PHARMACIA

Pharmacia 67(2): 71–81
DOI 10.3897/pharmacia.67.e48319

Research Article

Ailanthus altissima and *Amorpha fruticosa* – invasive arboreal alien plants as cheap sources of valuable essential oils

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Received 8 November 2019 ♦ **Accepted** 21 February 2020 ♦ **Published** 31 July 2020

Citation: Kozuharova E, Benbassat N, Berkov S, Ionkova I (2020) *Ailanthus altissima* and *Amorpha fruticosa* – invasive arboreal alien plants as cheap sources of valuable essential oils. Pharmacia 67(2): 71–81. https://doi.org/10.3897/pharmacia.67.e48319

Abstract

The high tolerance of various habitat conditions and potent propagation ability of *Ailanthus altissima* (Mill.) Swingle (Simaroubaceae) and *Amorpha fruticosa* L. (Fabaceae) promote their aggressive invasive behaviour. Additionally, they not only over-compete the local vegetation but suppress the seed development. In the newly invaded habitats they might not have suitable herbivores to control their populations. The aim of this review is to evaluate the potential of *A. altissima* and *A. fruticosa*, as cheap sources of valuable essential oils. The essential oils yield and compostion of both plant species vary significantly depending on plant parts, origin and time of collection. The main constituents of *A. altissima* essential oil are α-curcumene, α-gurjunene, γ-cadinene, α-humulene β-caryophyllene caryophyllene oxide, germacrene D etc. The main constituents of *A. fruticosa* are δ-cadinene, γ-cadinene, β-caryophyllene γ-muurolene +, ar-curcumene, myrcene etc. These essential oils have been reported to possess different activities such as antimicrobial, insect repellent, insecticidal and herbicidal activity. Due to the fact that these are aggressive invasive species, they can provide abundant and cheap resources. Additionally, future industrial exploitation of the biomass of these invasive plants for essential oils' extraction might contribute to biodiversity conservation by relieving their destructive impact on the natural habitats.

Keywords

essential oils, pharmacological activity, invasive plants, *Ailanthus altissima Amorpha fruticosa*

Introduction

Ailanthus altissima (Mill.) Swingle, (Simaroubaceae) and *Amorpha fruticosa* L. (Fabaceae), are alien plant species which have high tolerance of various habitat conditions and elevated propagation ability. They demonstrate aggressive invasive behaviour. They not only overcompete the local plants but also suppress their seed germination and seedling development. In the newly invaded habitats they practically do not have suitable herbivores to control their populations (DAISIE 2009, Monaco 2014, Global Invasive Species Database 2019). Therefore they can be regarded as cheap resources of bioactive compounds, especially essential oils. Additionally excessive harvesting might contribute to decrease their populations and reduce the destructive impact of these species on natural habitats.

In their native range of distribution these plants have been recognized as useful for remedial purpouses. In many parts of Asia including China the bark and the leaves of *A. altissima* have been used tradittionally against leucorrhoea, diarrhea; to treat cold, dysentery, endoparasites and gastric diseases (De Martino and De

Feo 2008). The Omaha have used *A. fruticosa* to cure wounds (Munson, 1981).

The aim of this mini review is to evaluate the potential of *Ailanthus altissima* and *Amorpha fruticosa*, as cheap sources of valuable essential oils.

Extraction methods of the essential oil

Material for essential oil of *Ailanthus altissima* is collected in September in Horacia, (Mastelić and Jerković 2002), in summer in Tunisia (Albouchi et al. 2013), and in summer in Tunisia (El Ayeb-Zakhama et al. 2014). The essential oil of different plant parts of *A. altissima* (roots, stems, leaves /young and old plants, flowers, and ripe fruits, all cut into small peaces), is extracted by hydrodistillation using a Clevenger-type apparatus (Mastelić and Jerković 2002, El Ayeb-Zakhama et al. 2014), or simple laboratory Quikfit apparatus (Albouchi et al. 2013). Identification of the components is accomplished by GC-FID and GC/MS analyses. For the repellent bioassays the essential oil of *A. altissima* is extracted by Soxhlet method with anhydrous diethyl ether from the bark Lu and Wu 2010).

The plant material of *Amorpha fruticosa* used for essential oil extraction is as follows: fresh or air-dried crushed fruits harvested at four stages of maturity (formation of fruits, unripe fruits, ripe fruits and ripe fruits after slight frost (Georgiev et al. 2000); air-dried crushed fruits stored for 6, 18, 30 and 40 months (Stoyanova et al. 2003); fresh flowers, fresh leaves, fresh crushed unripe fruits, fresh crushed ripe fruits and air-dried crushed ripe fruits (Lis and Góra 2001); ripe fruits collected in Bulgaria and in Poland (Lis et al. 2001); and air-dried crushed fruits collected during the period October to Novemnber (Ivanescu et al. 2014). The fruit surface of *A. fruticosa* is more or less heavily beset with conspicuous pustulate, resinous glands (Wilbur 1964, Straub 2010, Taft 2013, Reid 2019, Sevcik 2019). The essential oil is extracted usually by hydrodistillation (Georgiev et al. 2000, Lis and Góra 2001, Stoyanova et al. 2003, Ivanescu et al. 2014). A modified technique to separate essential oil from the fruit of *A. fruticosa* is developed using microwave-assisted hydrodistillation concatenated liquid-liquid extraction (MHD-LLE) (Chen et al. 2017). Microwave assisted hydro-distillation method provides important advantages over conventional method such as: accelerated extraction time, reduced energy consumption, and cleaner production (Akhbari. et al. 2018).

Ailanthus altissima (Mill.) Swingle

Distribution and invasion level

Ailanthus altissima (family Simaroubaceae, Tree of heaven) is a deciduous tree with a smooth, grey bark, alternate, odd-pinnate compound leaves with 11–25 lanceolate leaflets, numerous small flowers, clustered in panicles and seeds centred in a papery sheath (samara). It is native to Asia and globally invasive plant cultivated as ornamental and went out of control (Global Invasive Species Database 2019). According to Delivering Alien Invasive Species Inventories for Europe it is one of the three taxa together with *Ambrosia artemisiifolia* and *Robinia pseudacacia* which are considered most invasive alien species in Europe (DAISIE 2009, Monaco 2014, Sladonja et al. 2015). For example it is massively distributed in Bulgaria in all floristic regions between 0 and1800 m above sea level, and is extremely difficult to control due to its excessive seed set and germination, fast growing and significant regenerative abilities from stems and root fragments in addition to its vast tolerance to the environmental conditions (Petrova et al. 2012, Zahariev 2014). The plant contains a number of bioactive compounds with valuable pharmacological effects (Kozuharova et al. 2014, Al-Snafi 2015).

Composition of the essentiol oil

The essential oil of *Ailanthus altissima* varies considerably (Table 1). More than 130 constituents are identified in *A. altissima* essential oil (Mastelić and Jerković 2002, Albouchi et al. 2013, El Ayeb-Zakhama et al. 2014). The main of them are α-curcumene α-gurjunene, γ-cadinene, α-humulene β-caryophyllene caryophyllene oxide, germacrene D etc. (Table 1, Fig. 1). Both qualitatively and quantitatively the variability depends on the plant populations/ecological factors, extractable part, ontogenesis stage and the drying process (Mastelić and Jerković 2002, Albouchi et al. 2013, El Ayeb-Zakhama et al. 2014).

Antimicrobial activity

The high content of γ-cadinene (Fig. 1) in *A. altissima* essential oil indicates good antimicrobial activity. To γ-cadinene together wih thymol, carvacrol, eugenol, α-pinene, myrcene, α-terpineol, terpinen-4-ol, linalool, γ-muurolene, spathulenol, α-selinene is attributed significant antimicrobial activitiy such as antiseptic, antibacterial and antifungal (Isman 2000, Oliva et al. 2003, Hong et al. 2004, Behravan et al. 2007, Clarke 2009, Khomarlou et al. 2018). Experimenntally essential oil of *A. altissima* is poorly tested for antimicrobial activity. Methanolic extracts of *A. altissima* leaves and their hydrodistilled residues are efficient against Gram-positive bacteria, but not active against Gram-negative bacterial strains and the yeast *Candida albicans* (Albouchi et al. 2013).

Phytotoxicity Assay

The presence of α-humulene (Fig. 1) indicates potential of *A. altissima* essential to repel insects and to have contact, and fumigant insecticidal actions against specific pests. Such activity is attributed to α-humulene as well as to amphene, camphor, 1,8-cineole (eucaliptol), terpinen-4-ol, isoborneol, α-pinene and β-pinene, (-)-α-bisabolol (Shaaya et al. 1991, Isman 2000, Suthisut et al. 2011, Polatoğlu et al. 2013, Ortiz de Elguea-Culebras et al. 2017).

Table 1. Chemical composition (%) of *Ailanthus altissima* essential oil – a compilation of [1] - Mastelić and Jerković (2002): [2] – El Ayeb-Zakhama et al. (2014) and [3] – Albouchi et al. (2013) legend: Y-Young plant O-Old plant.

Components	**Quantyty of terpenes in the essential oil**							
Source of information	**[1]**		**[2]**	**[3]**				
	leaf Y mg/kg	**leaf O mg/kg**	**leaf %**	**root %**	**stem %**	**leaf %**	**flower %**	**samara%**
hexadecanal			0.17–0.40	22.60	4.50			0.30
(E)-2-hexenal	8.21	0.36				1.80		
(Z)-3-hexen-l-yl acetate	21.89	2.29						
(Z)-3-hexen-l-ol	40.63	12.18						
(Z)-3-hexen-l-yl butanoate	22.52	–						
(Z)-3-hexen-l-yl hexanoate	4.42	< 0.05						
hexadecanoic (palmitic) acid	13.05	< 0.05	0.29–0.85					
tetradecanol			0.1–10.65					
heneicosane			0.2–10.65					
docosane			0.96–11.7					
tricosane			0.61–8.63					
calarene	5.26	4.70						
(Z)-caryophyllene					10.90			
β-caryophyllene	19.37	24.60	0.81–2.14	1.20	18.90		0.80	2.90
α-curcumene			4.00–6.86					
α-gurjunene			3.36–3.89					
α-humulene	3.34	7.48	1.04–4.47		6.50		0.60	1.20
γ-cadinene	20.00	25.33	0.20–0.39	2.40	0.70		0.80	
δ-cadinene	3.79	3.26	1.15–1.63	2.80	3.10			
γ-cadinol				3.20	2.80	5.20	5.70	
α-cadinol				1.70	4.10	6.20	8.30	0.90
α-Terpinen-7-al			3.06–3.81					
caryophyllene oxide			0.19	0.80	8.30	22.70	42.50	2.60
germacrene D			0.24–0.26	15.50	10.30			2.00
hexahydrofarnesyl acetone				0.40	0.70	15.40	5.00	58.40
linalool	5.26	0.60	0.69–0.76	0.50	1.00	8.40	2.10	
β-cyclocitral	3.58	2.53	0.36–2.00					
α-terpineol	2.11	tr	0.11–0.36			1.90		
geraniol	1.05	0.36	0.41–1.67					
cis-jasmone	2.53	2.41						
neophytadiene	7.58	2.65						

It is shown experimentally that the essential oil of *Ailanthus altissima* negatively affects the seed germination and early stage development of the seedlings of the target species. The effect is dose dependant as well as it is greater in the light than in the dark. Also the phytotoc effect depends on the origin of the essential oil, as the oil extracted from flowers is most phytotoxic (Tsao et al. 2002, Albouchi et al. 2013, El Ayeb-Zakhama et al. 2014). The observed phytotoxic effect can be associated with caryophyllene oxide, b-caryophyllene, germacrene D, and hexahydrofarnesyl acetone (El Ayeb-Zakhama et al. 2014). Caryophyllene oxide and germacrene D are known for their phytotoxicity (Quintana et al. 2009, De Martino et al. 2010). Germination percentage is severely inhibited by the leaf hydrodistilled residues, where 400 to 600 µg/mL are sufficient to achieve complete inhibition of germination of the target plants (Albouchi et al. 2013).

Fumigant and repellent activity of the essential oil

The essential oil of *Ailanthus altissima* bark has a fumigant activity – it can be used to kill insects that damage stored foods or seeds. The tests show potent fumigant activity against *Oryzaephilus surinamensis* (Linnaeus) (Coleoptera: Silvanidae), *Sitophilus oryzae* (Linnaeus) (Coleoptera: Curculionidae) with 99.3 and 81.9% mortality within 24 h respectively and although fumigant activity is weak against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), and *Liposcelis paeta* Pearman (Psocoptera: Liposcelididae) adults it notably repells *T. castaneum* adults and *L. paeta* nymphae (Lü et al. 2006, Lü 2007, Lü and Wu 2010). *A. altissima* bark oil has extremely strong repellent activity against *Lasioderma serricorne* (Fabricius) (Coleoptera: Anobiidae) adults with the percentage repellency 93.7, 87.8 and 76.1% after 24, 48, and 72 h exposure, respectively. The oil also possesses high fumigant activity against *L. serricorne* adults with the corrected percentage mortality 100% at 8 µL/L air within 48 h exposure (Lü and Shi 2012). It is also found to have activity towards nematodes of the *Meloidogyne* genus (Caboni et al. 2012). There is a high mortality rate of aphyds, pests of peas when treated with ailanton (Polonsky et al. 1989). (Z)-3-hexen-l-ol which is one of the main components of the essential oil (Table 1) is known as a key herbivore-induced plant volatile. In spite of the conflict functions of (*Z*)-3-hexenol in direct and indirect plant defenses – attraction or repellent for various herbivore insects, there is no doubt for its role

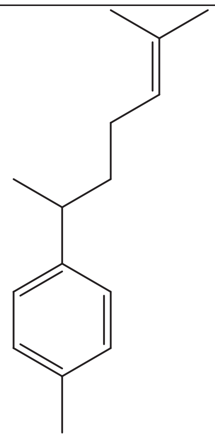
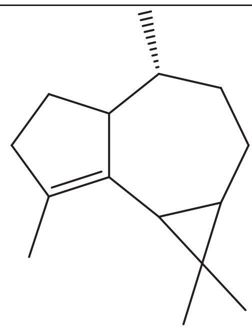
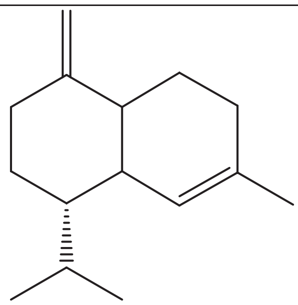
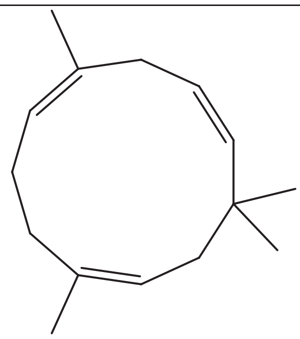
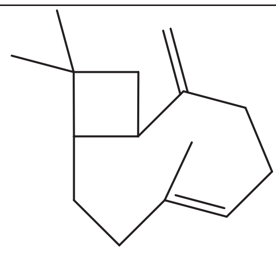
The main constituents of *Ailanthus altissima* essential oil	
α-curcumene	
α-gurjunene	
γ-cadinene	
α-humulene	
β-caryophyllene	

Figure 1. The main constituents of *Ailanthus altissima* and *Amorpha fruticosa* essential oils.

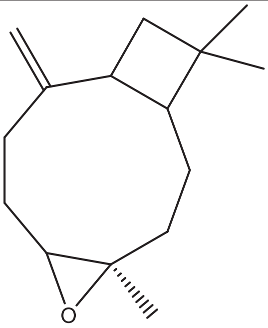
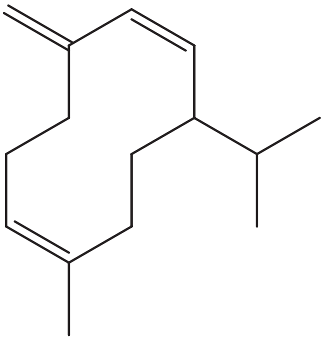
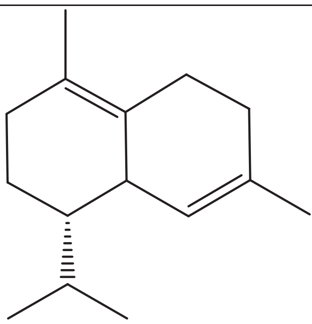
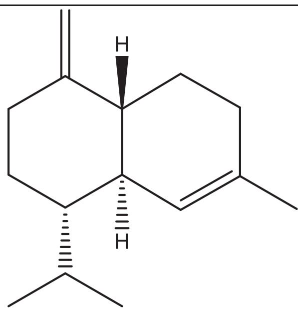
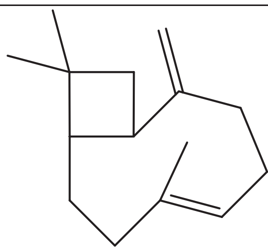
caryophyllene oxide	
germacrene D	
The main constituents of *Amorpha fruticosa* essential oil	
δ-cadinene	
γ-cadinene	
β-caryophyllene	

Figure 1. Continued.

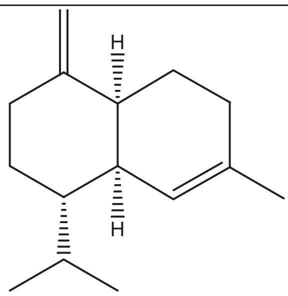
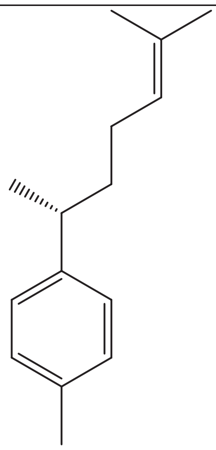
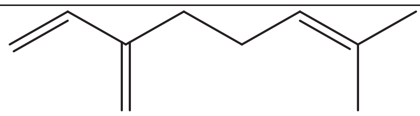
γ-muurolene	
ar-curcumene	
myrcene	

Figure 1. Continued.

as an indirect defense. This compound is agood candidate for novel insect pest control strategies (Wei and Kang 2011). Additionally caryophyllene, and caryophyllene oxide which are main constituents of the essential oil of *A. altissima* (Table 1) are attractive to green lacewings which are important predators of many insect pests and thus are part of the biological control. The activity mechanism of these compounds is expressed by increased adult oviposition and thereby increased larval predation against pest insects. (Flint et al 1979).

Amorpha fruticosa L.

Distribution and invasion level

Amorpha fruticosa L. (family Fabaceae, indigo bush) is a shrub with a stem of 1–3 m, extensive root system, with odd-pinnate compound leaves with stipules. Leaflets 9–35 ovate or elliptical, entire. The purple flowers are clustered in racemes. The fruit is an indehiscent pod of 8–9 mm coverd with glands and containing 1 or 2 seeds. The plant is native to North America, and it is widely distributed in the US, southern Canada and northern Mexico (Wilbur 1975, USDA NRCS 2009, USDA, ARSNPGS 2019). *A. fruticosa* was introduced in Europe as an ornamental, honey and protective against erosion plant (Kozuharova et al. 2017, CABI 2019) but turned into agressive invasive species and now is included in the list of "Worst invasive alien species threatening biodiversity in Europe" (Petrova et al. 2012, Monaco 2014, CABI 2019). It is widely distributed in Bulgaria along roadsides, and it forms large monodominant, dense groups, particularly along Danube river, along the major rivers in Strandzha Natural Parkand also reservoir banks, replacing native species and altering the structure of native plant communities. *A. fruticosa* is difficult to control as it propagates by seeds, which are produced in large quantities and have high germination rate. Additionally there is considerable vegetative propagation. The seeds are driven by the water to the moist places, which the plant prefers but it also tolerates both prolonged droughts and prolonged flooding, as well as wide range of light and soil conditions including salinity (Petrova et al. 2012, Zahariev 2014, Ciuvat et al. 2016). *A. fruticosa* contains number of bioactive compounds with valuable pharmacological effects such as antimicrobial, wound healing, hepatoprotective and osteoclast inhibitory effects, anticancer properties etc, and its potential against diabetes and metabolic disease is rather high (Kozuharova et al. 2017). It is attacked only by several more or less specialized insects (Petrova et al. 2012).

Table 2. Chemical composition (%) of *Amorpha fruticosa* essential oil – a compilation of [1]– Georgiev et al. 2000, [2]– Lis et al. 2001, [3] – Lis and Góra 2001, [4]– Stoyanova et al. 2003, [5]– Ivanescu et al. 2014, [6] – Chen et al. 2017.

Components	Quantyty of terpenes in the essential oil											
Source of information	[1]	[2]		[3]	[4]	[5]			[6]			
Flower oil												
α-eudesmol				15.80								
β-eudesmol				7.80								
δ-cadinene				6.20								
(E)-nerolidol				6.10								
Leaf oil												
α-eudesmol				13.90								
(E)-β-ocimene				11.90								
α-pinene				11.80								
Fruit oil	Formation to ripe fruit	Bulgaria ripe fruit	Poland ripe fruit	Unripe to dried ripe fruit	6 to 40 months storage	Loc. S1 Nov. 2011	Loc. S2 Oct. 2011	Loc. S3 Oct. 2014	MHD-LLD EOf	MHD-LLD EOs	MHD	HD
Yield	**0.32–0.72**	**0.60**	**1.40**	**0.45–1.36**	**0.83–0.76**	**1.30**	**1.50**	**1.80**				
α-pinene	1.20–4.10	4.90	19.55	25.80–19.60	5.70–4.50	3.82	10.86	0.91	5.55		4.75	5.07
myrcene	0.80–1.60	1.59	17.90	13.30–18.70	0.50–0.40	4.62	1.38	0.66				
α-copaene		4.84	2.25	1.70–2.30	4.20–4.00				7.65	1.70	8.11	6.80
bicyclosesquiphellandrene									8.98		7.44	8.09
γ-cadinene	7.90–10.70	9.53	3.41	2.10–2.80	6.70–6.70	5.34	3.19	7.21	6.97	9.84	7.99	6.65
δ-cadinene	14.40–17.30	14.93	6.89	5.10–6.90	11.40–12.50	5.72	5.82	7.77	12.10	3.32	12.22	15.01
β- caryophyllene	11.5–5.20	5.20	4.08	2.30–4.10	6.00–5.400	4.79	3.20	2.66				
caryophyllene									9.62		8.74	10.67
γ-muurolene +	13.20–18.10	13.10	4.50	4.30–5.30	16.70–10.60	7.17	7.30	9.85	10.61	9.61	13.88	11.52
ar-curcumene	12.20–18.10	12.10	7.00	4.80–6.00	0.00–8.60							
α-zingiberene	2.40–6.90	5.76	1.73	1.20–1.70		7.63	5.79	6.88				
α-eudesmol	2.30–0.90	1.17	2.40	3.60–2.40	4.30–4.00	7.89	5.65	6.51				
γ-eudesmol	0.6-<1.00	<1.00	0.58	0.70–0.60	2.40–2.20				2.11	15.12	2.28	2.02
isolongifolene										7.51		
isolongifolol									0.51	8.34	0.63	0.50
Monoterpene hydrocarbons	3.00–8.00	8.00	46.00	46.00		12.73	21.1	5.72				
Oxygenated monoterpenes	<1.00	1.00	1.00	0.00		0.96	6.57	1.22				
Sesquiterpene hydrocarbons	86.00–94.00	84.00	46.00	46.00		47.35	41.38	57.08				
Oxygenated sesquiterpenes	2.00–9.00	4.00	7.00	8.00		18.58	11.21	13.41				

Composition of the essential oil

The essential oil of *Amorpha fruticosa* fruits varies qualitatively and quantitatively (Table 2) depending on the maturity stage, drying process and storage, as well as location of the plant populations/ecological factors (Georgiev et al. 2000, Lis and Góra 2001, Lis et al. 2001, Stoyanova et al. 2003, Ivanescu et al. 2014, Chen et al. 2017). Flowers and leaves also produce essential oil with different composition (Table 2) compared to the fruits (Lis and Góra 2001). The yeald varies between 0.32–1.80 % (Table 2). Between 50 and 70 constituents are identified wih majors δ-cadinene, γ-cadinene, β-caryophyllene γ-muurolene +, ar-curcumene, myrcene etc. (Table 2, Fig. 1) but their quantitive content varies considerably. The odour of the oil is intensive, balsamic and long lasting therefore it can be used in parfumery (Lis and Góra 2001, Lis et al. 2001).

Antimicrobial and woundhealing activity

The high content of α-pinene, γ-muurolene, myrcene, γ-cadinene and δ-cadinene (Fig. 1) in *A. fruticos* essential oil indicates good antimicrobial activity. To these compounds together with thymol, carvacrol, eugenol, α-terpineol, terpinen-4-ol, linalool, spathulenol, α-selinene is attributed significant antimicrobial activitiy – antiseptic, antibacterial and antifungal (Isman 2000, Oliva et al. 2003, Hong et al. 2004, Behravan et al. 2007, Clarke 2009). It is shown that α-pinene affecs the integrity of the bacterial membrane (Toroglu 2007, Park and Lee 2011). Experimentally the antimicrobial activity of the essential oil of *Amorpha fruticosa* is studied using Gram positive bacteria (*Staphylococcus aureus* ATCC 25923, *Sarcina lutea* ATCC 9341, *Bacillus cereus* ATCC 14579, *B. subtilis*), Gram negative bacteria - *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and pathogenic yeasts *Candida albicans* ATCC 10231, *C. sake*, *C. glabrata* ATCC MYA 2950. The volatile oil manifests moderate antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus*, *Sarcina lutea*, *Bacillus cereus*, *B. subtilis*) and no antifungal activity (against three fungi *Candida albicans*, *C. glabrata*, *C. sake*) that can be explained by the absence of phenolic compounds and the low content of oxygenated monoterpenes (Ivanescu et al. 2014).

Phytotoxicity Assay

The essential oil of *Amorpha fruticosa* is not tested yet for its phytotoxicity effect. However it is known that α-pinene, caryophyllene, muurolene and cadinene possess phytotoxic activity (Angelini et al. 2003, De Martino et al. 2010, Wright et al. 2013, Polatoğlu et al. 2013, Amri et al.

2013). The high content of these components in the essential oil of *A. fruticosa* (Table 1, Fig. 1) indicates that such effect can be expected.

Fumigant and repellent activity of the essentiol oil

The essential oil of *Amorpha fruticosa* is poorly tested for its fumgant and repellent activity with few experimental research but the results are promising (Park and Shin 2005, Park et al. 2006). One of the essential oil's components related to insect repellent/fumigant effect is α-pinene (Shaaya et al. 1991, Isman 2000, Suthisut et al. 2011, Polatoğlu et al. 2013, Ortiz de Elguea-Culebras et al. 2017). The essential oil extracted from some populations and particularly from unripe fruits are rich in α-pinene (Table 1, Fig. 1). Also the high content of δ-cadinene (Table 1, Fig. 1) suggests a positive fumigant effect (Licciardello et al. 2013) as it has larvicidal effect against malaria, dengue and filariasis mosquitoes (Govindarajan et al. 2016a, 2016b). Adittionally δ-cadinene is responsible for the multiple defense responses of plants (Tan et al. 2000). However contradictively in some cases it can attract insects e.g the beetle which is a specialist on the elm tree (McLeod et al 2005).

Conclusion

Some plant essential oils repel insects. They even have contact and fumigant insecticidal actions against specific pests (Shaaya et al. 1991, Suthisut et al. 2011). Additionally essential oils are considered potential bio-herbicides, having different and selective herbicidal mechanisms in comparison to their synthetic herbicides (Dudai et al. 1999, Tworkoski 2002, Angelini et al. 2003, Kordali et al. 2008, Haig et al. 2009, Verdeguer et al. 2009, De Almeida et al. 2010, Pasdaran and Hamedi 2017) as they are active against germination and early radicle growth at different levels (De Almeida et al. 2010).

The *Ailanthus altissima* essential oil has phytotoxic and potent fumigant activity demonstrated by a number of research papers. It is prospective as a bio-pesticide because natural products are biodegradable and possibly less harmful to the humans' health. The fumigant and herbicide effects of *Amorpha fruticosa* essential oil is poorly studied experimentally. However there are some indications for such activity based on the main constituents of the essenial oil and it may appear a prospective bio-pesticide. The experimentally testesd antimicrobial activity of the essential oils of both plant species is evaluated as moderate.

Applcation of essential oils from these arboreal invasive plants agains pests and weeds can help to reduce the use the of synthetic pesticides which are known with their negative effects on the wild bees and honeybees (Potts et al. 2010, Goulson et al. 2013, 2018). However further research is necessary to test the effect of these essential oils on the pollinators.

Acknowledgements

This work has been carried out in the framework of the Grant Д-79/23.04.2019, Project 8276/20.11.2019 to Council of Medicinal Science at Medical University of Sofia. Our thanks to the graduate student Kristina Stoycheva for the assistance in the working process.

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