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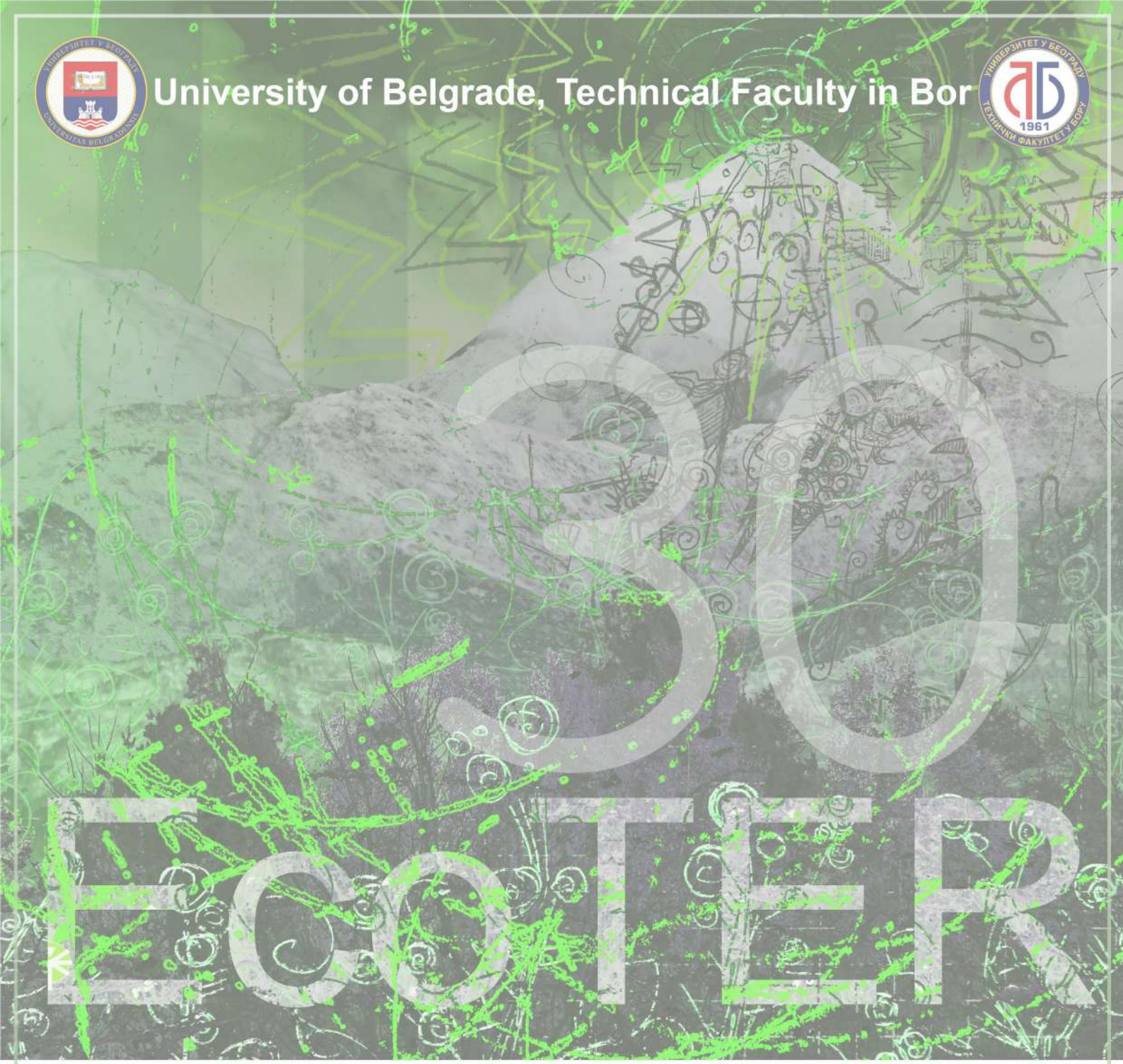

University of Belgrade, Technical Faculty in Bor

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THE EXTRACTION OF ACTIVE COMPOUNDS FROM PLANT WASTE: THE POTENTIAL IN HUMAN AND INDUSTRIAL APPLICATIONS AS THE CONCEPT OF ZERO WASTE IN THE CIRCULAR ECONOMY

Aleksandra A. Jovanović[1*]

[1]University of Belgrade, Institute for the Application of Nuclear Energy INEP, Banatska 31b, 11080 Zemun Belgrade, SERBIA

[*]*ajovanovic@inep.co.rs*

Abstract

Plant waste contains various active compounds that can be applied in food, pharmaceutical, cosmetic, or other industries. Polyphenols, carotenoids, pectin, aromas, fibers, or enzymes extracted from agri-food waste can be considered ready-to-use without demanding and expensive downstream isolation and purification steps. In the present study, plant extracts were prepared using Thymus serpyllum and Vaccinium myrtillus leaf dust and empty Aloe vera leaves (the matrix without aloe gel). The biological potential of T. serpyllum, V. myrtillus, and A. vera waste extracts with the highest polyphenol yield was investigated. T. serpyllum extract possessed the highest DPPH radical scavenging capacity (1.50±0.02 mg/mL). It also had antimicrobial potential against all examined strains, particularly against Enterococcus faecalis, and showed spasmolytic activities in isolated rat ileum models of spontaneous contractions, acetylcholine- and potassium chloride-induced contractions. V. myrtillus extract showed the highest ABTS radical scavenging potential (48.77±1.47 µmol Trolox equivalents/g of plant material) and significant antimicrobial and skin regeneration activity (percentage of wound healing was 29.2±1.8). However, A. vera extract had the lowest antioxidant capacity and did not show antimicrobial potential, while it showed a significant wound healing influence (30.9±1.7%). Due to showed the biological potential of all prepared waste extracts, they can be potentially used in the food, pharmaceutical, and cosmetic industries. In future experiments, T. serpyllum waste extract will be examined in terms of bronchodilatory activity, whereas all prepared waste extracts will be investigated via effects on enzymes, dying of textile, as well as anticorrosive effects.

Keywords: biological activities, extraction, industrial potential, plant waste.

INTRODUCTION

Plant waste or by-products possess plenty of bioactive compounds that may be applied in various food, functional food, pharmaceutical, or cosmetic formulations. According to Panić *et al*. [1], polyphenol extracts of grape and olive pomace (as food by-products) can be considered ready-to-use in pharmaceutical, food, and cosmetic industries without demanding and expensive processes of isolation and purification. Additionally, distillation liquid residues of several aromatic plants are used for the extraction of antioxidant and antimicrobial agents, including phenolic acids [2]. Due to the establishment of modern extraction techniques, dietary fibers from vegetable and fruit waste, such as onion layers, potato peels, cauliflower stems and florets, carrot and tomato pomace, apple, mango, orange, and peach peel, as well as polyphenol compounds from citrus, pear, apple, peach, and pomegranate peels, can be

successfully extracted and used [2,3]. Tea factories produce a higher amount of waste, including microfined tea dust, tea seeds, winnowings, and floor sweepings. Since the quantity of by-products generated from teas and aromatic plants manufacturing processes shows a significant increase with annual increment of their production over the world, the use of medicinal plants' waste as a low-cost material in different branches of industry has aroused more and more interest for recycling and reuse purposes. Tea dust represents particles lower than 0.5 mm which according to Regulations on the quality of tea, herbal tea, and their products of the Republic of Serbia cannot be an integral part of the tea products for the market, trade, and sale [4]. Examples of such plant waste are *Thymus serpyllum* and *Vaccinium myrtillus* leaf waste. Empty *Aloe vera* leaves (the matrix without aloe gel) also represent plant waste that is rich in carbohydrates, amino acids, lipids, organic acids, chromones, flavonoids, anthraquinones, minerals, vitamins, pigments, and volatile organic components [5].

The next step after the collection of plant waste is the extraction of the target compounds, i.e. the optimization of the extraction process *via* varying solvent types, solid-to-solvent ratio, extraction time, and technique. Furthermore, in recent studies, different methods for the extraction of bioactive compounds were established [6–8]. The extraction techniques vary in nature of the plant matrix, extraction medium, solvent-to-solid ratio, time, temperature, pressure, and pH. Considering that polyphenols, as the most biologically active plant metabolites, are various in structure, it is not simple to establish a standardized extraction process that would extract the majority of polyphenols from each plant matrix. However, traditional extraction procedures possess several disadvantages, such as low extraction yield, long extraction time, a large amount of plant material, high solvent consumption, and negative environmental impact. Hence, in recent time, application of the modern extraction methods have been evaluated, including heat-assisted extraction. Novel procedures provide numerous benefits, including solvent saving, shorter time of extraction, and high extract quality. Additionally, modern techniques support the concept of "green" solvent, which is aimed to minimize the negative environmental impact of the utilization of large amounts of solvents in the extraction process. It has also shown that simple alcohols (e.g. ethanol), as well as alcohol-water mixtures, are more environmentally favorable solvents [8].

Preparation of the extracts with the highest yield of target compounds under the optimized extraction conditions is further followed by their physicochemical characterization and investigation of the biological activities, including antioxidant, antimicrobial, enzyme inhibition, spasmolytic, skin regeneration potential (depending on the presence of the active compounds of the plants).

In this paper, the biological potential of *T. serpyllum*, *V. myrtillus*, and *A. vera* waste extracts with the highest polyphenol yield was represented.

MATERIALS AND METHODS

Plant materials and reagents

T. serpyllum and *V. myrtillus* leaf waste was herbal dust, the particle size of 0.3 mm or lower resulting from the grinding of the initial plant material in the Institute for Medicinal Plants Research “Dr Josif Pančić”, Pančevo, Serbia. *A. vera* was purchased in ASC Garden

d.o.o., Belgrade, Serbia. The aloe gel was removed from the leaves. Subsequently, clean and empty leaves that represent the waste were cut and freeze-dried in Beta 2-8 LD plus (Christ, Germany). Ethanol (Merck, Germany), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) or ABTS, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid or Trolox, and 2,2-diphenyl-1-picrylhydrazyl or DPPH (Sigma-Aldrich, Germany), dimethyl sulfoxide or DMSO, triphenyltetrazolium chloride or TTC, amoxicillin, fluconazole, sodium dodecyl sulfate, high-glucose Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), and thiazolyl blue tetrazolium bromide or MTT (Sigma-Aldrich, USA).

Extraction procedure

Heat-assisted extraction from all types of plant waste for (*T. serpyllum*, *V. myrtillus*, and *A. vera*) was performed at 80°C using the incubator shaker KS 4000i control (IKA, Germany) and 50% ethanol at a solid-to-solvent ratio of 1:30 g/mL for 30 min. The extracts were prepared in the Erlenmeyer flasks covered by aluminum foil to avoid light exposure and evaporation of the solvent. After the extraction, the sample was filtered using filter paper and stored at 4°C until further experiments.

Lyophilization

In order to obtain dried extracts for antimicrobial, skin regeneration, and spasmolytic potential analyses, liquid extracts were lyophilized. The ethanol from the extracts was evaporated using Heizbad Hei-VAP (Heidolph, Germany) at 40–50°C, a pressure of 50 mbar, and a rotation speed of 150 rpm. Subsequently, the sample was frozen in the freezer, at -80°C for 1 h and freeze-dried at -75°C and pressure of 0.011 mbar for 24 h and at -65°C and pressure of 0.054 mbar for one additional hour (Alpha 2-4 LSCplus, Christ, Germany).

Examination of the biological potential of the extracts

Antioxidant activity

The ABTS assay was based on the procedure described by Li *et al*. [9] with a slight modification and the absorbance was measured at 734 nm. The antioxidant activity was expressed as mmol Trolox equivalent per g of plant material (μmol TE/g). The DPPH assay was based on the procedure described by Xi and Jan [10] with a slight modification and the absorbance was measured at 517 nm. The results were expressed as IC_{50} (mg/mL), defined as the concentration of the extract required to scavenge 50% of DPPH free radicals.

All spectrophotometric measurements were performed in a UV-1800 spectrophotometer (Shimadzu, Japan).

Antimicrobial activity

The minimum inhibitory (MIC) and minimum bactericidal or fungicidal concentrations (MBC or MFC) of the extracts were determined by broth micro-dilution assay. Antimicrobial capacity was examined against *Bacillus cereus*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enterica*, and *Candida albicans*. The antimicrobial assay was performed using sterile 96-well microliter plates. The extract was dissolved in 5% DMSO aqueous solution, while 0.0075% TTC was used as a growth indicator. Positive growth control was a 5% DMSO in an appropriate medium. Plates with bacteria were incubated at 37°C for 24 h, whereas plates with fungus were incubated at 32°C for 48 h. The lowest

concentration of extract without any visible growth of microbial strains was considered as MIC value. MBC and MFC were determined by serial sub-cultivation of the samples taken from each well that showed no change in color into microplates containing the appropriate medium. The lowest concentration without any visible growth after repeated incubation was taken as MBC or MFC. Amoxicillin and fluconazole were used as positive control for bacterial and fungal strains, respectively. The antimicrobial analysis was done in triplicate and the highest value was taken as MIC and MBC/MFC, thus the results are not shown as average values of several measurements with standard deviation, but a "stricter criteria" rule was applied, common in antimicrobial assays.

Spasmolytic activity

The examination of the spasmolytic activity of *T. serpyllum* extract was performed using isolated rat ileum and three experimental models, including spontaneous contractions, acetylcholine- and potassium chloride-induced contractions. All experimental procedures were on eighteen male Wistar albino rats. The segments of the ileum (2 cm) were mounted in 10 mL of Tyrode's solution in the organ bath (37°C, pH 7.4, a mixture of 5% carbon dioxide and 95% oxygen), between two stainless steel hooks with continuous air-bubbling. Six segments of isolated rat ileum were tested in each experimental model. The change in intestinal activity was determined using transducer TSZ-04-E and analyzed with a SPEL Advanced ISOSYS Data Acquisition System. The extract and control compounds were added directly to the organ bath; the area under the curve was estimated. First, the influence of extract on spontaneous contractions of isolated rat ileum was examined. After the stabilization period, the segment of the ileum was exposed to the extract, whereas papaverine was used as a positive control. The spasmolytic activity of the sample was expressed as a percentage of the control contractility without extract. Additionally, the increasing concentrations of acetylcholine were added to the organ bath cumulatively in order to obtain the maximum contractile response curve. Further, the acetylcholine induced-contractions were registered in the presence of extract, whereas atropine was used as a positive control. In the third experimental model, the rat ileum contractions were induced using 80 mM KCl solution, while verapamil was used as a positive control; the extract was cumulatively added to the organ bath. The relaxation of the ileum segment in the presence of extract or antagonist was expressed as a percentage of the maximum contractile response induced by acetylcholine or KCl. After each experimental model, the intestinal preparation was flushed with fresh Tyrode's solution and left to adapt for 10 min.

Skin regeneration activity (cell viability and wound-scratch healing assays)

The viability of spontaneously immortalized keratinocyte cells (HaCaT) in the presence of *V. myrtillus* and *A. vera* waste extracts was assessed using the MTT assay. The cells (10^4/well) were seeded in 96-well plates in 100 μL of the complete medium and allowed to adhere overnight at 37°C in a 5% CO_2 incubator. After 24 h of incubation, cells were rinsed with warm, sterile phosphate-buffered saline (PBS) and further cultured in a complete medium in the absence and presence of the extract. The cells were incubated for 24 h at 37°C. and the medium was removed and replaced with 100 μL of fresh complete medium containing MTT and incubated for 3 h at 37°C. Subsequently, 100 μL of 10% sodium dodecyl sulfate was added to each well and the plate was further incubated at 37°C overnight. The absorbance

was measured at 570 nm using a microplate reader. The results were expressed as IC_{50} value, indicating 50% of cell viability when compared with untreated control. Namely, the criterion used to categorize the cytotoxicity of preparations in HaCaT cell line was as follows: IC_{50}≤20 µg/mL=highly cytotoxic, IC_{50} 21–200 µg/mL=moderately cytotoxic, IC_{50} 201–400 µg/mL=weakly cytotoxic, and IC_{50}>401µg/mL=no cytotoxicity.

The HaCaT cells were grown to confluency. The cell monolayer was scraped using a 200 µL tip. The floating cells were washed and grown in DMEM with 1% FBS, 2 mM L-glutamine, 1% antibiotic-antimycotic, and 250 g/mL or IC_{25} concentrations of the extracts. Cell migration was studied after 24 h. Non-treated cells were used as a control. The percentages of wound closure during the extract exposure were utilized to present the results.

Statistical analysis

The statistical analysis (except for the results from the antimicrobial assay) was done by using analysis of variance (one-way ANOVA) and Duncan's *post hoc* test in STATISTICA 7.0. The differences were considered statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

According to the literature data, oxidative stress induced by free radicals has an important role in the development of various chronic diseases, cancer, degenerative neuronal damage, diabetes mellitus, and coronary heart disease [11,12]. Reactive oxygen species (ROS) are a class of unstable chemical compounds that are produced in all cells during normal physiological and biochemical reactions. Excessive free radical generation can induce cellular and tissue damage by nonspecific alteration and disruption of lipids, proteins, and nucleic acids [13]. Therefore, the antioxidant capacity of *T. serpyllum*, *V. myrtillus*, and *A. vera* waste extracts was examined using two antioxidant assays (ABTS and DPPH methods). The results of the ABTS and DPPH radical scavenging capacity of all prepared extracts are shown in Figures 1a and 1b, respectively.

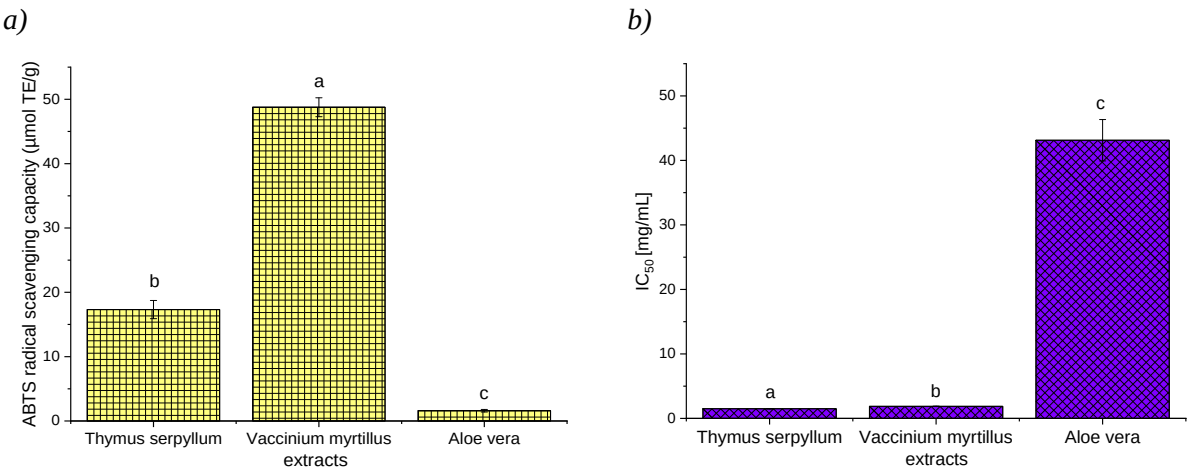

Figure 1 *a) ABTS and b) DPPH radical scavenging activity of Thymus serpyllum, Vaccinium myrtillus, and Aloe vera waste extracts; TE, Trolox equivalent, IC_{50}, concentration of the extract requires to scavenge 50% of free DPPH radicals; values with different letters (a-b) showed statistically significant differences ($p<0.05$; n=3; analysis of variance, Duncan's post-hoc test)*

According to the results from Figure 1a, it can be noticed a statistically significant difference in ABTS radical scavenging potential between *T. serpyllum* and *V. myrtillus* extracts (17.32±1.41 and 48.77±1.47 μmol TE/g, respectively), while *A. vera* extract showed significantly lower antioxidant activity (1.6±1.47 μmol TE/g).

As can be seen from Figure 1b, *A. vera* extract showed the highest IC_{50} value, i.e. the lowest DPPH radical scavenging capacity (43.13±3.21 mg/mL). *T. serpyllum* and *V. myrtillus* extracts had significantly lower IC_{50} values (1.50±0.02 and 1.87±0.03 mg/mL, respectively). However, *T. serpyllum* extract possessed statistically significantly higher DPPH antioxidant potential.

The presented results of ABTS antioxidant potential did not follow the trend of the results obtained in DPPH assays, which can be explained by the fact that various plant secondary metabolites and their synergism significantly influence the overall antioxidant activity of herbal extracts [14]. The mentioned phenomenon can be also explained by the fact that free radical scavenging potential depends on the polyphenol concentration in the extracts and their chemical structure as well. Furthermore, the presence of synergistic or antagonistic reactions among flavonoids should be taken into consideration for the overall antioxidant capacity of the extracts [15,16]. Differences between the antioxidant activity of *T. serpyllum* and *V. myrtillus* extracts can be explained by different reactivity of free radicals, as well as different mechanisms of the reactions. Hence, ABTS free radicals are more reactive compared to DPPH radicals. Additionally, DPPH radicals have a role in the transfer of hydrogen atoms, whereas ABTS radicals interact *via* electron transfer. Thus, the higher reactivity of ABTS radicals resulted in the high radical scavenging potential of *V. myrtillus* extract, while *T. serpyllum* extract possessed the highest DPPH antioxidant potential, i.e. the lowest IC_{50} value. Jovin *et al*. [17] have reported that flavonoid derivates in herbal extracts provide better biological effects. According to the literature data, the extracts with a higher concentration of flavonoid compounds possessed a higher DPPH radical scavenging ability [18] which can be the case with *T. serpyllum* extract. Hirano *et al*. study [19] also showed that the neutralization of DPPH radicals can be due to the reducing ability of flavonoids.

The investigation of antimicrobial properties of the extracts against five bacterial strains and one fungal strain was done and the results are presented in Table 1 (except for *A. vera* samples). *T. serpyllum* and *V. myrtillus* extracts have shown antibacterial activity against all investigated bacterial stains, while *A. vera* extract did not have antibacterial potential. *T. serpyllum* and *V. myrtillus* extracts were the most effective in inhibiting the growth of *E. faecalis* (MIC of 0.313 mg/mL). *V. myrtillus* extract exhibited significant activity against *B. cereus* with a MIC value of 0.625 mg/mL, whereas MIC was higher for *T. serpyllum* extract (1.25 mg/mL). MIC value against *S. aureus* was the same for both extracts and amounted to 1.25 mg/mL). Both extracts showed moderate activity against *E. coli* (MIC of 5 mg/mL). *V. myrtillus* extract inhibited growth of *S. enterica* with MIC of 2.5 mg/mL, while *T. serpyllum* extract exerted the same effect at higher concentrations, 5 mg/mL. According to the results of antifungal potential, both extracts showed inhibition of *C. albicans* strains in relatively high concentration, MIC of 20 mg/mL.

Table 1 *Antimicrobial activity of Thymus serpyllum and Vaccinium myrtillus waste extracts expressed as minimum inhibitory (MIC, mg/mL) and minimum bactericidal or fungicidal concentration (MBC or MFC, mg/mL)*

Microbial strain	*Thymus serpyllum* extract		*Vaccinium myrtillus* extract		Amoxicillin/fluconazole	
	MIC [mg/mL]	MBC/MFC [mg/mL]	MIC [mg/mL]	MBC/MFC [mg/mL]	MIC [mg/mL]	MBC/MFC [mg/mL]
Bacillus cereus	1.25	2.5	0.625	2.5	5.42	21.68
Enterococcus faecalis	0.313	5	0.313	5	0.34	2.71
Staphylococcus aureus	1.25	10	1.25	10	0.17	1.36
Escherichia coli	5	10	5	10	5.42	21.68
Salmonella enterica	5	5	2.5	5	2.71	5.42
Candida albicans	20	/	20	/	12.5	50

Besides growth inhibitory capacity, *T. serpyllum* and *V. myrtillus* extracts also exerted bactericidal potential at a relatively low concentration. The strongest bactericidal capacity of both extracts was observed against *B. cereus* (MBC value of 2.5 mg/mL). Furthermore, both extracts exhibited the same bactericidal potential against *E. faecalis* and *S. enterica* (MBC of 5 mg/mL), whereas bactericidal capacity against *S. aureus* and *E. coli* has been at the higher tested concentration, 10 mg/mL. Both extracts did not show fungicidal activity even at the highest tested concentration. According to the literature data, aromatic plant extracts showed strong antibacterial activity against *S. aureus* and only moderate activity against *E. coli* [20,21]. Several studies have shown that the antibacterial capacity of plant extracts can be attributed to the presence of various types of components, including phenolic acids, flavonoids, and tannins, which possess hydroxyl groups and the ability to form hydrogen bonds with water molecules in bacterial cell [20–22]. Polyphenol compounds can also coagulate the proteins in bacteria cells destroying enzymes involved in bacterial metabolism [20]. However, other compounds apart from polyphenols can be also responsible for the overall antimicrobial potential of plant extract. Due to the increasing incidence of resistant bacteria, including methicilin-resistant *S. aureus* and multi-resistant *E. coli* and *E. faecalis*, in food and clinical settings, these findings can be valuable. Therefore, this research opens the door for medicinal plant extracts to be considered natural, safe, and effective food, cosmetic, and pharmaceutical preservatives.

T. serpyllum plant has traditionally been used in various gastrointestinal disorders, thus the spasmolytic potential of the extract was tested in a model of isolated rat ileum. The results of the spasmolytic activity of *T. serpyllum* extract in spontaneous contractions, acetylcholine- and potassium chloride-induced contractions are shown in Table 2.

Table 2 *Spasmolytic activity of Thymus serpyllum waste extract on spontaneous contractions and acetylcholine- and potassium chloride-induced contractions of the isolated rat ileum*

Samples	Spontaneous contractions	Acetylcholine-induced contractions	Potassium chloride-induced contractions
	EC_{50} [µg/mL]	EC_{50} [nM]	EC_{50} [µg/mL]
Thymus serpyllum extract	$9.16 \cdot 10^3$	$1.25 \cdot 10^3$	$9.17 \cdot 10^3$
Papaverine	0.06	/	/
Atropine	/	28	/
Verapamile	/	/	0.94

T. serpyllum extract significantly inhibited the spontaneous contractions of isolated rat ileum. The EC50 value was $9.16 \cdot 10^3$ mg/mL (Table 2). Papaverine, as a positive control, showed an EC50 value of 0.06 µg/mL (Table 2) due to the inhibition of phosphodiesterase and calcium influx [23]. With the aim to investigate the possible mechanisms of the spasmolytic ability of the tested extract, the contractions of isolated rat ileum were induced by acetylcholine. The extract showed a significant reduction of acetylcholine-induced contractions (Table 2). The extract caused a change in the value of the EC_{50} of acetylcholine from 0.25 nM (in the absence of the extract) to $1.25 \cdot 10^3$ nM (in the presence of the extract).

Acetylcholine causes gastrointestinal smooth muscle contractions by the stimulation of muscarinic receptors, through two different mechanisms: 1) the stimulation of M_2 muscarinic receptors and activation of cationic channels through pertussis toxin-sensitive G proteins, causing membrane depolarization and the influx of Ca^{2+} ions [24], and 2) the activation of M_3 muscarinic receptors and induction of the G_q protein transduction signal, resulting in the activation of phospholipase C, formation of inositol trisphosphate and diacylglycerol, and increment of intracellular calcium concentration, membrane depolarization, and intestinal musculature contraction [25]. The inhibitory effect of the extract on acetylcholine-induced contractions was significantly lower in comparison to atropine, as a non-selective muscarinic receptor antagonist. Additionally, after rinsing, the contractility of the isolated ileum was the same as at the beginning. Thus, the spasmolytic ability of the extract can be attributed to the reversible blockade of muscarinic receptors.

Potassium chloride causes depolarization and tonic contraction of isolated rat ileum by inducing membrane depolarization and opening of voltage-dependent Ca^{2+} channels [23]. The extract has induced significantly the reduction of the potassium chloride-induced contractions with EC_{50} value of $9.17 \cdot 10^3$ µg/mL (Table 2). Verapamil, as an antagonist of Ca^{2+} influx through calcium channels, has shown a significant activity on potassium chloride-induced contractions with an EC_{50} value of 0.94 µg/mL (Table 2). According to Gilani *et al*. study [26], herbal extracts inhibit potassium chloride-induced contractions acting as antagonists of the influx of Ca^{2+} ions. Since the extract possessed a significant effect on smooth muscle relaxation of isolated rat ileum in the presence of a high concentration of K^+ ions, it can be concluded that its spasmolytic activity is related to Ca^{2+} channel blockade.

Since *V. myrtillus* and *A. vera* plants possess skin regeneration potential, both extracts were subjected to cytotoxicity and scratch wound healing assays. As can be seen from Table 3, none of the extracts had an unfavorable effect on the cell line's growth rate. The results

revealed that the extracts were not cytotoxic and their IC_{50} values were larger than 400 μg/mL. The presented results indicate the lack of toxicity of both extracts against skin cells (no harmful impact on HaCaT cells was detected) suggesting their potential use in pharmaceutical and cosmetic industries.

Table 3 *Cytotoxic and scratch wound healing activity of Vaccinium myrtillus and Aloe vera waste extracts on immortalized keratinocyte cells*

Samples	IC_{50} [μg/mL]	Wound healing [%]
Vaccinium myrtillus extract	>400	29.2±1.8[a*]
Aloe vera extract	>400	30.9±1.7[a]
Control	>400	0.10

[*]values with the same letter showed no statistically significant differences (p<0.05; n=3; analysis of variance, Duncan's *post-hoc* test).

Wound healing represents an important component of the skin's defensive and protective activities, thus *V. myrtillus* and *A. vera* waste extracts were included in the wound healing experiment and results are presented in Table 3. When both extracts were applied to immortalized keratinocyte cells with scratched wound gaps, it led to a significant improvement in the wound gap closure, compared to the control. Both extracts exhibited similar effectiveness in helping wound closure (30.9±1.7 and 29.2±1.8%). Wound healing varies in its duration and contains four sequentially overlapped phases, including homeostasis, inflammation, proliferation, and remodeling [27]. The results presented in Table 1 confirmed that *V. myrtillus* and *A. vera* extracts can stimulate keratinocyte growth and migration. Flavonoids and terpenoids are known to aid wound healing due to their antioxidant and antibacterial properties. According to Tsuchiya *et al*. [28], the mentioned abilities can be responsible for wound contraction and an increased rate of epithelialization. Therefore, these findings indicate that both extracts can help in skin wound healing.

CONCLUSION

The presented study investigated the biological potential of *T. serpyllum*, *V. myrtillus*, and *A. vera* waste extracts. *T. serpyllum* extract possessed the lowest IC_{50} value in DPPH radical scavenging assay, i.e. the highest antioxidant capacity, as well as antibacterial, bactericidal, and antifungal potential in the case of all tested bacteria and fungus. Additionally, the mentioned extract significantly inhibited spontaneous contractions and acetylcholine- and potassium chloride-induced contractions. *V. myrtillus* extract showed the highest ABTS radical scavenging capacity and the same antimicrobial activity as *T. serpyllum* extract. Furthermore, *V. myrtillus* extract had no harmful influence on HaCaT cells and exerted a significant wound-healing effect. On the other hand, *A. vera* extract possessed the lowest antioxidant activity in ABTS and DPPH assays and did not show antimicrobial potential against tested strains. However, *A. vera* extract also had no harmful effect on HaCaT cell lines and showed a significant wound-healing effect. In future experiments, *T. serpyllum* waste extract will be examined in terms of bronchodilatory activity, whereas all prepared waste

extracts will be investigated *via* .effects on enzymes, dying of textile, as well as anticorrosive effects.

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