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# *SESELI RIGIDUM* WALDST. & KIT: SECONDARY METABOLITES AND ANTIOXIDANT ACTIVITY OF METHANOL, ETHYL ACETATE AND ACETONE EXTRACTS

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**Abstract:** Representatives of the Seseli genus are recognized for their biological activities. The objective of this study was to analyze the phenolic and flavonoid content, along with the antioxidant effects, of methanol, ethyl acetate, and acetone extracts obtained from the roots, leaves, stems, and fruits of *S. rigidum.* Through spectrophotometric measurements of phenols and flavonoids, the results revealed that the polar (methanol) solvent extract from the leaves exhibited the highest concentrations of phenols (98.66 ± 2.64 mg GA/g) and flavonoids (20.74 ± 0.55 mg RU/g), surpassing those obtained from the stem. Specifically, the methanol extract from the leaves demonstrated the highest antioxidant activity at 25.87 ± 0.05 μg/mL, while the fruit extract exhibited the lowest antioxidant activity at 638.5 ± 0.01 μg/mL. Notably, the total phenol content displayed a strong positive correlation with the antioxidant activity of the extracts, unlike the total flavonoid content.

**Keywords**: *S. rigidum*, phenols, flavonoids, DPPH

## Introduction

Seseli rigidum Waldst. & Kit. (Apiaceae) is a subendemic perennial plant, commonly known as "devesilje" or "devesilj" (Ilić, 2016). Previous research has identified essential oils, coumarins, flavonoids, lignans, sesquiterpene lactones, and polyacetylenes in species of the Seseli genus (Abbaskhan et al., 2012). Traditionally, Seseli genus species are used, with roots employed to alleviate pain in bones, muscles, and joints, leaves used in culinary practices, and fruits utilized as a remedy for gastrointestinal issues (Gonçalves, 2012). The essential oil extracted from Seseli genus species has demonstrated antioxidant,

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antibacterial, antifungal, anti-inflammatory, and moderate cytotoxic activities (Marčetić, 2012).

Various studies have explored the antifungal, antigenotoxic, genotoxic, and antimicrobial properties of the *S. rigidum* plant in previous research (Živković, 2016; Marčetić, 2019). The current study aims to investigate the phenolic and flavonoid content, as well as the antioxidant effects, of methanol, ethyl acetate, and acetone extracts obtained from the roots, leaves, stems, and fruits of *S. rigidum.*

## Materials and methods

### Plant material and extracts preparation

Plant material, *S. rigidum* was collected in July 2022 from the locality Ovcar-Kablar Gorge (43°55′07″ N; 20°13′17″ E) in the flowering phase, (fruit, stem, leaves, and root). Collected materials was cleaned, and dried in a dark room at room temperature. The dried and finely ground fruit, stem, leaves, and root (50 gr) were extracted with methanol, ethyl acetate, and acetone (250 mL). After the 24 hours plant material was filtrated, and then 250 mL of adequate solvents were added. After next 24 hours, the procedure was repeated. After extraction, the material was evaporated to dryness using a rotary evaporator at 40°C. The obtained extracts (twelve) were stored in sterile tubes at 4°C till the chemical analysis.

### Determination of total phenols content of the extracts

The total phenol content of the sample's methanol solutions was measured by the Folin-Ciocalteu assay as described by Peter et al. (2011). The absorbance was measured using a spectrophotometer at $\lambda$ max = 725 nm. Gallic acid (GA) was used to construct the standard curve, which showed the linear regression of $r^2 > 0.99$, and the values obtained for the concentration of total phenols are expressed as GA/g of extract.

### Determination of total flavonoid content of the extracts

The concentration of flavonoids in the sample's methanol solutions was measured by the method of Quettier-Deleu et al. (2000). The absorbance was measured using a spectrophotometer at $\lambda$ max = 430 nm. Rutin (RU) was used

to construct the standard curve, which showed the linear regression of $r^2 > 0.99$, and the values obtained for the concentration of total flavonoids are expressed as RU/g of extract.

### Determination of the antioxidant activity of the extracts

The antioxidant activity of the plant extracts against DPPH was determined using the method proposed by Takao et al. (1994). Ascorbic acid was used as the standard. The absorbance was measured at $\lambda$ max =517 nm. The percent DPPH inhibition was calculated by the equation (Eq. 1). Antioxidant activity was expressed as the 50% inhibitory concentration ($IC_{50}$ values in μg/mL).

*%inhibition=(( A of control-A of sample)/(A of control))x100* (1)

### Statistical analysis

The content of phenols, flavonoids and antioxidant activity were carried out in triplicate and expressed as the average value, ± standard deviation. Statistical analyses of data was made by using IBM SPSS Statistics 21.0 (2012). The collected data were analyzed by the one-way ANOVA and Tukey post-hoc test. Statistically significant difference was defined as $p < 0.05$.

## Results and discussion

### *Total contents* phenols *of the extracts*

Results of the total amount of phenols in fruits, stem, leaf and root of species *S. rigidum*, are shown in (Table 1.).

Table 1. The total amount of phenols determined in different *S.rigidum* extracts presented as equivalents of gallic acid (mg of GA/g extract)

| | **Fruits** | **Stem** | **Leaf** | **Root** |
|---|---|---|---|---|
| **Methanol** | $61.94 \pm 0.25^{b}$ | $98.66 \pm 2.64^{a}$ | $95.06 \pm 0.54^{a}$ | $75.38 \pm 2.83^{b}$ |
| **Ethyl-acetate** | $71.06 \pm 1.42^{a}$ | $79.94 \pm 3.09^{b}$ | $59.67 \pm 5.36^{c}$ | $64.83 \pm 3.61^{c}$ |
| **Acetone** | $61.89 \pm 0.25^{b}$ | $80.72 \pm 1.13^{c}$ | $81.845 \pm 1.36^{b}$ | $85.11 \pm 1.71^{a}$ |

Values are mean ± standard deviation of triplicate analyses. Values within the row followed by the same letter (a, b, c), are not significantly different according to Tukey's test ($p<0.05$).

The highest concentration of phenols was extracted with polar solvents - methanol (98.66 ± 2.64 mg GA/g) from stem, and (95.06 ± 0.54 mg GA/g) from leaves, and the lowest concentration of phenols was measured in extracts obtained with non-polar solvent (ethyl-acetate) (59.67 ± 5.36 mg GA/g) from leaves. Differences in phenol content among extracts made by different solvent were significant ($p < 0.05$). Compared to the results published by Ilić, (2017), in the polar solvent acetone constated the highest amount of phenol (151.03 ± 4.47) was found in the root, and the lowest concentration coincides with the results of our study, (ethyl-acetate) (3.96 ± 0.12 mg GA/g) from leaves.

### *Total contents flavonoids of the extracts*

Results of the total amount of flavonoids in fruits, stem, leaf and root of species *S. rigidum*, are shown in (Table 2.).

Table 2. The total amount of flavonoids determined in different *S.rigidum* extracts presented as equivalents of rutin (mg of RU/g extract)

| | **Fruits** | **Stem** | **Leaf** | **Root** |
|---|---|---|---|---|
| **Methanol** | 11.38 ± 0.18$^{a}$ | 20.74 ± 0.55$^{a}$ | 20.07 ± 0.63$^{a}$ | 14.33 ± 0.14$^{a}$ |
| **Ethyl-acetate** | 2.86 ± 0.38$^{c}$ | 14.98 ± 0.48$^{c}$ | 18.29 ± 0.38$^{c}$ | 14.83 ± 0.27$^{a}$ |
| **Acetone** | 9.45 ± 0.32$^{b}$ | 17.67 ± 0.22$^{b}$ | 19.95 ± 0.72$^{b}$ | 12.24 ± 0.39$^{b}$ |

Values are mean ± standard deviation of triplicate analyses. Values within the row followed by the same letter (a, b, c), are not significantly different according to Tukey's test ($p<0.05$).

The highest concentration of flavonoids was extracted with polar solvent - methanol (20.74 ± 0.55 mg RU/g) from stem, and (20.07 ± 0.63 mg RU/g) from leave, while the lowest concentration of flavonoids was measured in extracts obtained with non-polar solvent (ethyl-acetate) (2.86 ± 0.38 mg RU/g) from fruit. Differences in flavonoid content among extracts made by different solvent were significant ($p < 0.05$). Compared to the results published by Ilić, (2017), the highest concentration of flavonoids was extracted with polar solvent methanol (20.74 ± 0.55 mg RU/g) from fruit, and the lowest concentration coincides with the results of our study, (ethyl-acetate) (0.15 ± 0.01 mg RU/g) from leaves.

### *The antioxidant activity of the extracts*

The antioxidant activity is expressed in terms of $IC_{50}$ (μg/mL) values (Table 3). The lower $IC_{50}$ value reflects greater activity. The highest antioxidant activity was measured in methanol (25.87 ± 0.05; 28.25 ± 3.56 μg/mL, respectively) from leave, while the lowest capacity to neutralized DPPH radicals was measured in the fruit methanol extract (638.5 ± 0.01 μg/mL). Unlike total flavonoid content, the total content of phenol is highly correlated with the antioxidant activity of extracts. Differences between antioxidant activity of extracts gained by different extraction solvents were significant ($p<0.05$). Compared to the results published by Maleki (2023), he methanolic extract also showed significant antioxidative activity (98.95 μg/ml).

**Table 3**. Antioxidant activity of investigated *S.rigidum* extracts, $IC_{50}$ values (μg/mL)

| | **Fruits** | **Stem** | **Leaf** | **Root** |
|---|---|---|---|---|
| **Methanol** | 638.5 ± 0.01$^{a}$ | 69.05 ± 0.45$^{c}$ | 25.87 ± 0.05$^{c}$ | 173.95 ± 0.05$^{a}$ |
| **Ethyl-acetate** | 455.65 ± 0.01$^{b}$ | 130.4 ± 0.45$^{a}$ | 28.25 ± 3.56$^{b}$ | 138.05 ± 3.56$^{b}$ |
| **Acetone** | 375.31 ± 1.92$^{c}$ | 75.55 ± 1.21$^{b}$ | 59.21 ± 2.36$^{a}$ | 72.57 ± 2.36$^{c}$ |

Values are mean ± standard deviation of triplicate analyses. Values within the row followed by the same letter (a, b, c), are not significantly different according to Tukey's test ($p<0.05$)

## Conclusions

Polar solvents showed to be more efficient in the extraction of active components, and the highest amount of tested compounds was extracted with methanol. The results showed that the leaves contains the most extracted phenols and have hight antioxidant potential, while the lowest amount flavonoids constated in from fruit. Unlike total flavonoid content, the total content of phenol is highly correlated with the antioxidant activity of extracts.

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