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ANTIMICROBIAL ACTIVITY OF THE *Asclepias syriaca* L. ROOT EXTRACT

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ABSTRACT

***Asclepias syriaca* L. is an invasive plant in Serbia which threatens the biodiversity and ecosystem functionality with its high production of wind-dispersed seeds and the rapid proliferation by the lateral rhizomes. The mechanism that allows its competitiveness is allelopathy - the release of the chemical compounds identified in the roots and leaves which have negative effects on the other plants. These allelochemicals not only affect the development of the neighboring plants, but also are a potential source of the antioxidant and antimicrobial compounds, which increase the immunity of the plant itself, protecting it from the pests and the pathogens. The present study aimed to screen the antifungal activity of the *A. syriaca* water and methanol extracts. In vitro antimicrobial activity was analyzed by the radial growth assays against the three phytopathogenic fungi isolates: *Alternaria alternata, Fusarium avenaceum* and *Discula platani*. The results were processed by factorial ANOVA and the statistically significant differences were determined by Duncan's multiple range test using the software STATISTICA 13.5. The obtained results suggest that the *A. syriaca* water extract has a significant fungistatic and potential fungicidal effect towards the tested phytopathogenic fungi and thus can be considered as a potential tool for their biological control.**

Keywords: biological control, *Alternaria alternata, Fusarium avenaceum, Discula platani,* antioxidant activity.

INTRODUCTION

The pesticides' side-effects on human health and the environment (Harris et al., 2001), as well as the resistance occurrence in pest organisms (Hawkins, et al., 2019), boost the interest for phytochemicals of the native plants and their use as the biological control tools for the plant disease management (Dellavalle et al., 2010).

The weeds are often resistant to the most of the microbial diseases when compared with the crops (Prakash et al., 2012). This partially explains the mechanism of their invasiveness. The antimicrobial activity of some plant species has been known for a long time. Higher plants produce more than 100,000 different compounds with low molecular weight or different secondary metabolites (Walker et al., 2003). It is assumed that the production of a wide range of secondary metabolites is the plants response to the selection pressure and that it improves the resistance mechanisms of plants to the pathogenic microorganisms, pests, as well as the other plants (Zeng et al., 2008). The most commonly produced secondary metabolites in plants have a protective role which can be expressed prior to or after the pathogen infection (Pedrol et al., 2006). The phenols, as well as the alkaloids, are the most significant bioactive substances in most natural products (Edeoga et al., 2005). The interest in eco-friendly plant disease control alternatives has been increasing and researches in this area are intensified worldwide (Zaker, 2016; Gurjar et al., 2012). The promising antimicrobial activity of the secondary plant metabolites as a potential eco-friendly alternative to the synthetic pesticides is currently widely used in agriculture.

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In this paper the antimicrobial activity of the *Asclepias syriaca* L. root extract has been studied. The inhibitory effects of the extracts derived from the plant parts of the genus *Asclepias* has been recorded for many human bacterial pathogens, such as: *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Streptococcus faecium* (Ignat et al., 2013; Olufemi et al., 2014). Currently, the literature data considering the *A. syriaca* antifungal properties against the phytopathogenic fungi is scarce. Therefore, the high antimicrobial potential against some human pathogens justifies the need to examine the *A. syriaca* potential against the plant pathogens.

MATERIAL AND METHODS

Sampling and preparation of the extracts

The *A. syriaca* root samples were collected in the city of Novi Sad, Republic of Serbia. The roots were dried at 40°C for 5 days in a dryer, milled to a fine powder and kept refrigerated at 2°C until use. The 40 g of the dried root powder was mixed with 1 L of the distilled water for 24 h in the shaker in the dark. The obtained extract with the concentration of 80 g L^{-1} was filtered according to the method described by Chon et al. (2003). The water extract was diluted to the final concentration of 40 g L^{-1}. The methanol solution was obtained by mixing 80 g of the root powder in 1 L of the 95% methanol at 24°C for 24 h in the same shaker in the dark. The mixing was followed by the filtration according to the same method. After that, the methanol as the solvent was vacuum-evaporated at 40°C and the dry residue of 3.30332 g 100 mL^{-1} was obtained.

Phenolic compounds and the antioxidant activity of the extracts

One gram of the dry plant material was extracted overnight with 50 mL of the 70% methanol, 70% ethanol and 70% acetone. The water extracts were prepared with the boiling distilled water. The extracts were centrifuged, filtered and kept refrigerated until the analysis.

The total phenolic content (TP) and total tannin content (TT) were determined using a Folin-Ciocalteu colorimetric method (Nagavani and Raghava Rao, 2010) and the results were expressed in milligrams of the gallic acid equivalents per 1 g of the plant material (mg GAE g^{-1}).

The ferric reducing antioxidant power (FRAP) assay was carried out according to the procedure described by Valentão et al. (2002). The FRAP assay is based on the antioxidants' ability to reduce Fe^{3+} into Fe^{2+} in the presence of 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), forming a blue Fe^{2+}-TPTZ complex. The ABTS [2,2'anizonbis (3-ethylbenzothiazoline-6-sulfonic acid)-diammonium salt)] assay was based on a method developed by Miller et al. (1993). The scavenging of the free radicals was evaluated in a DPPH (2,2-diphenyl-1-picrylhydrazyl) acetone solution (Lai and Lim, 2011). The decoloration degree of the solution indicates the scavenging efficiency of the added substance. The reducing power assay (total reduction capacity-TRC) was tested by the method of Saha et al. (2013). The total antioxidant activity (TAA) assay of the plant extracts was performed by the phosphomolybdenum method as reported by Kalaskar and Surana (2014). The standard curves for FRAP, ABTS, DPPH, TRC and TAA assays were plotted using the trolox solution. The standard curve was constructed using different concentrations of a trolox acetone solution and the results were expressed as mg trolox equivalents per gram of the dry plant material (mg TE g^{-1}). The superoxide-free radical ($O2\cdot-$) scavenging activity was carried out by the NBT (nitroblue tetrazolium) test (Kalaskar and Surana, 2014). The results were expressed as the inhibition percentage (%) of the $O_2\cdot-$ production during 15 minutes.

The data are reported as the means for at least three independent replications in case of all the performed assays.

The antifungal activity assay

The antifungal activity of the *A. syriaca* water and methanol extracts was analyzed by the radial growth assays against the three

phytopathogenic fungi isolates: *Alternaria alternata*, *Fusarium avenaceum* and *Discula platani*. The isolates were obtained from the apple fruits, with the exception of *D. platani* which was derived from the infected London Plane Tree leaves (*Platanus acerifolia*).

The different volumes of the water and methanol extracts were aseptically added to the potato dextrose agar medium (PDA), cooled to 50°C and homogenized by a magnetic stirrer in order to obtain two concentrations (0.04 and 0.08 g mL^{-1}). The PDA medium with the *A. syriaca* extract was poured into Petri dishes. In the control treatments the sterile distilled water was homogenized with the PDA medium.

The mycelial plugs (diameter 3 mm) were obtained from the margin of the seven days old cultures of the isolates: *A. alternata*, *F. avenaceum* and *D. platani*. The plugs were subcultured in the centre of the Petri dishes containing the PDA with the *A. syriaca* extract or sterile distilled water. The diameter of the mycelial growth was measured in two perpendicular directions after three and six days of the incubation at 25°C in the dark. The experiment was carried out twice in three replicates.

Statistical analysis

The results were processed by factorial ANOVA and the statistically significant differences were determined by Duncan's multiple range test using the software STATISTICA 13.5.

RESULTS AND DISCUSSION

Phenolic compounds and the antioxidant activity of the extracts

Regarding the solvent extraction, the significant differences were observed in most of the measured chemical parameters content of the *A. syriaca* root extracts ($p<0.05$). No significant differences were observed in case of the TP content and the TAA of different extracts.

It is observed that the water extract possesses significantly higher ($p<0.05$) TT content and a higher superoxide free radical ($O_2 \cdot -$) scavenging activity measured by the NBT assay (80.30%) compared with all the other solvents: 70% methanol (51.07%), 70% ethanol (13.39%) and 70% acetone (6.31%). The TT contents in the examined extracts ranged from 0.113 mg GAE g^{-1} (acetone extract) to 0.812 mg GAE g^{-1} (water extract). The acetone extract of the *A. syriaca* roots possesses higher antioxidant capacity in comparison with the other extraction solvent systems measured by the FRAP, DPPH, TRC and ABTS assays (Table 1).

Table 1. Content of phenolic and tannins compounds and antioxidant capacity of *A. syriaca* root

Solvent	Water	70% Methanol	70% Ethanol	70% Aceton
Total phenolics (mg GAE g^{-1})	2.055 ± 0.014^{a}	2.079 ± 0.089^{a}	2.178 ± 0.129^{a}	2.199 ± 0.143^{a}
Total tannins (mg GAE g^{-1})	0.812 ± 0.060^{a}	0.279 ± 0.092^{b}	0.399 ± 0.116^{b}	0.113 ± 0.024^{c}
FRAP (mg TE g^{-1})	2.418 ± 0.042^{a}	3.067 ± 0.074^{b}	3.293 ± 0.070^{b}	3.612 ± 0.185^{c}
DPPH (mg TE g^{-1})	0.872 ± 0.240^{a}	1.540 ± 0.044^{b}	1.675 ± 0.103^{bc}	1.806 ± 0.171^{c}
ABTS (mg TE g^{-1})	2.532 ± 0.166^{a}	2.988 ± 0.114^{b}	3.127 ± 0.355^{bc}	3.613 ± 0.333^{c}
TAA (mg TE g^{-1})	99.674 ± 10.974^{a}	93.387 ± 3.364^{a}	99.792 ± 3.724^{a}	104.980 ± 1.418^{a}
TRC (mg TE g^{-1})	0.953 ± 0.291^{a}	9.403 ± 0.943^{b}	10.472 ± 0.668^{b}	11.629 ± 0.989^{b}
NBT assay (%)	80.30 ± 1.47^{a}	51.07 ± 1.60^{b}	13.39 ± 4.66^{c}	6.31 ± 2.04^{dd}

The antifungal activity assay

According to the results obtained after three and six days of the incubation, the factorial ANOVA showed that the fungal isolates, type of extract and extract concentration had a highly significant effect on the mycelial growth diameter of the tested isolates ($p<0.01$).

Asclepias syriaca significantly inhibited the mycelial growth of all the tested isolates compared with the control, regardless of the type or concentration of the applied extract and incubation period. A higher concentration of the *A. syriaca* water extract caused complete inhibition of the mycelial growth, regardless of the incubation period.

After three days of the incubation (Table 2), the water extract caused more significant inhibition of the mycelial growth of all the tested isolates than the methanol extract, regardless of the applied concentration. The higher application rate of the methanol extract did not cause significantly stronger inhibition compared with the lower concentration. However, the lower application rate of the water extract had a significantly weaker effect compared with the higher concentration, in case of the *Alternaria* sp. and *Fusarium* sp. isolates (Figure 1).

Table 2. Duncan's multiple range test: Influence of *A. syriaca* extracts on diameter of mycelial growth of the tested isolates after three days of incubation

Isolate	Type of *A. syriaca* extract	Concentration (g mL^{-1})	Diameter of mycelial growth (mm)
T1Jg3 (*A. alternata*)	Water	0.08 0.04	3.00^{a} 11.50^{d}
	Methanol	0.08 0.04	16.00^{gh} 16.50^{h}
	Control (sterile water)	-	40.00^{k}
KA-13 (*F. avenaceum*)	Water	0.08 0.04	3.00^{a} 11.00^{cd}
	Methanol	0.08 0.04	15.00^{fg} 13.75^{e}
	Control (sterile water)	-	43.50^{l}
NSPlat 5 (*D. platani*)	Water	0.08 0.04	3.00^{a} 3.75^{a}
	Methanol	0.08 0.04	10.00^{c} 10.00^{e}
	Control (sterile water)	-	24.50^{i}

* The values followed with the same letter are at the same level of significance.

The results obtained after six days of the incubation (Table 3) also suggest that the water extract caused more significant inhibition of the mycelial growth of all the tested isolates than the methanol extract, regardless of the applied concentration. However, the *Fusarium* sp. lower concentration of the water extract was at the same level of the significance as the higher concentration of the methanol extract. After three days of the incubation, a higher application rate of the methanol extract did not cause significantly stronger inhibition compared with the lower concentration. However, the lower application rate of the water extract had a significantly weaker effect compared with the higher concentration, in case of all the tested isolates.

The obtained results suggest that the *A. syriaca* water extract expresses significant

antifungal activity against a range of economically significant phytopathogenic fungi, particularly when applied at the concentration above 0.04 g mL^{-1}. The application rate of 0.08 g mL^{-1} completely inhibits the mycelial growth of all the tested isolates, while the effect is probably not only fungistatic, but also fungicidal, which should be verified in subsequent studies. On the other hand, the *A. syriaca* methanol extract, although it significantly inhibited the mycelial growth, did not cause complete inhibition, while an increase in its application rate did not necessarily result in stronger inhibition. Hemavani and Thippeswamy (2012) reported high antifungal and antibacterial activity of the extracts of another plant from the *Asclepiadaceae* family (*A. curassavica*). However, the antifungal activity depended on the type of the extract and no antifungal activity was recorded in case of the methanol extract. In this study, the methanol extract also exhibited lower antifungal activity compared with the water extract. The high antifungal activity of the *A. syriaca* water extract achieved in this study is probably due to its total tannins content, considering that many authors (Zhu et al. 2019; Anttila et al., 2013) found the antifungal activity of the tannins. In this study, the total tannin content was also higher compared with the methanol extract and therefore it is probably the main source of the antifungal activity of the *A. syriaca* water extract.

Table 3. Duncan's multiple range test: Influence of *A. syriaca* extracts on diameter of mycelial growth of the tested isolates after six days of incubation

Isolate	Type of *A. syriaca* extract	Concentration (g mL^{-1})	Diameter of mycelial growth (mm)
T1Jg3 (*A. alternata*)	Water	0.08 0.04	3.00^{a} 29.50^{h}
	Methanol	0.08 0.04	27.00^{g} 27.00^{gh}
	Control (sterile water)	-	67.50^{l}
KA-13 (*F. avenaceum*)	Water	0.08 0.04	3.00^{a} 26.50^{fg}
	Methanol	0.08 0.04	25.25^{f} 20.50^{e}
	Control (sterile water)	-	58.25^{k}
NSPlat 5 (*D. platani*)	Water	0.08 0.04	3.00^{a} 5.50^{b}
	Methanol	0.08 0.04	13.50^{d} 12.00^{d}
	Control (sterile water)	-	31.50^{ij}

* The values followed with the same letter are at the same level of significance.

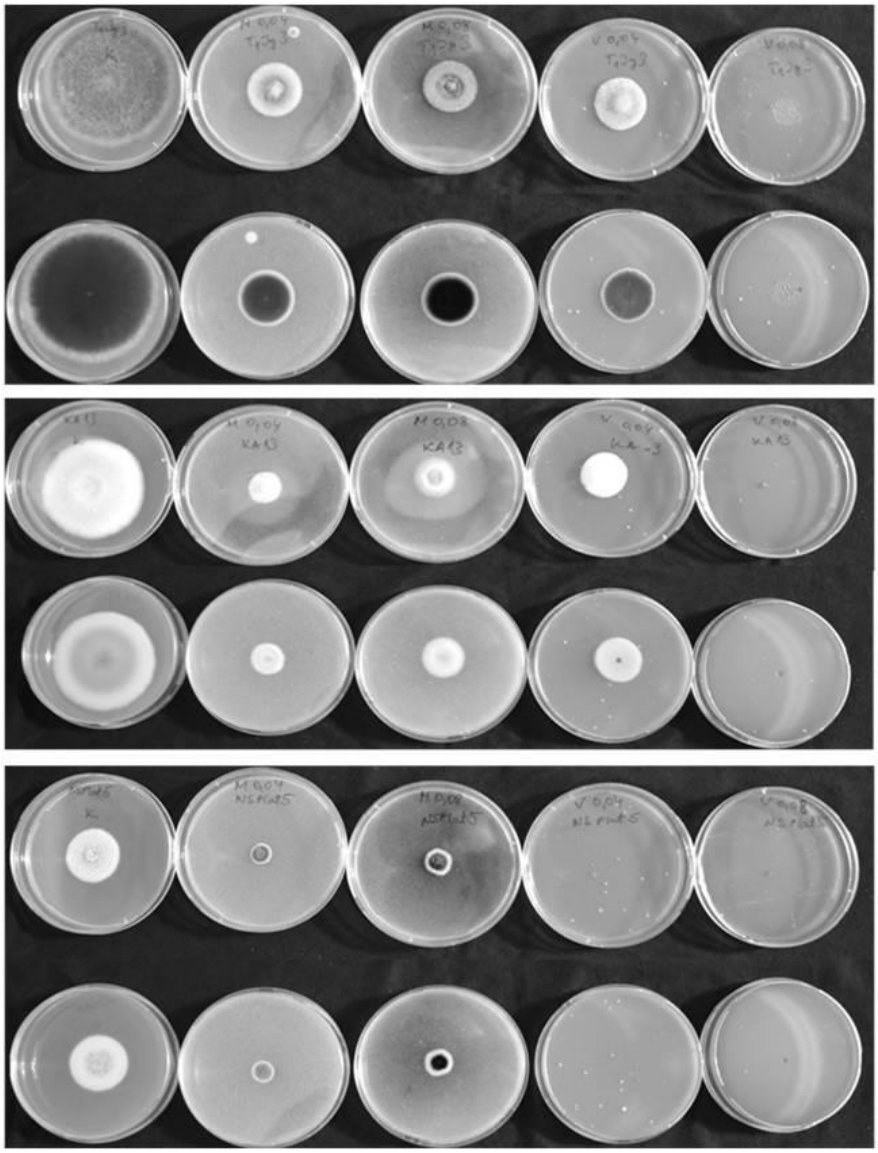

Figure 1. The influence of the *A. syriaca* extracts on the mycelial growth diameter of the tested isolates after six days of the incubation

CONCLUSIONS

It can be concluded that the *A. syriaca* water extract has a significant fungistatic and the potentially fungicidal activity against the tested *A. alternata*, *F. avenaceum* and *D. platani* and can be considered as a potential tool for the biological control of the plant diseases caused by these phytopathogenic fungi.

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