



UDC: 615.322:582.783:615.35

PHYTOCHEMICAL AND ANTIMICROBIAL STUDIES OF AN INTRODUCED *AMPELOPSIS BREVIPEDUNCULATA* (MAXIM.) TRAUTV.

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Yavorska, H., & Vorobets, N. (2026). Phytochemical and antimicrobial studies of an introduced *Ampelopsis brevipedunculata* (Maxim.) Trautv. *Studia Biologica*, 20(1), 83–102. doi:[10.30970/sbi.2001.867](https://doi.org/10.30970/sbi.2001.867)

Background. *Ampelopsis brevipedunculata* (Maxim.) Trautv (AB) is recognized for its potential in the treatment of hepatotoxic conditions, atopic dermatitis, and skin inflammation, which may be due to the synergism of certain bioactive compounds. The aim was to evaluate the ability of AB leaf extracts as antimicrobial agents against *Escherichia coli*, *Bacillus subtilis*, *Candida parapsilosis*, and to improve the understanding of the influence of polyphenols, flavonoids, hydroxycinnamic acids, carotenoids, and chlorophylls in their composition on antimicrobial activity.

Methods. The AB leaf aqueous ethanol (AE) extracts were obtained using the reflux condition and maceration methods. Spectrophotometric analysis was used for the calculation of phenolic compounds, flavonoids, and hydroxycinnamic acids total content, chlorophyll, and carotenoids content in the extracts. The antimicrobial activity of the extracts of AB (ABEs) were assessed by the agar diffusion method.

Results. Distilled water, 20 %, 60 % AE, and 96 % E provided high extraction of polyphenols by both methods, and 96 % E for carotenoids and chlorophylls. *E. coli* and *C. parapsilosis* were not sensitive to the aqueous extract and the extract prepared with 20 % AE by maceration. At the same time, ABEs prepared with 60 % AE and 96 % E had a certain inhibitory effect on *E. coli* and *C. parapsilosis*. *B. subtilis* was inhibited by the aqueous extract. Correlation analysis confirmed the relationships between the studied biological active substances and the diameter of the growth inhibition zones of the tested cultures. Two-factor analysis of variance confirmed the reliability of the proportions of the influence on the zones of inhibition of the studied cultures in the control and ABEs when using the maceration method, as well as ABEs obtained when using 20 % AE.



Conclusion. ABEs are a rich and diverse source of biochemicals that have inhibitory effects against *E. coli*, *B. subtilis*, and *C. parapsilosis*. ABEs can be used as a rich source of phenols, flavonoids, hydroxycinnamic acids, chlorophylls and carotenoids for further research, and use as antimicrobial agents.

Keywords: *Ampelopsis brevipedunculata*, green extracts, antimicrobial activity, biologically active substances

INTRODUCTION

Acclimatization of new plant species in Ukraine contributes to an increase in the biodiversity of the flora. Among the introduced plants are edible, decorative, and medicinal ones. At the same time, under favorable climatic conditions and the absence of natural enemies, there is a possibility of an introduced species “spreading too much” and acquiring the status of invasive. Therefore, it is important to approach the introduction of new species with caution, as well as to study the possibilities of using introduced plants for certain economic purposes, for example, as medicinal raw plant materials. *Ampelopsis brevipedunculata* (Maxim.) Trautv. (AB) is a deciduous liana, widely distributed in Asia, as well as in the eastern part of North America, Mexico, and Guatemala, introduced in the botanical gardens of Ukraine. In the regions of natural distribution of AB its roots, stem, leaves, and fruits have long been used as a folk remedy with anti-inflammatory, antioxidant, and antibacterial properties (Yabe *et al.*, 1998; Wu *et al.*, 2004; Choi *et al.*, 2019).

Most of the scientific studies on the screening of the action of extracts and compounds from AB carried out on model animal objects, cell-free and cell culture systems have revealed their potential therapeutic effects. Evaluation of the antihepatotoxic effect of the water-ethanol extract of the fruits of AB, which was carried out in mice, indicates inhibition of the progression of liver damage caused by a non-lethal dose of carbon tetrachloride (Yabe & Matsui, 2000). Studies demonstrated strong reducing power and scavenging effects of hydroxyl radicals and 2,2-diphenyl-1-picrylhydrazyl free radicals by the extract, while pretreatment or co-treatment of HepG2 cells with the extract of *A. brevipedunculata* can significantly protect cells from H₂O₂-induced oxidative stress (Wu *et al.*, 2004).

Ethanol extract of AB in an *in vivo* rat fibrosis model significantly inhibited the activation and altered the morphology of hepatic stellate cells; thus it is regarded as a promising therapeutic agent for the treatment of hepatofibrosis (Yum *et al.*, 2017).

Research has revealed the ability of ABE to prevent bone loss by inhibiting osteoclastogenesis *in vitro* and *in vivo* (Kim *et al.*, 2014). Data of Choi *et al.* (2019) demonstrated the pharmacological role and signaling mechanism of ABE in the regulation of skin allergic inflammation, supporting the suggestion that it could be developed as a potential therapeutic agent for the treatment of atopic dermatitis (Choi *et al.*, 2019). Oral administration of an ethanolic extract of AB rhizomes improves atopic skin dermatitis, which could be due to the synergism of individual bioactive compounds with potential anti-inflammatory and anti-allergic activity (Choi *et al.*, 2019). It is believed that ABE could be a candidate material that can be used to enhance skin immunity, health, and beauty (Bak *et al.*, 2023).

Our previous studies and other authors have shown that AB has excellent antibacterial activity against a number of strains (Kundaković *et al.*, 2008a; Kundaković *et al.*, 2008b).

Among the 17 compounds in the extract of AB, which has a protective effect against several inflammatory diseases, betulin has a significant activity (Jang *et al.*, 2018). The anti-Alzheimer effect of ABE is due to its content of flavonoid glycosides, which was confirmed both *in vivo* and *in vitro* (Rashed *et al.*, 2015).

A number of studies have reported that plant-based biologically active substances (BAS), particularly phenolic compounds, are associated with antimicrobial properties (Martillanes *et al.*, 2017; Teodoro *et al.*, 2015; Liu *et al.*, 2025). The search for antibacterial and antifungal agents remains an urgent task, in particular, from plant raw materials. The aim of this study was to determine the relationship between the concentrations of phenolic compounds, chlorophylls, and carotenoids in prepared green extracts of AB leaves and the antimicrobial activity of these extracts.

MATERIAL AND METHODS

The material for our study was AB leaves collected in the flowering phase.

Sample collection: The shoots of AB were obtained from Dr. Maria Skybitska, who introduced *Ampelopsis brevipedunculata* (Maxim.) Trautv. in The Ivan Franko National University of Lviv Botanical Garden. The leaves were separated from the stem, dried in the shade, and stored in airtight boxes until use.

Sample preparation: Before preparing the extracts, the dry raw material was ground with an electric mill (for grinding coffee beans) from Bosch to obtain powder. The work used “green extraction”, that is, solvents (extractants) were used – water and aqueous ethanol (AE) of various concentrations. AE extracts of the leaves were prepared with 20 %, 60 % AE and 96 % E by the reflux method at 60–80 °C for 30 min and by the maceration method – infusion in the dark at 25 °C for 14 days. Extraction was carried out in accordance with the requirements of the State Pharmacopoeia of Ukraine: (ratio of sample: extractant = 1:20 (mass, g/volume, mL). After cooling or infusing, each extract was filtered through a paper filter. The ABEs thus obtained were used in the experiment. Exposure to sunlight was avoided to prevent the loss of active components.

The total polyphenol content (TPC) in ABEs was determined according to the method with Folin-Ciocalteu reagent as described by K. K. Chew *et al.* (2011) and H. V. Yavorska *et al.* (2023). To measure the extinction, a ULAB 102UV (measuring range 190–1100 nm) was used and measured at a wavelength of 650 nm. A control sample was prepared identically by replacing 1 mL of extract with 1 mL of bidistilled water.

The total flavonoid content (TFC) of the ABEs was determined according to the method described by H. V. Yavorska *et al.* (2023).

The content of hydroxycinnamic acids (HCA) was determined by the spectrophotometric method (Koyro *et al.*, 2009). The content of the sum of HCA (X) in percent (%) was calculated in terms of caffeic or chlorogenic acid and dry raw materials accordingly.

Chlorophylls (Chl) and carotenoids (Car) were extracted with 96 % E. The optical density of the ABEs was determined at wavelengths corresponding to the absorption maxima of chlorophylls *a* and *b* and Car. Calculations of the Chl content in the extract were carried out according to the formula proposed by Wintermans and de Mots (1965).

The total carotenoid content in the ABEs prepared with 96 % E was determined by the method described in Natividad and Rafael (2014).

Bacteria: *Escherichia coli* (ATCC 25922), *Bacillus subtilis* (VKM B-408), and yeast *Candida parapsilosis* (ATCC 22019 = UKM Y-73τ) were used as test cultures obtained

from the culture museum of the Department of Microbiology of the Ivan Franko National University of Lviv. Test cultures of bacteria were grown on trypsin-soy agar (TSA), and yeast on glucose-peptone agar (Sabureau medium) for 24 and 48 hours, respectively, in a thermostat at a temperature of 28 ± 1 °C. Ciprofloxacin, 0.3 % (1 drop/well) was used as a control for bacterial cultures, and fluconazole (150 mg) – for fungi.

The antimicrobial and antifungal effect of the ABEs was detected by diffusion into a dense medium, in which 0.2 mL of aqueous and aqueous-ethanolic extract from the wells diffused into agar. Approximately 20 mL of nutrient medium (TSA or Sabureau agar) was poured into sterile Petri dishes. After the medium solidified, the Petri dishes were inoculated with prepared thick suspensions standardized to 0.5 McFarland; 4–5 wells with a diameter of 6 mm were cut with a flamed drill, maximally spaced from each other. The dishes with ABEs in the wells were placed in a thermostat at a temperature of 28 ± 1 °C for one-two days, respectively (Gudz *et al.*, 2014).

After cultivation, the diameter of the zone of inhibition (ZI) was measured with a ruler. Extractants (20 %, 60 %, 96 % aqueous ethyl alcohol) were used as controls.

To evaluate antimicrobial activity, the criteria according to G. Cappelli & F. Mariani (2021) were used.

Statistical analysis of the results was performed using the Microsoft Office Excel 2010 software package. The statistical program Jamovi 2.3.21 was used for correlation analysis of the obtained data. The scales were consistent with the normal distribution. To test the hypothesis of the relationship between the diameter of the ZI and the content of TPC, TFC, HCA, Chl, and Car, Pearson's linear pairwise correlation was used. Two-Way ANOVA of Excel software was used to determine the share of influence of ABEs and cultures and extracts and extraction methods. The experiments were conducted in five replicates.

RESULTS

Extracts were prepared from AB leaf powders using maceration and reflux methods with AE concentrations of 20 %, 60 %, and 96 % in the extraction solvent. An aqueous extract was also prepared using the reflux method.

Total phenolic, flavonoid, hydroxycinnamic acid, chlorophyll and carotenoid contents in ABEs. The TPC of the leaf ABEs of the studied AB was determined by the Folin–Ciocalteu method. Despite the solubility of phenolic compounds in water and aqueous ethanol of various concentrations, the content of polyphenols in AB leaf extracts varied within quite wide limits (**Table 1**). AB leaf extracts obtained by maceration with 60 % AE and 96 % E showed approximately equally high TPC.

The results of the study showed that the highest concentration of total polyphenols was found in extracts prepared with 60 % AE and 96 % E. That is, 60 % AE and 96 % E are effective solvents for the extraction of phenolic compounds from AB leaves.

In general, distilled water, 20 %, 60 % AE, and 96 % E as extraction solvents, provided high extraction of polyphenols by the maceration and reflux methods, and they were used further.

The TFC of the samples was estimated by the flavonoid-aluminum chloride conjugation method. **Table 2** reports the results of TFC analyses of the AB leaf extracts. The estimation of TFC in different samples revealed values from 11.27 ± 0.82 to 22.33 ± 1.21 mg·g⁻¹ DW in quercetin equivalent, and 10.55 ± 0.52 to 28.76 ± 0.53 mg·g⁻¹ DW in quercetin equivalent for the maceration and reflux method, respectively.

Table 1. Total polyphenol content in *Ampelopsis brevipedunculata* leaf extracts prepared using different extractants and methods of extraction ($M \pm \sigma$, $n = 5$)

Method of the extraction	Extractant	mg·g ⁻¹ DW in gallic acid equivalent
Maceration	20 % AE	11.70 ± 0.04
	60 % AE	17.96 ± 0.70
	96 % E	19.04 ± 1.09
Reflux	Distilled H ₂ O	9.60 ± 0.01
	20 % AE	7.25 ± 0.01
	60 % AE	11.57 ± 0.38
	96 % E	9.38 ± 0.03

Note: AE – aqueous ethanol; E – ethanol; DW – dry weight

Table 2. Total flavonoid content in *Ampelopsis brevipedunculata* leaf extracts prepared using different extractants and methods of extraction ($M \pm \sigma$, $n = 5$)

Method of the extraction	Extractant	mg·g ⁻¹ DW in quercetin equivalent
Maceration	20 % AE	11.84 ± 0.76
	60 % AE	11.27 ± 0.82
	96 % E	22.33 ± 1.21
Reflux	Distilled H ₂ O	10.55 ± 0.52
	20 % AE	28.76 ± 0.53
	60 % AE	16.55 ± 1.83
	96 % E	16.73 ± 1.30

Note: AE – aqueous ethanol; E – ethanol; DW – dry weight

The values of the TFC differ by 30–170 % depending on the extraction method and extractant.

HCA are the most abundant phenolic compounds in plants. Their content in AB leaves is given in **Table 3**.

Table 3. Total hydroxycinnamic acid contents of *Ampelopsis brevipedunculata* leaf extracts prepared using different extractants and types of extraction, ($M \pm \sigma$, $n = 5$)

Method of the extraction	Extractant	% in terms of DW in equivalent to the	
		chlorogenic acid	caffeic acid
Maceration	20 % AE	8.63 ± 0.83	11.59 ± 0.40
	60 % AE	14.08 ± 1.18	20.76 ± 1.80
	96 % E	12.18 ± 0.64	17.20 ± 0.12
Reflux	H ₂ O	9.17 ± 0.49	5.94 ± 0.10
	20 % AE	12.37 ± 0.28	18.45 ± 0.49
	60 % AE	18.61 ± 1.25	24.29 ± 2.72
	96 % E	15.87 ± 0.72	23.38 ± 0.81

Note: AE – aqueous ethanol; E – ethanol; DW – dry weight

Overall, the HCA content was lower in the aqueous extract compared to the AE ones. The content of HCA in extracts made with 60 % AE was the highest, regardless of the extraction method and the method of recalculation.

Table 4 presents the quantitative content of photosynthetic pigments (Chl *a*, Chl *b*, and Car) in the ethanol extract of AB leaves. The results of total Car content in ABEs were recorded in the range of 101.29 to 374.54 $\mu\text{g}\cdot\text{g}^{-1}$ DW depending on the extraction method used.

Table 4. Total content of photosynthetic pigments in ethanol extract of *Ampelopsis brevipedunculata* leaves using two extraction methods, ($M \pm \sigma$, $n = 5$)

Method of the extraction	Chlorophyll <i>a</i> , $\text{mg}\cdot\text{g}^{-1}$ DW	Chlorophyll <i>b</i> , $\text{mg}\cdot\text{g}^{-1}$ DW	Carotenoids, $\mu\text{g}\cdot\text{g}^{-1}$ DW
Maceration	9.92 ± 0.07	2.03 ± 0.06	374.54 ± 0.23
Reflux	1.13 ± 0.01	0.91 ± 0.02	101.49 ± 0.35

Note: DW – dry weight

The maceration method extracted 2–9 times more chlorophyll than the reflux method. Chlorophyll *a* content in the leaves was 1.2 or 4.9 times higher than that of chlorophyll *b*, depending on the extraction method.

Antimicrobial activity of the ABEs. The antimicrobial activity of the extracts of AB were assessed. The ABEs inhibited the growth of the tested microorganisms to varying degrees. The antimicrobial activity of the AB leaf extracts tested against different selected microorganisms is described in **Table 5**.

Table 5. Effect of *Ampelopsis brevipedunculata* (Maxim.) Trautv. leaf extracts on microorganisms (well method), ($M \pm \sigma$, $n = 5$)

Type of the extraction	Extractant	Test cultures, diameter of growth inhibition zone, mm		
		<i>Escherichia coli</i>	<i>Bacillus subtilis</i>	<i>Candida parapsilosis</i>
Control	20 % AE	10.33 ± 0.47	6.33 ± 0.27	7.33 ± 0.27
	60 % AE	13.00 ± 0.71	6.67 ± 0.27	8.00 ± 0.47
	96 % E	15.20 ± 0.83	8.33 ± 0.27	12.33 ± 0.27
Control, ciprofloxacin, 0.3 % (1 drop/well)		50.0 ± 0.70	52.23 ± 0.47	-
Control, fluconazole, 150 mg (0.2 mL/ well)		-	-	41.21 ± 6.91
Maceration	20 % AE	$8.41 \pm 0.55^{***}$	6.18 ± 0.37	8.0 ± 0.67
	60 % AE	$14.83 \pm 0.84^{**}$	$9.66 \pm 0.53^{***}$	$11.2 \pm 0.81^{***}$
	96 % E	$19.0 \pm 0.84^{***}$	$15.84 \pm 2.37^{***}$	$19.64 \pm 0.47^{***}$
Reflux	Distilled water	6.76 ± 0.84	21.00 ± 1.00	6.17 ± 0.37
	20 % AE	$14.66 \pm 1.82^{***}$	$11.04 \pm 0.73^{***}$	$10.38 \pm 0.47^{***}$
	60 % AE	$14.80 \pm 1.48^*$	$16.03 \pm 0.73^{***}$	$13.00 \pm 1.23^{***}$
	96 % E	14.42 ± 0.55	$13.64 \pm 1.08^{***}$	$23.57 \pm 1.08^{***}$

Note: AE – aqueous ethanol; E – ethanol; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

E. coli was not sensitive to aqueous and prepared with 20 % AE extract by the maceration method. At the same time, the extracts prepared with 60 % AE and 96 % E had some inhibitory effect on *E. coli*. The aqueous extract had high inhibitory activity against the tested *B. subtilis*. Other ABEs showed a much weaker effect of suppressing the growth of *B. subtilis* (on the scale we used – moderate), and the solvents themselves did not affect it. Extracts prepared with 20 % AE and distilled water had no inhibitory activity against the tested *C. parapsilosis*. At the same time, the zones of inhibition growth of *C. parapsilosis* under the action of extracts made with 60 % AE and 96 % E were significantly larger than in the control with the solvents. Since controls of extractants showed no inhibition, it proves that solvents did not act as antibacterial and anticandidal agents. All the tested strains of microorganisms were highly sensitive to the reference synthetic antimicrobial drugs – ciprofloxacin and fluconazole (**Table 5**).

To determine the relationships between the studied groups of BAS and antimicrobial activity, a correlation analysis was performed, the results of which are presented in **Table 6**.

Table 6. Results of correlation analysis of growth inhibition zones of microorganisms with *Ampelopsis brevipedunculata* (Maxim.) Trautv. biologically active substances in extracts (n = 5)

Type of the extraction	Extragent	r between the diameter of the growth inhibition zone and the BAS		
		<i>Escherichia coli</i>	<i>Bacillus subtilis</i>	<i>Candida parapsilosis</i>
Maceration	20% AE	HCHA -0.667	TPC 0.607	TPC -0.758 HCHA 1.000***
	60% AE	TPC -0.801	-	TPC 0.750
	96% E	TPC -0.801 TFC -0.677 HCHA 0.762	TFC 0.665 Car 0.884* HCHA 0.885	TPC 0.629 TFC 0.881* Car 1.000*** HCHA 0.671
Reflux	Distilled water	TFC 0.518	TFC 0.518	-
	20 % AE	HCCA 0.760	TPC -0.645 HCHA -0.599	TPC -0.667 HCCA -0.996** HCHA -0.722
	60 % AE	TPC 0.744 TFC -0.662 HCHA -0.847	HCCA 0.766 HCHA -0.780	HCCA -0.742
	96 % E	-	TPC 0.769 TFC 0.943 HCCA 0.895* Chl b 0.681 Car 0.835 HCHA 0.885*	TFC 0.910* HCCA 0.843 Car 0.614 HCHA -0.733

Note: AE – aqueous ethanol; E – ethanol; TFC – total flavonoid content; TPC – total polyphenol content; HCHA – hydroxycinnamic (chlorogenic acid); HCCA – hydroxycinnamic (caffeic acid); Chl – chlorophyll; Car – carotenoids; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Two-factor analysis of variance used to determine the share of the influence of ABEs and cultures (**Fig. 1**) revealed that the growth inhibition zones of the studied cultures are most influenced: in the controls, the cultures themselves (63.56 %, $p < 0.001$), when the reflux extraction method was used – unaccounted factors (59.85 %), and when the maceration method used – the ABEs (87.18 %, $p < 0.001$).

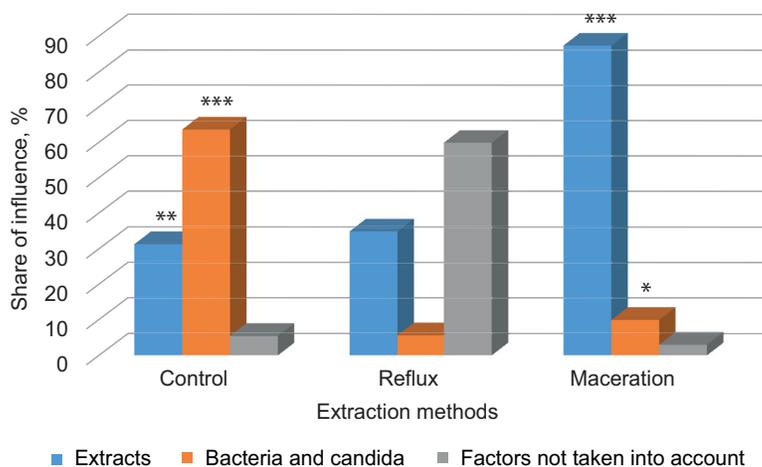


Fig. 1. The proportion of the effect of extracts and cultures on growth inhibition zones, %; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Using a two-factor analysis of variance to determine the proportion of influence of extracts and extraction methods, it was found that the growth inhibition zones of the studied crops are most affected: when using 20% AE – extracts (62.39 %, $p < 0.01$); when using 60 % AE – approximately 1:1 extracts:extraction methods and unaccounted factors; when using 95 % AE – 41.88 extracts (**Fig. 2**).

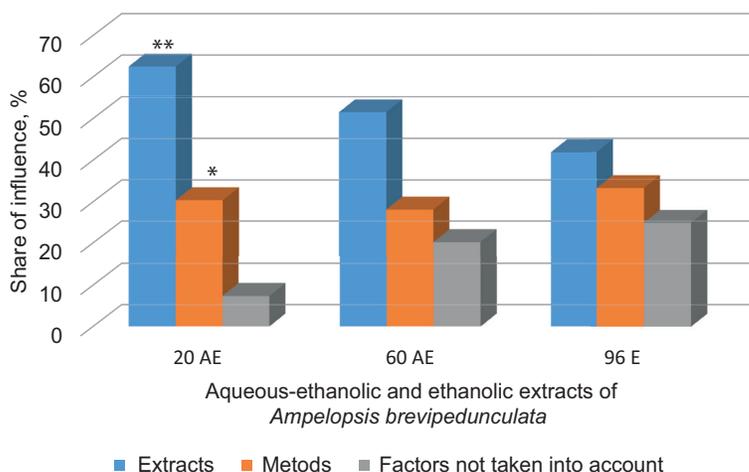


Fig. 2. The share of the influence of extracts and extraction methods on the zones of inhibition of bacterial and candida growth, %; * $p < 0.05$, ** $p < 0.01$

The obtained results indicate that the studied AB green extracts, having different BAS content, had different effects on microorganisms.

The studied *A. brevipedunculata*, under the cultural conditions of the Botanical Garden, is characterized by high shoot-forming ability, bears fruit annually, and its seeds are normally developed. The shoots are rarely frost-hardy and can grow up to 4–5 m. That is, it is possible to obtain a sufficient amount of raw materials for use annually.

DISCUSSION

The increased search for alternative antimicrobial agents from natural sources, especially from plants, has also been prompted by reports of hepatotoxic and carcinogenic effects of synthetic antimicrobials (synthetic antioxidants) (Mavrommatis *et al.*, 2021; Yueha *et al.*, 2014; Kyriakidis *et al.*, 2016). Medicinal plants (MPs) represent a huge resource for new drugs to combat bacterial and fungal diseases. MP research has usually focused on the study of plant extracts. Standard antimicrobial studies involve the extraction of the MP in a suitable solvent followed by the analysis of this crude extract against a specific pathogen using standard methods. The antimicrobial plants' compounds may inhibit bacteria by a different mechanism than the presently used synthetic antibiotics and may have preventive or clinical value in the treatment of resistant microbial strains (Eloff *et al.*, 1998). It can be considered proven that if the extraction is intended to screen plants for antimicrobial components, the influence of the extractant is not important (but the extractant should not interfere with the bioassay procedure), and the choice of solvent also depends on what the extract is intended for (Eloff *et al.*, 1998). The use of green solvents for the production of plant extracts with pharmacological activity has a number of advantages – above all, safety and efficiency.

According to previous reports (Wagner & Ulrich-Merzenich, 2009), the potentiation of the antimicrobial activity by a natural product can be achieved by different mechanisms. Synergistic effects can be produced if the constituents of an extract affect different targets or interact with one another in order to improve the solubility and thereby enhance the bioavailability of one or several substances of an extract. Green solvents such as ethanol are an environmentally friendly choice for medical and pharmaceutical applications. In addition, these extractants can be used to produce teas, tinctures, etc. Ethanol is one of the least toxic solvents for humans, and furthermore, its extraction capacity can be easily modulated by the addition of water, making it an ideal solvent for the extraction of a wide range of plant bioactive compounds with variable polarity (Lee *et al.*, 2024). Ethanol can be used to prepare tinctures as a solvent in various ratios with water for use in herbal medicine. AE of various concentrations can be prepared at home, and the maceration (infusion) method can be used for extraction.

The reflux extraction method for BAS extraction from MP is efficient, cost-effective, and widely used in both laboratory and industrial settings. Maceration and reflux methods with E or AE as solvents can extract various biologically active compounds from plants. E and AE are effective in extracting various types of compounds found in plant tissues and possessing various properties, including antimicrobial ones. The extraction methods used may not yield sufficient amounts of certain phytochemicals. However, they can easily be used to produce a herbal medicinal product or herbal preparation that meets the requirements of the basic documents for pharmaceutical development, research, and registration of herbal preparations and herbal medicinal products in Ukraine and the European Union (European Medicines Agency, n.d.; Hadzhieva *et al.*, 2023).

To have a complete picture of the bioactivity of crude extracts, and to achieve the extraction of the widest possible range of phytochemicals, we used two methods: maceration (cold infusion) and heating in a water bath with a reflux condenser (reflux method). The effect of the type of solvent used on the extraction of polyphenols from AB leaves is shown in **Table 1**, indicating that extracts obtained with 60% and 96% E had high TPC. Despite the fact that it is difficult to achieve complete extraction of BAS using these methods, they are simple, sufficiently reproducible if the extraction conditions are observed, and can be used for initial research into the properties of plants species extracts, in particular antimicrobial ones. The use of the same sets of extracts using identical methods for determining the content of various compounds and common standards allows us to compare the obtained results.

Diffusion methods are qualitative methods and provide insight into the presence or absence of antimicrobial substances in the tested extract, and are still widely used in many studies (Balouiri *et al.*, 2016). Statistical methods allow us to correlate the content of BAS with antimicrobial activity, therefore statistical processing of the obtained material is used to correlate the metabolomic profile of the extracts and their bioactivity. When processing the obtained results, the possible effects of standard commercial drugs (fluconazole and ciprofloxacin) and BAS extractants on the studied strains of microorganisms were taken into account.

In this study, AB leaves were examined for the content of polyphenols, flavonoids, HCAs, chlorophylls, and carotenoids as part of their metabolites with antimicrobial activity. Amongst phenolic compounds, flavonoids and HCAs have been widely recognized for their antibacterial property due to their tendency to retard the growth of a wide range of pathogenic microorganisms, including multidrug-resistant bacteria (Shamsudin *et al.*, 2022).

Phenolic compounds, which have a broad range of chemical additions and exhibit potent antimicrobial activities, can target multiple sites in bacteria, leading to a much higher sensitivity of cells towards these natural compounds (Efenberger-Szmechtyk *et al.*, 2021; Lobiuc *et al.*, 2023).

Flavonoids and HCAs are known for their antimicrobial effects. A number of flavonoids isolated in pure form or in extracts have been described as antimicrobial agents (Cushnie & Lamb, 2005; Górnai *et al.*, 2019; Biharee *et al.*, 2020). Flavonoids show antimicrobial effect by various mechanisms: inhibiting virulence factors, efflux pump, membrane disruption, cell envelope synthesis, nucleic acid synthesis, and bacterial motility inhibition (Biharee *et al.*, 2020; Shamsudin *et al.*, 2022; Liu *et al.*, 2025). These compounds are believed to have a lower probability of bacterial resistance occurrence (Borges *et al.*, 2013), provide protective actions against different deleterious agents and biofilm formation (Oteiza *et al.*, 2005). In addition, certain flavonoids have been reported to reverse antibiotic resistance and improve the efficacy of the present antibiotics (Biharee *et al.*, 2020; Song *et al.*, 2021; Ruddock *et al.*, 2011; Abreu *et al.*, 2015; Guan *et al.*, 2022).

HCAs have demonstrated efficacy against various bacteria, including both Gram-positive and Gram-negative strains, as well as fungi (Taofiq *et al.*, 2017). Some phenolic acids are known to cause disruption of bacterial and fungal cell membranes, and, finally, cell death (Teodoro *et al.*, 2015; Martins *et al.*, 2015). Some previous studies reported that *C. parapsilosis* was able to metabolize phenolic acids (Middelhoven *et al.*, 1992; Middelhoven, 1993) and to grow in the presence of some phenolic acids after 3 days of cultivation (Heleno *et al.*, 2015).

In this study, we demonstrated that ABEs with polyphenols, flavonoids, and HCAs inhibit *E. coli*, *B. subtilis*, and *C. parapsilosis* to some extent, suggesting that each of these BAS may contribute to this inhibition as has been supported by statistical analysis.

Given that Chl and Car are always present in photosynthetic organs and parts of plants, as well as their good solubility in E, they are also present in ethanol extracts. Extraction conditions and other substances present can significantly affect their content in the extract, and therefore their antimicrobial activity, which requires investigation in each individual case (Ebrahimi *et al.*, 2023). In view of this, the content of Chl and Car in ABE was studied. There are also individual studies concerning antimicrobial effects of chlorophyll. Antibacterial properties of Chl isolated from *Medicago sativa* were found against *Listeria* and *Staphylococcus* (Ahmadi *et al.*, 2022). It was found that the high chlorophyll content in *Punica granatum* leaves inhibits not only bacteria, but also yeasts and fungi (Elbatanony *et al.*, 2019). At the same time, chlorophyll content in *Punica granatum* leaves was comparable to that obtained by us in AB leaves (Ebrahimi & Lante, 2021). Antimicrobial activity of extracts from other plant species has also been associated with high chlorophyll content (Dziedziński *et al.*, 2020; Ilieva *et al.*, 2024; Bhagavathy *et al.*, 2011) in particular regarding *E. coli* and *B. subtilis* (Pothiraj *et al.*, 2021; Bhagavathy *et al.*, 2011).

In addition, Car have also been identified as antimicrobial molecules (Karpiński *et al.*, 2022). There are several papers that have presented the antimicrobial activity of higher plants with high content of Car (eg. Ahmad Nejhad *et al.*, 2023; Mavrommatis *et al.*, 2022; Molnár *et al.*, 2005; Natividad *et al.*, 2014; Tao *et al.*, 2010; Miljković *et al.*, 2022), and most of them used algae extracts or bacterial or yeasts resources (Tran *et al.*, 2019; Vargas-Sinisterra *et al.*, 2021; Villaró *et al.*, 2021; Ambati *et al.*, 2014; Ilieva *et al.*, 2024). Taking into account the available studies, both ours and those of other authors, we can assume a positive role of Car as antimicrobial substances.

It should also be noted that there is a significant gap in research on the metabolism of Car in the colon and their interactions with the gut microbiota, i.e. the microorganisms that inhabit the gut (Bohn, 2017). The bacterial community in the large intestine may promote carotenoid bioavailability by completing the digestion of plant cell walls and modulating intestinal permeability, thereby providing high serum carotenoid levels known to be associated with a reduced risk of chronic diseases (Djuric *et al.*, 2018). The above features (the effects of Car and the intestinal microbiota, their interactions with other BAS, etc.) determine the need to investigate these effects in further studies.

Some studies suggest that the presence of phenolic compounds within the pigment-rich extracts could be a synergist of antimicrobial activity (Dziedziński *et al.*, 2020; Elbatanony *et al.*, 2019), and even enhance the action of synthetic antimicrobials (Álvarez-Martínez *et al.*, 2021). However, there are reports of the possibility of Car and flavonoids reducing the efficacy of some antimicrobial drugs against clinical isolates of *E. coli* and *Staphylococcus aureus* (dos Santos *et al.*, 2015). This indicates a complex interaction between BAS included in the extracts during their application and the need for careful study of each extract, in particular, toxicological studies need to be conducted.

The extracts obtained by maceration with 60 % AE and 96 % E showed the highest content of total TPC polyphenols (**Table 1**). This directly correlates with their highest inhibitory activity against *E. coli* and *C. parapsilosis* (**Table 5**). Correlation analysis (**Table 6**) confirms this relationship, but it may also indicate the dominance of other components in this activity or a complex interaction.

The highest flavonoid content was found in the 96 % E extract obtained by maceration and in the extract obtained with 20 % AE by reflux (**Table 2**). The activity of the 96 % E sample (maceration) against *C. parapsilosis* was the highest, and correlation analysis indicated a strong relationship between TFC and this activity ($r = 0.881$, $p < 0.05$). In contrast, the extract with 20 % AE (refluxed) had the highest TFC, but its activity against *C. parapsilosis* was negligible, indicating that not only the total TFC content, but also the type (structure) of flavonoids or their synergy with other BACs determines the final activity. The highest HCA content (calculated as chlorogenic and caffeic acids) was recorded in the extract obtained by refluxing with 60 % AE (**Table 3**). This extract showed moderate but significant activity against all three microorganisms, including *B. subtilis*.

The aqueous extract had the lowest TPC, TFC, and HCA values (**Tables 1–3**). Its extremely high activity against *B. subtilis* compared to other extracts may indicate the presence of highly polar, water-soluble compounds other than the main phenols studied, which are potent inhibitors of Gram-positive bacteria. This requires further identification of these specific water-soluble BAS. The correlation analysis (**Table 6**) shows the multifaceted activity of the extracts against *B. subtilis*.

The content of photosynthetic pigments (**Table 4**) was estimated in 96 % ethanol extracts, as it is an effective green solvent for lipophilic compounds. Maceration was found to be much more effective for pigment extraction than reflux, releasing 2–9 times more chlorophyll and almost 4 times more carotenoids (**Table 4**). It was the extract prepared by maceration with 96 % E, containing the highest concentrations of Chl *a*, Chl *b*, Car, as well as the highest TPC and TFC, that demonstrated the broadest spectrum and the highest overall activity among all tested extracts (especially against *E. coli* and *C. parapsilosis*). Correlation analysis (**Table 6**) for 96 % E (maceration) shows a high positive correlation between carotenoid content (Car) and activity against *C. parapsilosis* ($r = 1.000$, $p < 0.001$) and *B. subtilis* ($r = 0.884$, $p < 0.05$). This may indicate a significant contribution of lipophilic pigments, which are often underestimated, to the overall antimicrobial activity.

The obtained results support the hypothesis that the highest antimicrobial activity of the extract obtained with 96 % E (maceration) is a consequence of a synergistic effect between high levels of phenolic compounds (TPC, TFC) and high levels of lipophilic pigments (Chl, Car), which can affect different cellular targets (membrane, intracellular structures). Two-way ANOVA (**Figs 1 and 2**) further confirmed the importance of the choice of extract/method. In the case of the maceration method, the proportion of the effect of the extract itself (type of solvent) on the inhibition zones was dominant (87.18 %, $p < 0.001$) (**Fig. 1**). This emphasizes that it was the phytochemical composition formed by the solvent, and not the type of crop or other factors, that was decisive for maceration.

Overall, the results clearly indicate that 96 % ethanol, when applied by the maceration method, is the most effective green extractant for BAS in *A. brevipedunculata* with a broad spectrum of antimicrobial activity. At the same time, the aqueous extract (reflux) is a valuable source of specific compounds active against *B. subtilis*.

When determining the role of various BAS as antimicrobial agents, their high antioxidant activity is often emphasized, which is decisive in this effect. However, it is the reactive oxygen species, among which singlet oxygen is the cytotoxic agent, that can destroy bacteria (Huang *et al.*, 2012).

CONCLUSIONS

This study showed that the leaves of *A. brevipedunculata* are rich in phenolic compounds and pigments and have antimicrobial activity against *E. coli*, *B. subtilis*, *C. parapsilosis*. The results obtained by us using the above methods can be used as a basis for the creation of extemporaneous preparations, as well as various galenic preparations. Additional studies can further clarify the efficacy of AB extracts (e.g., as a mouthwash, for prevention of skin diseases or prevention of food contamination, etc.). The potential antimicrobial activity of the studied AB leaf extracts, as well as the BAS isolated from these extracts, convincingly confirms the prospects of their further research for widespread use. The ability to obtain a sufficient amount of plant raw materials when growing AB in culture is one of the positive factors for its prospective use. However, a more complete phytochemical and pharmacological characterization of this plant is required before application. Other *in vitro* studies, including the assessment of cytotoxicity and mutagenicity and their effects on biofilm models, are also needed for the next stage of research.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: the authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Animal Rights: this article does not contain any studies with animal subjects performed by the any of the authors.

AUTHORS CONTRIBUTIONS

Conceptualization, [V.N.; Y.H.]; methodology, [V.N.; Y.H.]; validation, [V.N.; Y.H.]; formal analysis, [Y.H.]; investigation, [Y.H.; V.N.]; resources [V.N.; Y.H.]; data curation [V.N.; Y.H.]; writing original draft preparation [Y.H.; V.N.]; writing [V.N.]; visualization [Y.H.]; supervision [V.N.; Y.H.]; project administration [V.N.; Y.H.].

All authors have read and agreed to the published version of the manuscript.

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ФІТОХІМІЧНІ Й АНТИМІКРОБНІ ДОСЛІДЖЕННЯ ІНТРОДУКОВАНОГО *AMPELOPSIS BREVIPEDUNCULATA* (MAXIM.) TRAUTV.

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Обґрунтування. *Ampelopsis brevipedunculata* (Maxim.) Trautv (AB) визнаний своїм потенціалом у лікуванні гепатотоксичних станів, atopічного дерматиту і запалення шкіри, що може бути пов'язано зі синергізмом певних біоактивних сполук із протизапальною та протиалергічною активністю. Метою було оцінити здатність

екстрактів листя *A. brevipedunculata* як антимікробних засобів проти *Escherichia coli*, *Bacillus subtilis*, *Candida parapsilosis*, а також покращити розуміння впливу поліфенолів, флавоноїдів, гідроксикоричних кислот, каротиноїдів і хлорофілів у їхньому складі на антимікробну активність.

Методи. Екстракти листя АВ готували методами рефлюксу та мацерації з 20- і 60 % водним етанолом (АЕ) та 96 % етанолом (Е). Водний екстракт отримували методом рефлюксу. Спектрофотометричний аналіз використовували для визначення загального вмісту фенольних сполук, загального вмісту флавоноїдних сполук і загального вмісту гідроксикоричних кислот, вмісту хлорофілу та каротиноїдів у екстрактах. Антимікробну активність екстрактів *A. brevipedunculata* оцінювали методом дифузії в агар.

Результати. Дистильована вода, 20-, 60 % АЕ та 96 % Е забезпечили високий рівень екстракції поліфенолів методами мацерації та рефлюксу, а 96 % Е – каротиноїдів і хлорофілів. *E. coli* та *C. parapsilosis* не були чутливими до водного екстракту й екстракту, приготованого з 20 % АЕ мацерацією. Водночас екстракти, приготовані з 60 % АЕ та 96 % Е, мали певний інгібуючий вплив на *E. coli* та *C. parapsilosis*. *B. subtilis* інгібував водний екстракт. Кореляційний аналіз підтвердив взаємозв'язки між досліджуваними біологічно активними речовинами (БАР) та діаметром зон інгібування тестованих культур. Двофакторний дисперсійний аналіз підтвердив достовірність часток впливу на зони затримки досліджуваних культур у контролі та екстрактах за використання методу мацерації для їхнього приготування; а також екстрактів, отриманих за використання 20 % АЕ.

Висновок. Екстракти листя АВ є багатим і різноманітним джерелом біохімічних речовин, що мають інгібуючу дію проти *E. coli*, *B. subtilis* та *C. parapsilosis*. Зелені екстракти індродукованого АВ можна використовувати як багате джерело фенолів, флавоноїдів, гідроксикоричних кислот, хлорофілів та каротиноїдів для подальших досліджень і застосування як антимікробних засобів.

Ключові слова: *Ampelopsis brevipedunculata*, зелені екстракти, антимікробна активність, біологічно активні сполуки