

Phytochemical profiles, antioxidant and antimicrobial activity of *Actinidia polygama* and *A. arguta* fruits and leaves

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Plants of two species of *Actinidia* genus grown in an adverse steppe climate were examined in terms of secondary metabolites' accumulation, antioxidant potential, and antimicrobial ability. The aim of the work was to reveal whether the introduced plants *A. arguta* and *A. polygama* retain their well-known health benefits. Total content of polyphenols (549.2 and 428.1 mg GAE/100 g FW, respectively), flavonoids, and phenolic acids as well as total antioxidant activity and reducing power of the fruit isopropanol extracts were found to be equal or even higher than the reported data on kiwifruit varieties cultivated in China and other regions. Antioxidant potential and phenolic compounds' content in the fruit peel of both species were higher when compared to pulp, while corresponding indices of leaves exceeded those of the fruit. Disc-diffusion assays showed low to moderate antibacterial activity of *A. arguta* and *A. polygama* fruit and leaf extracts against collection Gram-negative and Gram-positive strains. Clinical strains of *P. aeruginosa* and *E. coli* resistant to the action of ofloxacin were notably inhibited by *A. arguta* and *A. polygama* fruit and leaf crude extracts. Inhibiting effects of plant extracts on clinical strains of *K. pneumoniae* and *A. baumannii* were comparable with the effect of ofloxacin. GC-MS assays identified 23 and 36 chemical constituents, respectively in *A. arguta* and *A. polygama* fruit isopropanol extracts. The main compounds in both extracts were 2-propenoic acid, penta-decyl ester followed by squalene, 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-dien-2,8-dione, octadecanoic acid, 2-oxo-methyl ester, ethyl-isoallocholate, and phytol having known bioactivities. Our findings confirmed the preservation of useful properties by the introduced plants and also indicated the rich health-promoting abilities and expediency of cultivating *A. arguta* and *A. polygama* in a steppe climate.

Keywords: kiwiberry; secondary metabolites; GC-MS assay; bioactivity.

Introduction

Species of the genus *Actinidia* Lindl (Actinidiaceae Hutch.) are widely distributed in eastern Asia, with most taxa in Central and Southwest China (Huang & Ferguson, 2007). These plants are perennial vine fruit trees, of which a large number are dioecious, but a very small number are monoecious. Currently, the genus *Actinidia* consists of 76 species and about 125 known taxa, which along with *Chaenomeles* (Lykholat et al., 2021) have become an important horticultural fruit crop worldwide (Wang et al., 2018b). The main commercial cultivation is associated with the species *Actinidia chinensis*, *A. deliciosa*, and less commonly *A. eriantha* and *A. arguta* (Williams et al., 2003; Kim et al., 2009). The species *A. chinensis* Planch is native to Southern China, while *A. kolomikta* and *A. arguta* are widely consumed in the region of Northeastern China (Zuo et al., 2012). *A. chinensis*, commonly known as Chinese kiwifruit, is a native Chinese fruit, which is becoming popular due to its outstanding health benefits, nutritional and economic properties (Bekhradnia et al., 2011). The whole plant including fruits, leaves, vines, and roots of *A. chinensis*, is served as food and is a rich source for the folk medicine in China (He et al., 2019). In accordance with data of Satpal et al. (2021), *A. deliciosa* is one of the most commercialized fruits whose nutrients and medicinal and therapeutic properties against diseases have been studied most completely. The health promoting properties of kiwifruit were traditionally associated with the cardiovascular system, diabetes, kidney problems, cancer, digestive disorders, bone, and eye problems.

Recently, extensive research has revealed a significant source of bioactive constituents in kiwi fruit which provide a strong antimicrobial, antiviral efficacy and immunomodulatory effect and contribute to the rich pharmacological profile of kiwifruit. McGhie (2013) reported more than 500 metabolites in green kiwifruit (*A. deliciosa*) and gold kiwifruit (*A. chinensis*); concentrations of several compounds have been docu-

mented, including vitamins, carotenoids (lutein and β -carotene), folate, and antioxidant phenolic compounds. Du et al. (2009) found great variability of the fruit total polyphenols of eight *Actinidia* genotypes, among which the wild *A. eriantha* and *A. latifolia* species have significantly higher antioxidant capacity than the cultivars of *A. chinensis* and *A. deliciosa*. Zuo et al. (2012) discovered notable differences in the antioxidant and antiproliferative properties of ethanol extracts from three *Actinidia* species with *A. kolomikta* exerting the highest antioxidant activity, but *A. arguta* had the highest inhibitory effect on cancer cell growth.

Despite the wide diversity of *Actinidia* fruits in both form and composition, high contents of vitamins C and E, organic acids, actinidin (Drummond, 2013), and dietary fiber (Li & Zhu, 2019) are found to be invariably present in *Actinidia*. However, the growing conditions can significantly affect the accumulation of nutrients and alter bioactivity of the fruit (Khromykh et al., 2018). Research has shown that antioxidant ability and bioactive molecules content (ascorbic acid, total polyphenols, carotenoids, and tocopherols) in *A. deliciosa* fruits differed notably depending on the growing region in Italy (D'Evoli et al., 2015). In the steppe zone of Ukraine, climate demonstrates sharp temperature changes in winter, which determines the expediency of growing only the more frost-resistant *Actinidia* species. Within the *Actinidia* genus, *A. arguta* (kiwiberry) has such cold-resistant properties (Pinto et al., 2021). Cultivation of kiwifruit in Poland showed that *A. arguta* accumulated a higher level of polyphenols, flavonoids, flavanols, tannins, vitamin C, lutein, zeaxanthin and dietary fibers than *A. deliciosa* (Leontowicz et al., 2016). However, the main problem of *Actinidia* cultivation in the steppe zone is the summer heat and dry air; shading and regular watering of plants can provide the necessary conditions for growth. Nevertheless, the question of the introduction success and development of health benefits of non-traditional fruit plants in general (Lykholat et al., 2019) and *Actinidia* in particular, remains little explored. The present work aimed to characterize the potential

of two *Actinidia* species in terms of the secondary metabolites' accumulation and component composition of the fruits and leaves, as well as their antioxidant and antimicrobial activity.

Materials and methods

Plants of the genus *Actinidia* were introduced on the territory of the Botanical Garden of Oles Honchar Dnipro National University (48°26'07" N, 35°02'34" E; Dnipro city, steppe zone of Ukraine) in 2002–2003. The regional climate has continental traits with sharp changes during year, including the periods of strong frosts in winter and summer heat with dry winds, and low precipitation (473 mm average, but 265 mm in dry years). In addition, the polluted air in the metropolis can serve as a stress factor for plants (Alexeyeva et al., 2016). However, the *Actinidia* plants today are in a satisfactory state and have been bearing fruit for the past few years. The mature leaves and ripe fruits of *A. arguta* (Siebold & Zuccarini) Miquel (cv. 'Veresneva') and *A. polygama* (Siebold & Zuccarini) Maxim were collected at the end of September 2021, packed in plastic containers and transferred to the laboratory for preparing plant extracts.

Evaluation of the content of phenolic compounds, profiling of phytochemicals and study of bioactivity were carried out using isopropanol-water (80:20, v/v) extracts from the plant leaves and fruits (both whole fruits and separated peel and pulp). Briefly, a 2.0 g weighed portion of fresh plant material was triturated with 20 mL of isopropanol solution and kept for 24 hours at room temperature in dark with occasional shaking after which the extracts were filtered through the paper filters. Total polyphenols' content (TPC), total flavonoid content (TFC), free phenolic acids' content (PAC), total antioxidant capacity (TAC), and reducing power (RP) were determined in the crude extracts obtained. For the phytochemicals' profiling and antimicrobial assays, crude extracts were dried at 45 °C using rotary evaporator IKA® RV 10 (IKA®-Werke GmbH & Co. KG, Germany), and a corresponding amount of solid residue was dissolved in isopropanol solution.

Total polyphenol content in the fruit and leaf extracts was determined with Folin-Ciocalteu reagent (Slinkard & Singleton, 1977); the absorbance was measured at 726 nm; the results were calculated using a calibration graph and expressed as mg Gallic acid (GA) equivalents per 100 g of fresh weight (mg GA/100 g FW). Total flavonoid content was evaluated by aluminum chloride spectrophotometric method (Pełkal & Pyszynska, 2014) at 425 nm; results were calculated using a calibration graph and expressed as Rutin equivalents (mg Ru/100 g FW). Free phenolic acids' content was determined by spectrophotometric method (Gawron-Gzella et al., 2012) with Arnov's reagent (10.0 g sodium molybdate, 10.0 g sodium nitrite in 100.0 mL water) at 490 nm was measured; the results were expressed in caffeic acid (CA) equivalents (mg CA/100 g FW). Reducing power of the plant samples was studied by potassium ferricyanide method (Pulido et al., 2000); the absorbance was measured at 700 nm, and the results were expressed in mg Ascorbic acid (AA) equivalents (mg AA/100 g FW). Total antioxidant capacity of fruits and leaves was determined in accordance with Prieto et al. (1999) at 695 nm; the results were calculated using a calibration graph prepared on the solutions of ascorbic acid, and expressed as mg AA equivalents (mg AA/100 g FW).

Fruit isopropanol extracts were subjected to gas chromatography – mass spectrometry (GC-MS) analysis using Shimadzu GCMS-QP 2020 El equipped with Rxi®-5ms column (30 m × 0.25 mm, film thickness 0.25 µm) containing 5% diphenyl/95% dimethyl polysiloxane as a fixed liquid phase. The column temperature 50 °C, with 5 min initial hold, and then programmed temperature gradient increased to 300 °C at a rate of

15 °C per min, and kept constant at 300 °C for 10.5 min. The carrier gas helium passed at a flow rate 54 mL/min. Injector temperature was 300 °C; sample volume was 1 µL. Mass Spectrum Library 2014 for GC-MS (O2125401310) was used to identify the separated compounds by comparing the mass spectra obtained with those stored in the library database (National Institute of Standards and Technology library similarity index, NIST14.lib, NIST14s.lib). The content of individual compounds was estimated using the corresponding peak area and expressed as a percentage of the total sum of identified compounds.

Antimicrobial activity of *Actinidia* fruit and leaf crude isopropanol extracts were tested using the disc diffusion method (Bhimba et al., 2012). The test strains of microorganisms were from the culture collection of Microbiology, Virology and Biotechnology Department of Oles Honchar DNU. Of these, there were four Gram-negative bacterial strains (*Erwinia dissolvens* 170, *Escherichia coli* B906, *Pseudomonas aeruginosa* B907, *Klebsiella pneumoniae* B920), and five Gram-positive strains (*Micrococcus lysodeikticus* 2665, *Staphylococcus aureus* B904, *S. aureus* B209, *S. epidermidis* ATCC149, and *S. epidermidis* B919). Additionally, crude extracts from *Actinidia* air-dried fruits were tested against clinical bacterial strains, namely *Pseudomonas aeruginosa* (two strains), *Klebsiella pneumoniae* (two strains), *Acinetobacter baumannii* (two strains) and *Escherichia coli* (two strains), and one fungus (*Candida albicans*). In each case, Petri plates containing meat-peptone agar (MPA) medium were seeded with 10⁹ cfu (colony forming units) suspension of microorganisms. Sterile paper discs (6 mm diameter) were impregnated with 10 µL of crude isopropanol fruit and leaf extracts and placed on the agar surface; plates incubated at 37 °C for 24 h. Ofloxacin (5.0 µg per disc) was used as the positive control for the bacterial strains; itraconazole 10.0 µg was used as the positive control for the fungal strains. Antimicrobial activity of the fruit and leaf extracts was expressed as the diameter of the inhibition zone (mm) around the discs along with disc diameter.

All bioassays were carried out in five replications. Statistical processing of experimental results was based on analysis of variance (ANOVA). The data obtained were expressed as the mean ± standard deviation, and the differences between means were compared with Tukey's HSD. The groups of values were compared by U-criterion Mann-Whitney. This is a statistical criterion used to assess differences between two independent samples, which allows us to identify differences in the parameter value between small samples. All differences were considered statistically significant at P < 0.05.

Results

Content of phenolic compounds in the fruit peel of both *A. arguta* cv. 'Veresneva' and *A. polygama* exceeded the corresponding indices in the fruit pulp (Table 1). The highest excess was in total flavonoid content (4.5 and 5.2 times, respectively for *A. arguta* and *A. polygama* fruits) followed by total polyphenol content (2.3 and 2.1 times respectively). Free phenolic acids' content differed sharply in *A. arguta* fruits (peel content 3.1 times higher than pulp), while it was almost the same in the fruits of the other species. Antioxidant potential of the fruit peel of both *A. arguta* and *A. polygama* was also higher when compared to pulp (respectively, 1.6 and 1.4 times for RP, and 1.5 and 1.7 times for TAC).

Total content of the polyphenolic compounds along with free phenolic acids' content and the reducing power were found to be higher in the fresh whole fruits of *A. arguta* (cv. 'Veresneva') when compared to *A. polygama* fruits (respectively, 1.3, 1.1, and 3.3 times). However, the total content of flavonoids and total antioxidant capacity were greater in the fruits of *A. polygama* (1.7 and 1.4 times respectively).

Table 1

Phenolic compounds content and antioxidant activity of *Actinidia* fruits (x ± SD, n = 5)

Index	Indicator unit	<i>A. arguta</i> (cv. 'Veresneva')		<i>A. polygama</i>	
		peel	pulp	peel	pulp
Total polyphenol content	mg GA/100 g FW	374.0 ± 7.7 ^a	175.2 ± 5.1 ^b	291.9 ± 6.4 ^c	136.2 ± 3.9 ^d
Total flavonoid content	mg Ru/100 g FW	101.6 ± 3.3 ^a	22.66 ± 0.89 ^b	180.0 ± 3.9 ^c	34.36 ± 1.3 ^d
Free phenolic acids content	mg CA/100 g FW	31.92 ± 2.35 ^a	10.23 ± 0.74 ^b	19.63 ± 1.53 ^c	18.24 ± 1.27 ^c
Reducing power	mg AA/100 g FW	286.1 ± 9.9 ^a	178.5 ± 5.8 ^b	83.7 ± 4.5 ^c	58.2 ± 4.3 ^d
Total antioxidant capacity	mg AA/100 g FW	884.0 ± 20.8 ^a	594.9 ± 12.4 ^b	1269.1 ± 21.6 ^c	736.2 ± 16.1 ^d

Note: different letters indicate the values significantly differing one from another within a line of the Table on the results of comparison using the Tukey test (P < 0.05).

The average fresh weight of the whole fruit of *A. arguta* cv. 'Veresneva' (11.73 ± 0.81 g) was 3.3 times higher ($P < 0.05$) than the index of *A. polygama* fresh fruit (3.60 ± 0.55 g).

As for the plant leaves (Table 2), *A. polygama* showed slightly higher levels of total polyphenols, total flavonoids, and total antioxidant capacity, while *A. arguta* (cv. 'Veresneva') had the stronger reducing power (1.3 times higher than the first species leaves).

In general, leaves of the both *A. arguta* and *A. polygama* exhibited greater levels of most indices in comparison with plant fruits: total phenolic compounds (1.9–2.7 times higher), total flavonoids (2.1–3.5 times), and total antioxidant capacity (1.2–1.5 times). The exception was the indicator of reducing power, being higher in the fruits of both *Actinidia* plants than in the leaves (in the range 1.2–1.9 times).

Correlation analysis of the indices of fresh whole *Actinidia* fruits revealed a strong positive relationship between the reducing power and total polyphenol content ($r = 0.99$, $P < 0.0001$) as well as between total antioxidant capacity and total flavonoid content ($r = 0.96$, $P < 0.0001$). In the

plant leaves, there was high correlation ($r = 0.76$, $P < 0.0001$) between total polyphenol content and the reducing power.

Table 2
Phenolic compounds' content and antioxidant activity of *Actinidia* leaves ($x \pm SD$, $n = 5$)

Index	Indicator unit	<i>A. arguta</i> (cv. 'Veresneva')	<i>A. polygama</i>
Total polyphenol content	mg GA/100 g FW	1032.2 ± 27.6	1138.9 ± 19.2*
Total flavonoid content	mg Ru/100 g FW	433.4 ± 29.3	441.9 ± 20.7 ^{ns}
Reducing power	mg AA/100 g FW	162.2 ± 15.7	122.0 ± 8.5*
Total antioxidant capacity	mg AA/100 g FW	2203.3 ± 114.1	2487.4 ± 92.2*

Note: * $P < 0.05$, ns – not statistically significance (according to the Mann-Whitney test).

Isopropanol extracts from both the fruits and leaves of *Actinidia* plants showed low to moderate antimicrobial activity against all collection pathogenic bacterial strains tested in the disc diffusion bioassays (Table 3).

Table 3
Diameter of inhibition zones (mm) caused by *Actinidia* isopropanol extracts in the collection bacterial strains ($x \pm SD$, $n = 5$)

Test-culture	<i>A. arguta</i> cv. 'Veresneva'		<i>A. polygama</i>		Positive control ¹
	fruits	leaves	fruits	leaves	
<i>Erwinia dissolvans</i> 170	9.18 ± 0.15 ^a	11.51 ± 0.13 ^b	11.06 ± 0.15 ^c	11.28 ± 0.12 ^{bc}	25.45 ± 0.44 ^d
<i>Escherichia coli</i> B906	13.18 ± 0.32 ^a	10.82 ± 0.21 ^b	9.95 ± 0.26 ^c	8.63 ± 0.10 ^d	27.51 ± 0.30 ^e
<i>Pseudomonas aeruginosa</i> B 907	12.20 ± 0.09 ^a	8.71 ± 0.10 ^b	12.12 ± 0.12 ^a	10.52 ± 0.18 ^c	15.63 ± 0.28 ^d
<i>Micrococcus lysodeikticus</i> 2665	11.86 ± 0.18 ^a	8.04 ± 0.09 ^b	8.28 ± 0.16 ^{bc}	8.38 ± 0.12 ^c	16.72 ± 0.24 ^d
<i>Klebsiella pneumoniae</i> B 920	9.06 ± 0.10 ^a	9.20 ± 0.04 ^{ac}	6.19 ± 0.04 ^b	9.31 ± 0.05 ^c	19.79 ± 0.13 ^d
<i>Staphylococcus aureus</i> B904	10.22 ± 0.12 ^a	7.36 ± 0.21 ^b	11.16 ± 0.10 ^c	8.40 ± 0.14 ^d	17.79 ± 0.20 ^e
<i>S. aureus</i> B209	8.51 ± 0.11 ^a	7.37 ± 0.23 ^b	8.19 ± 0.19 ^a	7.39 ± 0.19 ^b	21.52 ± 0.23 ^c
<i>S. epidermidis</i> ATCC149	8.92 ± 0.11 ^a	8.01 ± 0.12 ^b	8.30 ± 0.11 ^{bc}	8.46 ± 0.09 ^c	20.92 ± 0.31 ^d
<i>S. epidermidis</i> B919	9.60 ± 0.18 ^a	7.69 ± 0.18 ^b	8.09 ± 0.07 ^c	7.67 ± 0.19 ^b	21.37 ± 0.30 ^d

Notes: ¹ – ofloxacin (5.0 µg) was used as positive control; the diameter of the inhibition zones (mm), including the disc diameter (6 mm), are given as $x \pm SD$; different letters indicate the values significantly differing one from another within a line of the Table based on the results of comparison using the Tukey test ($P < 0.05$).

Table 4
Inhibition zones diameter (mm) caused by *Actinidia* isopropanol extracts in the clinical pathogenic strains ($x \pm SD$, $n = 5$)

Test-culture	<i>A. arguta</i> cv. 'Veresneva'		<i>A. polygama</i>		Positive control ¹
	fruits	leaves	fruits	leaves	
<i>Pseudomonas aeruginosa</i>	11.37 ± 0.11 ^a	10.36 ± 0.08 ^b	10.16 ± 0.09 ^c	8.43 ± 0.04 ^d	NA
<i>Klebsiella pneumoniae</i>	10.17 ± 0.07 ^a	10.61 ± 0.07 ^b	13.75 ± 0.21 ^c	12.14 ± 0.11 ^d	12.11 ± 0.10 ^d
<i>Acinetobacter baumannii</i>	12.05 ± 0.11 ^a	10.78 ± 0.09 ^b	9.95 ± 0.06 ^c	11.64 ± 0.09 ^d	12.75 ± 0.07 ^e
<i>Escherichia coli</i>	14.64 ± 0.15 ^a	12.79 ± 0.08 ^b	15.46 ± 0.15 ^c	11.61 ± 0.09 ^d	NA
<i>Candida albicans</i>	7.81 ± 0.17 ^a	8.31 ± 0.17 ^b	7.90 ± 0.24 ^a	8.41 ± 0.12 ^b	21.94 ± 0.25 ^c

Notes: ¹ – ofloxacin (5.0 µg) and itraconazole (10.0 µg) were used as positive control for bacteria and fungus, respectively; the diameter of the inhibition zones (mm), including the disc diameter (6 mm), are given as $x \pm SD$; different letters indicate the values significantly differing one from another within a line of the Table based on the results of comparison using the Tukey test ($P < 0.05$); NA – no activity.



Fig. 1. Effects of *Actinidia* extracts on *P. aeruginosa* (a), *K. pneumoniae* (b), *A. baumannii* (c), and *E. coli* (d): 1, 4 – *A. arguta* var. *purpurea* fruit and leaf extracts, respectively; 2, 5 – *A. arguta* cv. 'Veresneva' fruit and leaf extracts; 3, 6 – *A. polygama* fruit and leaf extracts; 7 – ofloxacin

In general, antibacterial activity of both *Actinidia* fruit extracts was more prominent when compared to leaf extracts. The highest inhibiting activity of *A. arguta* fruit extracts was against the Gram-negative strains *E. coli* B906 and *P. aeruginosa* B 907, followed by Gram-positive strains *M. lysodeikticus* 2665 and *S. aureus* B904. The fruit extracts of *A. polygama* were also most active against Gram-negative strains, namely *P. aeruginosa* B 907, *E. dissolvans* 170, and *E. coli* B906. Leaf extracts of both plant species generally showed the higher inhibition of Gram-negative strains; the lowest activity was against *P. aeruginosa* (effect of

A. arguta cv. 'Veresneva') and *E. coli* (effect of *A. polygama* extract). Some of the clinical bacterial strains tested in the disc diffusion bioassays showed equal or unexpectedly higher sensitivity to the action of fruit and leaf extracts of both *Actinidia* species when compared to positive control (Table 4).

Pathogenic clinical strains *P. aeruginosa* and *E. coli* were resistant to the action of the known antibiotic ofloxacin, while they were notably inhibited by *Actinidia* fruit and leaf crude extracts (Fig. 1). Inhibiting effects of *A. polygama* fruit extract on the growth of *K. pneumoniae* as well

as *A. arguta* fruit extract on the *A. baumannii* strains were comparable with the ofloxacin action. Isopropanol extracts from the whole fruits of *A. arguta* (cv. 'Veresneva') when assayed by GC-MS analysis showed the presence of 23 identified constituents which accounted for 97.7% of the separated compounds total amount (Table 5).

Most of the phytochemical constituents from the isopropanol extracts of *A. arguta* cv. 'Veresneva' belonged to the class of fatty acids esters (76.8% of total, in the range C₁₆–C₃₄) followed by alkanes and their derivatives (9.6% of total), ketones and their derivatives (4.5%), terpenoids

(2.9%), phenolic compounds (2.1%), and carbohydrates (1.1% of total). The less abundant classes were alcohols (1.0% of total), aldehydes (0.4%), and fatty acids (0.4% of total). The main compounds were 2-propenoic acid, pentadecyl ester (63.3%), eicosane, 2-methyl (5.5%), 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-dien-2,8-dione (4.5%), Heptadecanoic acid, heptadecyl ester (4.1%), dibutyl phthalate (3.0%), 9-octadecenoic acid (Z), phenylmethyl ester (2.5%), fumaric acid, pent-4-en-2-yl undecyl ester (2.3%), octadecanoic acid, 2-oxo-methyl ester (1.7%), phytol (1.7%), and heptacosane (1.6% of total).

Table 5

Chemical constituents of isopropanol extracts from the whole fruits of *A. arguta* cv. 'Veresneva'

RT, min	Compound name	Area, %	Formula	MW	Class of compounds
2.751	alpha-Ketostearic acid	0.40	C ₁₈ H ₃₄ O ₃	298	fatty acid
2.832	pyrrolidine,1-(1,6-dioxooctadecyl)	0.18	C ₂₂ H ₄₁ NO ₂	351	pyrrolidine derivate
3.605	phenol, 2-(4-diethylaminophenyl)iminomethyl)	0.35	C ₁₇ H ₂₉ N ₂ O	288	phenolic
3.979	3H-cycloocta[c]pyran-3-one,5,6,7,8,9,10-hexahydro-4-isopropyl-phenyl	0.71	C ₂₀ H ₂₄ O ₂	296	phenolic
5.261	octadecanoic acid, 2-oxo-methyl ester	1.65	C ₁₉ H ₃₆ O ₃	312	fatty acid ester
5.463	phenol, 2-(1,1-dimethyl)-4-(1-methyl-1-phenylethyl)	0.89	C ₁₉ H ₂₄ O	268	phenolic
5.778	fumaric acid, pent-4-en-2-yl undecyl ester	2.29	C ₂₀ H ₃₄ O ₄	338	fatty acid ester
6.312	eicosane, 2-methyl	5.52	C ₂₂ H ₄₈	298	alkane derivate
6.337	octadecane, 1,1[(1-methyl-1,2-ethanediyl)bis(oxy)]bis	1.21	C ₃₀ H ₆₀ O ₂	580	alkane derivate
6.642	4(3H)-pteridinone, 2-amino-7,8-dihydro-8-methyl-6,7-diphenyl	0.15	C ₁₉ H ₁₇ N ₃ O	331	phenolic
6.819	2-propenoic acid, pentadecyl ester	63.29	C ₁₈ H ₃₄ O ₂	282	fatty acid ester
7.076	5-octadecenal	0.42	C ₁₈ H ₃₄ O	266	aldehyde
7.328	2-methyltetracosane	1.32	C ₂₅ H ₅₂	352	alkane derivate
7.452	d-mannitol-1-decylsulfonyl	1.05	C ₁₆ H ₃₄ O ₇ S	370	carbohydrate deriv.
7.523	e-3-pentadecen-2-ol	1.24	C ₁₅ H ₃₀ O	226	sesquiterpenoid
7.539	3,7,11,15-tetramethyl-2-hexadecen-1-ol (syn. phytol)	1.65	C ₂₀ H ₄₄ O	296	diterpenoid
7.652	z,z-3,13-octadecadien-1-ol	1.03	C ₁₈ H ₃₄ O	266	alcohol
8.021	9-octadecenoic acid (Z), phenylmethyl ester	2.49	C ₂₅ H ₄₀ O ₂	372	fatty acid ester
8.163	dibutyl phthalate	2.99	C ₁₆ H ₂₂ O ₄	278	fatty acid ester
8.358	heptadecanoic acid, heptadecyl ester	4.13	C ₃₄ H ₆₈ O ₂	508	fatty acid ester
8.550	1,4-benzenediol, 2,5-bis(1,1-dimethylethyl)	1.01	C ₁₄ H ₂₂ O ₂	222	benzene derivate
8.521	7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-dien-2,8-dione	4.51	C ₁₇ H ₂₄ O ₃	276	diketone derivate
8.965	heptacosane	1.55	C ₂₇ H ₅₆	380	alkane

Note: RT – retention time; MW – molecular weight; data on compounds content are expressed as peak area (% of total).

Table 6

Chemical constituents of isopropanol extracts of the fruits of *A. polygama*

RT, min	Compound name	Area, %	Formula	MW	Class
2.066	4-aminocyclohexanone, N-acetyl	1.26	C ₈ H ₁₃ NO ₂	155	ketone derivate
2.256	furan, 2,5-dihydro, 2,5-dimethyl	0.13	C ₈ H ₁₀ O	98	furan derivate
2.277	d-lyxo-d-manno-nononic-1,4-lactone	0.28	C ₉ H ₁₆ O ₉	268	carbohydrate deriv.
2.365	butanoic acid, 3-hydroxy-3-methyl	0.24	C ₈ H ₁₀ O ₃	118	carboxylic acid
2.741	1-pentanol, 2,3-dimethyl	0.18	C ₇ H ₁₆ O	116	alcohol derivate
2.790	2-formyl-9-[beta-d-ribofuranosyl]hypoxanthine	0.16	C ₁₁ H ₁₂ N ₄ O ₆	296	carbohydrate deriv.
3.047	cyclohexanone, 3-hydroxy	0.44	C ₆ H ₁₀ O ₂	114	ketone derivate
3.622	L-gala-1-ido-octose	0.25	C ₈ H ₁₆ O ₈	240	carbohydrate deriv.
3.638	1-t-butyl-4-(adamanty-1)benzene	0.10	C ₂₀ H ₂₈	268	benzene derivate
3.964	carbonic acid, decyl vinyl ester	0.50	C ₁₃ H ₂₄ O ₈	228	fatty acid ester
3.973	octadecane, 9-ethyl-9-heptyl	0.31	C ₂₇ H ₅₆	380	alkane derivate
4.311	5-hydroxymethylfurfural	1.94	C ₆ H ₆ O ₃	126	aldehyde derivate
4.449	2-heptenal, 2-methyl	0.60	C ₈ H ₁₄ O	140	aldehyde derivate
4.563	4-isopropyl-1-phenyl-5,6,7,8,9,10-hexahydro-3H-cycloocta[c]-pyran-3-one	0.50	C ₂₀ H ₂₄ O ₂	296	phenolic
4.662	3,4-anhydro-d-galactosane	0.24	C ₈ H ₁₆ O ₄	144	carbohydrate deriv.
5.266	octadecanoic acid, 2-oxo-methyl ester	2.55	C ₁₉ H ₃₆ O ₃	312	fatty acid ester
5.463	phenol, 2-(1,1-dimethyl)-4-(1-methyl-1-phenylethyl)	0.62	C ₁₉ H ₂₄ O	268	phenolic
5.496	alpha-ketostearic acid, ethyl ester	3.31	C ₂₀ H ₃₈ O ₃	326	fatty acid ester
5.773	1-hexadecanol	0.27	C ₁₆ H ₃₄ O	242	alcohol
6.065	melezitose	0.36	C ₁₈ H ₃₂ O ₁₆	504	trisaccharide
6.318	octadecane, 1,1[(1-methyl-1,2-ethanediyl)bis(oxy)]bis	0.91	C ₃₀ H ₆₀ O ₂	580	alkane derivate
6.336	eicosane, 2-methyl	0.91	C ₁₈ H ₃₄ O ₂	298	alkane derivate
6.567	d-glucitol, 2,5-anhydro-1-0-octyl	0.11	C ₁₄ H ₂₈ O ₅	276	carbohydrate deriv.
6.813	2-propenoic acid, pentadecyl ester	36.78	C ₁₈ H ₃₄ O ₂	282	fatty acid ester
7.091	e-3-pentadecen-2-ol	0.12	C ₁₅ H ₃₀ O	226	alcohol
7.305	ethanol-2-(octadecyloxy)	1.17	C ₃₀ H ₆₂ O ₂	314	alcohol derivate
7.321	2-methyltetracosane	0.66	C ₂₅ H ₅₂	352	alkane derivate
7.668	z,z-3,13-octadecadien-1-ol	4.21	C ₁₈ H ₃₄ O	266	alcohol
8.014	9-octadecenoic acid (Z), phenylmethyl ester	1.15	C ₂₅ H ₄₀ O ₂	372	fatty acid ester
8.163	dibutyl phthalate	3.53	C ₁₆ H ₂₂ O ₄	278	fatty acid ester
8.212	docosanoic acid, docosyl ester (syn. behenyl behenoate)	0.64	C ₄₄ H ₈₈ O ₂	648	fatty acid ester
8.342	estra-1,3,5-(10)trien-17.beta-ol (syn. estradiol, 3-deoxy)	2.35	C ₁₈ H ₂₄ O	256	steroid
8.516	7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-dien-2,8-dione	6.02	C ₁₇ H ₂₄ O ₃	276	diketone derivate
8.611	9,9-dimethoxybicyclo(3.3.1)nona-2,4-dione	1.02	C ₁₁ H ₁₆ O ₄	212	diketone derivate
8.781	ethyl-isallocholate	2.82	C ₂₆ H ₄₄ O ₅	436	steroid
12.208	squalene	23.42	C ₃₀ H ₅₀	410	triterpenoid

Note: RT – retention time; MW – molecular weight; data on compounds content are expressed as peak area (% of total).

Isopropanol extracts from *A. polygama* fresh whole fruits assayed by GC-MS analysis showed the presence of 36 identified constituents, which accounted for 98.4% of the separated compounds total amount (Table 6).

Fatty acids esters having both even and odd carbon atoms in the range C₁₆–C₃₄ represented the most abundant class of phytochemical compounds in the isopropanol extracts from *A. polygama* fruits (48.5% of total). The next most abundant classes were terpenoids (23.4%), ketones and their derivatives (8.7%), alcohols and their derivatives (6.0%), alkanes and their derivatives (2.8%, in the range C₁₈–C₃₉), aldehydes (2.5% of total), carbohydrates (1.4%), and phenolic compounds (1.1% of total). The main constituents of *A. polygama* fruit extracts were 2-propenoic acid, pentadecyl ester (36.8% of total), squalene (23.4%), 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-dien-2,8-dione (6.0%), z,z-3,13-octadecadien-1-ol (4.2%), dibutyl phthalate (3.5%), alpha-ketostearic acid, ethyl ester (3.3%), ethyl-isoallochololate (2.8%), estradiol, 3-deoxy (2.4%), 5-hydroxymethylfurfural (1.9%), octadecanoic acid, and 2-oxo-methyl ester (1.7% of total).

Discussion



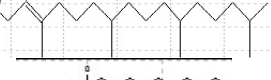

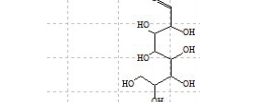
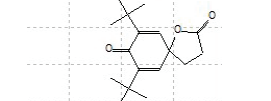
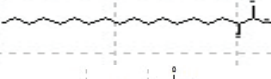
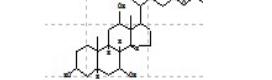
The results of the study revealed moderate to high phenolic compound accumulation and antioxidant activity in the leaf and fruit extracts of both *A. arguta* cv. 'Veresneva' and *A. polygama*. The data obtained are consistent with the reported level of phenolic compound in fruits of *Actinidia* spp. which are grown in other environmental conditions. As for the natural range of *Actinidia* plants, Wang et al. (2018b) showed that fruit total phenolic content varies significantly among different *Actinidia* genotypes grown in China, ranging from 78.17 to 461.12 mg GAE per 100 g FW. Of these, two different *A. arguta* genotypes exhibited 315.51 and 330.69 mg GAE·100 g⁻¹ FW total phenolic content, while total flavonoid content was 169.61 and 181.99 mg catechin equivalents (CE) 100 g⁻¹ FW respectively. An et al. (2016) reported that kiwi berry (*A. arguta*) contains

118.2–191.6 mg GAE/100 g FW of phenolic compounds, including 28.8–40.4 mg CE /100 g FW of flavonoids. In the fruit extracts of *A. kolomieta*, *A. arguta* and *A. chinensis* grown in China, Zuo et al. (2012) determined the total phenolic content as 430.03, 362.18, and 115.76 mg GAE/100 g FW, respectively; total flavonoid content was 68.05, 188.43, and 67.63 mg CE/100 g FW, respectively. According to the data about eight kiwi berry varieties cultivated in China (Zhang et al., 2021), total phenolic content ranged from 223.09 mg to 451.16 mg GAE/100 g FW; total flavonoid content of eight kiwi berry varieties ranged from 49.52 to 74.35 mg CE/100 g FW.

The kiwi berry (*A. arguta*) cultivated in Japan was reported (Mikami-Konishide et al., 2013) as having phenolic content of up to 426 mg GAE/100 g FW. The fruits of different *Actinidia* species grown in Korea (Lee et al., 2015) contained 775.3 mg GAE per 100 g FW of the total phenolic and 13.1 mg catechin equivalents per 100 g FW of flavonoid compounds. In the kiwifruit grown in India, Pal et al. (2015) revealed a decrease in total phenolic content (from 215.0 to 84.0 mg GAE/100 g FW), but an increase in total flavonoid content (from 23.45 to 32.54 mg CE/100 g FW) during ripening. In accordance with the average assessment, the total content of polyphenols in the fruit of *A. arguta* grown in Poland can be as high as 360 mg GAE/100 g fresh weight (Baranowska-Wójcik & Sz wajgier, 2019). At the same time, Wojdyło and Nowicka (2019) found in dried *A. arguta* fruits only 845.54 mg/100 g of total polyphenols and 29.63 mg/100 g of phenolic acids, which is lower than what we have shown for fresh fruit. The above comparative data confirms the rather rich content of metabolites and antioxidant activity level achieved by *Actinidia* species introduced in the steppe climate. The high amount of the phenolic metabolites found in the fruits of *A. arguta* cv. 'Veresneva' is consistent with the definition of the kiwi berry (Latocha, 2017; Wang et al., 2018b) as 'health food' or 'superfood'.

Table 7

Bioactivity of the phytochemicals identified by GC-MS in the isopropanol fruit extracts of *Actinidia* plants

Compound name	Compound structure	Bioactivity	Reference
2-Propenoic acid, pentadecyl ester		antimicrobial and antioxidant properties	Mujeeb et al. (2014)
Squalene		antioxidant agent	Oh et al. (2021)
Phytol		anticancer, anti-inflammatory, antimicrobial, diuretic effects	Bharathy et al. (2012)
d-Mannitol-1-decylsulfonyl		anticancer, antimicrobial activity. Non-steroidal inhibitor of cyclooxygenase-2 and potential anti-inflammatory drug	Muthukrishnan & Thinakaran, (2012); Dr. Duke's Phytochemical and Ethnobotanical Databases, 1992–2016
L-Gala-1-ido-octose		important compound for production of memory drugs, preventing the cognitive deficits associated with dementias	Jun et al. (2015)
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-dien-2,8-dione		antioxidant agent	Merlin et al. (2009)
Octadecanoic acid, 2-oxo-methyl ester		antimicrobial activity	Kannan & Kannan (2019)
Ethyl-isoallochololate		anti-inflammatory activity	Sosa et al. (2016)

In our work, sufficient differences were found between the fruit peel and pulp in terms of the phenolic compound content and antioxidant ability with the predominance of most indicators in the peel. This pattern seems to be general, and has been noted in many studies. In eight typical varieties of kiwi fruit in China, Wang et al. (2018a) revealed higher polyphenol content and the antioxidant activity in fruit peel compared to flesh and seeds. The significantly greater abundance in polyphenols and flavo-

noids, and the antioxidant and antibacterial activity was also observed by Alim et al. (2019) in kiwifruit (*A. chinensis*) peel when compared to the flesh. Dias et al. (2020) showed higher antioxidant activity as well as cytotoxicity and anti-inflammatory activity of the extracts from *A. deliciosa* fruit peel than that of pulp. In general, fresh peel of the kiwifruit has a wide range of compounds leading to distinct flavours in the fruit (Atkinson & Macrae, 2007); in particular, the greater amount of polyphenols

determines the stronger astringency of kiwifruit peel taste (Kim et al., 2009). Composition of the chemical compounds identified in *A. arguta* cv. 'Veresneva' and *A. polygama* fruit extracts by GC-MS analysis impresses with its diversity. The main compound classes of both *Actinidia* fruits represented by esters, alcohols, and aldehydes, coincide with the dominant components of eating-ripe *A. chinensis* fruit (Wang et al., 2011) and the most abundant volatile organic compounds from *A. kolomikta* fruits (Cesoniene et al., 2020). The main constituents of both *A. arguta* and *A. polygama* fruit extracts (first of all, 2-propenoic acid, pentadecyl ester) were the compounds which have known biological activities, including health-promoting properties (Table 7).

The notable amount of terpenoids in both studied fruits looks like a general property of *Actinidia* plants, because researchers (Xu et al., 2010; Wei et al., 2018) reported the isolation of different new triterpenoids from the roots of *A. chinensis*, which showed positive cytotoxic activity against cancer cell lines.

Antibacterial activity developed by both fruit and leaf extracts of *A. arguta* cv. 'Veresneva' and *A. polygama* against Gram-positive and Gram-negative strains confirmed the known high bioactivity of *Actinidia* plants. Extract from *A. chinensis* seeds achieved better antibacterial activities against Gram-positive than Gram-negative bacteria, including *E. coli* strains (Deng et al., 2013). Fruit peel extracts of *A. deliciosa* showed activity against the bacterial (*B. subtilis*, *S. aureus*, *E. coli*, and *P. aeruginosa*) and fungal (*A. fluves*, *S. cerevisiae*, and *C. albicans*) strains (Salama et al., 2018). Notable inhibition of clinical bacterial strains having resistance to ofloxacin demonstrated by the crude extracts of *A. arguta* (cv. 'Veresneva') and *A. polygama* indicated the high antimicrobial activity and health-promoting ability of the introduced plants.

Conclusion

Leaves and fruits of *A. arguta* cv. 'Veresneva' and *A. polygama* plants introduced in the steppe zone accumulated high content of polyphenols, flavonoids and free phenolic acids and showed notable antimicrobial activity. Antioxidant potential and phenolic compound content in the fruit peel of both species were higher when compared to pulp, while corresponding indices of leaves exceeded those of the fruit (excepting the level of reducing power). High amounts of 2-propenoic acid, pentadecyl ester, squalene, 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-dien-2,8-dione, octadecanoic acid, 2-oxo-methyl ester, ethyl-isoallocholate, phytol, and some phenolic compounds which also have well-known bioactivities were identified in the fruit extracts by GC-MS assays. Antibacterial effects of *A. arguta* and *A. polygama* fruit extracts were revealed against both collection and clinical pathogenic strains including those resistant to ofloxacin action. The results of our research confirmed that fruits and leaves of *A. arguta* cv. 'Veresneva' and *A. polygama* are a potential source of natural biological active compounds with health-promoting abilities, indicating the preservation of useful properties by the introduced plants and the expedience of their cultivation in a steppe climate.

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