The invasive alien species *Amorpha fruticosa* in Bulgaria and its potential as economically prospective source of valuable essential oil

Ekaterina Kozuharova¹, Niko Benbassat¹, Iliana Ionkova¹

¹ Medical University of Sofia, Department of Pharmacognosy, Faculty of Pharmacy

Corresponding author: Ekaterina Kozuharova (ina_kozuharova@yahoo.co.uk)

Received 20 February 2020 • Accepted 25 March 2020 • Published 27 November 2020

Citation: Ekaterina Kozuharova E, Niko Benbassat N, Ionkova I (2020) The invasive alien species *Amorpha fruticosa* in Bulgaria and its potential as economically prospective source of valuable essential oil. Pharmacia 67(4): 357–362. https://doi.org/10.3897/pharmacia.67.e51334

Abstract

The high tolerance of various habitat conditions and potent propagation ability of *Amorpha fruticosa* L. (Fabaceae) promote its aggressive invasive behaviour. The aim of this study is to evaluate 1) several populations of this plant by the potential yield of the fruit, 2) the approximate yield of the essential oils, and 3) composition of the essential oil.

The potential yield of fruit is evaluated based on extrapolations of weight and number of fruits per infrutescence, number of infrutescences per plant and number of individuals per population. Steam extraction of the essential oil was performed and GS/MS analysis of the composition of the essential oil.

The populations on our key plots are big enough for harvesting with fruit/infrutescence 152 ±15 (n = 20) and infrutescences/shrub 436 ±157 (n = 20). The yield is 0.83 ml/100g. We identified 22 components with major constituents caryophyllene (17.64%) α-guaiene (14.70%), naphthalene (6.75%), γ-muurolene + (5.98%).

Keywords

*Amorpha fruticosa*, chemical composition, essential oil, yield

Introduction

Indigo bush, *Amorpha fruticosa* L. (family Fabaceae) is a 1–3 m tall shrub with odd-pinnate compound leaves with stipules and 9–35 ovate or elliptical, entire leaflets. The purple flowers are clustered in racemes. The fruit is an indehiscent pod of 8–9 mm. 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 (Petrova et al. 2012; Kozuharova et al. 2017; CABI 2019) but turned into aggressive 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). *A. fruticosa* is widely distributed in Bulgaria along roadsides where it is often planted, and it forms large monodominant, dense groups along rivers and reservoir banks, replacing native species and altering the structure of native plant communities (Petrova et al. 2012; Zahariev 2014). *A. fruticosa* not only overcompete the local plants. It contains compounds that suppress their seed germination and seedling developments (Pavićević 2013; Hovanet et al. 2015; Martinović 2018) and its juglon index is 1.11–2.00 depending on extract concentrations (Csiszár 2009). Typically for a leguminous
plant, *A. fruticosa* contains a set of family marker classes such as rotenoids (Kozuharova et al. 2018). Namely, the rotenoid compounds (e.g. amorfigenin, rotenone etc.) are known to have phytotoxicity (Simin et al. 2002). Additionally *A. fruticosa* is attacked only by several more or less specialized insects (Petrova et al. 2012) because the plant contains compounds with insecticide activity (Brett 1946). These compounds are the rotenoids (Moring and McChesney 1979; Berenbaum 1989; Konoshima et al. 1993; Fang and Casida 1998; Ivanescu et al. 2014; Kariuki et al 2014; Mingshan et al. 2015). In fact rotenone is a botanical insecticide, which has been used for centuries and is still used worldwide (Gupta 2007).

*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; Ciuvat et al. 2016).

*Amorpha 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). The essential oil of *A. fruticosa* fruits varies qualitatively and quantitatively 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 compared to the fruits (Lis and Góra 2001).

The aim of this study is to evaluate 1) the potential fruit yield of several populations of *Amorpha fruticosa*, 2) the approximate yield of the essential oil, 3) the composition of the essential oil from Pasarel locality.

### Material and methods

#### Amorpha fruticosa resources estimation

In order to evaluate exploitation potential of *Amorpha fruticosa* we selected key plots at four localities (harvesting areas) in Bulgaria. These are as follows: 1) the banks and adjusted area of Ivailovgrad reservoir, 2) the banks and adjusted area of Koprinka reservoir, 3) the banks of Struma river between the towns of Blagoevgrad and Simitly, 4) the banks and adjusted area of Iskar river above village Pasarel, 5) The roadside in the vicinity of Djulino village (Table 1; Figure 1).

![Amorpha fruticosa key plots](image)

**Figure 1.** Key plots.

### Table 1. Estimated resources of *Amorpha fruticosa* at four key plots in Bulgaria.

| Locality           | Area of the yield gathering territory [ha] | Projective cover of *A. fruticosa* in the key plot [%] | Area taken by *A. fruticosa* in the key plot [ha] | Fruit/infrutescence; infrutescences/shrub | Average resource of the key plot [kg] |
|--------------------|-------------------------------------------|------------------------------------------------------|--------------------------------------------------|------------------------------------------|--------------------------------------|
| Ivailovgrad reservoir | ~ 10                                      | 95                                                   | 0.000005                                          | 152 ±35 (n = 20); 436 ±157 (n = 20)       | 612.5                                |
| Koprinka reservoir   | ~ 4                                       | 90                                                   | 0.00003                                          | 165 ±24 (n = 20); 440 ±149 (n = 20)       | 375.0                                |
| Struma river         | ~ 15                                      | 94                                                   | 0.00006                                          | 148 ±35 (n = 20); 449 ±150 (n = 20)       | 800.0                                |
| Village Pasarel      | ~ 1                                       | 91                                                   | 0.00001                                          | 155 ±37 (n = 20); 420 ±160 (n = 20)       | 62.5                                 |
| Village Djulino      | ~ 4                                       | 92                                                   | 0.00003                                          | 142 ±34 (n = 20); 415 ±152 (n = 20)       | 187.4                                |
Fig. 1). We followed the “Methodology for assessment of medicinal plant resources” developed by Shroeter and co-authors (Shroeter et al. 1986) modified and adapted to the specifics of this invasive species. For A. fruticosa fruit yield assessment we chose the method of “key plots”. We did transects within each key plot with 1–10 measuring plots of a size 10×10 m along transects in accordance to the “key plot” area. The “key plot” approach is used for plant species specialized to particular habitats. According to this methodological approach we determined the area where individuals of A. fruticosa are distributed within each key plot as a projective cover [%] (Table 1). We used GPS and “Garmin Etrex Vista CX” receiver to measure the total area of the gathering territory, the area of each key plot, and based on that we calculated the area occupied by the individuals of A. fruticosa (Table 1). The potential expected yield of fruit (kg/ha) is evaluated based on extrapolations of number of fruits per infrutescence, number of infrutescences per plant and number of individuals per population, calculated by the projective cover of a shrub. The weight of 10 samples of 100 fruits each was measured and the average was calculated. Based on the average fruit set per plant, number of individuals and their weight the extrapolations were performed. Additionally we double checked the expected resources of fruit for each of the key plots by measuring the weight of entire fruit yield of a shrub with cover of 4 m².

**Essential oil extraction and analysis**

*Amorpha fruticosa* fruit was collected at Passarel locality in third decade of October 2018. It was kept at room temperature until the extraction in May 2019. Steam extraction of the essential oil was performed using Clevenger apparatus for 4h. GS/MS analysis of the composition of the essential oil was performed. Gas chromatography-mass spectroscopy (GC/MS) analysis was conducted according to Ph. Eur. 9th. The GC-MS analysis of diluted (1:1000) *A. fruticosa* essential oils was performed on Exactive Orbitrap GC-MS system (ThermoFisher Scientific) operating at 70 eV, ion source temperature 230 °C, interface temperature 280 °C with split injection (1 μL, ratio 20:1) at 270 °C injector temperature. A fused silica capillary column, 5% phenyl/95% methyl polysiloxane (TG-5SILMS 30 m × 0.25 mm × 0.25 μm, Thermo) was used.

**Results and discussion**

**Amorpha fruticosa** resources

The extrapolation of the data reveals that: *Amorpha fruticosa* forms dense patches (with >90% cover) in shape of belts following the banks (Table 1; Fig. 2). There are hundreds of inflorescence/infrutescences (Fig. 2). The propagation is basically by seed. Numerous small seedlings were observed in the key plots. Also seedlings were detected to appear immediately after the water level dropped and opened the soil, as the bottom of reservoir Ivailovgrad was completely dry in August 2019 (Fig. 3). *A. fruticosa* over competes the local vegetation on the banks (Fig. 2). The high local resource of *Amorpha fruticosa* is due to the lavish fruit set, numerous fruits per infrutescence and numerous infrutescences per plant as well as the dense cover of this plant in the localities. The populations on our key plots are big enough for harvesting (Table 1; Figs 2, 4) with average fruit/infrutescence 155 (n = 100) and infrutescences/shrub 432 (n = 100).
The essential oil of *Amorpha fruticosa* basically is not difficult to extract. The fruit surface is more or less heavily beset with conspicuous pustulate, resinous glands (Fig. 4). The yield is 0.83 ml/100g. We identified 22 components (Table 2; Fig. 5) with major constituents: caryophyllene (17.64%) α-guaiene (14.70%), naphthalene (6.75%), γ-muurolene + (5.98%).

A considerable qualitative and quantitative composition variability of the *Amorpha fruticosa* essential oil is observed (Table 3). The yield varies between 0.32% and 1.50% and depends on the locality, possibly the extraction method and definitely the ripe fruit produces more essential oil (Table 3). Only α-pinene, myrcene, caryophyllene and γ-muurolene + are universal components for all *A. fruticosa* essential oil samples but even they were presented in different quantitative values (Table 3).

### Table 2. Chemical composition (%) of essential oil from *Amorpha fruticosa* fruit collected at Passarel locality in third decade of October 2018.

| Major constituents | Rt (min) | Exat Mass | Formula | % |
|--------------------|---------|-----------|---------|----|
| 1. 3-Carene         | 6.73    | 136.1252  | C10H16  | 4.17 |
| 2. β-Pinene         | 8.50    | 136.1252  | C10H16  | 1.98 |
| 3. α-pinene         | 10.28   | 204.1878  | C10H16  | 4.17 |
| 4. α-Guaiene         | 18.74   | 204.1878  | C15H24  | 1.81 |
| 5. γ-Muurolene       | 18.89   | 204.1878  | C15H24  | 5.98 |
| 6. Caryophyllene     | 19.91   | 204.1878  | C15H24  | 1.74 |
| 7. Arromandendrene   | 20.33   | 204.1878  | C15H24  | 1.11 |
| 8. cis-b-Farnesene   | 20.60   | 204.1878  | C15H24  | 2.81 |
| 9. Humulene          | 20.73   | 204.1878  | C15H24  | 2.49 |
| 10. trans-β-Ocimene  | 21.43   | 204.1878  | C15H24  | 2.49 |
| 11. α-Copaene        | 21.24   | 202.172151| C15H22  | 4.23 |
| 12. γ-Cadinene       | 21.56   | 204.1878  | C15H24  | 14.70|
| 13. Ylangene         | 21.74   | 204.1878  | C15H24  | 2.13 |
| 14. 1-Methyl-4-(6-methylhept-5-en-2-yl)cyclohexa-1,4-diene | 21.87   | 204.1878  | C15H24  | 6.75 |
| 15. Caryophyllene oxide | 22.46   | 220.1727  | C15H26O | 1.11 |
| 16. 1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene | 22.99   | 204.1878  | C15H24  | 11.00|
| 17. α-Guaiene         | 23.46   | 222.1984  | C15H26O | 1.69 |

### Table 3. Comparison of the major constituents of *Amorpha fruticosa* fruit essential oil detected by various researchers.

| Major constituents |
|---------------------|
| Our results (Bulgaria 18 ripe fruit [%]) | Georgev et al. 2000 (Bulgaria ripe fruit formation to ripe fruit) | Lis et al. 2001 (Poland ripe fruit) | Lis et al. 2001 (Romania Loc. S2 formation to ripe fruit) | Lis and Góra 2001 (Poland ripe fruit) | Ivanescu et al. 2014 (Romania 2011 ripe fruit [%]) |
| Yield | 0.80 | 0.32–0.72 | 0.00 | 1.40 | 0.45–1.36 | 0.50 |
| α-pinene | 10.86 | 1.20–4.10 | 4.90 | 19.55 | 25.80–19.60 | 10.86 |
| β-pinene | 1.38 | 0.80–1.60 | 1.59 | 17.90 | 13.30–18.70 | 1.38 |
| γ-Muurolene | 5.98 | 13.20–18.10 | 13.10 | 4.50 | 4.30–5.30 | 7.30 |
| γ-cadinene | 2.22 | 204.1878 | 7.93 | 3.41 | 2.10–2.80 | 3.20 |
| δ-cadinene | 2.22 | 204.1878 | 7.93 | 3.41 | 2.10–2.80 | 3.20 |
| α-Guaiene | 22.56 | 200.1565 | 204.1878 | C15H24O | 0.63 |
| 1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene | 22.99 | 204.1878 | C15H24O | 1.11 |
| γ-Cadinene | 22.56 | 200.1565 | 204.1878 | C15H24O | 0.63 |

**Table 2.** Comparative analysis of the essential oil isolated from *Amorpha fruticosa* fruit samples collected at Passarel locality in third decade of October 2018.

**Table 3.** Comparison of the major constituents of *Amorpha fruticosa* fruit essential oil detected by various researchers.
our results confirm the statement of other researchers (Georgiev et al. 2000; Lis and Góra 2001; Stoyanova et al. 2003; Ivanescu et al. 2014; Chen et al. 2017). The intensive balsamic, long lasting odour the oil can be used in perfumery. Also it possesses antimicrobial and wound healing activity. (Das et al. 2007; Qu et al. 2013; Ivanescu et al. 2014). The high content of δ-cadinene in some origins (Table 3, Lis et al. 2001) suggests fumigant and repellent effects (Licciardello et al. 2013). Fumigant and repellent effects possess also α-pinene (Angelini et al. 2003; Wright et al. 2013; Polatoğlu et al. 2013) which is presented in all samples (Table 3). The high content of caryophyllene/β-caryophyllene (Table 3) suggest anti-inflammatory and local anesthetic activity (Ghelardini et al. 2001; Gertsch et al. 2008). It also can be efficient for biological pest control as it is attractant for the green lacewing (Flint et al. 1997), a well known beneficial insects, as with lady beetles, these natural enemies are important predators.

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.

References

Angelini LG, Carpanese G, Cioni PL, Morelli I, Macchia M, Flamini G (2003) Essential oils from Mediterranean Lamiaceae as weed germination inhibitors. Journal of Agricultural and Food Chemistry 51(21): 6158–6164. https://doi.org/10.1021/jf0210728

Berenbaum MR (1989) North American ethnobotanicals as sources of novel plant-based insecticides. https://doi.org/10.1021/bk-1989-0387.ch002

Brett CH (1946) Insecticidal properties of the indigobush (Amorpha fruticosa). Journal of agricultural research 73(3): 81–96.
Kozuharova E et al.: Amorpha fruticosa in Bulgaria as a source of essential oil

CABI (2019) *Amorpha fruticosa* [original text by D. Iamonico] – Invasive Species Compendium. CABI International, Wallingford. http://www.cabi.org/isc

Cao YP, Lu CY, Bai G (2004) Separation and identification of pesticide components of *Amorpha fruticosa*. Chemical Research and Application 16(5): 719–720.

Caiszár A (2009) Allelopathic effects of invasive woody plant species in Hungary. Acta Silvestra et Lignaria Hungarica 5: 9–17.

Fang N, Casida JE (1998) Anticancer action of cubé insecticide: Correlation for rotenoid constituents between inhibition of NADH:ubiquinone oxidoreductase and induced ornithine decarboxylase activities. Proceedings of the National Academy of Sciences 95(7): 3380–3384. https://doi.org/10.1073/pnas.95.7.3380

Flint HM, Salter SS, Walters S (1979) Caryophylline: an attractant for the green lacewing. Environmental Entomology 8(6): 1123–1125.

Gertsch J, Leonti M, Raduner S, Racz I, Chen JZ, Xie XQ, Altmann KH, Karsak M, Zimmer A (2008) Beta-caryophylline is a dietary cannabinoid. Proceedings of the National Academy of Sciences 105(26): 9099–9104. https://doi.org/10.1073/pnas.0803601105

Ghelardini C, Galeotti N, Mannelli LDC, Mazzanti G, Bartolini A (2001) Local anaesthetic activity of β-caryophyllene. Il Farmaco 56(5–7): 387–389.

Chen F, Jia J, Zhang Q, Gu H, Yang L (2017) A modified approach for isolation of essential oil from fruit of *Amorpha fruticosa* Linn using microwave-assisted hydrodistillation concatenated liquid-liquid extraction. Journal of Chromatography A 1524: 254–265. https://doi.org/10.1016/S0014-827X(01)01092-8

Georgiev EV, Stoianova AS, Lis A, Göra J (2000) Seasonal variation of the fruit essential oil of *Amorpha fruticosa* L. Herba Polonica 46(4): 220–225.

Gupta R (2007) Rotenone. In: Gupta R (Ed.) Veterinary Toxicology Basic and Clinical Principles. Academic Press, 499–501. https://doi.org/10.1016/B978-012370467-2/50139-5

Hovanet MV, Marinas IC, Dinu M, Oprea E, Chifiriuc MC, Stavropoulou E, Lazar V (2015) The phytotoxicity and antimicrobial activity of *Amorpha fruticosa* L. leaves extract. Romanian Biotechnological Letters 20(4): 10670–10678.

Ivanescu B, Lungu C, Spac A, Tuchilus C (2014) Essential oils from *Amorpha fruticosa* L. fruits-chemical characterization and antimicrobial activity. Analele Stiintifice ale Universitatii”Al. I. Cuza” din Iasi 60(1): 33–33.

Kariuki DK, Kariuki DN, Mugweru J (2013) Synergistic bio-pesticide combination of pyrethrins and rotenoids for the control of the cockroach *Americana periplaneta*. International Journal of Humanities, Arts, Medicine and Sciences 2(3): 43–48.

Kole RK, Satpathi C, Chowdhury A, Ghosh MR, Adityachaudhury N (1992) Isolation of amorpholone, a potent rotenoid insecticide from *Tephrosia candida*. Journal of Agricultural and Food Chemistry 40(7): 1208–1210. https://doi.org/10.1021/jf00019a026

Konoshima T, Terada H, Kokumai M, Kozuka M, Tokuda H, Estes JR, Lee KH (1993) Studies on inhibitors of skin tumor promotion, XII. Rotenoids from *Amorpha fruticosa*. Journal of Natural Products 56(6): 843–848. https://doi.org/10.1021/np50069a006

Kozuharova E, Matkowski A, Wozacuñenak D, Simeonova R, Naychov Z, Malainer C, Mocan A, Nabavi SM, Atanasov AG (2017) *Amorpha fruticosa* – a noxious invasive alien plant in Europe or a medicinal plant against metabolic disease? Frontiers in Pharmacology 8(333): 1–17. https://doi.org/10.3389/fphar.2017.00333

Liciardello F, Muratore G, Suma P, Russo A, Nerín C (2013) Effectiveness of a novel insect-repellent food packaging incorporating essential oils against the red flour beetle (*Tribolium castaneum*). Innovative Food Science Emerging Technologies 19: 173–180. https://doi.org/10.1016/j.ifset.2013.05.002

Lis A, Stoianova A, Georgiev E, Göra J (2001) Essential oil composition of *Amorpha fruticosa* L. From Bulgaria and Poland. In: Rothe M (Ed.) Flavour 2000 – Proceedings of the 6th Wurtzburg Aroma Symposium isenach April 10–13, 2000, Eigenverlag Bergholz-Rechbrücke, 376–379. https://doi.org/10.1080/1429300.2001.9712227

Lis A, Göra J (2001) Essential oil of *Amorpha fruticosa* L. Journal of Essential Oil Research 13(5): 340–342.

Martinović A (2018) Effects of indigo bush on development and germination of wheat and clover. PhD Thesis, University of Zagreb, Zagreb.

Mingshan J, Liang Y, Gu Z, Li X (2015) Inhibitory effects of amorphigen-in on the mitochondrial complex I of *Culex pipiens pallens* Coquillet (Diptera: Culicidae). International Journal of Molecular Sciences 16: 19713–19727. https://doi.org/10.3390/ijms160819713

Monaco A (2014) European Guidelines on Protected Areas and Invasive Alien Species. Council of Europe, Rome, 60 pp.

Moring SE, McChesney JD (1979) High pressure liquid chromatographic separation of rotenoids from plant extracts [*Tephrosia virginiana*, *Amorpha canescens*, used in insecticidal preparations in the United States]. Journal of the Association of Official Analytical Chemists 62(4): 774–781. https://doi.org/10.1093/aoac/62.4.774

Pavičević M (2013) Alelopatsko djelovanje ekstrakta listova običnog oraha i nekih invazivnih biljnih vrsta na klijanje pšenice (*Triticum aestivum* L.) i gorušice (*Sinapis alba* L.). PhD Thesis, University of Zagreb, Zagreb.

Petrova A, Vladimirov V, Georgiev V (2012) Invasive Alien Plant Species in Bulgaria. Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia. [in Bulgarian]

Polatoğlu K, Karakoç OC, Gören N (2013) Phytotoxic, DPPH scavenging, insecticidal activities and essential oil composition of *Achillea vermicularis*, *A. tereftillia* and proposed chemotypes of *A. biebersteinii* (Asteraceae). Industrial Crops and Products 51: 35–45. https://doi.org/10.1016/j.indcrop.2013.08.052

Shroeter IA, Krilova I, Borisova N, Luchkov L, Bocharov L (1986) Methodology for assessment of medicinal plant resources in USSR. State Committee of Forestry, Moscow. [in Russian]

Somin K, Ali Z, Khaliq-Uz-Zaman SM, Ahmad VU (2002) Structure and biological activity of a new rotenoid from Pongamia pinnata. Natural Product Letters 16(5): 351–357. https://doi.org/10.1080/10575630290033114

USDAs [Agricultural Research Service, National Plant Germplasm System] (2019) Germplasm Resources Information Network (GRIN – Taxonomy). National Germplasm Resources Laboratory, Beltsville, Maryland. https://www.ars-grin.gov/gringlobal/taxonomydetail.aspx?id=2937 [Accessed 17 August 2019]

USDAs, NRCS (2009) The PLANTS Database. National Plant Data Center, Baton Rouge. http://plants.usda.gov [24 June 2009]

Wilbur RL (1975) A revision of the North American genus *Amorpha* (Leguminosae–Psoraleae). Rhodora 77(811): 337–409.

Wright C, Chhetri BK, Setzer WN (2013) Chemical composition and phytotoxicity of the essential oil of *Eucalyptus* *ferruginea* growing in the Sonoran Desert. American Journal of Essential Oil and Natural Products 1: 18–22.

Zahariév D (2014) Invasive Plant Species Along the Major Rivers in Strandža Natural Park. Seminar of Ecology – 2014, Proceedings, Union of scientist in Bulgaria, IBER – BAS, 148–158.