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Phytochemicals and Antimicrobial Potential of Dry Ethanol Extracts from Ailanthus altissima – An Invasive Plant Species for the Bulgarian Flora

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Abstract. Dry ethanol extracts of flowers, leaves, and stem bark of *Ailanthus altissima* were subject to the determination of the chemical components by GC/MS analysis. Fresh plant material was used for obtaining the plant extracts (*in vacuo)*. Forty-seven compounds were identified from different plant parts. The oxygenated aliphatics were the best represented group in the extracts (flower – 55.44%, leaf – 51.57%, bark – 33.19%), followed by oxygenated monoterpenes (bark – 25.42%; leaf – 15.87%), and diterpenes (flower 14.22%). The major constituent in the flower and leaf extracts was (3Z)-hexenyl hexanoate (28.59%, 12.61%, respectively), while in the bark extract - *α*-terpinyl acetate (15.55%). The distribution by functional groups, in relation to the total oxygen-containing components, pointed out that the esters were predominated in the three extracts analyzed, followed by alcohols and acids. The EtOH extracts of *A. altissima* were studied for *in vitro* antimicrobial activity against gram-positive and gram-negative bacterial strains. The study results showed that the leaf and bark extracts of *A. altissima* inhibited the growth of six of the tested pathogenic strains. The largest zones of inhibition were measured of ethanol leaf extracts against *Bacillus subtilis* ATCC 6633 (24.00 ± 0.11 mm) and *Klebsiella* (clinical isolate – 23.00 ± 1.24 mm). To the best of our knowledge, this is the first study that investigates the phytochemical composition and the antimicrobial activity of the extracts obtained from *Ailanthus altissima* from Bulgaria. Furthermore, our results revealed that the leaf and bark ethanol extracts could be used as natural antimicrobial agents.

Key words: *Ailanthus altissima,* aerial parts, ethanol dry extract, GC/MS, antimicrobial activity, Simaroubaceae.

Introduction

Ailanthus altissima (Mill.) Swingle (Simaroubaceae) is a tree from the Far East, introduced in Europe in the 18th century for decorative purposes and now it is found on all continents except Antarctica (Kowarik &

Säumel, 2007; Sladonja et al., 2015). Today it is considered one of the worst invasive plant species, both in Europe and in various regions of the world. For Bulgaria, the species is in the "top 10" among the most problematic alien plant species (Petrova et al., 2012).

On the other hand, it has been found that the tree contains several valuable phytochemicals such as alkaloids, flavonoids, quassinoids, phenylpropanoids, triterpenoids, volatile oils, sterols and others, which are responsible for the many proven biological activities - antimicrobial, antifungal, antiviral, antitumor, herbicidal, anti-inflammatory, and others (De Martino & De Feo, 2008; Kundu & Laskar, 2010; Albouchi et al., 2013; Kožuharova et al., 2014; Sladonja et al., 2015; Li et al., 2021). The subject of research is different plant parts of *A. altissima* (mostly bark, leaves, fruits), with various extraction methods and identification of the chemical components. All this is the reason for the differences in the quantitative and qualitative composition of the studied plant products - extracts and essential oils (Andonova et al., 2021; Hadadi et al., 2020; Chouhan et al., 2017).

There are many studies on the biological activity of *A. altissima* extracts, but data for component determination by gas chromatography-mass spectrometry analysis are scarce. Caboni et al. (2012) identified a total of 14 components of wood, leaf, bark, and root methanol extracts, and they found that unsaturated aldehydes are responsible for the nematicide activity. Fewer components (twenty-seven) in leaf ethanol extracts of *A. altissima* determined Lungu et al. (2016) when studying their antioxidant and phytotoxic activity. Forty-one chemical components in fruit methanol extracts and thirty-five components from fresh leaves of *A. altissima* were identified by Panasenko et al. (2020). They point out the presence of compounds with antioxidant and anti-inflammatory activities, such as α-tocopherol in fruits.

Increasing microbial resistance to drugs due to excessive use of antibiotics, and on the other hand, the negative perception of synthetic preservatives increase the interest in the search for new natural alternatives (Swamy et al., 2016; Chouhan et al., 2017). Many studies have focused on the antibacterial properties of plant extracts and their possible use as reliable inhibitors of bacterial and fungal growth (Zazharskyi et al., 2020). Aissani et al. (2018) prove the antimicrobial activity of *A. altissima* wood aqueous extract against *Pseudomonas aeruginosa* and conclude that this extract can be used as a natural antimicrobial agent. Other authors report that the methanol extracts of *A. altissima* leaves and their hydro-distilled residues are efficient against Gram-positive bacteria, but not active against Gram-negative bacterial strains and the yeast *Candida albicans* (Albouchi et al., 2013). The composition of ethanol fruit extracts (*in vacuo*) of *A. altissima* and their antimicrobial activity was investigated by Zhao et al. (2005) and they found that individual ingredients (stigmasterols) showed moderate activity against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Salmonella typhimurium*. The N-butanol fraction of methanol fruit extract is well active against *P. aeruginosa* and *S. typhi* and moderately active against the fungus *Microsporum canis* (Khan et al., 2016). Significant inhibitory activity against *E. coli* and *S. aureus* is demonstrated in the green synthesis of zinc oxide and copper oxide nanoparticles with water extracts of *A. altissima* fruit and leaves (Awwad & Amer, 2020; Awwad et al., 2020).

In Bulgaria, the first chemical investigations on *A. altissima* species belong of our team. They are related to the carotenoid content of stem bark (Zhelev et al., 2016) and on the essential oil composition of its various plant parts (Andonova et al., 2021). The results of our previous research necessitated our interest in investigating other possible sources of

phytochemicals of this plant species. Therefore, the aim of the present study was to evaluate the chemical composition of dry ethanol extracts obtained from the bark, leaves, and flowers of *A. altissima*, and their antimicrobial activity against human pathogenic strains.

Materials and Methods

Plant material and obtaining the plant extracts

Flowers, leaves, and stem bark of *A. altissima* were collected from the city of Plovdiv, Bulgaria, in June-July 2020, and were identified by Prof. Ivanka Dimitrova-Dyulgerova at the Department of Botany, University of Plovdiv "Paisii Hilendarski" (Fig.1). Fresh plant parts (100 g flowers, leaves, and bark) were grounded and soaked in an Erlenmeyer flask with 1l (96%) ethanol (1:10 w/v), to complete exhaustion of the herb for 10 days with intermittent stirring. The extracts were filtered using Whatman filter paper no.1, and the filtrates were evaporated under reduced pressure and dried using a rotary evaporator (Buchi, Rotavapor R-300) at 50°C. All plant samples were stored in the dark at 4°C before analysis. For sample preparations for microbiological analysis, 2 g of each extract was dissolved in distilled water in a ratio of 1:2.

(a) (b) (c)

Fig. 1. Analyzed plant parts of *Ailanthus altissima*:
(a) - flowers, (b) - leaves, and (c) - stem bark (the photos taken by the authors).

Gas Chromatography/Mass Spectrometry (GC/MS) and GC-FID analyses

Extract of 10.0 mg, dissolved in 1.0 ml absolute alcohol, was injected into a gas chromatograph Agilent GC 7890A and in a mass spectral detector Agilent MD 5975C, column HP-5MS with parameters: length – 30 m, diameter - 0.32 mm, and film-coating thickness - 0.25 µm. The temperature program was as follows: initial temperature – 100°C, 2 min retention, increase to 180°C with 15°C/min, 1min retention, increase to 300°C with 5°C/min, 10min retention; injector and detector temperatures – 250°C. Helium was used as a carrier gas, at flow speed - 1.0 ml/min; mass-detector scan range - m/z=50-550; injected sample volume - 1 µL in flow split ratio 20:1. The compounds were identified by comparing retention times and relative Kovats (RI) indices with those of standard substances and mass spectral data from The Golm Metabolome Database - GMD (Hummel et al., 2010) and NIST'08 - National Institute of Standards and Technology, USA.

Antibacterial activity assay

Test microorganisms

Strains of pathogenic bacteria that are reported as causing infections, toxic infections, and toxicosis were used. The strains were supplied by the National Bank for Industrial Microorganisms and Cell Cultures (NBIMCC). The following Gram-positive bacteria were used: *Listeria*

monocytogenes NCTC 11994, *Staphylococcus aureus* ATCC 25093, *Bacillus subtilis* ATCC 6633, *Bacillus cereus*; and the Gram-negative bacteria - *Escherichia coli* ATCC 8739, *Salmonella enterica subsp. Enterica serovar abony* NCTC 6017, *Pseudomonas aeruginosa* ATCC 6027, *Proteus vulgaris* ATCC 6380, *and Klebsiella* (clinical isolate).

Determination of the titer of microbial suspensions

From two consecutive tenfold dilutions of the suspensions in saline, surface sowing was done on the relevant selective media for the test cultures - 1 mL of an appropriate dilution of each suspension was evenly distributed in four plates with previously poured and dried media. The titer of the suspension was determined by the formula:

$N = \Sigma C / V \times (n1 + 0,1 \times n2) \times d$, cfu / cm^3 (1)

where:

V – sowing material volume; ΣC – sum of the listed colonies;

n1 – number of plates from the first dilution in which colonies are listed;

n2 – number of plates from the second dilution in which colonies are listed;

d – dilution factor corresponding to the first dilution in which colonies are listed.

The antibacterial activity was determined by a modification of the agar diffusion method, through measuring the zones of pathogen growth inhibition around metal rings, in which a certain amount of the tested material was imported. Selective media for the test cultures were inoculated with pathogen suspensions prepared from a 24-hour culture on oblique PCA. From a suitable tenfold dilution of the suspension, the molten and cooled to 45 - 50°C selective media were inoculated. The current concentration of the cells in the culture medium was equated to the concentration of the dilution suspension because 1 mL of suspension was inoculated into 99 mL of medium. After solidification of the media, sterilized metal rings of diameter Ø = 6 mm were placed on their surfaces, in which 0,10 and 0,15 µL of filtrate from the extract were imported, respectively. The test cultures were incubated at 37°C. The diameter (cm) of the growth inhibition zones of the test cultures was measured at the 24^{th} and 48^{th} hours, and a comparative assessment of their antibacterial activity was made. The final DMSO content was 5% (v/v), and this solution was used as a negative control. Chlorhexidine (100 µL) was used as a positive control.

Results and Discussion

Chemical composition of ethanol extracts from aerial parts of Ailanthus altissima

The extracts' yields are as follows: bark - 0.608 g, leaf – 0.630 g, flower – 0.573 g. They are viscous liquids of dark-brown and dark-green color, with a specific odor. A total of 47 components were identified in the three extracts tested (Table 1) and their chemical composition is as follows:

In flower extract, 31 components were identified (representing 99.27% of the total content). Twenty-one of them were in a concentration above 1%, and the other ten – were less than 1%. The major compounds (over 3%) were: (3Z)-hexenyl hexanoate (28.59%), oleic acid (11.04%), dihydro-eudesmol (6.66%), (3Z)-hexenyl butanoate (5.46%), phytol (5.23%), phenyl ethyl 2-methylbutanoate (4.42%), (6E,10E)-pseudo phytol (3.25%) and linoleic acid (3.09%).

There is a lack of literature data on the composition of plant's flower extracts. A parallel could be drawn with our previous study on volatiles in *A. altissima* flowers (Andonova et al., 2021), in which 44 components were identified, 8 of them were major compounds (over 3%): *β*-caryophyllene (16.98%), germacrene D (16.24%), n-tricosane (8.33%), methyl hexadecanoate (7.87%), linoleic acid (6.84%), *α*-humulene (5.30%), n-pentacosane (3.70%), and linolenic acid (3.38%). As can be seen, only linoleic acid is among the main constituents of the essential oil and the ethanol extract.

In leaf extract, 31 components were identified, which represents 98.45% of the total composition. Twenty-two of them were in a concentration above 1%, and the other nine – were less than 1%. The main components in the extract (over 3%) were: (3Z)-hexenyl hexanoate (12.61 %), isopropyl hexadecanoate (10.77%), lavandulyl acetate (9.65%), (3Z)-hexenyl 2-methyl butanoate (8.05%), β-caryophyllene (5.88%), dihydro-eudesmol (5.57%), phenyl ethyl butanoate(5.20%), (2E,4E)-nonadienol (5.07%), linoleic acid (4.61%), and palmitic acid (4.39%). Lungu et al. (2016) identify 27 components in 70% ethanol extracts of dry grounded leaves of *A. altissima*, growing spontaneously in Romania (obtained by the reflux and ultrasound methods), among which are: saturated fatty acids, sterols, vitamin E, neophytadiene, phytol, and others. In methanol leaf extract, Caboni et al. (2012) identify four plant metabolites: acetic acid, 5-hydroxymethylfurfural, nonanal and hexanoic acid. Panasenko et al. (2020) found a greater variety of phytochemicals in *A. altissima* methanol leaf extracts from Ukraine (35 biologically active compounds), as eleven of them – were over 3%, and the main ones among them were phytol – 21.15%, hexadecanoic acid – 8.53%, α-tokospiro A – 8.14%, 2-C-methyl-myo-inositol – 7.78%. In comparison with our results, the difference in the main components is obvious (only palmitic acid is present in both types of extracts). Our previous GC/MS analysis (Andonova et al., 2021) on the essential oil of *A. altissima* leaves identify 41 components with fatty acids predominating (oleic acid is the best-represented compound – 22.94%).

In stem bark extract, 42 components were identified, which represents 98.76% of the total composition. Twenty-two of them were found to be in a concentration above 1%, and the other twenty – less than 1%. The main components in the extract (over 3%) were: α-terpinyl acetate (15.55%), oleic acid (9.99%), tetracosane (9.04%), linoleic acid (8.85%), α-curcumene (5.75%), palmitic acid (3.71%), nerol (3.42%), β-eudesmol (3.36%), and phenyl ethyl butanoate (3.02%). Caboni et al. (2012) determined in methanol bark extract by GC/MS-analysis only three metabolites: acetic acid, nonanal and hexanoic acid. Andonova et al. (2021) found 41 chemicals in stem bark essential oil, among which five are the major compounds - oleic acid (30.21%), β-caryophyllene (6.7%), γ-cadinene (5.94%), palmitaldehyde (5.87%) and palmitic acid (4.31%).

The distribution of the components by chemical groups (Fig. 2) showed that aliphatic oxygen derivatives predominated in the three extracts, followed by oxygenated monoterpenes (in bark and leaves), diterpenes (in flowers), sesquiterpene hydrocarbons (in bark), and the other groups were less represented and their distribution can be seen in Fig. 2.

Oxygenated aliphatics also predominated in the essential oils of leaves and bark, while sesquiterpene hydrocarbons predominated in the essential oil of flowers (Andonova et al., 2021).

The distribution of the components by functional groups in the ethanol extracts showed the highest content of esters (33.83% in bark – 61.33% in leaves), followed by alcohols (14.88% in leaves – 24.89% in flowers), acids, and hydrocarbons (Fig. 3). Aldehydes and ketones were poorly represented, lactones were present only in bark extracts, and phenols were not identified in the extracts.

The differences between the quantities of the identified components in the extracts and the literature data are due to the plant's growing conditions, the technological parameters of the extraction, and its analysis.

Antibacterial activity of Ailanthus altissima ethanol extracts

The zones of growth inhibition of the test cultures are presented in Table. 2 and Fig. 4. The study results showed that *A. altissima* leaves had the most substantial inhibitory effect against *Bacillus subtilis* ATCC 6633 – 24.00 ± 0.11 mm and *Klebsiella* (clinical isolate) – 23.00 ± 1.24 mm.

Table 1. Chemical composition of the ethanol extracts of *Ailanthus altissima* aerial parts. *Legend:* RT - Retention Time; RI - Kovats Retention Index, calculated by authors; TIC - Total Ion Current; nd - not detected.

Peak	RT	RI	Compound	*Ailanthus altissima*, % of TIC		
				flower	leaf	stem bark
1	4.49	1174	Furfuryl butanoate	nd	1.28	nd
2	4.68	1190	(3Z)-Hexenyl butanoate	5.46	0.34	0.68
3	5.20	1218	(2E,4E)-Nonadienol	nd	5.07	0.39
4	5.30	1233	Nerol	nd	0.55	3.42
5	5.41	1231	(3Z)-Hexenyl 2-methyl butanoate	0.99	8.05	0.56
6	5.60	1288	Lavandulyl acetate	0.78	9.65	1.29
7	6.11	1316	δ-Terpinyl acetate	nd	1.04	0.82
8	6.23	1345	α-Terpinyl acetate	1.17	2.49	15.55
9	6.55	1366	α-Methyl benzyl butyrate	2.02	0.73	0.96
10	6.64	1375	(3Z)-Hexenyl hexanoate	28.59	12.61	1.42
11	6.82	1386	Isobutyl phenylacetate	1.10	2.35	0.73
12	7.01	1401	n-Tetradecane	0.48	2.10	1.85
13	7.08	1416	β-Caryophyllene	1.23	5.88	1.91
14	7.16	1439	Phenyl ethyl butanoate	1.79	5.20	3.02
15	7.26	1453	Geranyl acetone	0.74	1.28	1.37
16	7.41	1465	Linalool isovalerate	nd	nd	1.84
17	7.55	1476	α-Curcumene	0.55	2.27	5.75
18	7.64	1481	γ-Curcumene	0.48	0.89	0.42
19	7.72	1486	Phenyl ethyl 2-methylbutanoate	4,42	1.50	nd
20	7.76	1488	β-Selinene	nd	nd	2.86
21	7.97	1497	α-Selinene	nd	nd	1.77
22	8.15	1511	β-Curcumene	1.07	0.21	0.49
23	8.26	1556	Lauric acid	nd	nd	1.05
24	8.41	1562	Geranyl butanoate	nd	nd	0.56
25	8.50	1568	Octyl hexanoate	2.73	1.64	0.42
26	8.56	1576	Decyl butyrate	0.20	0.58	0.34
27	8.71	1611	Tetradecanal	1.54	1.43	0.77
28	9.07	1640	Phenyl ethyl hexanoate	2.59	0.67	0.91
29	9.17	1649	β-Eudesmol	1.92	1.32	3.36
30	9.26	1654	α-Eudesmol	2.01	2.50	1.43
31	9.37	1662	dihydro-Eudesmol	6.66	5.57	2.70
32	9.61	1671	epi-β-Bisabolol	nd	nd	1.11
33	9.67	1675	β-Bisabolol	nd	nd	0.69
34	9.86	1690	(Z)-α-trans-Bergamotol	nd	nd	0.86
35	10.21	1699	(2Z,6Z)-Farnesol	nd	nd	0.74
36	10.50	1706	δ-Dodecalactone	nd	nd	0.82
37	10.82	1718	Methyl eudesmate	0.33	0.87	0.28
38	11.93	1826	(E)-Nerolidyl isobutyrate	0.17	0.61	0.25
39	14.49	1953	Phytol	5.23	nd	nd
40	14.61	1971	Palmitic acid	1.39	4.39	3.71
41	14.73	1992	Ethyl palmitate	nd	nd	2.80
42	16.67	2018	(6E,10Z)-Pseudo phytol	5.64	nd	nd

43	16.85	2026	Isopropyl hexadecanoate	nd	10.77	0.98
44	16.94	2055	(6E,10E)-Pseudo phytol	3.25	nd	nd
45	18.05	2133	Linoleic acid	3.09	4.61	8.85
46	18.28	2142	Oleic acid	11.04	nd	9.99
47	21.58	2400	Tetracosane	0.61	nd	9.04
			Total identified,%	**99.27**	**98.45**	**98.76**

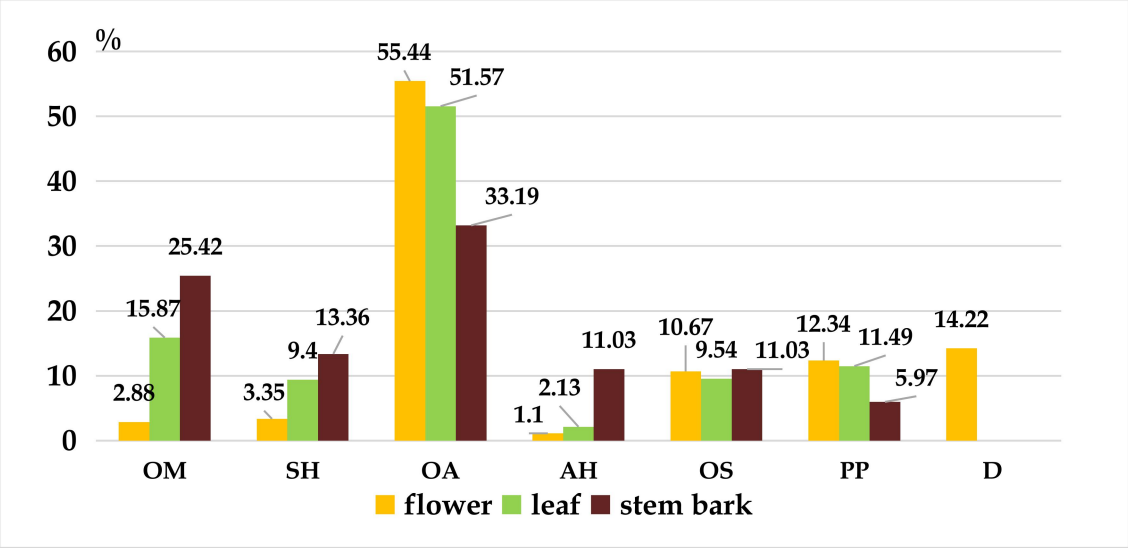

Fig. 2. Composition by chemical groups of ethanol extracts, obtained from fresh aerial parts of *Ailanthus altissima* (%). *Legend:* OM – Oxygenated monoterpenes; SH - Sesquiterpene hydrocarbons; OA - Oxygenated aliphatics; AH - Aliphatic hydrocarbons; OS - oxygenated sesquiterpenes; PP - Phenyl propanoids; D – Diterpenes.

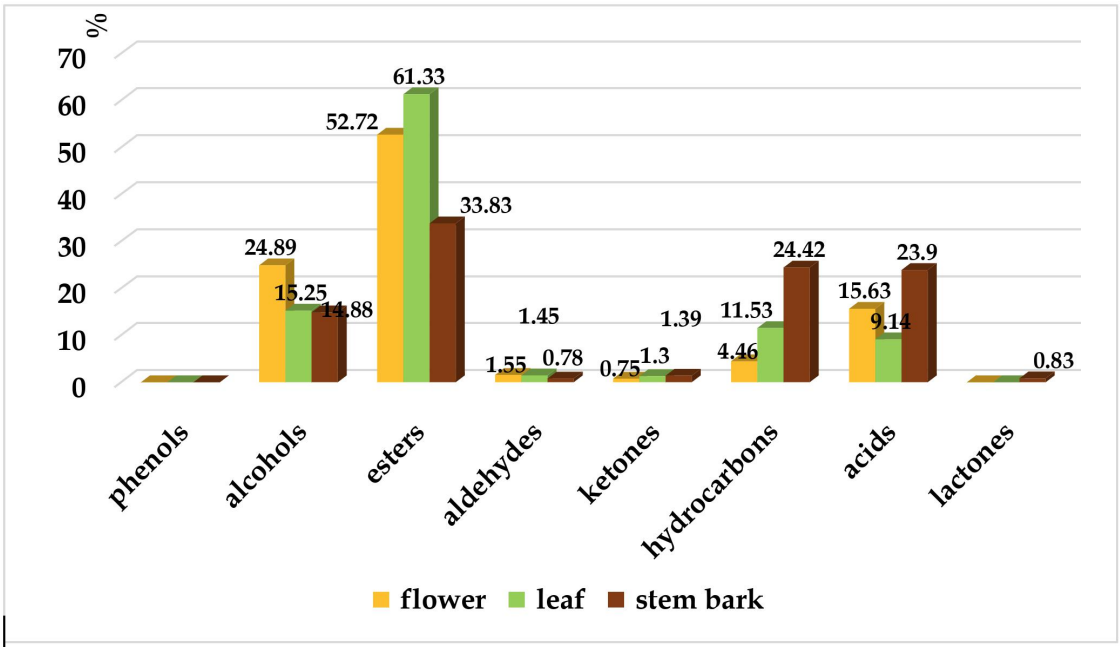

Fig. 3. Composition by functional groups of ethanol extracts, obtained from fresh aerial parts of *Ailanthus altissima* (%).

The most susceptible to the effect of the *A. altissima* leaves was *B. subtilis* which was still lower than that of the positive control (39.00 ± 0.12). The inhibitory activity of the plant's leaf extract against *Klebsiella* was determined as closest, even higher than that of the positive control sample (21.00 ± 0.81). It is known that Gram (+) bacteria are generally more sensitive to plant extracts than Gram (-). Gram (-) bacteria have an outer membrane and a unique periplasmic space that is not found in Gram (+). Gram (-) bacteria's resistance to antibacterial substances is due to their outer membrane, rich in hydrophilic molecules, serves as a barrier to the penetration of many antibiotic molecules. Resistance also binds to enzymes in the periplasmic space that can destroy molecules that have entered from the outside. Some plant extracts do not fully follow this trend. The antimicrobial activity of plant extracts was widely studied by several authors. The methanol leaf extract and its different polar subfractions inhibited significantly the growth of gram-positive bacteria (*Listeria monocytogenes, Staphylococcus aureus, Bacillus subtilis*) and gram-negative bacteria (*Pseudomonas aeruginosa, Escherichia coli)* (Rahman et al., 2009). The methanol extracts of *A. altissima* leaves and their hydrodistilled residues were effective against Gram-positive bacteria (*S. aureus, Enterococcus feacium, Streptococcus agalactiae* and *Bacillus subtilis*). Neither the Gram-negative bacteria (*E. coli, Enterococcus feacalis* and *Salmonella typhimurium*) nor the yeast (*Candida albicans*) were inhibited by these extracts (Albouchi et al., 2013). The *A. altissima* acetone leaf extract was active against *E. coli*. Both acetone and methanol: dichloromethane extracts had higher activity against *C. albicans* than a standard drug amphotericin B (Poljuha et al., 2017). Aissani et al. (2018) studied the antimicrobial activity of aqueous and methanol extracts of wood and bark of *A. altissima* against a set of Gram-positive and Gram-negative bacteria using disc diffusion and broth microdilution methods and reported antimicrobial activity of the wood extract against *P. aeruginosa* with a MIC of 10.46 mg/mL. The volatile oil and phenolic constituents of *A. altissima* leaves were efficient against Gram-positive bacteria, but not active against Gram-negative bacterial strains and the yeast *C. albicans* (Aissani et al., 2018). The results obtained in this study might be attributable to the occurrence of some specific components such as linoleic acid, *β*-caryophyllene, germacrene D, and methyl hexadecanoate. The antimicrobial potential of ethanol leaf extracts was found by Zazharskyi et al. (2020), but the zone of inhibition of *E. coli* and *Listeria monocytogenes* is weaker compared to our results.

Table 2. Inhibition zones of the pathogenic bacteria growth, mm. *Legend:* VF- Value of the filtrate; FE - Flower extract; LE - Leaf extract; SBE - Stem bark extract; CH – Chlorhexidine; *- There was no inhibitory activity.

Test microorganism	VF	Inhibition zone/mm			
		FE	LE	SBE	CH
Gram-negative bacteria					
Escherichia coli ATCC 8739	150 µL	-*	9.00 ± 0.47	6.00 ± 0.81	28.00 ± 0.02
	100 µL	-	-	-	
Salmonella enterica NCTC 6017	150 µL	-	-	-	24.00 ± 0.09
	100 µL	-	-	-	
Klebsiella (clinical isolate)	150 µL	-	23.00 ± 1.24	-	21.00 ± 0.81
	100 µL	-	-	-	

Pseudomonas aeruginosa ATCC 6027	150 µL	-	8.00 ± 0.09	5.00±1.01	18.00 ± 0.24
	100 µL	-	5.00 ± 0.24	-	
Proteus vulgaris ATCC 6380	150 µL	-	8.00 ± 1.24	8.00±1.47	-
	100 µL	-	7.00 ± 0.12	7.00±0.24	
Gram-positive bacteria					
Staphylococcus aureus ATCC 25093	150 µL	-	-	-	25.00 ± 0.18
	100 µL	-	-	-	
Bacillus subtilis ATCC 6633	150 µL	-	24.00 ± 0.11	14.00 ± 1.24	39.00 ± 0.12
	100 µL	-	10.00 ± 0.19	10.00 ± 0.18	
Bacillus cereus	150 µL	-	-	-	-
	100 µL	-	-	-	
Listeria monocytogenes NCTC 11994	150 µL	-	-	11.00 ± 0.09	21.00 ± 0.81
	100 µL	-	-	5.00 ± 0.51	

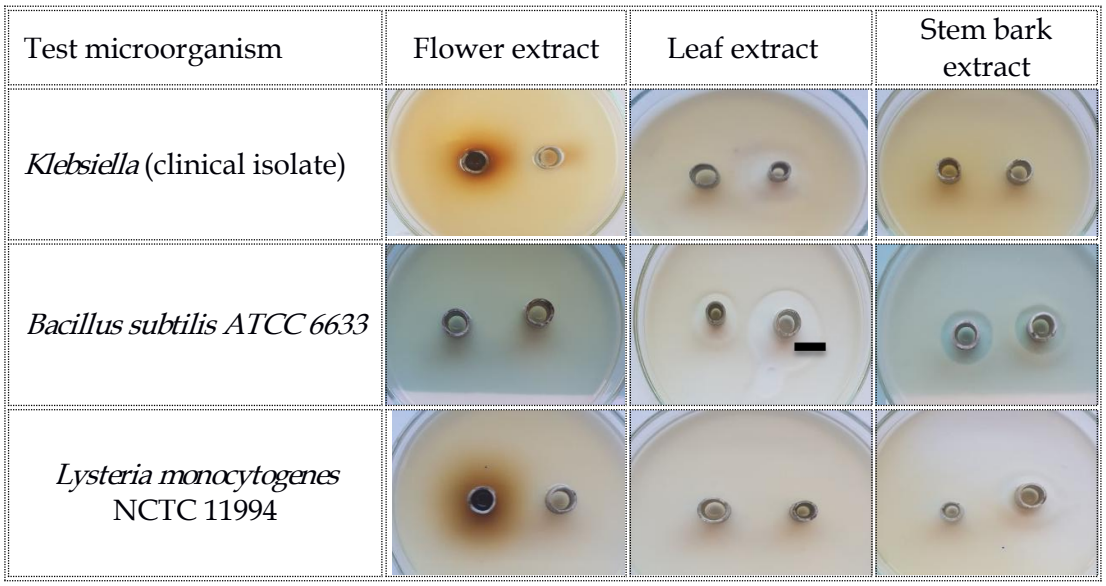

Fig. 4. Photos of the growth inhibition zones (over 10 mm) (Scale bar indicated 20 mm).

Our study confirms the antimicrobial activity of leaf and stem bark extracts. The flower extracts were not effective at all. As it is known, the activity of the main components of essential oils is in the following sequence: *phenols > alcohols > aldehydes > ketones > esters > hydrocarbons* (Gabrielli et al., 1988). In the extracts studied, the content of esters predominates, which would explain the lower antimicrobial activity against some of the strains studied.

Conclusions

Bringing clarity to the composition of the extracts of *A. altissima* is an important step for discovering new compounds into the composition of various natural products. The results obtained can add valuable information to the knowledge available about the species. In Bulgaria, the composition and antimicrobial activity of dry ethanol extracts of various aerial parts of the invasive alien tree *A. altissima* were studied for the first time. Forty-seven compounds were identified from different plant parts. The oxygenated aliphatics were the best-represented group in the extracts, followed by oxygenated monoterpenes, diterpenes, while other groups were poorly represented. Our findings show that the leaf

and bark ethanol extracts of *A. altissima* exhibited antibacterial activity (especially of ethanol leaf extract against *Bacillus subtilis* ATCC 6633 and *Klebsiella*, clinical isolate). The extracts could be used as a natural medicinal resource, which requires further research.

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