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PHYTOCHEMICAL RESEARCH ON AERIAL PARTS OF *Raphanus raphanistrum* subsp. *landra* (Moretti ex DC.) Bonnier & Layens

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Abstract

Raphanus raphanistrum subsp. landra is a wild annual plant from Brassicaceae family. Aerial parts of the plant can be used as food: the leaves can be used raw or cooked, flowers and young seedpods can be used raw in various dishes. In our country, it is considered a weed and it is not paying attention to its edible and pharmaceutical uses. The aim of this study is to evaluate the antioxidant, total polyphenols and flavonoid content of the ethanol extract of aerial parts (flowers, silique, and leaves) from this species. The plants were collected from ruderal areas. Ethanolic extracts from the flowers, silique, and leaves were analysed spectrophotometric. These preliminary studies on the phytochemical characteristics of Raphanus raphanistrum subsp. landra bring new and important information about nutraceutical importance and the possibility to be used in pharmaceutical industry.

Key words*: Raphanus raphanistrum subsp. landra, polyphenols, flavonoids, antioxidant capacity.*

INTRODUCTION

Plants for spontaneous flora play a very important role in the discovery of active principles that can lead to the development of drugs for the treatment of many diseases. Today it is a very great interest to use natural antioxidants in food and in pharmaceutical ingredients, that can replace successfully the synthetic ones (Soare et al., 2015; Taghvaei and Jafari, 2015; Eça et al., 2014).

The phenolic compounds from plants, vegetables, fruits and cereals like polyphenols and flavonoids (Cercel et al., 2017; Bălan et al., 2014), have the proprieties to reduce oxidative stress, and in this way they can be used in prevention and treatment in many diseses including cancer, neurological, cardiovascular, diabetes, hepatitic, immune deficiency diseses, and they also can be used in antiaging therapy (Yaseen et al., 2017; Nichita et al., 2016).

The phenolic compounds also influence anthocyanins stability during food processing and storage. Thus, the addition of phenolic compounds can be a valuable tool for improvement of nutritive value of functional food products (Alexe and Dima, 2014; Kopjar et al., 2009).

This study was carried out to investigate the antioxidant, total polyphenols and flavonoid content of the ethanol extract of aerial parts (flowers, silique, and leaves) from *Raphanus raphanistrum* subsp. *landra* with posibilities to use in food and pharmaceutical industry.

Raphanus raphanistrum subsp. *landra* is a spontaneos weed plant that can grow both in winter and in spring crops, ruderal areas, from steppe to forests (Georgescu et al., 2016; Ionescu and Ionescu, 2014). It is believed that it is the ancestor of cultivated radish (*Raphanus sativa*) (Nishio and Kitashiba, 2017).

This plant has edible uses, young leaves, flowers and seeds can be used in salads, deep-dish, raw or in powder form as spice (Maldini et al., 2017; Conforti et al., 2012).

The aerial parts of the plant are used in traditional medicine for their purifing, antiinflammatory effects, anti-rheumatic and hypoglicemic activity and for the treatment of various ailments such as gastrointestinal diseases (Conforti et al., 2008).

Marelli et al. (2015), showed that ethanolic extract of *Raphanus raphanistrum* subsp.

landra exerted antioxidant and antiproliferative proprieties and contain the highest amount of sterols and highest content of linolenic acid (9.4%) of the studied species. Jdei et al. (2017) showed that ethanolic extract of *R. raphanistrum* subsp. *landra***,** according to the phenolic compositions have antioxidant, antibacterial, and anti-tyrosinase activities. Küçükboyaci et al. (2012) demostrated that aquous extract of this plant, have a potential source of antioxidant and minerals of natural origin. Anti-inflammatory and antioxidant activity has been also demonstrated by Conforti et al. (2011) and El and Karakaya (2004).
In our country *R. raphanistrum* subsp. l*andra* it is consider a weed and there are not studies regarding the nutraceutical potential of this plant.

MATERIALS AND METHODS

Plant material

The plants were collected from ruderal area in October 2017 at flowering stage (Figure 1). The plants were authenticated at horticulture botany department of U.A.S.V.M of Bucharest. Plants were separated into leaves, flowers and fruits.

Figure 1. *Raphanus raphanistrum* subsp. *landra* at flowering stage

Preparation of plant extracts

The ethanolic extracts were prepared after Romanian pharmacopoeia method.
1 g of the aerial plant fresh material was weighed accurately on analytical balance and extracted by 100 ml of ethanol 50% (v/v) for 30 minutes at boiling temperature in water bath. A whole content of flask was quantitatively percolated through paper filter into a calibrated flask and filled up to 100 ml with ethanol 50% (v/v).

Determination of total phenolic content

Total phenolic contents were determined spectrophotometrically using a modified method after Mitic et al. (2014), Tukun et al. (2014), Rakcejeva et al. (2012) and Abdelhady et al. (2011) using the Folin-Ciocalteu reagent and expressed as gallic acid equivalents per g fresh material (mg GAE/g FW). To each sample 200 µl of sample extracts, were added 1200 µl distilled water and 300 µl Folin-Ciocalteu reagent and was mixed thoroughly. After 5 min, 1500 µl of 2% Na_2CO_3 was added. After 15 min. of incubation at room temperature in the dark, the absorbances were measured against the reagent blank at 750 nm and compared to a gallic acid calibration curve (R^2 = 0.996). The same procedure was repeated for all standard gallic acid solutions at different concentrations (0, 50, 100, 150, 250, 500 mg/L). The experiment was caried out in triplicate.

Determination of total flavonoid content

The $AlCl_3$ modified assay after Asănică et al. (2016) and Agbo et al. (2015) was used for quantification of the total flavonoid content of the ethanolic plant extracts. 300 µl of the sample extract or standard solutions of rutin (0.005, 0.010, 0.015, 0.020, 0.025, 0.030, 0.035 mg/ml) was mixed with 1200 µl distilled water and 90 µl of 5% $NaNO_2$ was added. After 5 min, 90 µl of 10% $AlCl_3.6H_2O$ solution was added. After another 5 minutes 600 µl NaOH 1M was added, followed by 720 µl distilled water. The mixture was vigorously shaken.

The absorbance of the standards

The absorbance of the standards and samples extracts were measured against a blank at 510 nm with a UV-spectrophotometer. Total flavonoid content was expressed as mg rutin equivalents/g fresh weight (mg RE/g FW). The determination of total flavonoid content was done in triplicate.

Radical scavenging activity (RSA) assay

The measurement of the DPPH radical scavenging activity was performed according to a modified methodology described by Ibraheim et al. (2015), Garcia et al. (2012) and Brand-Williams et al. (1995) as follows : 0.5 ml hydro alcoholic plant extract was mixed with 3 ml Ethanol ≥99.8% absolute grade and 0.3 ml of ethanol solution of 0.1 mM DPPH. After 100 min of reaction, the absorbance of the samples was measured at 517 nm. The blank is represented by the mixture of 3.3 ml ethanol and 0.5 ml sample extract. The control solution is the mixture between 3.5 ml ethanol and 0.3 ml sample extract.

The scavenging activity was determined using the following formula:

$$AA\%=100-\frac{(\text{Abs sample-Abs blank})x100}{\text{Abs control}}$$

All the solvents used in all the experiments were of analytical grade. All the samples were analyzed in triplicates. All the absorbances were measured using Specord 210 Plus UV/VIS spectrophotometer

Chlorophyll a, b, total carotenoids content

The method was adapted after Asanică et al. (2017), Burducea et al. (2016), Pandia et al. (2013), Lichtenthaler and Wellburn (1983) and Arnon (1949) as follows: 1g of the samples (flowers, leaves, silique) were ground with 80% acetone (v/v). The extract was filtered with a vacuum pump until the residue becomes colorless and completed to volume to 50 ml. (Figure 3). The absorbance of the samples extracts was read at 663, 646 and 470 nm against the blank (acetone). The following formulas were used to calculate the chlorophyll a, b, and total carotenoids content of the samples:

Ca µg/ml = 12.21A663-2.81A646

Cb µg/ml = 20.13A646-5.03A663

$$Cx+c\ \mu g/ml=\frac{1000A470-3.27Ca-104Cb}{229}$$

All the solvents used in the experiment were of analytical grade. All the samples were analyzed in duplicates. All the absorbances were measured using Specord 210 Plus UV/VIS spectrophotometer.

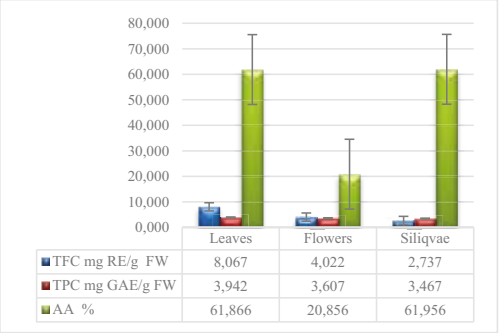
Dry matter content

The *Raphanus raphanistrum* var. *landra* parts (flowers, leaves and silique) were dry at 105°C in drying oven until they reach constant weight (AOAC, 2000).

RESULTS AND DISCUSSIONS

The results for total phenolic, total flavonoid content and radical scavenging activity presented in Figure 2, showed that the leaves have the highest total flavonoid content: 8.06 ± 0.028 mg RE/g FW and total phenolic content: 3.94 mg ± 0.003 GAE/g FW. The lowest content in TFC was recorded by the siliqvae: 2.73 ± 0.003 mg RE/g FW and the flowers had the lowest content in TPC: 3.61 ± 0.001 mg GAE/g FW and radical scavenging activity 20.85 ± 0.002%. The highest radical scavenging activity had the siliquae with 61.95% ± 0.001.

The total phenolic content was similar with Jovancevic et al. (2011) showed in the wild bilberrys (*Vaccinium myrtillus*) that ranged from 3.92-5.24 mg GAE/g FW.



	Leaves	Flowers	Siliqvae
TFC mg RE/g FW	8,067	4,022	2,737
TPC mg GAE/g FW	3,942	3,607	3,467
AA %	61,866	20,856	61,956

Figure 2. TFC, TPC and AA from aerial parts of *R. raphanistrum* subsp. *landra*

There was find also a positive correlation between total flavonoid, total phenols content, radical scavenging activity of all the aerial parts from *Raphanus raphanistrum* subsp. *landra* (Table 1).

Also, it was found a positive correlation between leaves, flowers and siliquae regarding the total flavonoid, total phenols content and radical scavenging activity (Table 2).

In the Figure 3 the highest values regarding the chlorophyll a, b, total carotenoids, total chlorophyll content and chlorophyll a/b ratio

were recorded by *R. raphanistrum* subsp. *landra* leaves. The flowers had the lowest values.

Table 1. Correlation between TFC, TPC and AA from aerial parts of *R. raphanistrum* subsp. *landra*

	TFC mg RE/g FW	*TPC mg GAE/g FW*	*AA %*
TFC mg RE/g FW	1		
TPC mg GAE/g FW	0.998	1	
AA %	0.285	0.228	1

Table 2. Correlation between leaves, flowers and silique of *R. raphanistrum* subsp. *landra*

	Leaves	Flowers	Siliqvae
Leaves	1		
Flowers	0.999	1	
Siliqvae	0.997	0.999	1

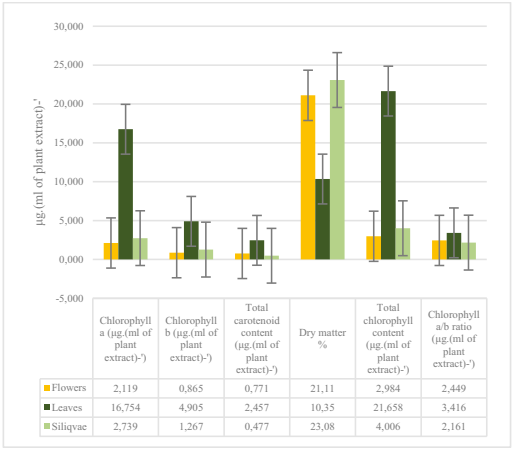


Figure 3. Chlorophyll a, b, total carotenoid and dry matter content of aerial parts from *R. raphanistrum* subsp. *landra*

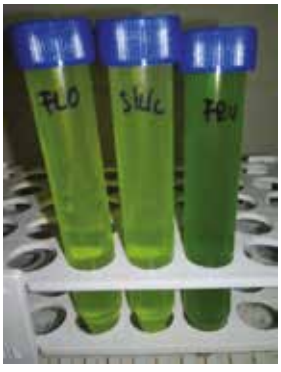


Figure 4. Chlorophyll acetone extract from flowers, siliquae and leaves of *R. raphanistrum* subsp. *landra*

Table 3 shows a positive correlation between chlorophyll a, chlorophyll b, total carotenoids of the flowers, leaves and siliquae.

Correlation to determine the relationship between the variables were calculated using MS Excel software.

Table 3. Correlation between chlorophyll a, chlorophyll b and total carotenoids of aerial parts from *R. raphanistrum* var. *landra*

	Chlorophyll a (µg.(ml of plant extract)-')	Chlorophyll b (µg.(ml of plant extract)-')	Total carotenoid content (µg.(ml of plant extract)-')
Chlorophyll a (µg.(ml of plant extract)-')	1		
Chlorophyll b (µg.(ml of plant extract)-')	0.998	1	
Total carotenoid content (µg.(ml of plant extract)-')	0.985	0.974	1

CONCLUSIONS

This preliminary work reveals that *Raphanus raphanistrum* susp. *landra* harvested in October can be a very interesting source of antioxidants principles similar to wild bilberries with great potential use in food and pharmaceutical industry. Future studies are needed on chemical composition and nutritional value of this plant.

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