sweet taste. Furthermore, they exhibit significant antibacterial effect on Gram-positive and Gram-negative bacteria. The major constituents of the volatile oils were 3-butanedione, acetophenone, palmitic acid and oleic acid. In addition to the above chemical compositions, there are some other ingredients/components that have been isolated and identified from *S. baicalensis,* including benzoic acid, β-sitosterol, lutein, β-carotene, and benzyl alcohol [21]. Extracts of *S. baicalensis* display a wide spectrum of antibacterial and antiviral activity [23,24]. Błach-Olszewska [23] proved the antiviral effect of baicalein and wogonin preparations by showing that an extract from *S. baicalensis* containing these two compounds regulated the innate antiviral immunity of the host by the modulation of cytokine production and the stimulation of human leukocyte resistance.

Glycyrrhiza glabra L. (licorice) belongs to the *Fabaceae* family [25]. *G. glabra* is a perennial herb, native to central and South-Western Asia, as well as to the Mediterranean region and cultivated in temperate and sub-tropical regions of the world, including Europe and Asia [26]. The plant is well-documented as having many pharmacological activities, such as expectorant, antitussive, anti-inflammatory, anticancer, antiulcer and antimicrobial effects [25]. The root of the plant, when dried and processed, is called licorice and has a characteristic odour and sweet taste [26]. The raw plant material contains saponins with expectorant, emollient and coating activity. Several medicines in the form of syrups, extract concentrates, as well as thick and dry extracts, were formulated based on the biologically active substances of *G. glabra* [27]. The main chemical constituents of licorice root are triterpene saponins (4–20%), mostly glycyrrhizin, a mixture of potassium and calcium salts of glycyrrhizic acid (also known as glycyrrhizic or glycyrrhizinic acid, and a glycoside of glycyrrhetinic acid) which is 50 times as sweet as sugar. Glycyrrhizin is a major component of *G. glabra* extracts, with a concentration varying between 1%–9%, depending on the species, geographical location and extraction method [26]. Many extracts of *G. glabra* were also found to contain proteins, carbohydrates, lipids and a small amount of essential oil. Glycolipids, sterols, fatty acids, their esters, phospholipids and higher aliphatic hydrocarbons have also been found [26,27]. Biologically active constituents, including lipids, were isolated from a *G. glabra* raw material using various solvents, such as ethanol or $CHCl_3$. All extracts were brown, possibly due to the presence of flavonoids [27]. It was demonstrated that the extracts of *G. glabra* showed significant fungicidal activity against phytopathogenic fungal strains, and the pronounced antifungal activity of licorice extracts has been attributed to the presence of phenolic compounds, such as isoflavonoids, flavonoids, chalcones and bibenzyls [25]. Several flavonoids with C_5 aliphatic residues which were isolated in the extracts of this plant were found to be effective constituents of licorice against methicillin-resistant *Staphylococcus aureus* (MRSA) and restored the effects of β-lactam and oxacillin antibiotics against MRSA. The ether–water extracts of *G. glabra* were found to be active against Gram-negative bacteria such as *E. coli*, *K. pneumoniae* and

Gram-positive bacteria, e.g., *B. subtilis* [28]. Additionally, glycyrrhizic acid and glycyrrhizin from *G. glabra* extracts have exhibited antiviral effects on the influenza virus and the SARS-associated coronavirus (SARS-CoV) [26,28].

To summarize, all of the plants described above are well known pharmaceutical plants with high antibacterial properties and some also contain antiviral and active compounds. This means that they can be used as active additives to a carrier to obtain internal and external coatings with antimicrobial properties. Prior research carried out by the authors demonstrated that there was a synergistic effect between extracts which were introduced into the active coatings [2]. The main aim of this research was based on these assumptions, which was to develop an active packaging material covered with active coatings (with antibacterial and antiviral properties) containing selected plant extracts as active compounds. Additionally, the synergistic effect of these active substances in the extracts was also analysed.

2. Materials and Methods

2.1. Materials

The test microorganisms used to perform experiments in this research were purchased from a collection from the Leibniz Institute Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany). The organisms used in this study were Gram-positive bacteria: a *B. subtilis* DSMZ 1090 and a *S. aureus* strain DSMZ 346. Gram-negative strains, such as *E. coli* DSMZ 498 and *P. syringae* van Hall 1902 DSM 21482 were also used and the microorganism was used as bacteriophage host. Phage phi 6 DSM-21518 was used as a SARS-CoV2 surrogate.

Polyethylene foil (A4, 50 µm) (KB FOLIE, Warsaw, Poland) and methylhydroxypropyl cellulose (MHPC, Chempur, Piekary Śląskie, Poland) were used as a coating carrier in the tests. Caprylyl/capryl glucoside and decyl glucoside (Greenaction Sp. z o.o., Kielce, Poland) were used as emulsifiers. *Astragulus* L., *Uncaria tomentosa, Fomitopsis betulina, Glycyrrhiza* L., *Verbascum* L. (Planteon, Borków Stary, Poland) and *Scutellaria baicalensis* (MyVita, Białystok, Poland) were used as active compounds. To analyse the antibacterial and antiviral activity of the coatings, MacConkey agar, TSA, Luria-Bertani (LB) and TSB broths (Merck, Darmstadt, Germany) were used. All mediums were prepared in accordance with the manufacturer's protocols (all mediums were weighed according to Merck instructions, suspended in 1 L of distilled water and autoclaved at 121 °C for 15 min).

2.2. Extracts Preparation

Dry plants: *Astragulus* L., *Uncaria tomentosa, Fomitopsis betulina, Glycyrrhiza* L., *Verbascum* L. and *Scutellaria baicalensis* were introduced (separately) into a TM6 Thermomix (VORWERK, Wrocław, Poland). The plants were ground to a powder (7600 rpm, 20 s). Later, 50 g of each plant sample were introduced (separately) into 100 mL of 70% ethanol. The samples were then introduced into a microwave oven (Amica, Wronki, Poland). Extraction was carried out for 5 min at 70 °C according to [29,30]. Next, the samples were introduced into a shaker (Ika, Staufen im Breisgau, Germany) and extracted for 1 h at 70 °C @150 rpm. The crude ethanol extracts were filtered through a Büchner funnel containing a cellulose filter. The 70% aqueous ethanol extracts were then concentrated at 40 °C. After the evaporation of ethanol, the samples were filtered through a 0.2 µm filter and used at the next stage of the tests. The dry mass of each extract was determined via a moisture analyser (Radwag, Puszczykowo, Poland).

2.3. The Analysis of Antimicrobial Properties of the Extracts

An antibacterial analysis was performed using the methods described in previous papers [31–33]. As a first step, an antibacterial property analysis was carried out, the *B. subtilis, S. aureus, P. syringae* cells were pre-grown on TSA and *E. coli* cells were pre-grown on MacConkey agar for 24 h. All strains with the exception of *P. syringae,* were pre-grown at 37 °C. *P. syringae* was incubated at 28 °C. After incubation, the biomass was

suspended separately in a sterile 0.85% NaCl solution to obtain 1.5 × 10^8 CFU/mL. Next, 5%, 10%, 25% and 50% solutions of *Astragulus* L., *Uncaria tomentosa, Fomitopsis betulina, Glycyrrhiza* L., *Verbascum* L. and *Scutellaria baicalensis* extracts in 10 mL of TSB medium in flasks were prepared separately. The samples were mixed with a magnetic stirrer (250 rpm, Ika, Legnica, Poland) for 15 min. The mixture of the extracts and TSB were then introduced into test tubes. The suspended biomass of the microorganisms (100 µL of each sample) was added to sterile test tubes, which contained TSB with extracts at concentrations of 5%, 10%, 25%, 50% and was further mixed in a vortex mixer (Ika, Legnica, Poland) for 1 min. The medium containing biomass which did not contain any extracts was designated as the control sample. After stirring, the samples were incubated for 24 h in a shaker (150 rpm, Ika, Staufen im Breisgau, Germany) at 37 °C (*B. subtilis, S. aureus, E. coli*) and 28 °C (*P. syringae*). After incubation, 100 µL of each sample (and its decimal dilutions) was plating on TSA or/and on MacConkey agar and incubated at 37 °C and 28 °C for 24 h. The cell concentration was expressed as colony-forming units (CFU) per mL. All tests were performed in triplicate.

To determine the synergistic effect between extracts, the most active extracts were selected. *Glycyrrhiza* L. and *Scutellaria baicalensis* were selected and these extracts were mixed at a ratio of 1:1; 1:2 and 2:1 using a vortex (Ika, Warsaw, Poland). The next step was to prepare 5%, 10%, 25% and 50% solution mixtures (in a TSB medium). An antibacterial activity analysis of all of the mixtures was performed in accordance with the method described above.

To analyse the *S. aureus, B. subtilis* and *E. coli* growth rate in real time, after their incubation with the 5% solutions of extract mixtures of *Glycyrrhiza* L. and *Scutellaria baicalensis* (in 1:2; 1:1 and 2:1 ratio), the LB broth, and LB broth with a mixture of the extracts was introduced into BioSan bioreactors (BS-010160-A04, BioSan, Riga, Latvia). Later, overnight bacterial culture (each strain separately) was added to 30 mL of LB broth and incubated at 37° or 28° (depending on the analysed strain) until OD = 0.2 was met (optical density). Then, mixtures of *Glycyrrhiza* L. and *Scutellaria baicalensis* extracts (in 1:2; 1:1 and 2:1 ratio) were added to a bacterial culture (OD = 0.2) to obtain 5% solutions of the extract mixtures in LB medium, and the samples were then incubated for 24 h at 37° or 28°. LB medium devoid of bacterial culture but containing a mixture of extracts and LB medium devoid of any extract mixture, though containing bacterial culture were set as control samples.

2.4. Coatings Preparation

Three 25% dispersions of coating carrier containing the mixtures of *Glycyrrhiza* L. and *S. baicalensis* extracts (in 1:2; 1:1 and 2:1 ratio) were prepared. Initally, *Glycyrrhiza* L. and *S. baicalensis* extracts were mixed at a ratio of 1:1; 1:2 and 2:1 in beakers using a magnetic stirrer (500 rpm, Ika, Warsaw, Poland). Then, 2 g of Decyl Glucoside and 2 g of Caprylyl/Capryl glucoside were introduced into each beaker and mixed for 5 min using a magnetic stirrer at 1000 rpm (Ika, Warsaw, Poland). Then, 12 g of MHPC was introduced into 288 mL of water. The dispersion was mixed for 1 h using a magnetic stirrer (Ika, Warsaw, Poland) at 1000 rpm. 75 g of 4% MHPC was then introduced into 25 g of each mixture containing emulsifiers. Mixtures I, II, and III were mixed separately for 15 min using a magnetic stirrer (Ika, Warsaw, Poland) at 1000 rpm.

A polyethylene (PE) foil sample was coated using Unicoater 409 (Erichsen, Hemer, Germany) at a temperature of 25 °C with a 40 µm diameter roller. The coatings were dried for 10 min at 50 °C. 1.6 g layers of MHPC containing mixtures of extracts per 1 m^2 of PE were obtained. PE foil that was not coated was the control sample (K). The film samples were then cut into square shapes (3 cm × 3 cm) and left for the next analysis.

2.5. Antibacterial Analysis

The antibacterial effect of the coatings obtained against Gram-negative bacteria *P. syringae, E. coli* and Gram-positive *B. subtilis, S. aureus* strains, compared to the non-coated

PE foil samples was performed according to the ASTM E 2180-01 standard and based on the previous study [2,4,8,34].

2.6. Antiviral Analysis

As a first step of the antiviral properties analysis, the bacteriophage phi6 was purified according to Bhetwal et al. [35]. The phage lysate obtained was then prepared according to Bonilla et al. [36]. The antiviral effect of the coatings containing the extracts mixtures was compared to the non-coated films and this was carried out in accordance to a modified ISO 22196-2011 standard [37].

To determine the *P. syringae* growth rate in real time during its incubation with phage particles, phi 6 bacteriophage lysate was incubated with covered foil squares (each active coated foil square separately) according to the ISO 22196-2011 standard, while at the same time phage lysate was incubated with PE squares devoid of coating. The LB broth was added to Biosan bioreactors (BS-010160-A04, Biosan, Riga, Latvia). The overnight bacterial cell culture was then added to 30 mL of LB medium and incubated at 28° until OD = 0.2. Four phi 6 bacteriophage lysates were amplified in respective *P. syringae* culture (one lysate—after incubation with a control sample, three phi 6 bacteriopfage lysates separately—after their incubation with active coatings/layers containing the mixtures of extracts as active compounds). Next, 11 µL of phi6 lysate (MOI = 1, 1 phage per 1 bacterial cell) were introduced to a host culture (OD = 0.2) and incubated for 24 h at 28°. Four tests were performed simultaneously.

2.7. FT-IR

To determine the similarities and differences in two extracts chemical composition, fourier transform infrared (FT-IR) analysis was performed. FT-IR spectra of the *Glycyrrhiza* L. and *S. baicalensis* extracts was measured using a Spectrum 100 FT-IR spectrophotometer (Perkin Elmer, Waltham, MA, USA), operated at a resolution of 4 cm^{-1}, over four scans. Each extract sample was placed directly at the ray-exposing stage separately. The spectrum was recorded at a wavelength of 650–4000 cm^{-1}.

2.8. Statistical Analysis

Statistical significance was evaluated using a variance analysis (2way ANOVA). The values were considered significantly different when $p < 0.05$. All analyses were performed with GraphPad Prism 8 (GraphPad Software, Version 9, San Diego, CA, USA).

3. Results

Fifty g of each plant powder was introduced into 100 mL of 70% ethanol. After an extraction process, each extract was recovered and purified by the use of a filtration method. After ethanol evaporation, each extract was filtrated with a 0.2 µm filter and dry mass of each extract was evaluated. The results of this process showed that the highest dry extract mass was noted in the case of *U. tomentosa.* The lowest dry mass was observed in the case of the *Verbascum* L. extract. Comparing the dry mass of *Glycyrrhiza* L. and *S. baicalensis*, it was noted that the *S. baicalensis* dry mass was only 8.35% lower than the *Glycyrrhiza* L. dry mass extract (Table 1).

Table 1. Dry mass of plant extracts.

Extract of the Plant	Dry Mass of Crude Extracts (%)
Fomitopsis betulina	13.74
Verbascum L.	3.75
Uncaria tomentosa	21.82
Astragulus L.	11.86
Glycyrrhiza L.	18.69
Scutellaria baicalensis	17.13

3.1. Antibacterial Analysis of Extracts

The results of the study demonstrated that *Fomitopsis betulina* extract did not inhibit the growth of *S. aureus*. A low reduction in the number of bacterial cells was observed for 5% and 10% plant extracts. A greater than 2 log reduction in the number of *S. aureus* cells was only noted in the case of a 50% extract of *Fomitopsis betulina.* Similar results were observed for *Astragulus* L. and *Verbascum* L. extracts (Table 2). When comparing the activity of the extracts against *S. aureus*, it should be added that *Uncaria tomentosa* extract had a much lower effect on the bacterial strain than the *Astragulus* L. and *Verbascum* L. extracts. The highest activity was observed for *Glycyrrhiza* L. and *S. baicalensis* extracts. This is emphasized in Table 2, where 5% extracts of these plants indicated a bacteriostatic effect on *S. aureus* cells resulting in a higher than 2 log reduction in the number of bacterial cells. It should be noted that a bacteriolytic effect on Gram-positive bacterial cells was determined for the 10%, 25% and 50% extracts of *Glycyrrhiza* L. and *S. baicalensis.*

Table 2. The influence of plant extracts on *S. aureus* growth.

Extract of the Plant	Extract Concentration (%)			
	5	10	25	50
Fomitopsis betulina				
Verbascum L.				
Uncaria tomentosa				
Astragulus L.				
Glycyrrhiza L.				
Scutellaria baicalensis				

Black—complete inhibition; dark grey—>2 log reduction; light gray—<2 log > 1 log reduction; dark blue—<1 log reduction; light blue—not active.

The results of this research have shown that *F. betulina, Verbascum* L., *Uncaria tomentosa* and *Astragulus* L. extracts had no effect on the growth of *E. coli*. A reduction in the number of Gram-negative bacteria was not noted, even for the 50% extracts of the analysed plants. In the case of *Glycyrrhiza* L., *a* higher than 2 log reduction in the number of bacterial cells was observed for the 10%, 25% and 50% plant extracts (Table 3). Analysing the activity of the *S. baicalensis* extract, a bacteriostatic effect was only determined in the case of the 50% solution.

Table 3. The influence of plant extracts on *E. coli* growth.

Extract of the Plant	Extract Concentration (%)			
	5	10	25	50
Fomitopsis betulina				
Verbascum L.				
Uncaria tomentosa				
Astragulus L.				
Glycyrrhiza L.				
Scutellaria baicalensis				

Black—complete inhibition; dark grey—>2 log reduction; light gray—<2 log > 1 log reduction; dark blue—<1 log reduction; light blue—not active.

While analysing the activity of *F. betulina* extracts on Gram-positive bacilli it was demonstrated that all of the extracts showed lower that 2 log reduction, but higher than a 1 log reduction in the number of *B. subtilis* cells. The concentration of extract had no influence on its antibacterial effect. Similar results were also observed for the *U. tomentosa* and *Verbascum* L. extracts (Table 4). The concentration of the extract had no effect on its activity, but a reduction in the number of microorganism cells was lower than 1 log. *Astragulus* L. extracts demonstrated similarly low activity against *B. subtilis* cells, except in the case of a 50% extract, which caused a higher than 2 log reduction in the number of the bacteria. The highest activity was also observed for *Glycyrrhiza* L. and *S. baicalensis* extracts. As shown in the Table 4, a 5% extract of *Glycyrrhiza* L. and 5–25% concentrations of *S. baicalensis* extracts had a bacteriostatic effect on *B. subtilis* cells. A complete inhibition in of the growth of Gram-positive bacilli was determined in the case of 10%, 25% and 50% extracts of *Glycyrrhiza* L. and a 50% extract of *S. baicalensis.*

Table 4. The influence of plant extracts on *B. subtilis* growth.

Extract of the Plant	Extract Concentration (%)			
	5	10	25	50
Fomitopsis betulina				
Verbascum L.				
Uncaria tomentosa				
Astragulus L.				
Glycyrrhiza L.				
Scutellaria baicalensis				

Black—complete inhibition; dark grey—>2 log reduction; light gray—<2 log > 1 log reduction; dark blue—<1 log reduction; light blue—not active.

The results of this research showed that 5–50% extracts of *F. betulina;* 5–25% extracts *Verbascum* L., *Uncaria tomentosa* and *Astragulus* L. extracts; 5–10% extracts of *Glycyrrhiza* L. and *S. baicalensis* did not decrease the number of *P. syringae* cells. A higher than 1 log reduction in the number of Gram-negative bacteria was not seen, even in the case of the 50% extracts of *Verbascum* L., *Uncaria tomentosa* and *Astragulus* L. plants. In the case of *Glycyrrhiza* L. *S. baicalensis* extracts, a greater than 2 log reduction in the number of bacterial cells was observed for the 25% extracts of these plants (Table 5). 50% extracts of these two plants demonstrated the complete inhibition of *B. subtilis* cells.

Table 5. The influence of plant extracts on *P. syringae* growth.

Extract of the Plant	Extract Concentration (%)			
	5	10	25	50
Fomitopsis betulina				
Verbascum L.				
Uncaria tomentosa				
Astragulus L.				
Glycyrrhiza L.				
Scutellaria baicalensis				

Black—complete inhibition; dark grey—>2 log reduction; light gray—<2 log > 1 log reduction; dark blue—<1 log reduction; light blue—not active.

Summarizing the results of the experiments it could be determined that the most active substances were *Glycyrrhiza* L. *S. baicalensis* extracts. These extracts inhibited the growth of *S. aureus* (10–50% extracts of both plants) and *B. subtilis* (10–50% extracts of *Glycyrrhiza* L. and 50% extract of *S. baicalensis*) completely. In the case of *E. coli and P. syringae*, *Glycyrrhiza* L. extract was also found to be more active than *S. baicalensis* extract, but the concentration of extract which caused the inhibition of bacterial growth was 50%.

3.2. Antibacterial Analysis of Mixtures of Extracts

Based on the results obtained by the authors it was assumed that a synergistic effect between *Glycyrrhiza* L. and *S. baicalensis* extracts would be observed. This is why the mixtures (M1, M2, M3) of these two extracts were prepared (at a ratio of 1:1; 1:2 and 2:1). It was assumed that if there is a synergistic effect between active compounds, the 5% and 10% solutions of extract mixtures would inhibit the growth of Gram-positive and Gram-negative bacteria.

The results of this study demonstrated that the 25% and 50% solutions of the extract mixtures had a bacteriolytic effect on all of the analysed strains (Table 6). *Glycyrrhiza* L. and *S. baicalensis* extracts did not inhibit *E. coli* growth, but decreased the number of viable cells. Reverse results were found in the case of the extract mixtures. As clearly shown in Table 7, even 5% and 10% solutions of mixtures M1, M2, M3 were found to have a bacteriolytic effect on *E. coli* cells. The results illustrate that there is a synergistic effect between the two extracts. A one-way ANOVA test confirmed that there were significant differences. A synergistic effect between *Glycyrrhiza* L. and *S. baicalensis* extracts was also observed for the *P. syringae* strain. A 25% extract of *Glycyrrhiza* L. and a 25% extract of *S. baicalensis* decreased the number of *P. syringae* cells. In addition the extracts were not effective against the microorganisms at lower concentrations. The mixtures of two extracts were found to be more active than pure extracts. It was determined that 25% solutions of mixtures M1, M2 and M3 completely inhibited the growth of the Gram-negative bacteria (Table 7). Lower concentrations of mixtures of 5% and 10% were found to have bacteriostatic effect where a greater than 4 log reduction in the number of bacterial cells was noted (Figure 1). Statistical analysis demonstrated that the differences between the number of bacterial cells for the control sample and the number of *P. syringae* cells for the M1, M2 and M3 samples were significant ($p < 0.0001$).

Table 6. The influence of mixtures of *S. baicalensis* and *Glycyrrhiza* L. extracts on the viability of *S. aureus*, *E. coli*, *P. syringae* and *B. subtilis*.

Plant	The Number of Bacterial Cells of the Control Sample [CFU/mL]	Ratio of the Mixture	Mixture of Extract Concentration [%]	
			25	50
			The Number of Bacterial Cells after Incubation with Mixtures of Extracts [CFU/mL]	
Glycyrrhiza L. *Scutellaria baicalensis* *	*S. aureus* $4.75 \times 10^8 \pm 1.64 \times 10^7$ **	M1 = 1 *:2	*S. aureus* 0 ****	0 ****
Glycyrrhiza L. *Scutellaria baicalensis* *	*E. coli* $8.65 \times 10^8 \pm 9.31 \times 10^7$ **	M2 = 2 *:1	*E. coli* 0 ****	0 ****
Glycyrrhiza L. *Scutellaria baicalensis* *	*B. subtilis* $5.80 \times 10^7 \pm 1.10 \times 10^6$ **	M3 = 1 *:1	*B. subtilis* 0 ****	0 ****
	P. syringae $2.05 \times 10^8 \pm 7.67 \times 10^6$ **		*P. syringae* 0 ****	0 ****

*—*Scutellaria baicalensis* extract; **—Standard Deviation; One-way ANOVA test: ****—$p < 0.0001$.

Table 7. The influence of mixtures of *S. baicalensis* and *Glycyrrhiza* L. extracts on the viability of *S. aureus*, *E. coli*, and *B. subtilis*.

Plant	The Number of Bacterial Cells of the Control Sample [CFU/mL]	Ratio of the Mixture	Mixture of Extract Concentration [%]	
			5	10
			The Number of Bacterial Cells after Incubation with Mixtures of Extracts [CFU/mL]	
Glycyrrhiza L. *Scutellar ia baicalensis* *	*S. aureus* $4.75 \times 10^8 \pm 1.64 \times 10^7$ **	M1 = 1 *:2	*S. aureus* 0 ****	0 ****
Glycyrrhiza L. *Scutellaria baicalensis* *	*E. coli* $8.65 \times 10^8 \pm 9.31 \times 10^7$ **	M2 = 2 *:1	*E. coli* 0 ****	0 ****
Glycyrrhiza L. *Scutellaria baicalensis* *	*B. subtilis* $5.80 \times 10^7 \pm 1.10 \times 10^6$ **	M3 = 1 *:1	*B. subtilis* 0 ****	0 ****

*—*Scutellaria baicalensis* extract; **—Standard Deviation; One-way ANOVA test: ****—$p < 0.0001$.

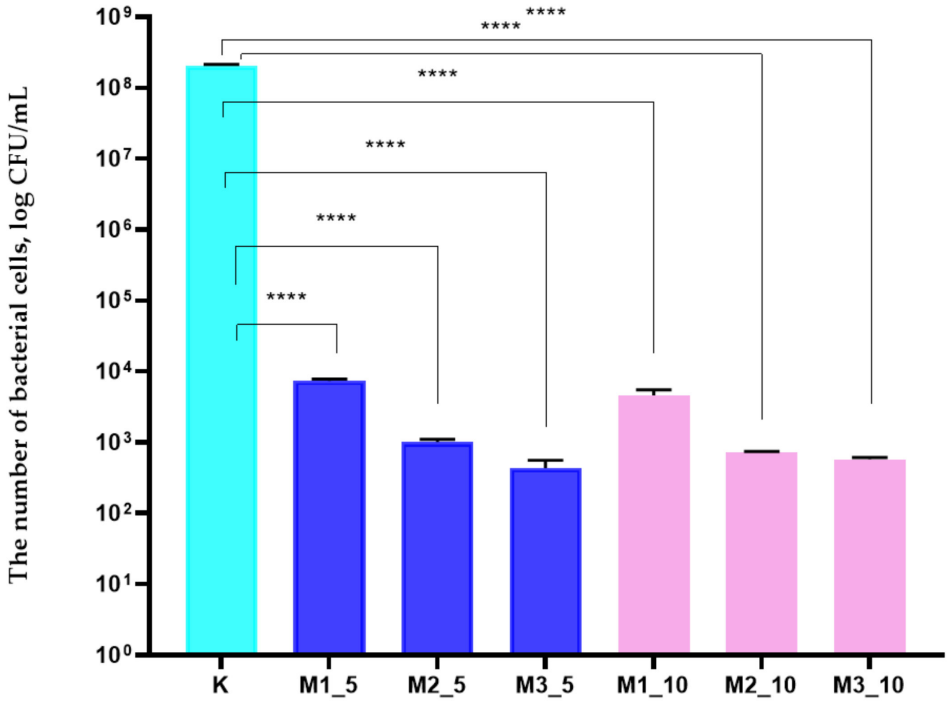

Figure 1. The influence of mixtures of *S. baicalensis* and *Glycyrrhiza L.* extracts on the viability of *P. syringae*. One-way ANOVA test: ****—$p < 0.0001$.

Analysing low concentrations of M1, M2 and M3, it can be assumed that 5% and 10% solutions of these mixtures were active against three of the four strains, resulting in the complete growth inhibition of the bacteria. In the case of *P. syringae*, bacteriostatic effect was only noticed for the 5% and 10% solutions of the extract mixtures. 25% solutions of M1, M2 and M3 were found to represent the Minimal Inhibition Concentration (MIC) for *P. syringae*. Consequently 25% mixture solutions should be used to prepare a coating which could be effective against this microorganism. Varying results were obtained for the *B. subtilis*, *E. coli* and *S. aureus* cells. A 5% concentration of M1, M2 and M3 was the MIC for these three strains. Additional tests were performed that would show if 5% solutions of these mixtures should be used to prepare coatings that would be active against the bacteria. The growth of *S. aureus*, *E. coli* and *B. subtilis* in real time which was in contact with a 5% solution of M1, M2 and M3 was analysed.

In relation to the control sample (the curve of the growth *E. coli* strain incubated in LB medium devoid of any extract), the highest bacteriostatic effect was observed in the case of an M1 mixture of *Glycyrrhiza* L. and *S. baicalensis* extracts. A gradual decrease in the growth of *E. coli* cells which were cultivated in LB medium with the addition of 5% of the M1 mixture was noted. This demonstrated that the effectiveness of the M2 and M3 mixtures decreased, respectively. In the case of the M3 mixture, a small increase in the growth curve of *E. coli* was observed and in the M2 mixture, much greater increase in the growth curve of the bacterial cells was determined than compared to the curve of the control sample or the curve of bacterial growth obtained for the M1 mixture (Figure 2).

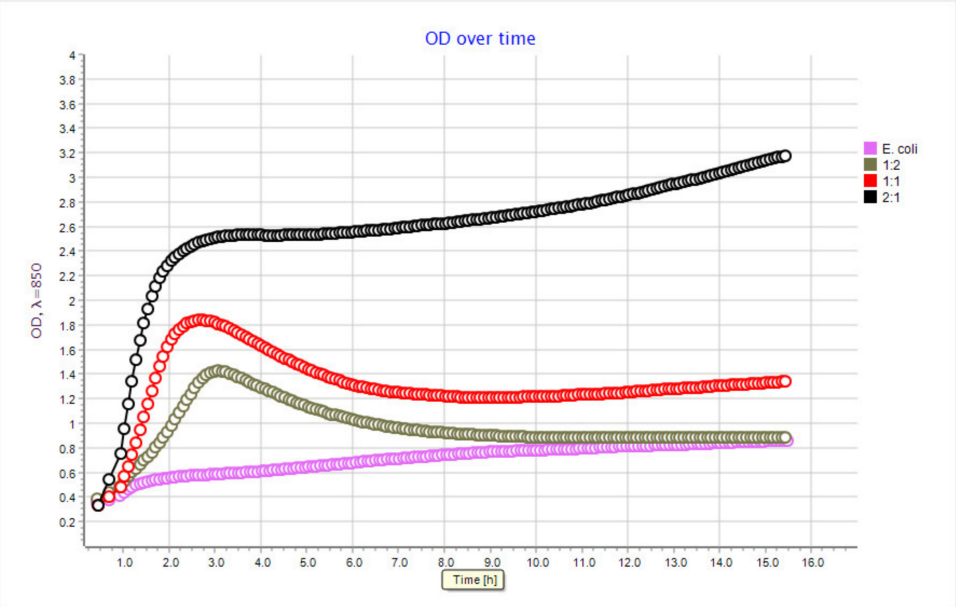

Figure 2. The influence of mixtures of *S. baicalensis* and *Glycyrrhiza* L. extracts on the growth of *E. coli*.

Comparing the growth curve of the *S. aureus* devoid of any extract (the control sample) with the growth curves of the strain exposed to 5% solutions of M1, M2 and M3 extract mixtures, the highest bacteriostatic effect was observed in the case of the M1 mixture. As clearly shown in Figure 3, a clear decrease in the growth curve of *S.aureus* was noted. It was observed that the activity of M2 and M3 mixtures were lower than the activity of mixture M1. In the case of the M3 mixture, a small increase in the growth curve of *S. aureus* was noted, and in the case of the M2 mixture, a greater increase in the growth curve of the microorganism (than for M3 mixture) was seen. Additionally, a slight decrease in the growth curve of these bacteria was observed after 22 h of cultivation with a 5% addition of the M2 mixture to the LB medium.

Comparing the growth curve of the *B. subtilis* devoid of any extract (the control sample) with the growth curves of the strain exposed to the 5% solutions of M1, M2 and M3 mixtures of extracts, the highest bacteriostatic efficiency was also observed in the case of the M1 mixture, as a clear decrease in the growth curve of the strain (cultivated in a LB medium with 5% of M1) was demonstrated. Additionally, a slight decrease in the growth of *B. subtilis* cells was noted after 22 h of their incubation in LB in the M1 mixture. On the other hand, the activity of M2 and M3 mixtures decreased respectively. In the case of the M3 mixtures a steady/constant growth of bacilli cells was noted, as well as in the M2 mixtures (Figure 4). In addition, a slight decrease in the curves of the growth of *B. subtilis*, in the case of the M2 and M3 mixtures was determined after 22 h of microorganism cultivation.

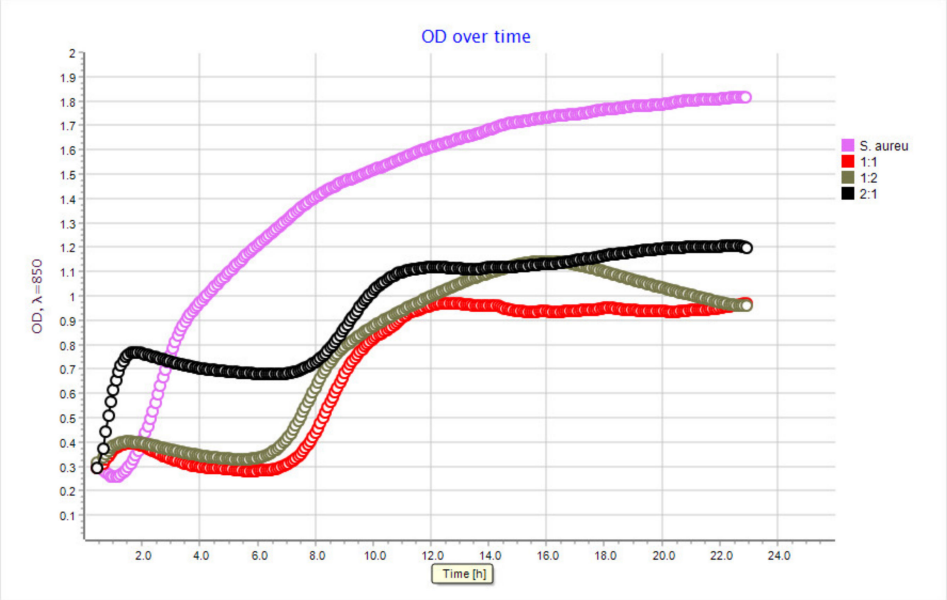

Figure 3. The influence of mixtures of *S. baicalensis* and *Glycyrrhiza* L. extracts on the growth of *S. aureus*.

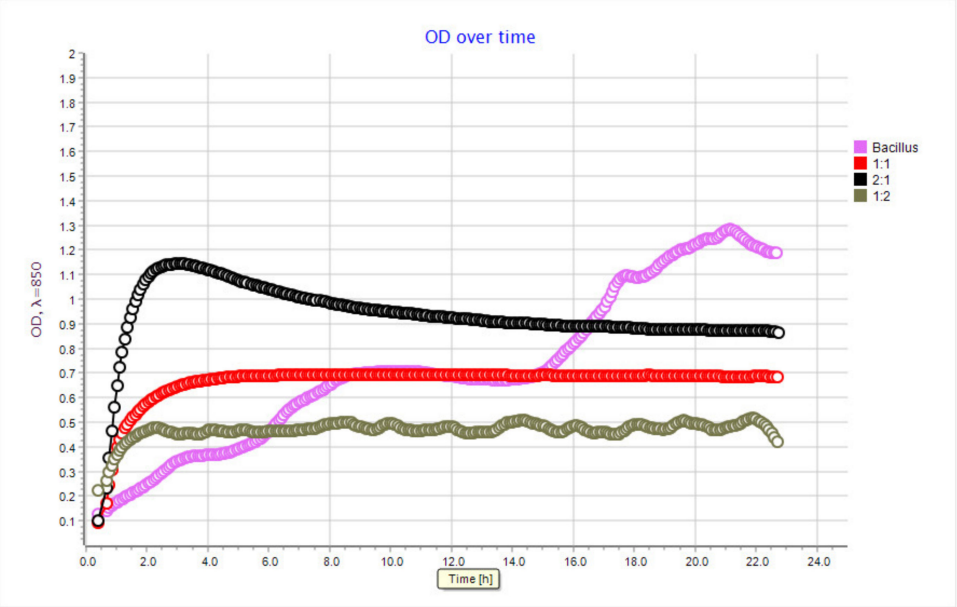

Figure 4. The influence of mixtures of *S. baicalensis* and *Glycyrrhiza* L. extracts on the growth of *B. subtilis*.

To summarise, it was clear that the most active mixture of the selected two extracts was the M1 mixture. An analysis of the growth of *B. subtilis*, *S. aureus* and *E. coli* strains in real time demonstrated that the influence of a 5% addition of each mixture to the LB medium had no bacteriolytic effect on these microorganisms. It was assumed that even if the previous experiment (Table 7) confirmed that 5% solutions of the mixtures were found to inhibit the growth of these microorganism, an analysis of the growth curves of all the strains led to the conclusion that the addition of 5% of each mixture (separately) to the coating carrier would not be enough to obtain a coating with bacteriolytic activity against all strains. This is why coating carriers containing 25% of each mixture (separately) were prepared to cover the PE films.

3.3. Antibacterial Analysis of Coatings

The results of the study showed that coatings containing M1, M2 and M3 mixtures as active compounds were transparent (Figure 5). They inhibited the growth of *B. subtilis* cells completely (Figure 6), showing conclusively that all of analysed coatings had a significant, bacteriolytic effect on bacilli cells, and this was confirmed by a one-way ANOVA test ($p < 0.0001$). In the case of the *S. aureus* strain, a decrease in the number of the bacterial cells was determined from 4.87×10^7 to 4.3×10^4 (CFU/mL) after their incubation with the coating containing the M1 mixture as an active additive. Similar results were obtained for a PE film covered with layers containing M2 and M3 mixtures as antibacterial substances. It was shown that the number of *S. aureus* cells decreased from 4.87×10^7 to 4.18×10^4 (CFU/mL) for M2 and from 4.87×10^7 to 5.75×10^4 (CFU/mL) for M3 mixture. Statistical analysis demonstrated that changes in the number of *S. aureus* cells were significant ($p < 0.0001$). In the case of *S. aureus cells*, the most effective coating was a layer containing the M2 mixture.

Figure 5. The active coatings containing M1 mixture as active compound in coating.

It was observed that all of the analysed coatings which contained the mixtures M1, M2 and M3 as an antibacterial agents did not inhibit the growth of *E. coli*, and did not even decrease the amount of these *E. coli* viable cells. The differences between the number of these bacteria cells were not significant statistically when compared to the control sample (see Figure 7). Similarly, the coatings described here were found to not be active against the *P. syringae* strain. A reduction in the number of the bacterial cells was not noted even when a statistical analysis determined that the differences between the number of *P. syringae* cells obtained in the case of the control sample were significant ($p < 0.0001$) comparing to the number of these microorganisms obtained for coatings containing mixtures M1, M2 and M3. To summarise, none of the analysed coatings with active mixtures M1, M2, M3 showed a bacteriolytic or even bacteriostatic effect on the Gram-negative bacteria described here.

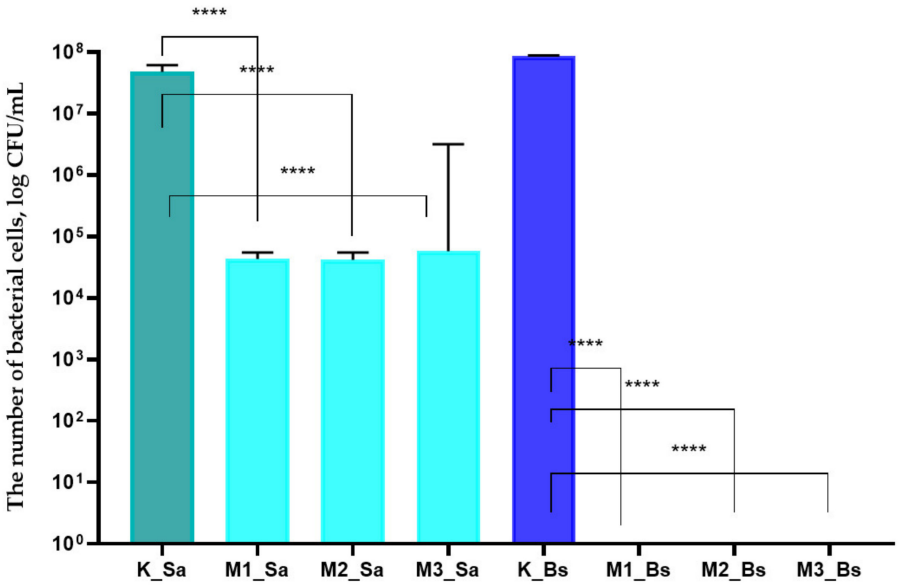

Figure 6. The influence of coatings on *S. aureus* and *B. subtilis* growth. K_Sa—PE film—control sample (Sa—incubated with *S. aureus*); M1_Sa—PE film covered with the MHPC coating containing 25% of mixture M1 as active compound; M2_Sa—PE film covered with the MHPC coating containing 25% of mixture M2 as active compound; M3_Sa—PE film covered with the MHPC coating containing 25% of mixture M3 as active compound; K_Bs—PE film—control sample (Bs—incubated with *B. subtilis*); M1_Bs—PE film covered with the MHPC coating containing 25% of mixture M1 as active compound; M2_Bs—PE film covered with the MHPC coating containing 25% of mixture M2 as active compound; M3_Bs—PE film covered with the MHPC coating containing 25% of mixture M3 as active compound. One-way ANOVA: ****—$p < 0.0001$.

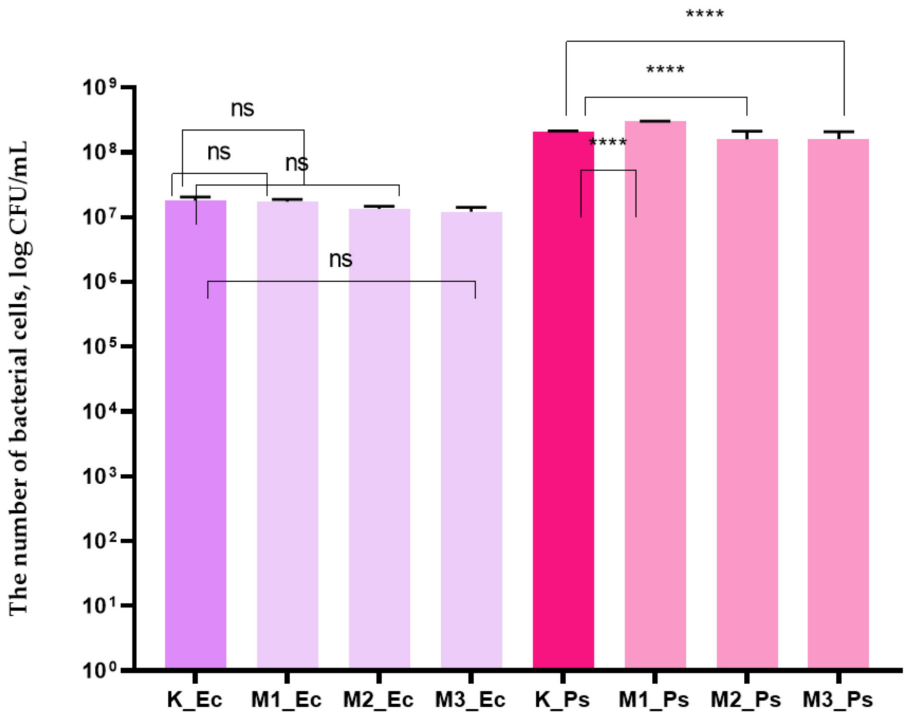

Figure 7. The influence of coatings on *E. coli* and *P. syringae* growth. One-way ANOVA: ns—not significant; ****—$p < 0.0001$.

3.4. Antiviral Analysis of Coatings

With an evaluation of the growth rate in real time, the OD of the Gram-negative *P. syringae* over time, cultivated with bacteriophage phi6 particles after its incubation with covered and uncovered polyethylene films was performed to determine the antiviral activity of the layers containing the M1, M2 and M3 mixtures as antiviral agents. Figure 8 indicates the OD of the *P. syringae* cultivation at 28 °C over time. An analysis of the OD of the host microorganism cultivated with the bacterial virus particles over time (after the incubation of the phi6 phage with the uncoated PE film squares as control samples) was carried out and an OD fall was noticed after 12 h of phi6 bacterial virus cultivation with the rod-shaped *P. syringae*. The OD fall confirmed that phi6 phage inhibited most of the host cells. This clearly demonstrates that the control sample, which was uncovered polyethylene did not have any effect on ph6 bacteriophages. The results that were obtained confirmed by an additional test which was performed according to a modified ISO 22196-2011 standard. This experiment showed (Table 8) that the number of bacterial virus particles after their incubation with the PE film control squares was 3.57×10^{-8} [PFU/mL].

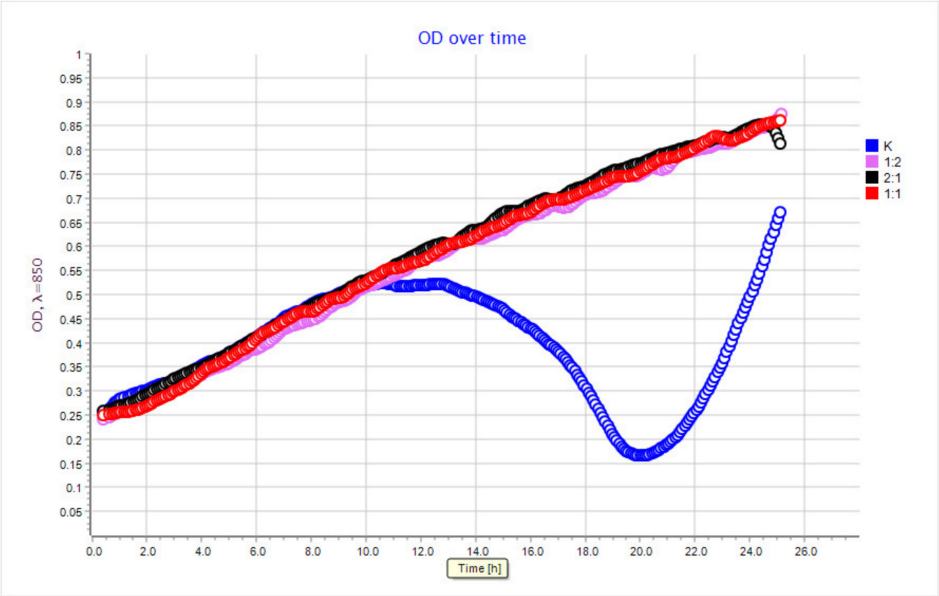

Figure 8. OD over time for host—*P. syringe* cells incubated with phi 6 bacteriophage particles after their incubation with the coatings (with M1, M2 and M3 mixtures of extracts). Phages particles were added when OD = 0.2, amount of phage MOI = 1.

Table 8. The influence of coated, active films on bacteriophage titer.

The Number of phi6 Phage Particles [PFU/mL]			
K	M1	M2	M3
$3.2 \times 10^{-9} \pm 4.64 \times 10^{-8}$	0 ****	0 ****	0 ****

One-way ANOVA: ****—$p < 0.0001$.

The results of the study clearly show (Figure 8) that the incubation of phi6 phage particles with polyethylene films coated with layers containing M1, M2, M3 mixtures inactivated the phi6 particles completely. An analysis of the OD of the host cells over time which were cultivated with phi 6 particles (after their incubation with PE foil squares coated with coatings containing the antiviral agents, respectively: I-III separately) was performed and an OD fall was not observed after 24 h of bacteriophage cultivation with the *P. syringae* strain (Figure 8). These results confirmed that phi6 phage particles did not inhibit any *P. syringae* cell growth. These results were clear evidence that the phi6 particles were not active after their incubation with coatings containing M1, M2 and M3 as active

compounds. The antiviral effect of all of the developed coatings/layers were confirmed using a modified ISO 22196-2011 standard. As emphasized in Table 8, the number of bacterial virus particles after incubation with each active coating was found to be zero, meaning that phi 6 did not decrease the number of *P. syringae* cells. To summarise, it can be assumed that coatings contained M1, M2 and M3 mixtures had very a high antiviral effect on SARS-CoV-2 surrogate particles.

3.5. FTIR Analysis of Extracts

The results of the study demonstrated that there were six regions viewed in the FT-IR spectroscopy, extending over (1) ranges from 3600 to 2800 cm^{-1}; (2) ranges from 2400 to 2000 cm^{-1}; (3) ranges from 1800 to 1500 cm^{-1}; (4) ranges from 1500 to 1200 cm^{-1}; (5) ranges from 1100 to 1000 cm^{-1}; and (6) ranges from 950 to 650 cm^{-1}. A peak consistent with the presence of O–H single bonds can be noted at 3347 cm^{-1}, confirming the presence of polyphenols/phenols in both extracts. Additionally, peaks at 2980 and 2929 cm^{-1} can be consistently observed, which correspond to an aliphatic C–H induced absorption (stretching vibration of aliphatic C–H in CH_3 groups). An asymmetric O–H-induced absorption at 1247, 1293 and 1360 cm^{-1} can be observed for glycyrrhizin, baicalin and saponin. A 1635 cm^{-1}–1611 cm^{-1} band was also observed for glycyrrhizin, baicalin and saponins. Absorptions at 1584, 1492 and 1468 cm^{-1} (C–C stretching vibrations from aromatic rings) were assigned to flavones. Peaks from 945 to a 684 cm^{-1} were observed only for *S. baicalensis* extract (characteristic vibrations for aromatic bonds and C–Cl stretching vibrations). These peaks were attributed to the presence of flavones, chloro-flavones and flavonols. The FTIR analysis thus confirmed that the analysed extracts contained the active compounds described above. Differences between the compositions of both extracts were noted. The spectral analysis also showed changes in absorbance intensity: an increase of the band indicating single O–H bonds (3347 cm^{-1}), as well as C=O stretching bonds (1611–1635 cm^{-1}) (Figure 9).

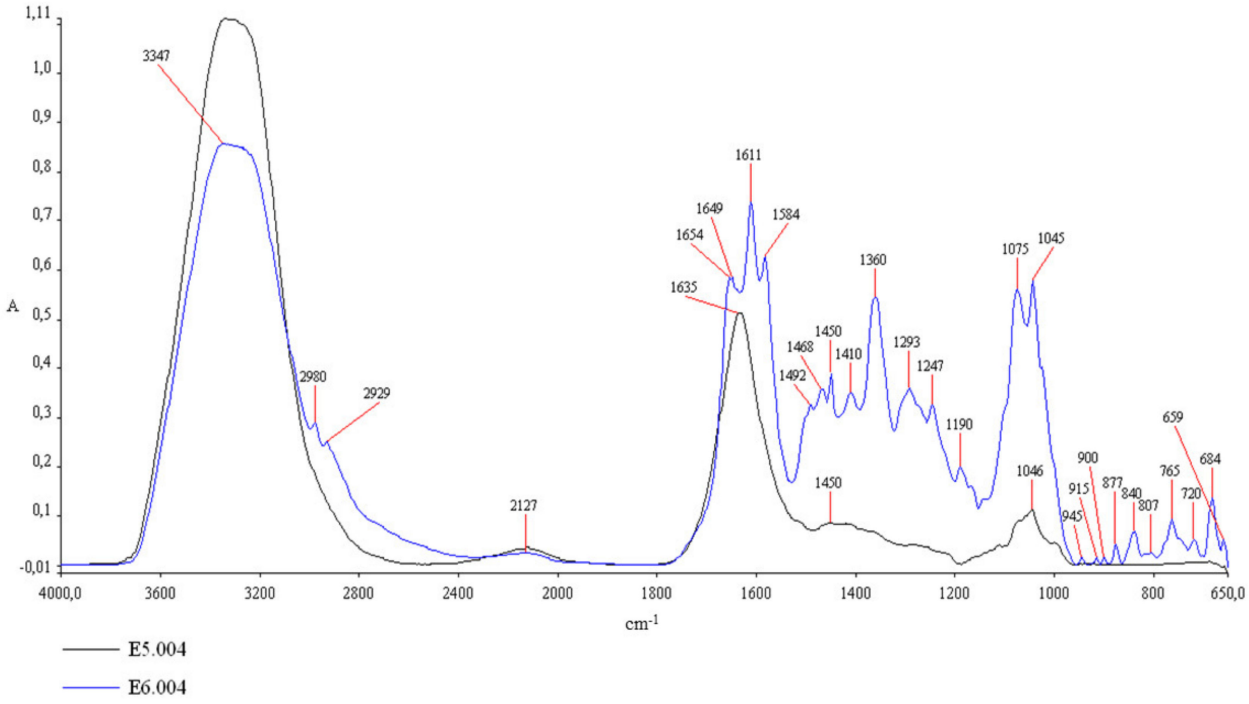

Figure 9. The FT-IR spectra of *Glycyrrhiza* L. extract (E5.004) and *S. baicalensis* extract (E6 004).

4. Discussion

The spread of SARS-CoV-2 has changed the world. Many retailers are focused on ensuring consumer safety and e-commerce was found to be an excellent replacement to traditional shopping, increasing the demand for disposable polymer packaging [4,5]. Additionally, the shelf life of food products has begun to be of vital importance. Polymer films used for disposable bags in food packaging production have to be improved [4,5,8]. Packaging producers and scientists have sought to extend the properties of packaging films, focusing especially on antimicrobial activity, to protect the products inside the packaging and prevent contamination by human hands on the outside. The development of packaging covered with active coatings has led to a search for new active compounds which could be introduced into coating carriers. Plant extracts are rich in active substances which may inhibit bacterial growth and inactivate selected virus particles. Plants and herbs used in traditional medicine are of special interest as they might also be useful as active compounds in functional coatings. Zhao et al. [21] confirmed that the active compounds of *S. baicalensis* exhibited very significant antibacterial activity against a range of Gram-positive and Gram-negative bacteria (*S. Typhimurium, E. coli, and B. coagulans, S. aureus, P. aeruginosa, E. faecalis, B. subtilis, B. cereus, B. megaterium, P. fluorescens, Sarcina lutea, S. paratyphi, S. typhi, Shigella boydii and S. dysenteriae*). Interestingly, El-Saber Batiha et al. [38] demonstrated that *G. glabra* extracts showed antibacterial properties toward *B. subtilis*, *B. cereus*, *B. megaterium*, *E. coli*, *S. aureus*, *Enterococcus faecalis*, *P. fluorescens*, *P. aeruginosa*, *Sarcina lutea*, *Salmonella paratyphi*, *S. typhi*, *Shigella boydii*, *S. dysenteriae*, *Vibrio parahaemolyticus* and *V. mimicus*. The results of this study indicated that as little as 10% of *Glycyrrhiza* L. and *S. baicalensis* extracts showed bacteriolitic activity against *S. aureus*. The presence of flavonoids, their flavonols, chalcones, tenaxin, dihydroflavones and their dihydroflavonols, viscudulin and biflavonoids [18–22] in *S. baicalensis* extracts and the presence of glabridin, glabrene and licochalcone from *G. glabra* extracts [28] could lead to the complete inhibition of bacterial growth. The most likely mechanism of the antimicrobial action of these substances is efflux pump inhibition or an increase in bacterial cell wall permeability, which are related to a bacteriolytic type/mechanism. Among the active compounds present in mixtures of *Glycyrrhiza* L. and *S. baicalensis* extracts, terpenes were found to be the most abundant antimicrobial forms, followed by polyphenols [39]. The results obtained in our study were confirmed by several authors [21,40] who showed that *S. baicalensis* extracts inhibited the growth of various microorganisms. The effectiveness of these two extracts against *E. coli, P. syringae* and *B. subtilis* were found to be weaker than their activity against *S. aureus*. The results of this study demonstrated that *Verbascum* L. extracts did not have any effect on Gram-positive and Gram-negative bacteria. Similar results were found by Marian et al. [41]. Contrary results were obtained by the authors of this work showing that *Verbascum* L. extracts exhibited good antibacterial activity against *S. aureus*, *S. epidermidis, E. faecalis* [42], L. *monocytogenes*, *B. anthracis*, *B. cereus, S. Typhimurium* [16], *S. pyogenes*, and *E. coli* [43]. The results of this research showed that *Astragalus* extracts had moderate antibacterial activity. Kanaan et al. [44] demonstrated that these plant extracts exhibited a bacteriostatic effect against *E. coli, P. aeruginosa* and pathogenic *S. epidermidis,* strains. Albayrak [11] tested the sensitivity of pathogenic and not pathogenic strains: *Aeromonas hydrophila, B. cereus*, *B. subtilis*, *E. coli*, *Klebsiella pneumoniae*, *Listeria monocytogenes*, *Mycobacterium smegmatis* RUT, *Morganella morganii*, *Proteus mirabilis, P. aeruginosa, S. Typhimurium*, *S. aureus* and *Yersinia enterocolitica on Astragalus* taxa extracts. We concluded that the extracts demonstrated no antibacterial activity, except against *P. aureginosa*. El-Sebakhy [12] demonstrated the weak activity of *Astralagus* extracts against *S. aureus, B. subtilis, K. pneumoniae.* The results of this research demonstrated that mixtures M1, M2, M3 had a bacteriolytic effect on Gram-positive and Gram-negative bacteria. Our findings proved that there was a synergistic effect between *Glycyrrhiza* L. and *S. baicalensis* extracts, as mixtures of these two extracts were more active than pure extracts. A synergistic effect was also noted in a previous study where it was found that the addition of a rosemary extract to the coating carrier containing pomegranate and raspberry seed extracts had an impact on an increase in the

coatings antimicrobial effectiveness [2]. Cho [45] underlined that synergy needs to be determined, not predicted, because it is easy to determine but difficult to predict. The synergistic effect of the active compounds may be quantified using the Chou-Talalay method [45], it may be also confirmed by the experiments. Based on these results, it was assumed that the mixtures of the most active plant extracts would be more effective against bacteria than a single plant extract. The results of this study showed that the coatings containing M1, M2 and M3 mixtures as active compounds did not inhibit the growth of *S. aureus*, but they had a bacteriolytic effect on *B. subtilis* cells and a bacteriostatic effect on the *S. aureus* strain. These coatings were not active against *E. coli* and *P. syringae* cells. Disturbance in protein and bacterial DNA metabolism by terpenes from the extract mixtures (as active additives in coatings) could have caused bacteriostatic activity [39]. Bacteriophage Phi6 is a dsRNA bacterial virus which is commonly used as a surrogate for tests of enveloped RNA viruses, such as SARS coronaviruses. These phages are very similar to SARS-CoV-2, with spike proteins and enveloped by a lipid membrane, even being similar in size (~80–100 nm) [46]. Due to these similarities it could be concluded that compounds that are effective against bacteriophage phi6 will be also effective against SARS-CoV-2. When considering the potential action mechanisms of active compounds on virus particles, they may be distinguished as either proteins, nucleic acids, (membrane proteins or capsid proteins) and an envelope membrane. Active compounds, such as flavonoids carry several phenolic OH groups. It is known that OH groups can form hydrogen and ion bonds with the outer groups of proteins, such as positively charged amino groups. When these active substances are incubated with viruses, these compounds bind to viral proteins in the envelope which very often prevents the virus from docking to host cells. These active additives can be antiviral substances. It should be also mentioned that these active additives have antiviral effect against free viral particles, but not, or to a lesser degree, when a virus has already entered the host cell [47]. The results of this research showed that active coatings containing M1, M2 and M3 extract mixtures inactivated the phi 6 phage particles completely and confirmed that the antiviral effect of the PE squares covered with an active layer was very high. It may be assumed that glycyrrhizin, baicalein, wogonin, flavonoids and their flavonols, dihydroflavones and their dihydroflavonols, chalcones, tenaxin, viscudulin, and biflavonoids from mixtures of extracts of *Glycyrrhiza* L. and *S. baicalensis* inactivated bacteriophage particles. It may be concluded that coatings containing M1, M2, M3 active additives, that inactivate surrogate particles, would be also active against eucariotic SARS-CoV-2. Comparing coatings with mixtures of extracts of *Glycyrrhiza* L. and *S. baicalensis* with those described in previous studies [2,4], it could be suggested that MHPC coatings containing M1, M2 and M3 mixture as active compounds in coatings offered similar antiviral activity to the coatings containing CO_2 extracts of rosemary, pomegranate seeds, raspberry seeds and their mixtures [2]. The coatings obtained in this work offered greater antiviral effect than the MHPC coatings containing ZnO nanoparticles, geraniol and carvacrol, despite the observed synergistic effect of nano ZnO, geraniol and carvacrol [4]. To summarize, the coatings described here had antimicrobial activity against Gram-positive bacteria. They could be used as internal coatings to protect food against Gram-positive microorganisms which may be responsible for food spoilage. In addition, packaging materials covered with all of active coatings/layers (containing M1, M2 and M3 mixtures) described here (as external coatings) may protect products from consumers when handling them and limit the spread of SARS-CoV-2 and pathogenic, Gram-positive bacteria transferred via human contact during the pandemic, especially when hands are often disinfected.

Food products have a high nutritional value and contain beneficial amounts of lipids, proteins and vitamins. However, many food products are often considered to be difficult to store because they are prone to spoilage from improper handling or incorrect storage. Spoilage of food is primarily caused by microbial growth which leads to unacceptable product quality. Shelf life of food is affected by several factors such as initial microbial contamination, storage temperature, and packaging conditions. Preservation of the high

quality of food products is significantly important. The preservation methods studied in the 20th century sought to preserve food products and extend their shelf life by the use of safe chemical preservatives such as potassium sorbate. Active packaging materials covered with the active coatings containing natural, plant extracts as active compounds are an innovative approach to maintain or prolong the shelf-life of food products while ensuring their quality, safety, and integrity.

5. Conclusions

Mixtures of *Glycyrrhiza* L. and *S. baicalensis* extracts (M1, M2, M3) had a bacteriolytic effect on Gram-positive and Gram-negative bacteria. The active coatings containing M1, M2 and M3 mixtures as active compounds did not inhibit the growth of *S. aureus*, but they had a bacteriolytic effect on *B. subtilis* cells and a bacteriostatic effect on the *S. aureus* strain. These coatings were not active against *E. coli* and *P. syringae* cells. They also inactivated the phi 6 bacteriophage particles completely, which confirmed that the antiviral effect of the PE foiln squares covered with an active layer was very high. To summarise, the coatings described in this research offered antimicrobial activity against Gram-positive bacteria. They could be used as internal coatings to protect food against typical Gram-positive microorganisms responsible for food spoilage. In addition, the packaging materials covered with all of active coatings/layers (containing M1, M2 and M3 mixtures), as external coatings may protect the hands of consumers from cross infection. They may also limit the spread of SARS-CoV-2 and pathogenic, Gram-positive bacteria transferred via humans' hands during the pandemic, especially where hands disinfection is often required.

Summarizing, the PE films covered with the active coatings will be used as packaging material and the storage tests using selected food product will be carried out. Additionally, the antiviral activity of the external coating after storage will be analysed.

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References

1. Sultan, M.S.; Elsayed, A.; El-Amir, Y.A. In Vitro Effect of Plant Parts Extract of *Senecio glaucus* L. on Pathogenic Bacteria, Biointerface. *Res. Appl. Chem.* **2021**, *12*, 3800–3810.
2. Ordon, M.; Nawrotek, P.; Stachurska, X.; Mizielińska, M. Polyethylene films coated with antibacterial and antiviral layers based on CO_2 extracts of raspberry seeds, of pomegranate seeds and of rosemary. *Coatings* **2021**, *11*, 1179. [CrossRef]
3. Nguyen, P.H.; De Tran, V.; Pham, D.T.; Tran Dao, T.N.P.; Dewey, R.S. Use of and attitudes towards herbal medicine during the COVID-19 pandemic: A cross-sectional study in Vietnam. *Eur. J. Integr. Med.* **2021**, *44*, 101328. [CrossRef]
4. Mizielińska, M.; Nawrotek, P.; Stachurska, X.; Ordon, M.; Bartkowiak, P. Packaging covered with antiviral and antibacterial coatings based on ZnO nanoparticles, geraniol and carvacrol. *Int. J. Mol. Sci.* **2021**, *22*, 1717. [CrossRef]
5. Mizielińska, M.; Kowalska, U.; Jarosz, M.; Sumińska, P. A comparison of the effects of packaging containing nano ZnO or polylysine on the microbial purity and texture of cod (*Gadus morhua*) fillets. *Nanomaterials* **2018**, *8*, 158. [CrossRef]
6. Zhang, W.; Jiang, H.; Rhim, J.W.; Cao, J.; Jiang, W. Effective strategies of sustained release and retention enhancement of essential oils in active food packaging films/coatings. *Food Chem.* **2021**, *367*, 130671. [CrossRef] [PubMed]
7. Akbar, A.; Anal, A.K. Zinc oxide nanoparticles loaded active packaging, a challenge study against *Salmonella typhimurium* and *Staphylococcus aureus* in readyto-eat poultry meat. *Food Control* **2014**, *38*, 88–95. [CrossRef]

8. Mizielińska, M.; Lisiecki, S.; Jotko, M.; Chodzyńska, I.; Bartkowiak, A. The antimicrobial properties of polylactide films covered with ZnO nanoparticles-containing layers. *Przem. Chem.* **2015**, *94*, 1000–1003.
9. Noshirvani, N.; Ghanbarzadeh, B.; Mokarram, R.R.; Hashemi, M. Novel active packaging based on carboxymethyl cellulose-chitosan-ZnO NPs nanocomposite for increasing the shelf life of bread. *Food Packag. Shelf Life* **2017**, *11*, 106–114. [CrossRef]
10. Prussin, A.J., II; Schwake, D.O.; Lin, K.; Gallagher, D.L.; Buttling, L.; Marr, L.C. Survival of the Enveloped Virus Phi6 in Droplets as a Function of Relative Humidity, Absolute Humidity, and Temperature. *Appl. Environ. Microbiol.* **2018**, *84*, 1–10. [CrossRef] [PubMed]
11. Albayrak, S.; Onur Kaya, O. Antioxidant and antimicrobial activities of four *Astragalus* species growing wild in Turkey. *Turk. J. Biochem.* **2018**, *43*, 1–10. [CrossRef]
12. El-Sebakhy, N.A.; Asaad, A.M.; Abdallah, R.M.; Toaima, S.M.; Abdel-kader, M.S.; Stermi-lzt, F.R. Antimicrobial isoflavans from *Astragalus* species. *Phytochemistry* **1994**, *36*, 1387–1389. [CrossRef]
13. Herrera, D.R.; Durand-Ramirez, J.E.; Falcão, A.; Nogueira da Silva, E.J.L.; dos Santos, E.B.; Figueiredo de Almeida Gomes, B.P. Antimicrobial activity and substantivity of *Uncaria tomentosa* in infected root, canal dentin. *Braz. Oral Res.* **2016**, *30*, 61–66. [CrossRef]
14. Cahuana-Vasquez, R.A.; Soléo Ferreira dos Santos, S.; Koga-Ito, C.Y.; Cardoso Jorge, A.O. Antimicrobial activity of *Uncaria tomentosa* against oral human pathogens. *Braz. Oral. Res.* **2007**, *46*, 46–50. [CrossRef] [PubMed]
15. Frezza, C.; Bianco, A.; Serafini, M.; Foddai, S.; Salustri, M.; Reverberi, M.; Gelardi, L.; Bonina, A.; Bonina, F.P. HPLC and NMR analysis of the phenyl-ethanoid glycosides pattern of *Verbascum thapsus* L. cultivated in the Etnean area. *Nat. Prod. Res.* **2019**, *33*, 1310–1316. [CrossRef] [PubMed]
16. Nofouzi, K.; Mahmudi, R.; Tahapour, K.; Ebrahim Amini, E.; Yousefi, K. *Verbascum speciosum* Methanolic Extract: Phytochemical Components and Antibacterial Properties. *J. Essent. Oil. Bear. Plants* **2016**, *19*, 499–505. [CrossRef]
17. Sofrenić, I.; Anđelković, B.; Todorović, N.; Stanojković, T.; Vujisić, L.; Novaković, M.; Milosavljević, S.; Tešević, V. Cytotoxic triterpenoids and triterpene sugar esters from the medicinal mushroom *Fomitopsis betulina*. *Phytochemistry* **2021**, *181*, 112580. [CrossRef] [PubMed]
18. Vrabec, R.; Vokurková, D.; Tůmová, L.; Cheel, J. Ex-vivo Immune-stimulating Activity of *Scutellaria baicalensis* and Its Major Flavonoids on Human Immune Cells. *Rec. Nat. Prod.* **2021**, *16*, 188–193.
19. Yurievna Stepanova, A.; Ivanovna Solov'eva, A.; Victorovna Malunova, M.; Andreevna Salamaikina, S.; Mikhailovich Panov, Y.; Aleksandrovich Lelishentsev, A. Hairy Roots of *Scutellaria* spp. (*Lamiaceae*) as Promising Producers of Antiviral Flavones. *Molecules* **2021**, *26*, 3927. [CrossRef]
20. Liu, G.; Jinyu, M.; Chen, Y.; Tian, Q.; Shen, Y.; Wang, X.; Chen, B.; Yao, S. Investigation of flavonoid profile of *Scutellaria bacalensis* Georgi by high performance liquid chromatography with diode array detection and electrospray ion trap mass spectrometry. *J. Chromatogr. A* **2009**, *1216*, 4809–4814. [CrossRef]
21. Zhao, T.; Tang, H.; Xie, L.; Zheng, Y.; Ma, Z.; Sun, Q.; Li, X. *Scutellaria baicalensis* Georgi. (*Lamiaceae*): A review of its traditional uses, botany, phytochemistry, pharmacology and toxicology. *J. Pharm. Pharm. Sci.* **2019**, *71*, 1353–1369. [CrossRef]
22. Makino, T.; Hishida, A.; Goda, Y.; Mizukami, H. Comparison of the major flavonoid content of *S. baicalensis*, *S. lateriflora*, and their commercial products. *J. Nat. Med.* **2008**, *62*, 294–299. [CrossRef] [PubMed]
23. Błach-Olszewska, Z.; Jatczak, B.; Rak, A.; Lorenc, L.; Gulanowski, B.; Drobna, A.; Lamer-Zarawska, E. Production of Cytokines and Stimulation of Resistance to Viral Infection in Human Leukocytes by *Scutellaria baicalensis* Flavones. *J. Interf. Cytok. Res.* **2008**, *28*, 571–582. [CrossRef] [PubMed]
24. Chu, M.; Lan Xu, L.; Zhang, M.; Chu, Z.; Wang, Y. Role of Baicalin in Anti-Influenza Virus A as a Potent Inducer of IFN-Gamma. *Biomed. Res. Int.* **2015**, *263630*, 1–11. [CrossRef]
25. Li, A.; Zhao, Z.; Zhang, S.; Zhang, Z.; Shi, Y. Fungicidal Activity and Mechanism of Action of Glabridin from *Glycyrrhiza glabra* L. *Int. J. Mol. Sci.* **2021**, *22*, 10966. [CrossRef] [PubMed]
26. Fiore, C.; Eisenhut, M.; Krausse, R.; Ragazzi, E.; Pellati, D.; Armanini, D.; Bielenberg, J. Antiviral Effects of *Glycyrrhiza* species. *Phytother. Res.* **2008**, *22*, 141–148. [CrossRef]
27. Asilbekova, D.T.; Bobakulov, K.M.; Sotimov, G.B.; Sagdullaev, S.S. Lipids and Essential oils From Roots of *Glycyrrhiza glabra* growing in Uzbekistan. *Chem. Nat. Compd.* **2021**, *57*, 2380241. [CrossRef]
28. Asi, M.N.; Hosseinzadeh, H. Review of Pharmacological Effects of *Glycyrrhiza* sp. and its Bioactive Compounds. *Phytother. Res.* **2008**, *22*, 709–724.
29. Kubra, I.R.; Kumar, D.; Rao, L.J.M. Effect of microwave-assisted extraction on the release of polyphenols from ginger (*Zingiber officinale*). *Int. J. Food Sci. Tech.* **2013**, *48*, 1828–1833. [CrossRef]
30. Mandal, V.; Mohan, Y.; Hemalatha, S. Microwave Assisted Extraction—An Innovative and Promising Extraction Tool for Medicinal Plant Research. *Pharmacogn. Rev.* **2007**, *1*, 1–12.
31. Salachna, P.; Grzeszczuk, M.; Meller, E.; Mizielińska, M. Effects of Gellan Oligosaccharide and NaCl Stress on Growth, Photosynthetic Pigments, Mineral Composition, Antioxidant Capacity and Antimicrobial Activity in Red Perilla. *Molecules* **2019**, *24*, 3925. [CrossRef]
32. Salachna, P.; Byczyńska, A.; Zawadzińska, A.; Piechocki, R.; Mizielińska, M. Stimulatory Effect of Silver Nanoparticles on the Growth and Flowering of Potted Oriental Lilies. *Agronomy* **2019**, *9*, 610. [CrossRef]

33. Salachna, P.; Mizielińska, M.; Płoszaj-Witkowska, B.; Jaszczak, A. Zinc Oxide Nanoparticles Enhanced Biomass and Zinc Content and Induced Changes in Biological Properties of Red *Perilla Frutescens*. *Materials* **2021**, *14*, 6182. [CrossRef]
34. *ASTME 2180-01 Standard Test Method for Determining the Activity of Incorporated Antimicrobial Agent(s) in Polymeric or Hydrophobic Materials*; American Society for Testing and Materials: West Conshohocken, PA, USA, 2001.
35. Bhetwal, A.; Maharjan, A.; Shakya, S.; Satyal, D.; Ghimire, S.; Khanal, P.R.; Parajuli, N.P. Isolation of Potential Phages against Multidrug-Resistant Bacterial Isolates: Promising Agents in the Rivers of Kathmandu, Nepal. *Hindawi BioMed Res. Int.* **2017**, *2017*, 1–11. [CrossRef] [PubMed]
36. Bonilla, N.; Rojas, M.J.; Cruz, G.N.F.; Hung, S.H.; Rohwer, F.; Barr, J.J. Phage on tap—A quick and efficient protocol for the preparation of bacteriophage laboratory stocks. *PeerJ* **2016**, *4*, e2261. [CrossRef]
37. ISO 22196-2011: Measurement of Antibacterial Activity on Plastics and Other Non-Porous Surfaces. Available online: https://www.iso.org/standard/54431.html (accessed on 3 August 2011).
38. El-Saber Batiha, G.; Beshbishy, A.M.; El-Mleeh, A.; Abdel-Daim, M.M.; Devkota, H.P. Traditional Uses, Bioactive Chemical Constituents, and Pharmacological and Toxicological Activities of *Glycyrrhiza glabra* L. (*Fabaceae*). *Biomolecules* **2020**, *10*, 352. [CrossRef]
39. Alvarez-Martínez, F.J.; Barraj'on-Catal'an, E.; Herranz-L'opez, M.; Micol, V. Antibacterial plant compounds, extracts and essential oils: An updated review on their effects and putative mechanisms of action. *Phytomedicine* **2021**, *90*, 153626. [CrossRef] [PubMed]
40. Do, H.T.T.; Nguyen, T.H.; Nghiem, T.D.; Nguyen, H.T.; Choi, G.J.; Ho, C.T.; Dang, Q.L. Phytochemical constituents and extracts of the roots of *Scutellaria baicalensis* exhibit in vitro and in vivo control efficacy against various phytopathogenic microorganisms. *S. Afr. J. Bot.* **2021**, *142*, 1–11. [CrossRef]
41. Marian, E.; Vicas, L.G.; Jurca, T.; Muresan, M.; Pallag, A.M.; Stan, R.L.; Sevastre, B.; Diaconeasa, Z.; Ionescu, C.M.L.; Hagan, A.C. *Salivia Officinalis* L. and *Verbascum Phlomoides* L. Chemical, Antimicrobial, Antioxidant and Antitumor Investigations. *Rev. Chim.* **2018**, *69*, 365–370. [CrossRef]
42. Sen-Utsukarci, B.; Dosler, S.; Taskin, T.; Abudayyak, M.; Ozhan, G.; Mat, A. An evaluation of antioxidant, antimicrobial, antibiofilm and cytotoxic activities of five *Verbascum* species in turkey. *Farmacia* **2018**, *66*, 1014–1020. [CrossRef]
43. Ghasemi, F.; Rezaei, F.; Araghi, A.; Tabari, M.A. Antimicrobial Activity of Aqueous-Alcoholic Extracts and the Essential Oil of *Verbascum thapsus* L. *Jundishapur J. Nat. Pharm. Prod.* **2015**, *10*, 23004.
44. Kanaan, H.; El-Mestrah, M.; Sweidan, A.; As-Sadi, F.; Bazzal, A.A.; Chokr, A. Screening for antibacterial and antibiofilm activities in *Astragalus angulosus*. *J. Intercult. Ethnopharmacol.* **2017**, *6*, 50–57. [CrossRef] [PubMed]
45. Chou, T.C. Drug Combination Studies and Their Synergy Quantification Using the Chou-Talalay Method. *Cancer Res.* **2010**, *70*, 440–446. [CrossRef]
46. Fedorenko, A.; Grinberg, M.; Orevi, T.; Kashtan, N. Survival of the enveloped bacteriophage Phi6 (a surrogate for SARS-CoV-2) in evaporated saliva microdroplets deposited on glass surfaces. *Sci. Rep.-UK* **2020**, *10*, 22419. [CrossRef]
47. Wink, M. Potential of DNA Intercalating Alkaloids and Other Plant Secondary Metabolites against SARS-CoV-2 Causing COVID-19. *Diversity* **2020**, *12*, 175. [CrossRef]