American Journal of Essential Oils and Natural Products

Available online at www.essencejournal.com

AkiNik

AJEONP

American Journal of Essential Oils and Natural Products

ISSN: 2321 9114
AJEONP 2016; 4(2): 23-26

Received: 05-02-2016
Accepted: 06-03-2016

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Some chemical properties, fatty acid composition and mineral contents of *Diplotaxis tenuifolia* seed and oil

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Abstract
While non-maturated seed contain 641 mg/kg P, 8834 K, 8523 Ca and 3477 mg/kg Mg, maturated seed of *D. tenuifolia* contained 4343 P, 82137 K, 10797 Ca and 4630 mg /kg Mg. Crude protein contents of seeds were found as 33% (non-maturated) and 32% (maturated). Oil content of maturated and non-maturated seeds was determined as 35% and 34%, respectively. Total flavonoids and phenolic contents of non-maturated and maturated seeds of *D.tenuifolia* were found as 875-1159 mg catechol /g and 661-856 mg Gallic Acid/ g (dw), respectively. While non-maturated contains 11.7% palmitic, 3.5% stearic, 5.3% palmitoleic, 30.6% oleic, 3.8% erucic, 31.1% linoleic and 7.2% linolenic, maturated seed oil contained 10.8% palmitic, 3.9% stearic, 2.3% palmitoleic, 30.1% oleic, 6.6% erucic, 34.9% linoleic and 8.3% linolenic acids.

Keywords: *D.tenuifolia*, mineral, ICP-AES, oil, phenol, flavonoid, fatty acid, GC

1. Introduction
Diplotaxis tenuifolia is widely cultivated in Italy and found endemic in most Mediterranean countries in Northern and Eastern Europe. Wild rocket is native to the southern and western parts of Turkey but not cultivated nor used as a vegetable [1, 2]. It is belong to a species of flowering plant in the Brassicaceae family. Brassica species are cultivated extensively to produce edible and industrial oils throughout the world [3]. In the past few years, an important increase in the cultivation of wild plant species has been carried out for the commercial production of traditional Mediterranean green salads [4]. Plants are good sources for both common and uncommon fatty acids. Many uncommon fatty acid types have been isolated from different species [5]. They contain high carbohydrate, vitamin and mineral contents [6]. It is known for the pungent taste of its young leaves, which makes it appreciated for the salads and in cooked culinary, preparations [7]. Rocket contains a range of health-promoting phytochemicals including carotenoids, vitamin C, fiber, polyphenols and glucosinolates [8, 9]. The aim of the current study was to determinate minerals, fatty acid compositions, protein contents and some chemical properties of *Diplotaxis tenuifolia* seeds.

2. Material and methods
2.1. Material
Wild rocket (*Diplotaxis tenuifolia*) seeds were provided from Department of Field Crop, Faculty of Agriculture plant seed herbarium, Selçuk University, Konya in Turkey.

2.2. Oil extraction
The oil content was determined according to the method ISO 659:1998 (ISO,1998). About 2 g of the seeds were ground in a ball mill and extracted with petroleum ether in a Twisselmann apparatus for 6 h [10]. The solvent was removed by a rotary evaporator at 40 °C and 25 mmHg. The oil was dried by a stream of nitrogen and stored at – 20 °C until used.

2.3. Determination of fatty acids
Fatty acid compositions for wild rocket seed oil were determined using a modified fatty acid methyl ester method as described by Hışıl [11]. The oil was extracted three times for 2 g air-dried seed sample by homogenization with petroleum ether. The oil samples (50-100 mg) was converted to its fatty acid methyl esters (FAME). The methyl esters of the fatty acids (1 µl) were analysed in a gas chromatography (HP 6890) equipped with a flame ionising detector (FID), a fused silica capillary column (60 m x 0.25 mm i.d.; film thickness 0.20 micrometer).

It was operated under the following conditions: oven temperature program. 175 ºC for 7 min. Raised to 250 ºC at a rate 5 ºC/min and then kept at 250 ºC for 15 min); injector and detector temperatures, 250 and 250 ºC; respectively, carrier gas. nitrogen at flow rate of 1.51 ml/min; split ratio. 1/50 µl/min.

2.4. Determination of mineral and protein contents

Provided wild rocket seed samples were dried at 70 ºC in a drying cabinet with air-circulation until they reached constant weight. Later, about 0.5 g dried and ground samples were digested by using 5ml of 65% HNO_3 and 2 ml of 35% H_2O_2 in a closed microwave system (Cem-MARS Xpress). The volumes of the digested flour samples were completed to 20 ml with ultra-deionized water, and mineral contents were determined by ICP AES (Varian-Vista, Australia). Measurements of mineral concentrations were checked using the certified values of related minerals in the reference samples received from the National Institute of Standards and Technology (NIST; Gaithersburg, MD, USA) [12]. The nitrogen combustion method was used for determination of crude protein. The protein determination was made in Leco combustion analyzer and 6.25 was used as the conversion factor. Crude protein values of samples were determined according to the Association of Official Analytical Chemists [13].

Working conditions of ICP-AES:

Instrument	: ICP-AES (Varian-Vista)
RF Power	: 0.7-1.5 kw (1.2-1.3 kw for Axial)
Plasma gas flow rate (Ar)	: 10.5-15 L/min. (radial) 15 "(Axial)
Auxilary gas flow rate (Ar):	1.5 "
Viewing height	: 5-12 mm
Copy and reading time	:1-5 s (max.60 s)
Copy time	: 3 s (max. 100 s)

2.5. Determination of total phenolic contentsand flavonoids

The phenols of the plant material were extracted with MeOH. Total phenolic content was assayed quantitatively by absorbance at 765 nm with Folin-Ciocalteau reagent according to the method of Madaan *et al.* [14]. Firstly, a standard curve of known concentrations of gallic acid was prepared to calculate the total phenolic content to be expressed as gallic acid equivalent (GAE). Ten mg of gallic acid was dissolved in 100 mL of 50% methanol (100 µg/mL) and then diluted to 12.5, 25, 50 or 100 µg/mL. 0.076 mL aliquot of each dilution was taken in a test tube and diluted to 0.76 mL of distilled water. Then 0.12 mL FolinCiocalteu's reagent (1 N) was added and allowed to incubate at room temperature for 5 min. 0.32 mL of 20% (w/w) Na_2CO_3 was added in each test tube, adjusted with distilled water up to the mark of 2 mL, vortexed and left to stand for 30 min at room temperature. Absorbance of the standard was measured at 765 nm using UV/VIS spectrophotometer (Schimadzu, Japan) against blank, i.e., distilled water. For measurement of plant samples, appropriately diluted methanolic extracts of 0.76 mL were taken in test tubes and then similar procedure was followed with the standards. Total flavonoids content was estimated according to Dewanto *et al*. [15]. Methanol extracts were properly diluted with distilled water. 5% $NaNO_2$ solution was added to each test tube; after five minutes, 10% $AlCl_3$ solution was added and then after six minutes 1.0 M NaOH was added. Finally total volume was filled up to 5 mL with water and the test tubes were mixed well. Absorbance of the resulting pink-colored solution was measured at 510 nm versus blank. Calibration curve was prepared using Catechol as standard. The flavonoid content was expressed as mg Catechol equivalents (CE) per g of dry weight (mg CE/g DW). The average is calculated by analyzing the fruits three times [16].

3. Results and Discussion

Mineral contents of maturated and non-maturated *D. tenuifolia* seeds are given in Table I. Both seeds contained P, K, Ca, Mg and S as major mineral. Macro and micro element contents of maturated seeds were found higher than that of non-maturated *D. tenuifolia* seeds. While non-maturated seed contain 641 mg/kg P, 8834 K, 8523 Ca and 3477 mg/kg Mg, maturated seed of *D. tenuifolia* contained 4343 P, 82137 K, 10797 Ca and 4630 mg /kg Mg. In addition, maturated seed contained 35 mg/kg Zn, 21 Mn, 29 mg/kg B and 7.3 mg/kg Cu, 0.4 mg/kg Mo (Table 1).

Crude protein, total phenol and total flavonoid contents of *D. tenuifolia* seeds are presented in Table 2. Crude protein contents of seeds were found as 33% (non-maturated) and 32% (maturated). Oil content of maturated and non-maturated seeds were determined as 35% and 34%, respectively. Tonguç and Erbaş [5] reported that *Diplotaxis tenuifolia* seed contained 23.40% oil and 25.2% crude protein. Total flavonoids and phenolic contents of non-maturated and maturated seeds of *D.tenuifolia* were found as 875-1159 mg catechol /g and 661-856 mg Gallic Acid/ g (dw), respectively (Table 2). In addition, both total flavonoids and phenolic contents of maturated seeds were determined higher than that of results of non-maturated seeds. Different light levels during the cultivation period of *D. tenuifolia* has a significant impact on the levels of flavonoids present in the crop at harvest with over 15-fold increase achieved flavonoid compounds [17]. Quercetin derivatives were the major group of phenolic compounds in this species [18].

Fatty acid composition of both seed oils were presented in Table 3. While non-maturated contains 11.7% palmitic, 3.5% stearic, 5.3% palmitoleic, 30.6% oleic, 3.8% erucic, 31.1% linoleic and 7.2% linolenic, maturated contained 10.8% palmitic, 3.9% stearic, 2.1% palmitoleic, 30.1% oleic, 6.6% erucic, 34.9% linoleic and 8.3% linolenic acids. Stearic, erucic, linoleic and linolenic acid contents of maturated *D. tenuifolia* seed oil increased compared with non-maturated oil. Large ranges for fatty acids were observed, and linolenic, eicosenoic (20:1) and erucic acids were found. The seed oil of *D. tenuifolia* plant contained 8.2% palmitic, 3.6% stearic, 22.2% oleic, 16.7% linoleic, 19.6% linolenic, 9.7% eicosenoic and 18.8% erucic acids [5]. Oil content of cultivated Brassica species is higher than 30% and could reach up to 45% [19]. Oil content are quantitatively inherited characters and they are influenced greatly by environmental and genetic factors [5]. While palmitic, oleic and linoleic contents of both seed oils are found higher compared with results of Tonguç and Erbaş [5], eicosenoic, erucic and linolenic acid contents were found low. As a result, wild rocket seeds are good sources for protein, mineral and oil. In addition, seed oils are good sources for both common and uncommon fatty acids. Wild vegetable are of good sources for mineral and other useful chemical for animal feeding.

Table 1: Mineral contents of *D.tenuifolia* seed (mg/Kg)

Macro Elements (mg kg^{-1})							
Samples		**P**	**K**	**Ca**	**Mg**	**S**	
Non maturated	Mean± Std D.	641± 36	8834± 134	8523± 304	3477± 273	943± 17	
Maturated	Mean± Std D.	4343± 12	28137± 1195	10797± 507	4630± 184	2518± 126	
Micro Elements (mg kg^{-1})							
Samples		**Fe**	**Zn**	**Mn**	**B**	**Cu**	**Mo**
Non maturated	Mean± Std D.	72± 6.7	6.7± 0.3	8.5± 0.6	14.5± 1.9	3.2± 0.2	0.3± 0.1
Maturated	Mean± Std D.	110± 15	35± 0.9	21.3± 1.5	28± 2.1	7.3± 0.1	0.4± 0.0

n:3

Table 2: Chemical properties of *D.tenuifolia* seed

Samples			**Total flavonoids content**	**Total phenolic content**
		Crude protein (%)	**(mg catechol g^{-1} DW)**	**(mg Gallic acid g^{-1} DW)**
Non-maturated	Mean±	33±0.4	875±93	661±18
	Std D.			
Maturated	Mean±	32±0.3	1159±76	856±29
	Std D.			

n:3

Table 3: Fatty acid composition of *D.tenuifolia* seed oils (%)

Fatty acids	**Non-maturated**	**Maturated**
	0.7	0.9
C8:0	0.7	0.1
C10:0	0.5	0.1
C12:0	0.4	nd
C14:0	0.3	0.1
C16:0	11.7	10.8
C17:0	0.4	nd
C18:0	3.5	3.9
C20:0	0.3	nd
C22:0	nd	0.6
C14:1	0.1	0.1
C16:1	5.3	2.1
C17:1	nd	nd
C18:1 (n-9)	30.6	30.1
C20:1	1.6	2.2
C22:1	3.8	6.6
C18:2 (n-6)	31.1	34.9
C18:3 (n-3)	7.2	8.3
TOTAL	97.3	99.8
∑SFA	17.6	15.6
∑MUFA	41.3	41.0
∑PUFA	38.3	43.2
∑UFA	79.6	84.2
∑Trans	nd	nd
P/S	2.2	2.8
n6/n3	4.3	4.2

Acknowledgements

This work was supported by Selçuk Üniversity Scientific Research Project (S.Ü.-BAP, Konya-TURKEY).

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