Pestic. Phytomed. (Belgrade), 37(1), 2022, 9–19
DOI: https://doi.org/10.2298/PIF2201009D

UDC 581.524.13:634.51+635.45:632.51
Original scientific paper

Antioxidant activity of *Juglans regia* L. and *Rumex obtusifolius* L. leaf extracts and screening for their allelopathic potential

Tijana Đorđević[1]*, Jelena Gajić Umiljendić[1], Marija Sarić-Krsmanović[1], Ljiljana Radivojević[1], Rada Đurović-Pejčev[1], Marija Stevanović[1] and Mara Vuković[2]

[1] Institute of Pesticides and Environmental Protection, Banatska 31b, 11080 Belgrade, Serbia
[2] Faculty of Technology and Metallurgy, Division of Biochemical Engineering and Biotechnology, University of Belgrade, Karnegijeva 4, 11000 Belgrade, Serbia
** Corresponding author: tijana.djordjevic@pesting.org.rs*

Received: 30 December 2021
Accepted: 10 February 2022

SUMMARY

Secondary plant metabolites with allelopathic activity or phytotoxicity could be biotechnologically important, serving as a source of allelochemicals, and thus contributing to the agro-industrial sector. The objective of this study was to use the obtained common walnut (*Juglans regia* L.) and bitter dock (*Rumex obtusifolius* L.) leaves extracts rich in phenolic compounds, i.e. with high antioxidant potential, and to identify their phytotoxicity to *Setaria glauca* (L.) P. Beauv. and *Sorghum halepense* (L.) Pers. weed seedlings grown *in vitro*. The obtained plant extracts had remarkably high affinity for scavenging free radicals, having DPPH IC_{50} values of 0.127 mg/ml for common walnut leaf extract and 0.194 mg/ml for bitter dock leaf extract. Ferric reducing antioxidant power of the extracts was also high, FRAP value of the common walnut leaf extract was 384.4 ± 8.1 μmol Fe^{2+}/g dry mass, and of the bitter dock leaf extract 321.6 ± 2.5 μmol Fe^{2+}/g dry mass. At the highest used concentration, common walnut leaf extract reduced germination of *S. glauca* by 67.3%, while bitter dock leaf extract reduced germination of that weed by 54.5%. Shoot length of *S. glauca* was inhibited 80.7% when subjected to common walnut leaf extract, and 78.2% under the influence of bitter dock leaf extract, and its root length was inhibited 96.4% and 93.1% respectively. Germination of *S. halepense* was inhibited 100% under the influence of the obtained common walnut leaf extract at its highest test concentration, and 79.2% when subjected to bitter dock leaf extract at the same concentration. Shoot length of this weed was reduced 100% after treatment with common walnut leaf extract, and 93.7% when subjected to bitter dock leaf extract. Root length was reduced 100% and 99.3%, respectively. Overall, the extracts demonstrated pronounced antioxidant activity and remarkable allelopathic potential.

Keywords: common walnut, bitter dock, weeds, antioxidant activity, allelopathy

INTRODUCTION

The fact of increasing world population has put some durable challenges before the food and agricultural sector through increased demand of total food availabilities coupled with higher quality standards (safety, environment, welfare and ethics). Along with increased crop production, pressure from weeds, insects and diseases has also risen. Among crop pests, weeds are regarded as a major one, responsible for 45% of crop yield

losses (Gnanavel & Natarajan, 2014). Weed infestation decreases crop productivity or reduces the quality of harvested products due to direct competition with crop plants for limited resources (Kaur et al., 2018).

For decreasing crop yield and product quality losses caused by weeds, control strategies are necessary and chemical control is still regarded as the most common and successful control method across the globe, despite problems and issues associated with the use of synthetic herbicides, (Hossen et al., 2021). However, extensive uses of herbicides in modern agriculture cause more and more problems, including development of weed resistance, increment of toxic residues in products, health concerns and environmental pollution. As a result, the tendency towards finding effective and safer alternatives for synthetic herbicides is rapidly increasing. Among non-chemical approaches for weed control, using the phenomenon of allelopathy to suppress weeds turns out to be one of the most effective weed management methods (Jabran & Farooq, 2013).

As allelopathy utilization in agro-ecosystems relies on allelochemicals, i.e., secondary metabolites produced and released by plants, and their effect on the germination, development, reproduction and survival of other nearby plants in the same population (Rice, 1984), it is precisely those phytochemicals that are at the focus of research in the field of bioherbicide development. Due to their destructive effects on plants (seed germination inhibition, shoot and root length restriction and photosynthesis and water/nutrient uptake disruption), various allelopathic compounds such as phenolic compounds (Arroyo et al., 2018), sterols and terpenes (Gaaliche et al., 2017), essential oils (Hazrati et al., 2018) and fatty acids (Qian et al., 2018) are already being tested for potential usage in weed control within organic agricultural systems. One of the advantages of using herbicides based on the mentioned natural compounds is that their half-lives are usually short, indicating that those bioherbicides will degrade quickly and leave no residues in the soil after harvest (Hazrati et al., 2018). Hence, literature data shows that secondary metabolites from plants could be a promising tool for weed control and their implementation in weed management would probably reduce chemical herbicide usage, which would further promote human health protection and environment preservation.

Among the identified chemicals with phytotoxic activity, phenolic compounds are shown to be one of the most important ones (Simões et al., 2008; Li et al, 2010; Mominul Islam & Kato-Noguchi, 2014). Thus a promising start for finding candidates for potential bioherbicides is to obtain plant extracts rich in phenolic compounds, i.e., with high antioxidant potential, and conduct quick testing of their phytotoxic activity by *in vitro* bioassays. Therefore, the main objective of this study was to test the antioxidant activity of extracts of common walnut (*Juglans regia* L.) and bitter dock (*Rumex obtusifolius* L.) leaves, and evaluate their impact on seed germination and seedling growth of *Setaria glauca* (L.) P. Beauv. (yellow foxtail) and *Sorghum halepense* (L.) Pers. (Johnson grass), two invasive weeds considered highly damaging to agricultural crops.

MATERIAL AND METHODS

Plant material and plant extraction

Fresh leaves of common walnut (*Juglans regia* L.) and bitter dock (*Rumex obtusifolius* L.) were collected in Vojvodina province, Serbia, in April 2019, then air dried under shade conditions, stored in paper bags in order to protect them from light and milled just before extraction.

A mix of methanol:acetone:distilled water (40:40:20 v/v) was used as extraction solvent. The powdery material was soaked in the solvent in 1:5 solid to volume ratio and sonicated for 15 min in an ultrasonic bath. Extraction was performed in triplicate and aliquots from three extractions were merged after centrifugation (3000 rpm, 10 min) and filtration (Whatman filter paper grade 1). Extract was evaporated to dryness at 50°C using a vacuum rotary evaporator. Residues were dissolved in distilled water, freeze dried, and kept at -20°C for future analysis.

In vitro evaluation of antioxidant activity

Antioxidant activity of the obtained extracts was tested by determining their radical scavenging activity (2,2-diphenyl-1-picrylhydrazyl [DPPH] scavenging capacity - DPPH assay) and by measuring their reducing potential (ferric ion reducing antioxidant power - FRAP assay).

DPPH assay: For evaluation of free radical scavenging activity, 0.25 g of dry leaf extract was dissolved in distilled water (25 ml), and series of dilutions were prepared from the stock solution in order to obtain concentrations within a range from 0.05 to 0.5 mg/ml. A volume of 0.5 ml of each dilution was mixed with 1 ml of DPPH solution (0.2 mM in methanol) and 3 ml of methanol in test tubes and vortexed well. Volume was adjusted up to 6 ml with methanol, and the solution was incubated for 30 minutes at 25 °C in the dark. The absorbance was measured by spectrophotometer at 517 nm. The solution containing only reagents, excepting the extract, was considered as a control, and antioxidant activity was calculated using the formula:

% inhibition = $[(Abs_{control} - Abs_{sample})/(Absc_{ontrol})] \times 100$. IC_{50} values (concentration of sample required to scavenge 50% of free radicals) were calculated. Ascorbic acid was used as a positive control.

FRAP assay: Acetate buffer (pH 3.6) (300 mM), TPTZ solution (10 mM in 40 mM HCl) and $FeCl_3 \times 6H_2O$ solution (20 mM) were mixed (10:1:1 v/v) into a FRAP solution. Dry leaf extract (150 µl, concentration 0.5 mg/ml in methanol) and 150 µl of distilled water were mixed with 3 ml of FRAP solution and incubated in the dark at 37 °C for 30 minutes. FRAP solution was used as a blank. Absorbance was measured at 593 nm by spectrophotometer. Ferrous sulphate heptahydrate ($FeSO_4 \times 7H_2O$) was used as equivalents for calibration curve preparation, and the results were expressed as µmol of ferric ion (Fe^{2+}) per g of dry extract. Ascorbic acid was used as a positive control.

Germination bioassay

A Petri-dish experiment was set up for *Setaria glauca* (L.) P. Beauv. (yellow foxtail) and *Sorghum halepense* (L.) Pers. (Johnson grass) under controlled conditions. Seeds of *S. glauca* were collected in fields around Batajnica (Belgrade, Central Serbia) in October 2017. Seeds of *S. halepense* were collected in fields around Zemun (Belgrade, Central Serbia) in October 2017. The seeds were cleaned and stored in paper bags in the laboratory at a temperature of 20-22 °C. Prior to the experiment the seeds were surface sterilized for 3 minutes in a 5% aqueous solution of sodium hypochlorite and washed several times with distilled water. Fifteen disinfected seeds were placed into each Petri dish (Ø = 9 cm) lined with sterilized filter paper disk. Common walnut and curly dock leaf extracts were diluted in distilled water to 0.5, 0.75 and 1% (w/v) concentrations and 5 ml was applied to each Petri dish. Distilled water served as a control. All dishes were sealed with parafilm to avoid evaporation. The dishes were placed in an incubator at 27±1 °C and kept in darkness. After a period of 7 days, the percentage of germination was calculated and early seedling growth (shoot and radical length) was measured. The experiment design was a randomized complete block with four replications, repeated twice, and data were combined for analysis.

Statistical analysis

All chemical measurements were performed in triplicates. The results of the FRAP assay were expressed as mean ± standard deviation. The IC_{50} values for DPPH assay were calculated using the GraphPad Prism statistical software package. Germination bioassay data were analyzed by a one-way analysis of variance (ANOVA), using the STATISTICA 8.0. software package. When F-values were statistically significant ($p<0.05$) treatments were compared using Fisher's Least Significant Difference (LSD) test.

RESULTS

Antioxidant activity of plant extracts

The scavenging effect of plant extracts on DPPH radical is shown in Figure 1. Both common walnut and bitter dock leaf extracts showed remarkably high DPPH reductions, compared with ascorbic acid at the same concentrations.

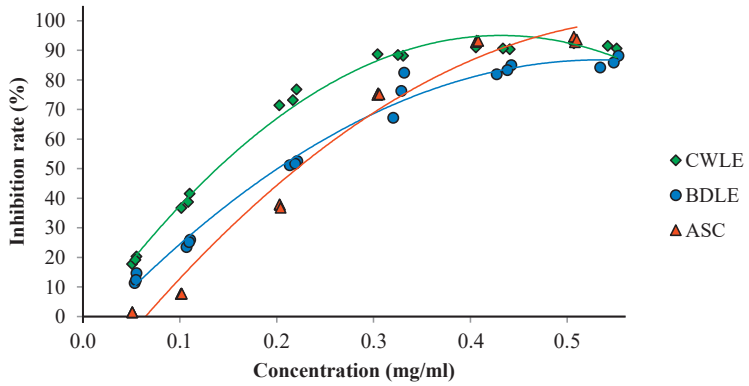

Figure 1. DPPH radical scavenging ability of common walnut (*J. regia*) and bitter dock (*R. obtusifolius*) leaf extracts (CWLE-common walnut leaf extract; BDLE-bitter dock leaf extract; ASC-ascorbic acid)

Common walnut leaf extract achieved approximately 20% inhibition already at its concentration of 0.05 mg/ml, and reached a little above 90% with the concentration of 0.5 mg/ml. Bitter dock leaf extract had somewhat lower scavenging ability as inhibition was approximately 13% at the concentration of 0.5 mg/ml, and reached around 85% at the concentration of 0.5 mg/ml. In comparison, the highest concentration (0.5 mg/ml) of ascorbic acid inhibited almost 94% of DPPH radicals, however, at lower concentrations this antioxidant had lower DPPH reduction activity than the obtained plant extracts. Powerful scavenging ability of the plant extracts is especially noticeable when comparing the calculated IC_{50} values. Thus, IC_{50} values were 0.127 mg/ml for common walnut leaf extract and 0.194 mg/ml for bitter dock leaf extract, both significantly lower compared with the IC_{50} value of ascorbic acid (0.228 mg/ml).

The ferric reducing antioxidant power (FRAP) of plant extract was also high. The results presented in Figure 2 demonstrate that the tested plant leaf extracts possess a significant ferric reducing capacity compared to the standard used (ascorbic acid), although pure antioxidant showed higher potency for this ability.

The FRAP value of common walnut leaf extract was 384.4 ± 8.1 μmol Fe^{2+}/g dry mass, the FRAP value of bitter dock leaf extract was 321.6 ± 2.5 μmol Fe^{2+}/g dry mass, while ascorbic acid had FRAP value of 2576.3 ± 21.5 μmol Fe^{2+}/g dry mass.

Germination bioassay

The effects of common walnut and bitter dock leaf extracts on *Setaria glauca* germination and seedling growth at different concentrations are presented in Figure 3.

Data show that over 90% of the seeds germinated in control petri dishes. Inhibition of *S. glauca* germination by both plant extracts was significant at all used concentrations, and especially high at the highest concentration. Common walnut leaf extract was overall more effective. Although this extract caused lower germination inhibition with its lowest concentration (0.5% w/v), compared to the same concentration (9.1% inhibition) of bitter dock leaf extract, inhibition of *S. glauca* germination caused by common walnut leaf extract at the concentration as low as 0.75% w/v was 34.5%, while it reached 67.3% at the highest test concentration (1% w/v). The lowest concentration of bitter dock leaf extract inhibited *S. glauca* germination by 18.2%, the 0.75% concentration achieved somewhat higher inhibition (23.6%), although not significant, while the highest used concentration reached 54.5% inhibition.

Considering seedling growth, both plant extracts significantly affected shoot and radical elongation of *S. glauca*. Reduction in shoot length was similarly high after treatments with common walnut and bitter dock leaf extracts. At the lowest and mid concentrations, inhibition of shoot length caused by walnut was 63.0 and 69.3%, while 59.4 and 68.3% was caused by dock, and the difference

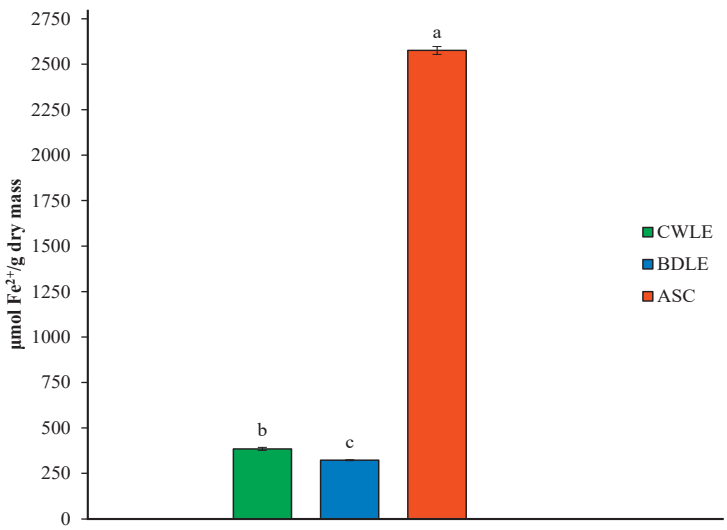

Figure 2. Ferric reducing antioxidant power (FRAP) of common walnut (*J. regia*) and bitter dock (*R. obtusifolius*) leaf extracts (CWLE-common walnut leaf extract; BDLE-bitter dock leaf extract; ASC-ascorbic acid). Data represent the mean values of three experiments (±SD). Values marked with different letters differ significantly ($p \leq 0.05$))

between these results was without statistical significance. As expected, the highest inhibition of shoot length occurred under the highest concentration of common walnut and bitter dock leaf extracts, and without statistical significance between the plant extracts – 80.7% and 78.2%, respectively. The extracts of test plants had even higher negative effect on radical elongation of *S. glauca*, and, although at lower concentrations the reduction in radical length caused by both plants was more or less similar (70.5-89.7% for common walnut; 86.1-89.9% for bitter dock), a significant difference was noted for the highest concentration, where common walnut leaf extract inhibited radical length by 96.4%, and bitter dock leaf extract by 93.1%.

The effects of common walnut and bitter dock leaf extracts on *Sorghum halepense* germination and seedling growth at different concentrations are presented in Figure 4.

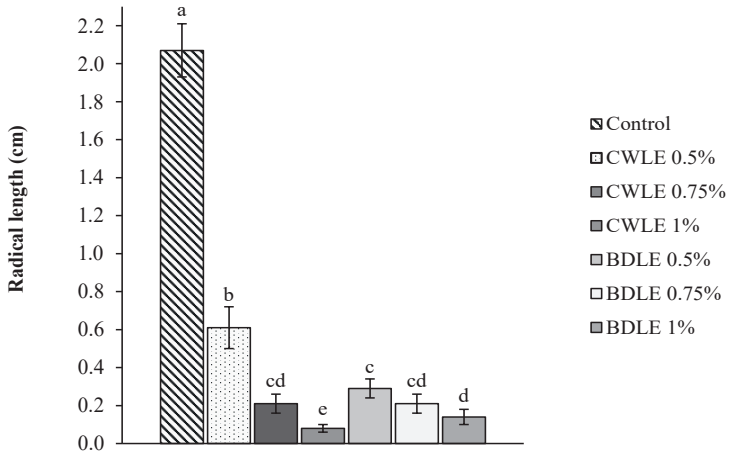

Figure 3. Effects of common walnut (*J. regia*) and bitter dock (*R. obtusifolius*) leaf extracts on germination (a), shoot elongation (b) and radical elongation (c) of *Setaria glauca* seed at different concentrations (CWLE-common walnut leaf extract; BDLE-bitter dock leaf extract). Data represent the mean values of experiments (±SD). Values marked with different letters differ significantly ($p \leq 0.05$)

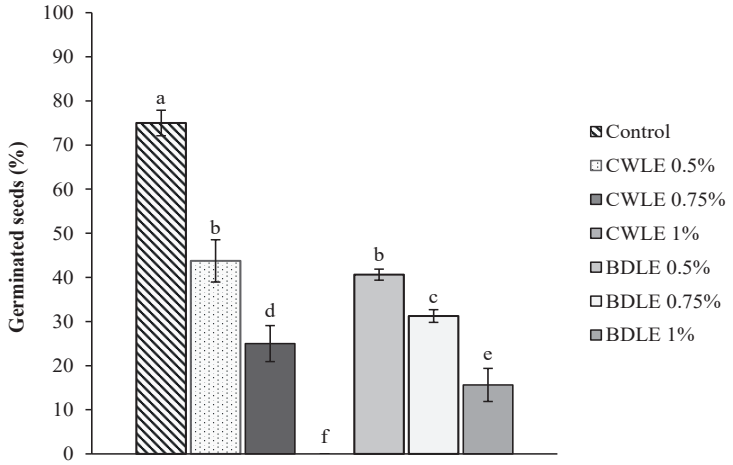

Figure 4. Effects of common walnut (*J. regia*) and bitter dock (*R. obtusifolius*) leaf extracts on germination (a), shoot elongation (b) and radical elongation (c) of *Sorghum halepense* seed at different concentrations (CWLE-common walnut leaf extract; BDLE-bitter dock leaf extract). Data represent the mean values of experiments (±SD). Values marked with different letters differ significantly ($p \leq 0.05$)

The results showed that seeds of *S. halepense* germinated also at high rate in the control - 75%. Both plant extracts highly inhibited the seed germination of this weed at all used concentrations. Inhibition by the lowest concentration, although high, was still under 50% and without a significant difference between the plants (41.7% for walnut and 45.8% for dock). However, the mid and highest concentrations of common walnut leaf extract showed extremely high effects on germination rate. Inhibition caused by the concentration of 0.75% w/v was 66.7%, while 1% w/v concentration of this extract prevented germination of *S. halepense* (100% inhibition). Bitter dock leaf extract did not cause total inhibition of germination of this weed, although the effect was still very high. Germination inhibition was 58.3% at the mid concentration and it reached 79.2% at the highest test concentration.

Seedling growth of *S. halepense* was significantly affected by both plant extracts. Common walnut leaf extract was overall more effective in reducing shoot length, as inhibition of shoot elongation was 90.4 and 92.0% already at the lowest and mid concentrations, respectively (without statistical differences). As mentioned before, germination inhibition at the highest concentration, and consequently shoot length inhibition, was 100%. Inhibition of shoot length caused by the three concentrations of bitter dock leaf extract from the lowest to the highest, was 72.0, 86.2 and 93.7%, respectively, and differences between those results were statistically significant.

As for *S. glauca*, negative effects of plant extracts on its radical length were even more pronounced in comparison with shoot length. At lower concentrations the reduction of radical elongation caused by both plants was without statistical differences and extremely high: 94.0-95.4% for common walnut and 92.9-96.1% for bitter dock. At the highest used concentration, as mentioned earlier, common walnut leaf extract inhibited germination, and consequently radical length, by 100%, while inhibition was 99.3% after treatment with bitter dock leaf extract.

DISCUSSION

Antioxidant activity of plant extracts

Walnut and dock leaves have been intensively used in medical practice, but also as a source of valuable compounds for various industrial applications based on their antioxidant properties. The varied biological activities of *Juglans* and *Rumex* species is due to the presence of various groups of biologically active substances in them, most belonging to phenolic compounds: tannins (galotannins and ellagitannins), a naphthoquinone derivative (juglone), anthraquinones, flavonoids (quercetin, kaempferol, etc.), phenolic acids (caffeic acid, p-coumaric acid, etc.) (Amaral et al., 2008; Zhou et al., 2015; Rusu et al., 2018; Jimoh et al., 2008; Wegiera et al., 2007; Litvinenko & Muzychina, 2008). Phenolic profiles of extracts obtained from these plants may differ quite significantly depending on the polarity of solvents used for extraction. In this study a mix of three solvents (acetone, methanol and water) was used in order to extract antioxidative phenolic compounds with a wide range of polarity. As antioxidants from plants respond in a different manner to different radical or oxidant sources (Prior et al., 2005), two methods based on different reaction mechanisms were used to determine the antioxidant activity of the obtained extracts.

Both common walnut and bitter dock leaf extracts obtained in this experiment showed to possess high antioxidant activities. Their affinity for scavenging free radicals was remarkably high, compared with ascorbic acid, with DPPH IC_{50} values being lower (higher antioxidant activity) than the IC_{50} value of ascorbic acid. Ferric reducing antioxidant power (FRAP) of plant extract was also significantly high. However, compared to the standard used (ascorbic acid), plant extracts showed lower ability to reduce transition metal ions.

The DPPH scavenging ability of the obtained common walnut leaf extract was higher than it was in bitter dock. Its IC_{50} value (0,127 mg/ml) was lower than those Pereira et al. (2007) found for walnut leaves aqueous extracts (0.151-0.202 mg/ml) or Carvalho et al. (2010) for walnut leaves methanol (0.199 mg/ml) and petroleum ether (2.921 mg/ml) extracts. On the other side, Santos et al. (2013) recorded even lower IC_{50} values (0.066 mg/ml) for walnut leaves methanol extracts from certain cultivars. Bitter dock leaf extract obtained in our present study had somewhat lower DPPH scavenging ability, compared to walnut, although still significantly higher than ascorbic acid. Its IC_{50} value (0.194 mg/ml) was significantly lower than those found for dock leaf extracts obtained by ultrasonic extraction, using methanol:water 80:20 solvent mix (approximately 40 mg/ml) (Wegiera et al., 2011), but higher than those obtained by Soxhlet extraction with methanol (0.078 mg/ml) (Harshaw et al., 2010). Inconsistencies in results are most likely due to the extraction methodology, which is important in any antioxidant assay as the yield of antioxidative phenolic compounds depends on the

solubility of natural products and choice of solvent. However, the importance of plant cultivar itself, as well as the growth environment and phase of plant ontogenetic development should not be underestimated. Extracts of common walnut and bitter dock leaves obtained in this experiment could be considered to have high radical scavenging ability.

Considering the ferric reducing antioxidant power, common walnut leaf extract showed a moderate ability to reduce metal ions. FRAP value obtained for this plant (384.4 μmol Fe^{2+}/g dry mass) was somewhat higher compared with bitter dock leaf extract. However, its potency regarding this ability was notably lower compared to ascorbic acid. A similar FRAP value for walnut leaves extract was obtained by Shah et al. (2018) (418.92 μmol Fe^{2+}/g dry mass), although this group of authors reported even higher values (up to 1067.94 μmol Fe^{2+}/g dry mass), depending on walnut genotype tested. The ferric reducing antioxidant power of bitter dock leaf extract was moderate, somewhat lower than the power of walnut, with a FRAP value 321.6 μmol Fe^{2+}/g dry mass. Antioxidant activity has been examined in a number of *Rumex* species, thus Jimoh et al. (2008) reported a significant ferric reducing antioxidant power of *Rumex ecklonianus* with FRAP value of 384.64 μmol Fe^{2+}/g dry mass for acetone leaf extract, 707.26 μmol Fe^{2+}/g dry mass for methanol leaf extract and 47.88 μmol Fe^{2+}/g dry mass for water leaf extract, while Chelly et al. (2021) tested bioproperties of methanol extracts of different parts of *Rumex roseus* and obtained a FRAP value for leaf of 700 μmol Fe^{2+}/g dry mass. Generally, regarding FRAP assay, it is difficult to make comparison with literature data primarily due to different interpretations of results, but also differences in plant cultivars, their phenological phase and plant growth conditions, as well as extraction solvents used and sample preparation procedures, especially considering that the extraction method has proved to have significant influence on FRAP.

Overall, bioactive compounds present in the obtained common walnut and bitter dock leaf extracts could be considered as interesting and economical sources of antioxidants with strong antioxidant capacity for various applications, including potential weed control.

Allelopathic potential of plant extracts

The results of our research indicate that common walnut and bitter dock leaf extracts were phytotoxic to both tested weeds - *S. glauca* and *S. halepense*.

For *S. glauca,* the allelopathic effect expressed through the inhibition of seed germination was more pronounced only after treatments with the highest used concentrations of extracts, reaching over 50% inhibition, and common walnut leaf extract was more efficient regarding this bioassay parameter. Regarding *S. halepense,* both extracts used already at their mid concentrations caused over 50% seed germination inhibition. At the highest concentration, bitter dock leaf extract extremely reduced the germination rate of *S. halepense*, while common walnut leaf extract fully stopped its germination (100% inhibition). Inhibition of seed germination by the tested extracts can be attributed to the presence of phenolic compounds and effects may be due to their synergistic effect rather than a single constituent. Based on the strong antioxidant capacity of the extracts it could be assumed that phenolic compounds (flavonoids, phenolic acids, tannins, quinines, etc.) are most probably present in large quantities in common walnut and bitter dock leaves extracts. As those phenolic compounds could interfere with the activities of respiratory enzymes in seed germination (Muscolo et al., 2001) or alter the activities of the growth hormone gibberellic acid, thereby causing inhibitory effect on its germination (Olofsdotter, 2001), their presence in the extracts could probably be the reason for the obtained inhibitions.

Considering seedling growth, both plant extracts significantly reduced shoot and radical length of both tested weeds. Regarding *S. glauca,* shoot length was similarly affected by the same concentrations of common walnut and bitter dock leaf extracts, and both tested extracts inhibited shoot elongation at almost the same rate without statistically significant difference. Radical elongation of this weed was more sensitive to bitter dock leaf extract at the lowest tested concentration but the highest concentration of common walnut leaf extract achieved higher inhibition of this parameter. *S. halepense* was even more sensitive to the tested plant extracts, and overall the common walnut leaf extract had a higher impact on its shoot and radical length than the bitter dock leaf extract. Radical elongation was for this weed also more intensively inhibited than shoot elongation. Generally, roots often show higher sensibility compared with shoots, as roots of plants are in fact the first plant organ to absorb allelopathic compounds from extracts (Nishida et al., 2005). Besides, root tissue is more permeable than shoot tissue (Mominul Mominul Islam & Kato-Noguchi, 2013). Phenolic compounds, namely flavonoids, have been shown to influence the expression of specific genes

associated with root tissue differentiation, decreasing root development (Franco et al., 2015), and generally many studies report suppressions of root growth due to decrease in mitotic cell division in root apex when exposed to different plant extracts (Levizou et al., 2002; Piyatida & Kato-Noguchi, 2010; Morikawa et al., 2012, Rob et al., 2021).

Overall, common walnut leaf extract showed greater efficacy in inhibiting germination and seedling elongation of the test weeds, compared to bitter dock leaf extract, and there is a consistency regarding antioxidant activities of the extracts and their phytotoxicity as the walnut leaf extract also had a higher antioxidant potential. Walnut is one of the most famous allelopathic plants (Ercisli et al., 2005) and its toxicity is associated mostly with the powerful napthoquinone juglone (Strugstad & Despotovski, 2012). However, juglone is not expected to be the only allelochemical present in *Juglans* species. Studies regarding the allelopathic potential of walnut are generally focused on the effect of juglone itself, while some studies have also investigated the effect of walnut leaf extracts with or without quantification of contents of juglone and/or other phenolic compounds in them, and all studies reported high inhibitory effects on germination and seedling growth of various cultivated or native plants (Babula et al., 2014; Zubay et al., 2021; Ercisli et al., 2005; Kocacë Aliskan & Terzi, 2001; Ercisli & Turkkal, 2005; Medic et al., 2021). Regarding *Rumex* species, less literature data is available dealing with its allelopathic potential. Zaller (2006) tested the effect of an aqueous extract of bitter dock leaves on seed germination of 14 plant species belonging to graminoids, non-leguminous forbs and leguminous forbs, and revealed that all tested grasses were heavily inhibited by the extracts, while herbs and legumes varied from unaffected to heavily inhibited. Another group of authors also pointed out strong inhibiting effects of *R. crispus* and *R. obtusifolius* leaf extracts on tested grassland grass species (Dragomir et al., 2017), while allelopathic effects of those extracts were severe for some grassland perennial legume seeds (*Medicago sativa*, *Trifolium pratense* and *Lotus corniculatus*) and moderate for others (*Trifolium repens*) (Camen et al., 2017). As indicated in numerous previous studies, the effects of identical extracts are not necessarily the same across various plants, i.e. each plant species has its own response to allelochemicals (Medic et al., 2021). Our research showed that *S. halepense* seeds were more sensitive to the tested common walnut and bitter dock leaf extracts than *S. glauca* seeds.

CONCLUSION

The results of the present study demonstrated that the obtained common walnut (*Juglans regia* L.) and bitter dock (*Rumex obtusifolius* L.) leaves extracts have pronounced antioxidant activity, and it could be inferred from the presented preliminary investigation that they possess remarkable allelopathic potential as both had significant negative impact on the germination and seedling growth of the tested weeds *Setaria glauca* (L.) P. Beauv. and *Sorghum halepense* (L.) Pers. The level of growth suppression varied with extract concentration and examined plant species. An association was noted between the antioxidative potential of extracts and their phytotoxicity, suggesting that phenolic compounds, the dominant antioxidant components in plants, could be responsible for their allelopathic potential. The result may be useful for future research in the field of bioherbicide development, but additional studies are required to validate the present results under field conditions and to test extract phytotoxicity to cultivated plants before proceeding towards development of herbicides based on natural products.

ACKNOWLEDGEMENT

This investigation was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grants No. 451-03-9/2021-14/200214 and 451-03-9/2021-14/200125).

REFERENCES

Amaral, J. S., Valentão, P., Andrade, P. B., Martins, R. C., & Seabra, R. M. (2008). Do cultivar, geographical location and crop season influence phenolic profile of walnut leaves? *Molecules*, *13*, 1321-1332. doi:10.3390/molecules13061321

Arroyo, A.I., Pueyo, Y., Pellissier, F., Ramos, J., Espinosa-Ruiz, A., Millery, A., & Alados, C.L. (2018). Phytotoxic effects of volatile and water-soluble chemicals of *Artemisia herba-alba*. *Journal of Arid Environments*, *151*, 1-8. doi:10.1016/j.jaridenv.2017.11.010

Babula, P., Vaverkova, V., Poborilova, Z., Ballova, L., Masarik, M., & Provaznik, I. (2014). Phytotoxic action of naphthoquinone juglone demonstrated on lettuce seedling roots. *Plant Physiology and Biochemistry*, *84*, 78-86. doi: 10.1016/j.plaphy.2014.08.027

Camen, D., Dragomir, N., Horablaga, M., Dragomir, C., Rechiţean, D., & Dragoş, M. (2017). Allelopathic aspects in *Rumex crispus* L. and *Rumex obtusifolius* L. II. Allelopathic effect on grassland legumes. *Romanian Journal of Grassland and Forage Crops, 15*, 19-24.

Carvalho, M., Ferreira, P.J., Mendes, V.S., Silva, R., Pereira, J.A., Jerónimo, C., & Silva, B.M. (2010). Human cancer cell antiproliferative and antioxidant activities of *Juglans regia* L. *Food and Chemical Toxicology*, *48*, 441-447. doi: 10.1016/j.fct.2009.10.043

Chelly, M., Chelly, S., Occhiuto, C., Cimino, F., Cristani, M., Saija, A. ... Siracusac, L. (2021). Comparison of phytochemical profile and bioproperties of methanolic extracts from different parts of tunisian *Rumex roseus*. *Chemistry and Biodiversity, 18*, e2100185. doi: 10.1002/cbdv.202100185

Dragomir, N., Horablaga, M., Camen, D., Dragomir, C., Rechiţean, D., & Dragoş, M. (2017). Allelopathic aspects in *Rumex crispus* L. and *Rumex obtusifolius* L. I. Allelopathic effect on grassland grasses. *Romanian Journal of Grassland and Forage Crops, 15*, 31-37.

Ercisli, S., Esitken, A., Turkkal, C., & Orhan, E. (2005). The allelopathic effects of juglone and walnut leaf extracts on yield, growth, chemical and PNE compositions of strawberry cv. Fern. *Plant*, *Soil and Environment*, *51*(6), 283-287. doi: 10.17221/3587-PSE

Ercisli, S., & Turkkal, C. (2005). Allelopathic effects of juglone and walnut leaf extracts on growth, fruit yield and plant tissue composition in strawberry cvs. 'Camarosa' and 'Sweet Charlie'. *Journal of Horticultural Science and Biotechnology*, *80*, 39-42. doi: 10.1080/14620316.2005.11511888

Franco, D.M., Silva, E.M., Saldanha, L.L., Adachi, S.A., Schley, T.R., Rodrigues, T.M. ... Rolim de Almeida, L.F. (2015). Flavonoids modify root growth and modulate expression of short-root and HD-ZIP III. *Journal of Plant Physiology*, *188*, 89-95. doi: 10.1016/j.jplph.2015.09.009

Gaaliche, B., Ladhari, A., Medeiros, A.G., Ben Mimoun, M., & Hajlaoui, M.R. (2017). Relationship between phytochemical profiles and phytotoxic proprieties of Tunisian fig leaf cultivars. *South African Journal of Botany*, *112*, 322-328. doi: 10.1016/j.sajb.2017.06.015Get

Gnanavel, I., & Natarajan, S. K. (2014). Eco-friendly weed control options for sustainable agriculture. *Agricultural Reviews*, *35*(3), 172. doi: 10.5958/0976-0741.2014.00904.0

Harshaw, D., Nahar, L., Vadla, B., Saif-e-Naser, G. M., & Sarker, S.D. (2010). Bioactivity of *Rumex obtusifolius* (Polygonaceae). *Archives of Biological Science*, *62*(2), 387-392. doi: 10.2298/ABS1002387H

Hazrati, H., Saharkhiz, M. J., Moein, M., & Khoshghalb, H. (2018). Phytotoxic effects of several essential oils on two weed species and tomato. *Biocatalysis and Agricultural Biotechnology*, *13*, 204-212. doi: 10.1016/j.bcab.2017.12.014

Hossen, K., Ozaki, K., Teruya, T., & Kato-Noguchi, H. (2021). Three active phytotoxic compounds from the leaves of *Albizia richardiana* (Voigt.) King and Prain for the development of bioherbicides to control weeds. *Cells*, *10*, 2385. doi: 10.3390/cells10092385

Jabran, K., & Farooq, M. (2013). Implications of potential allelopathic crops in agricultural systems. In Cheema, Z., Farooq, M., Wahid, A. (eds), *Allelopathy* (pp. 349-385). Berlin/Heidelberg, Germany: Springer. doi: 10.1007/978-3-642-30595-5_15

Jimoh, F.O., Adedapo, A.A., Aliero, A.A., &. Afolayan, A.J (2008) Polyphenolic contents and biological activities of *Rumex ecklonianus*. *Pharmaceutical Biology*, *46*(5), 333-340. doi: 10.1080/13880200801887765

Kaur, S., Kaur, R., & Chauhan, B.S. (2018). Understanding crop-weed-fertilizer-water interactions and their implications for weed management in agricultural systems. *Crop Protection*, *103*, 65-72. doi: 10.1016/j.cropro.2017.09.011

Kocacë Aliskan, I., & Terzi, I. (2001). Allelopathic effects of walnut leaf extracts and juglone on seed germination and seedling growth. *Journal of Horticultural Science and Biotechnology*, *76*, 436–440. doi: 10.1080/14620316.2001.11511390

Levizou, E.F.I., Karageorgou, P., Psaras, G.K., & Manetas, Y. (2002). Inhibitory effects of water-soluble leaf leachates from *Dittrichia viscosa* on lettuce root growth, statocyte development and graviperception. *Flora*: *Morphology*, *Distribution*, *Functional Ecology of Plants*, *197*, 152-157. doi: 10.1078/0367-2530-00025

Litvinenko, Y.A., & Muzychina, R. A. (2008). New antioxidant phytopreparation from *Rumex thyrsiflorus* roots. III. *Chemistry of Natural Compounds*, *44,* 239-240. doi: 10.1007/s10600-008-9026-y

Li, Z.H., Wang, Q., Ruan, X., Pan, C.D., & Jiang, D.A. (2010). Phenolics and plant allelopathy. *Molecules*, *15*(12), 8933-8952. doi: 10.3390/molecules15128933

Medic, A., Zamljen, T., Slatnar, A., Hudina, M., & Veberic, R. (2021). Is juglone the only naphthoquinone in *Juglans regia* L. with allelopathic effects? *Agriculture*, *11*, 784. doi: 10.3390/agriculture11080784

Mominul Islam, A.K.M., & Kato-Noguchi, H. (2013). Plant growth inhibitory activity of medicinal plant *Hyptis suaveolens*: Could allelopathy be a cause? *Emirates Journal of Food and Agriculture*, *25*, 692-701. doi: 10.9755/ejfa.v25i9.16073

Mominul Islam, A.K.M., & Kato-Noguchi H. (2014). Phytotoxic activity of *Ocimum tenuiflorum* extracts on germination and seedling growth of different plant species. *Scientific World Journal*, *2014*, 1-8. doi: 10.1155/2014/676242

Morikawa, C.I.O., Miyaura, R., de Lourdes Tapia y Figueroa, M., Liliana, E., Rengifo Salgado, E.L., & Fujii, Y. (2012). Screening of 170 Peruvian plant species for allelopathic activity by using the sandwich method. *Weed Biology and Management*, *12*, 1-11. doi: 10.1111/j.1445-6664.2011.00429.x

Muscolo, A., Panuccio, M.R., & Sidari, M. (2001). The effect of phenols on respiratory enzymes in seed germination. *Plant Growth Regulation*, *35,* 31-35. doi: 10.1023/A:1013897321852

Nishida, N., Tamotsu, S., Nagata, N., Saito, C., & Sakai, A. (2005). Allelopathic effects of volatile monoterpenoids produced by *Salvia leucophylla*: Inhibition of cell proliferation and DNA synthesis in the root apical meristem of *Brassica campestris* seedlings. *Journal of Chemical Ecology*, *31*, 1187-1203. doi: 10.1007/s10886-005-4256-y

Olofsdotter, M. (2001). Rice – A step toward use of allelopathy. *Agronomy Journal, 93,* 3-8. doi: 10.2134/agronj2001.9313

Pereira, J.A., Oliveira, I., Sousa, A., Valentao, P., Andrade, P.B., Ferreira, I.C.F.R. ... Estevinho, L. (2007). Walnut (*Juglans regia* L.) leaves: Phenolic compounds, antibacterial activity and antioxidant potential of different cultivars. *Food and Chemical Toxicology*, *45*(11), 2287-2295. doi: 10.1016/j.fct.2007.06.004

Piyatida, P., & Kato-Noguchi, H. (2010). Screening of allelopathic activity of eleven Thai medicinal plants on seedling growth of five test plant species. *Asian Journal of Plant Sciences*, *9*, 486-491. doi: 10.3923/ajps.2010.486.491

Prior, R.L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*, *53*(10), 4290-4302. doi: 10.1021/jf0502698

Qian, H., Xu, J., Lu, T., Zhang, Q., Qu, Q., Yang, Z., & Pan, X. (2018). Responses of unicellular alga *Chlorella pyrenoidosa* to allelochemical linoleic acid. *Science of the Total Environment*, *625*, 1415-1422. doi: 10.1016/j.scitotenv.2018.01.053

Rice, E.L. (1984). *Allelopathy*, 2nd ed. Orlando, FL, USA: Academic Press.

Rob, M.M, Hossen, K., Khatun, M.R., Iwasaki, K., Iwasaki, A., Suenaga, K., & Kato-Noguchi, H. (2021). Identification and application of bioactive compounds from *Garcinia xanthochymus* Hook. for weed management. *Applied Sciences*, *11*, 2264. doi: 10.3390/app11052264

Rusu, M.E., Gheldiu, A.M., Mocan, A., Moldovan, C., Popa, D.S., Tomuta, I., & Vlase, L. (2018). Process optimization for improved phenolic compounds recovery from walnut (*Juglans regia* L.) septum: phytochemical profile and biological activities. *Molecules*, *23*, 2814. doi: 10.3390/molecules23112814

Santos, A., Barros, L., Calhelha, R.C., Dueñas, M., Carvalho, A.M., Santos-Buelga, C., & Ferreira, I.C.F.R. (2013). Leaves and decoction of *Juglans regia* L.: Different performances regarding bioactive compounds and in vitro antioxidant and antitumor effects. *Industrial Crops and Products*, *51*, 430/436. doi: 10.1016/j.indcrop.2013.10.003

Shah, U.N., Mir, J.I., Ahmed, N., Jan, S., & Fazili, K.M. (2018). Bioefficacy potential of different genotypes of walnut *Juglans regia* L. *Journal of Food Science and Technology, 55*, 605-618. doi: 10.1007/s13197-017-2970-4

Simões, K., Du, J., Kretzschmar, F.S., Broeckling, C.D., Stermitz, F.S., Vivanco, J.M., & Braga, M.R. (2008). Phytotoxic catechin leached by seeds of the tropical weed *Sesbania virgata*. *Journal of Chemical Ecology*, *34*, 681-687. doi: 10.1007/s10886-008-9443-1

Strugstad, M., & Despotovski, S. (2012). A summary of extraction, synthesis, properties, and potential uses of juglone: A literature review. *Journal of Ecosystems and Management*, *13*, 1-16.

Wegiera, M., Grabarczyk, P., Baraniak, B., & Danuta Smolarz, H. (2011). Antiradical properties of extracts from roots, leaves and fruits of six *Rumex* L. species. *Acta Biologica Cracoviensia Series Botanica*, *53*(1), 125-131. doi: 10.2478/v10182-011-0018-z

Wegiera, M., Smolarz, H. D., Wianowska, D., & Dawidowicz, A. L. (2007). Anthracene derivatives in some species of *Rumex* L. genus. *Acta Societatis Botanicorum Poloniae, 76*, 103-108. doi: 10.5586/asbp.2007.013

Zaller, J.G. (2006). Allelopathic effects of *Rumex obtusifolius* leaf extracts against native grassland species. *Journal of Plant Diseases and Proctection, 20*, 463-470.

Zhou, Y., Yang, B., Jiang, Y., Liu, Z., Liu, Y., Wang, X., & Kuang, H. (2015). Studies on cytotoxic activity against HepG-2 cells of naphthoquinones from green walnut husks of *Juglans mandshurica* Maxim. *Molecules*, *20*(9), 15572–15588. doi: 10.3390/molecules200915572

Zubay, P., Kunzelmann, J., Ittzés, A., Németh Zámboriné, É., & Szabó, K. (2021). Allelopathic effects of leachates of *Juglans regia* L., *Populus tremula* L. and juglone on germination of temperate zone cultivated medicinal and aromatic plants. *Agroforestry Systems*, *95*, 431-442. doi: 10.1007/s10457-020-00572-9

Antioksidativna aktivnost i skrining alelopatskog potencijala ekstrakata lista *Juglans regia* L. i *Rumex obtusifolius* L.

REZIME

Alelopatski potencijal i fitotoksičnost sekundarnih metabolita biljaka značajni su sa aspekta biotehnologije imajući u vidu da ove fitohemikalije u svojstvu alelohemikalija mogu značajno da doprinesu razvoju agroindustrijskog sektora. Cilj rada bio je da se dobiju ekstrakti lista oraha (*Juglans regia* L.) i štavelja (*Rumex obtusifolius* L.) bogati fenolnim jedinjenjima odnosno sa visokim antioksidativnim potencijalom i da se ispita njihova fitotoksičnost prema korovskim vrstama *Setaria glauca* i *Sorghum halepense* kroz inhibiciju klijanja i rasta klijanaca. Dobijeni biljni ekstrakti pokazali su izražen potencijal u neutralisanju DPPH radikala, sa IC_{50} vrednostima od 0,127 mg/ml za ekstrakt lista oraha i 0,194 mg/ml za ekstrakt lista štavelja. Redukciona sposobnost jona metala dobijenih biljnih ekstrakata takođe je bila veoma visoka, FRAP vrednost za ekstrakt lista oraha iznosila je 384,4 ± 8.1 μmol Fe^{2+}/g suvog ekstrakta, dok je za ekstrakt lista štavelje iznosila 321,6 ± 2.5 μmol Fe^{2+}/g suvog ekstrakta. Pri najvećoj korištenoj koncentraciji ekstrakt lista oraha inhibirao je klijanje semena *S. glauca* za 67,3%, dok je ekstrakt lista štavelja inhibirao klijanje ovog korova za 54,5%. Porast stabaoceta *S. glauca* inhibiran je 80,7% nakon tretmana ekstraktom lista oraha, a 78,2% pod uticajem ekstrakta lista štavelja, dok je porast korenka inhibiran 96,4%, odnosno 93,1%. Klijanje semena *S. halepense* potpuno je inhibirano (100%) pod uticajem ekstrakta lista oraha, dok je ekstrakt lista štavelja pri najvećoj korišćenoj koncentraciji prouzrokovao 79,2% inhibicije. Porast stabaoceta ovog korova potpuno je redukovan (100%) nakon tretmana ekstraktom lista oraha, a ekstrakt lista štavelja prouzrokovao je 93,7% inhibicije ovog parametra. Porast korenka inhibiran je 100% odnosno 99.3% nakon tretmana ovim ekstraktima. Dobijeni rezultati ukazuju na postojanje značajne antioksidativne aktivnosti i izraženog alelopatskog potencijala dobijenih ekstrakata lista oraha i lista štavelja.

Ključne reči: orah, štavelj, korovi, antioksidativna aktivnost, alelopatija