

## **Antioxidant capacity and fatty acid composition of different parts of *Adenocarpus complicatus* (Fabaceae) from Turkey**

Adnan Berber<sup>1</sup>, Gokhan Zengin<sup>2</sup>, Abdurrahman Aktumsek<sup>2</sup>, Murad Aydin Sanda<sup>2</sup> & Tuna Uysal<sup>2</sup>

1. Necmettin Erbakan University, Seydisehir Ahmet Cengiz Engineering Faculty, Department of Mechanical Engineering, Konya, Turkey; aberber@selcuk.edu.tr
2. Selcuk University, Science Faculty, Department of Biology, Konya, Turkey; gokhanzengin@selcuk.edu.tr, aktumsek@selcuk.edu.tr, muradsanda@gmail.com, tuyosal@selcuk.edu.tr

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**Abstract:** *Adenocarpus complicatus* is distributed throughout the Anatolian peninsula and is widely used for human and animal nutrition. The purpose of this work was to study the antioxidant properties and fatty acid composition of different parts of this plant (fruits and mixed materials). The species was collected from Golyuzu village of the Seydisehir district near Konya province, Turkey. Fruit and mixed parts obtained from this species were ground and a 15g sample was used to prepare methanolic extracts. Powdered plant samples were extracted with 100mL methanol in a mechanical shaker. The obtained extracts were filtered and concentrated to dryness under reduced pressure and were subsequently stored at -20°C. Antioxidant components, namely total phenolic and flavonoid content, were detected for each extract using spectrophotometric methods. Antioxidant capacity was evaluated by various assays including phosphomolybdenum, DPPH free radical scavenging capacity, metal chelating activity, and ferric and cupric ion reducing power. The fatty acid profiles of plant parts were also determined by using gas chromatography. The total phenolic content of fruit (36.21mgGAE/g) was higher than that of mixed materials (13.79mgGAE/g). The methanolic extract of mixed material had higher amounts of flavonoid than fruit extract. The free radical scavenging activity of extracts was expressed as IC<sub>50</sub> value (µg/mL) (amount required to inhibit DPPH radical formation by 50%). The lower IC<sub>50</sub> value reflects better free radical scavenging action. The radical scavenging activity of the samples was compared with BHT, it showed the mixed material to be almost two times more potent than the fruit extract. However, BHT is an excellent free radical scavenger with an IC<sub>50</sub> of 34.061 µg/mL. The ferric and cupric reducing power potentials of the extracts were expressed as EC<sub>50</sub> value (the effective concentration at which the absorbance was 0.5). Fruit extract exhibited strong ferric reducing power with an EC<sub>50</sub> of 871.25 µg/mL. The metal chelating activity of the extracts increased with concentration. Chelating effect was 83.60% for fruit extract at 1mg/mL concentration. Oil content of fruit and mixed parts were detected as 6.71 and 6.14%, respectively. A total of 32 fatty acids were found in the oil. Essential fatty acids (linoleic and α-linolenic acid) were identified as the most abundant fatty acids in the oil. These results demonstrated that this plant species can be considered as an alternative to synthetic antioxidants. Likewise, the oil obtained from the plant can be used as a source of essential fatty acids for food and pharmacological applications. Rev. Biol. Trop. 62 (1): 337-346. Epub 2014 March 01.

**Key words:** *Adenocarpus complicatus*, antioxidant capacity, fatty acid composition, total flavonoid content, total phenolic content.

Free radicals are an atom or a group of atoms with an unpaired electron and are generally known as reactive oxygen species, which include hydrogen peroxide, hydroxyl radical, nitric oxide, peroxynitrite, singlet oxygen, peroxy radical and superoxide anion (Halliwell, 2001). These radicals are generated

by normal metabolic processes or exogenous factors such as ultraviolet light and cigarette smoke. Overproduction of these radicals is considered to be the main contributor to oxidative stress (Ellnain-Wojtaszek, Kruczynski & Kasprzak, 2003). Oxidative stress is involved in many acute and chronic diseases including



cancer, cardiovascular pathologies and neurodegenerative diseases (Bouayed et al., 2007). The human body has multiple mechanisms, especially enzymatic systems, to protect cellular components against damage-inducing reactive oxygen species (Anderson, 1999). However, the defense system may not be enough for persistent oxidative stress. Hence, intake of exogenous antioxidants is required in order to balance the reactive oxygen species in the human body. Antioxidants are known to play an important role in protection against oxidative damage. Epidemiological studies have shown that food rich in antioxidants plays an essential role in the prevention of certain diseases such as cancer and cardiovascular diseases (Ismail, Marjan & Foong 2004). However, synthetic antioxidants such as BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), TBHQ (tertiary butylhydroquinone) and PG (propyl gallate) have been restricted for use in food industry because of some side effects (Namiki, 1990). Therefore, there is great interest in finding new and safe antioxidants from natural sources. Several plant extracts and phytochemicals such as flavonoids, tannins and other phenolic compounds have strong antioxidant potential and are used as additives in the food industry (Salah, Miller, Paganga, Tijburg, Bolwell & Rice-Evans 1995; Van Acker et al., 1996).

Fabaceae is a family of flowering plants comprising about 269 genera and 5 100 species and is one of the largest plant families in the world. In Turkey, it is represented by 68 genera and more than 900 species (Davis, 1970). *Adenocarpus* is a genus of this family, and belongs to the sub family of Faboideae. The genus is solely represented with the perennial *Adenocarpus complicatus* in Turkey. The species is mainly distributed in Western, Central and Southern Anatolia (Mill & Tan, 1988).

Some Fabaceae species have been investigated for their antioxidant capacity and fatty acid composition (Akpinar, Akpinar & Turko glu, 2001; Bagci, 2006; Tung, Wu, Hsieh, Chen & Chang, 2009; Custodio et al., 2009). However, there is no data about antioxidant

potential and fatty acid composition for *Adenocarpus complicatus*. Thus, the purpose of this work was to investigate the total phenolic and total flavonoid contents, the antioxidant properties and the fatty acid profile of *Adenocarpus complicatus*.

## MATERIALS AND METHODS

**Plant material:** Root, stem, leaves, flowers and pods were collected and mixed from specimens obtained from a single location.

**Fruits:** Particularly, pods are selected and separately analyzed because of their dense and visible oil content. For this reason, mature fruits were collected from same location.

Both mixed and fruit materials were collected from Golyuzu village of the Seydisehir district near Konya province ( $37^{\circ}20'49.73''$  N -  $31^{\circ}55'22.68''$  E) between July and August 2009. The plants were identified by Dr. Tuna Uysal from Section of Molecular Systematics, Department of Biology, Science Faculty, Selcuk University. The voucher specimens have been deposited in KNYA herbarium at the department of Biology at Selcuk University (Voucher Number: KNYA 14 062, Collected number: Uysal 1639, Sanda and Berber).

**Preparation of methanolic extracts:** The different plant materials were dried under shade at room temperature and powdered to a fine grain using a laboratory mill (Simsek Labor technic, HD-702). For methanolic extraction, powdered plant samples (15g) were extracted with 100mL methanol in a mechanical shaker (Topo, MS300-HS) for 8h at room temperature. The extracts were filtered and evaporated under vacuum to dryness, using a rotary evaporator (Heidolph Laborota 4000). The extracts were stored in the dark at -20°C.

**Chemicals:** Potassium ferricyanide, ferric chloride, Folin-Ciocalteu's phenol reagent, trichloroacetic acid, methanol, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) were purchased from Merck (Darmstadt, Germany). 2, 2-diphenyl-1-picrylhydrazyl

(DPPH), ferrozine (3-(2-pyridyl)-5-6-diphenyl-1,2,4-triazine-4',4''-disulfonic acid sodium salt) and FeCl<sub>2</sub> were purchased from Sigma Chemical Co. (Sigma-Aldrich GmbH, Sternheim, Germany). All chemicals and solvents were analytical grade.

**Assay for total phenolics:** The total phenolic content of the extracts was determined using the Folin-Ciocalteu reagent (Slinkard & Singleton, 1977). A sample of 0.2mL of extract solution (1mg/mL) were mixed with 1mL Folin-Ciocalteu reagent and 2mL Na<sub>2</sub>CO<sub>3</sub> (7.5%). The final volume was brought up to 7mL with deionized water. After 2h of incubation at room temperature, the absorbance was measured at 765nm using a spectrophotometer (Shimadzu, UV-1800). Gallic acid was used as a standard for calibration curve. The total phenolic content was expressed as gallic acid equivalents (mgGAE/g extract).

**Total flavonoid analysis:** The total flavonoid content of the extracts was determined according to standard methods (Zou, Lu & Wei, 2004). An amount of 0.5mL of sample solutions (1mg/mL) was mixed with 2mL of distilled water and subsequently with 0.15mL 5% of NaNO<sub>2</sub> solution. After 6min incubation at the room temperature, 0.15mL of 10% AlCl<sub>3</sub> solution was added and allowed to stand for 6min; followed by addition of 2mL 4% NaOH solution to the mixture. The mixture was made up to 5mL with methanol, mixed well. The absorbance was measured at 510nm after incubation for 15min. The total flavonoid content was expressed in milligrams of rutin equivalents (RE) per gram of extracts.

**Determination of total antioxidant capacity by phosphomolybdenum assay:** The total antioxidant capacities of extracts were evaluated by the phosphomolybdenum method according to Prieto, Pineda & Aguilar (1999). A sample of 0.3mL of extract solution (1mg/mL) was mixed with 3mL reagent solution (0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The reaction

mixture was incubated at 95°C for 90 minutes. The absorbance of the solution was then measured at 695nm against a blank. Results were expressed as milligrams ascorbic acid (AE) and trolox equivalents (TE) per gram of extracts.

**Scavenging activity on DPPH (2,2-diphenyl-1-picrylhydrazyl) radical:** The free radical scavenging activity of plant extracts was determined with the method described by Sarikurkcü, Tepe, Daferera, Polissiou & Harmandar (2008) with slight modifications. A total of 0.5mL of various concentrations of the methanol extracts was added with 3mL (6.10<sup>-5</sup>M) of a methanol solution of DPPH. The solution was incubated for 30min in the dark at room temperature. After the incubation, the mixture absorbance was measured at 517nm. The Inhibition activity (I%) was calculated as follows:

$$I (\%) = (A_0 - A_1) / A_0 \times 100$$

Where A<sub>0</sub> is the absorbance of the control, A<sub>1</sub> is the absorbance of the extract/standard. The extract concentration providing 50% of radical scavenging activity (IC<sub>50</sub>) was calculated from the graph of inhibition percentage against extract concentration. BHT was used as standard.

**Reducing power activity (Iron (III) to iron (II) reduction):** The analysis of ferric reducing power followed Oyaizu (1986) with slight modifications. Different concentrations of extracts were mixed with 2.5mL of 0.2M phosphate buffer (pH 6.6) and potassium ferri-cyanide (1%). This was incubated at 50°C for 20min. After incubation, 2.5mL of 10% trichloroacetic acid was added. A total of 2.5mL of the reaction mixture was mixed with 2.5mL distilled water and 0.5mL of 0.1% ferric chloride. The solution absorbance was measured at 700nm. The same procedure was repeated with BHA and BHT. The reducing power of the tested samples increased with the absorbance values. The EC<sub>50</sub> value (the effective concentration at which the absorbance was 0.5) was calculated for each sample.



**Cupric ion reducing antioxidant capacity (CUPRAC assay):** The cupric ion reducing capacity of extracts was determined according to Apak, Guclu, Ozyurek, Karademir, & Ercag (2006). For this, 1mL each of 10mM CuCl<sub>2</sub>, 7.5mM neocuproine, and NH<sub>4</sub>Ac buffer (1M, pH 7.0) solutions were added into a test tube. Then, 0.5mL of extract at different concentrations was mixed, and the total volume was brought up to 4.1mL with deionized water. After 30min incubation at room temperature, the mixture absorbance was measured at 450nm and recorded against a blank. Ascorbic acid was used as a standard and the results of the assay were evaluated by using EC<sub>50</sub> values.

**Ferrous ions chelating activity:** The chelating properties of extracts were performed according to the method of Dinis, Madeira & Almeida (1994). For this, 2mL of different concentrations (1, 0.5, 0.2 and 0.05mg/mL) of extracts were mixed with 2mM FeCl<sub>2</sub> (0.05mL) and 5mM ferrozine (0.2mL). Then, the total volume was adjusted to 5mL with methanol (99%). The mixture was shaken vigorously and left standing at room temperature for 10min. Absorbance of the solution was measured at 562nm. The ferrous ion chelating property of extracts was calculated as a percentage using the following formula:

$$\text{Chelating activity (\%)} = (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100$$

Where A<sub>control</sub> is the absorbance of control and A<sub>sample</sub> is absorbance of extract. The control contained FeCl<sub>2</sub> and ferrozine.

**Oil extraction:** The oil extraction of the dried and powdered different plant parts (10g) were carried out at 60°C for 6h by Soxhlet extractor using petroleum ether as a solvent (>90%). The solvent was evaporated by rotary evaporator. The obtained oils were stored in the dark at -20°C. The fatty acids in the oils were esterified into methyl esters by saponification with 0.5N methanolic NaOH and transesterified with 14% BF<sub>3</sub> (v/v) in methanol (Iupac, 1979).

**Gas chromatographic analysis:** FAMEs were analyzed on a HP (Hewlett Packard) Agilent 6890N model gas chromatograph (GC), equipped with a flame ionization detector (FID) and fitted to a HP-88 capillary column (100m length, 0.2mm i.d. and 0.2μm thickness). Injector and detector temperatures were set at 240 and 250°C, respectively. The oven was held at 160°C for 2min. Thereafter the temperature was increased up to 185°C at rate of 4°C/min then increased at up to 200°C at a rate of 1°C/min and held at 200°C for 46.75min. Total run time was 70min. Helium was used as carrier gas (1mL/min).

Identification of fatty acids was carried out by comparing sample FAME peak relative retention times with those obtained for Alltech and Accu standards. Results were expressed as FID response area in relative percentages. Each reported result is given in the average value of three GC analyses. The results are offered as means±S.D. Atherogenic index (AI) and thrombogenicity index (TI) were calculated according to Ulbricht & Southgate (1991). AI = [12:0 + (4 x 14:0) + 16:0] / [(n6+n3) PUFA + 18:1 + other MUFA]; TI = [14:0 + 16:0 + 18:0] / [0.5 x 18:1 + 0.5x other MUFA + 0.5 x n6 PUFA + 3 x n3 PUFA + (n3 PUFA/n6 PUFA)].

## RESULTS

**Assay for total phenolics and flavonoids:** As seen in table 1, fruit of *A. complicatus* showed higher phenolic contents than the mixed material. However, the mixed sample contained a higher level of flavonoids. Total phenolic and flavonoid content of fruit of *A. complicatus* were found to be 32.21mgGAE/g and 8.89mgRE/g, respectively.

**Determination of total antioxidant capacity by phosphomolybdenum assay:** Higher activity of 105.75mgAE/g and 251.53mgTE/g was observed in mixed materials of *A. complicatus*. Total antioxidant capacity of fruit was determined to be 90.75mgAE/g and 207.53mgTE/g, respectively (Table 1).

TABLE 1

Total antioxidant capacities, total flavonoids and total phenolic contents of different parts of *Adenocarpus complicatus*

Sample	Yield (%)	mg AE/g <sup>a</sup>	mg TE/g <sup>b</sup>	mg RE/g <sup>c</sup>	mg GAE/g <sup>d</sup>
Fruit	13.56	90.75±0.19*	207.53±1.66	8.89±0.15	36.21±0.28
Mixed material	14.95	105.75±0.22	251.53±2.01	36.67±0.14	13.79±0.11

\* Values reported are as means ±SD.

a Total antioxidant capacity expressed as ascorbic acid equivalent (mg AE/g extract).

b Total antioxidant capacity expressed as trolox equivalent (mg TE/g extract).

c Total flavonoid content expressed as rutin equivalent (mg RE/g extract).

d Total phenolic content expressed as gallic acid equivalent (mg GAE/g extract).

**Scavenging activity on DPPH radical:** In the DPPH assay, IC<sub>50</sub> values of fruit and mixed material were detected as 843.38 and 474.95 µg/mL, respectively (Table 2). When compared to BHT (34.061 µg/mL), methanol extracts were found to less effective as a free radical scavenger than the synthetic antioxidant.

**Reducing power activity and cupric ion reducing antioxidant capacity:** EC<sub>50</sub> values of extracts were determined as 871.25 (fruit) and 901.25 µg/mL (mixed material) in ferric reducing power assay. A higher reducing power is associated with a lower EC<sub>50</sub> value. BHA and BHT exhibited excellent reducing power activities with EC<sub>50</sub> values of 17.807 and 24.348 µg/mL, respectively. According to EC<sub>50</sub> values, the extract of mixed material has stronger activity than fruit extract in the cupric reducing power assay (Table 2). The cupric reducing power of

mixed material (84.29 µg/mL) was about 6-fold less than that of ascorbic acid (13.725 µg/mL).

**Ferrous ions chelating activity:** Fig. 1 shows the metal chelating activities of *Adenocarpus* extracts at different concentrations. Both fruit and mixed material showed a concentration dependent metal chelating activity. The percentages of metal scavenging capacity of 1, 0.5, 0.2 and 0.05 mg/mL concentration of fruit were found to be 83.60%, 82.02%, 60.67% and 57.71%, respectively. The metal scavenging activity of the same concentration of mixed material were determined as 76.88%, 73.72%, 67.39% and 58.10%, respectively.

**Fatty acid composition:** The results of the fatty acid analyses are shown in Table 3. A total of 32 fatty acids were identified in the oil fraction. Oils extracted from different parts of *A. complicatus* showed a relatively close

TABLE 2

DPPH scavenging activities, ferric and cupric ion reducing powers of different parts of *A. complicatus* and standard antioxidants

Sample	IC <sub>50</sub> (µg/mL) <sup>a</sup>	EC <sub>50</sub> (µg/mL) <sup>b</sup>	EC <sub>50</sub> (µg/mL) <sup>c</sup>
Fruit	843.38±28.08*	871.25±100.28	127.00±0.01
Mixed material	474.95±77.47	901.25±13.97	84.29±1.45
BHA	—	17.807±1.054-	—
BHT	34.061±0.381	24.348±7.852-	—
Ascorbic acid	—	—	13.725±0.478

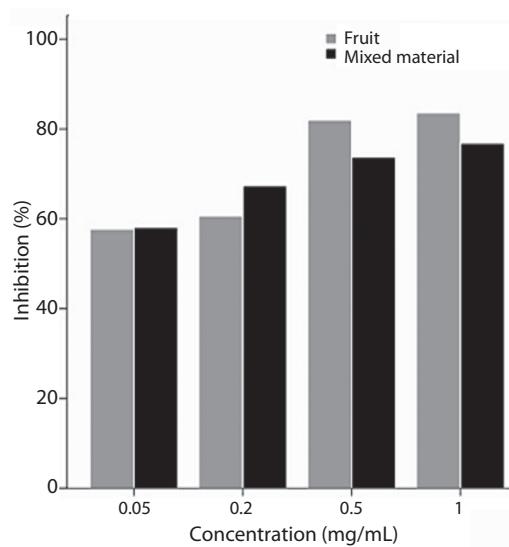
\* Values reported are as means ±SD.

a Results of DPPH assay.

b Results of ferric ion reducing power.

c Results of cupric ion reducing power.





**Fig. 1.** Ferrous ion chelating effects of different concentrations of different parts *A. complicatus*.

fatty acid profile. The predominant fatty acid components in the oils were C 16:0 (palmitic acid), C 14:0 (myristic acid), C 20:4 n6 (arachidonic acid), C 18:1 n9 (oleic acid) and C 18:0 (stearic acid). Polyunsaturated fatty acids were higher (50.33% in fruit, 55.46% in mixed material) than monounsaturated and saturated fatty acids in fatty acid composition of fruit and mixed material. The major fatty acid composition of MUFA is C 18:1 n9 (oleic acid) in the studied oils (8.07% in fruit and 6.63% in mixed material). Among saturated fatty acids, C 14:0 (myristic acid) and C 16:0 (palmitic acid) showed the highest percentages. These fatty acids accounted for more than 70% of the total saturated fatty acids. Both C 18:2 n6 (linoleic acid) (17.24% in fruit) and C 18:3 n3 ( $\alpha$ -linolenic acid) (27.67% in mixed material) were determined as the most abundant fatty acids in the oils. For fruit and mixed materials oils, n3/n6 ratios were determined as 0.52 and 1.07, respectively. According to Atherogenic (AI) and thrombogenicity (TI) values, *A. complicatus* oil obtained from the mixed material had higher potential health benefits than the fruit oil.

**TABLE 3**  
Fatty acid composition different parts  
*of Adenocarpus complicatus (%)*

Fatty acids	Fruit	Mixed material
C 8:0	0.02 $\pm$ 0.01 <sup>a</sup>	0.17 $\pm$ 0.01
C 10:0	0.03 $\pm$ 0.01	0.02 $\pm$ 0.01
C 11:0	0.01 $\pm$ 0.01	0.01 $\pm$ 0.01
C 12:0	3.44 $\pm$ 0.01	3.52 $\pm$ 0.02
C 13:0	0.37 $\pm$ 0.01	0.06 $\pm$ 0.01
C 14:0	13.91 $\pm$ 0.06	10.06 $\pm$ 0.04
C 15:0	0.21 $\pm$ 0.01	0.19 $\pm$ 0.01
C 16:0	13.11 $\pm$ 0.01	15.34 $\pm$ 0.01
C 17:0	1.30 $\pm$ 0.01	0.51 $\pm$ 0.01
C 18:0	5.38 $\pm$ 0.01	5.99 $\pm$ 0.01
C 19:0	0.01 $\pm$ 0.01	0.08 $\pm$ 0.02
C 20:0	0.07 $\pm$ 0.01	0.17 $\pm$ 0.01
C 21:0	0.66 $\pm$ 0.01	0.46 $\pm$ 0.01
C 22:0	0.13 $\pm$ 0.06	0.07 $\pm$ 0.05
<b>ESFA<sup>b</sup></b>	<b>38.62<math>\pm</math>0.12</b>	<b>36.63<math>\pm</math>0.11</b>
C 14:1 n5	1.91 $\pm$ 0.04	0.02 $\pm$ 0.01
C 15:1 n5	0.07 $\pm$ 0.01	0.12 $\pm$ 0.01
C 16:1 n7	0.10 $\pm$ 0.03	0.21 $\pm$ 0.01
C 17:1 n8	0.07 $\pm$ 0.01	0.07 $\pm$ 0.01
C 18:1 n9	8.07 $\pm$ 0.01	6.63 $\pm$ 0.01
C 18:1 n7	0.22 $\pm$ 0.01	0.36 $\pm$ 0.01
C 20:1 n9	0.57 $\pm$ 0.01	0.45 $\pm$ 0.01
C 22:1 n9	0.05 $\pm$ 0.01	0.06 $\pm$ 0.01
<b>ΣMUFA<sup>b</sup></b>	<b>11.06<math>\pm</math>0.06</b>	<b>7.92<math>\pm</math>0.05</b>
C 18:2 n6	17.24 $\pm$ 0.04	13.81 $\pm$ 0.02
C 18:3 n6	4.89 $\pm$ 0.02	5.45 $\pm$ 0.01
C 18:3 n3	16.14 $\pm$ 0.04	27.67 $\pm$ 0.04
C 20:2 n6	0.10 $\pm$ 0.01	0.05 $\pm$ 0.01
C 20:4 n6	10.74 $\pm$ 0.01	7.47 $\pm$ 0.04
C 20:5 n3	0.19 $\pm$ 0.01	0.20 $\pm$ 0.03
C 22:2 n6	0.05 $\pm$ 0.01	0.01 $\pm$ 0.01
C 22:3 n3	0.02 $\pm$ 0.01	0.01 $\pm$ 0.01
C 22:4 n6	0.21 $\pm$ 0.04	0.01 $\pm$ 0.01
C 22:6 n3	0.77 $\pm$ 0.02	0.80 $\pm$ 0.01
<b>ΣPUFA<sup>b</sup></b>	<b>50.33<math>\pm</math>0.05</b>	<b>55.46<math>\pm</math>0.06</b>
<b>ΣUFA<sup>b</sup></b>	61.39 $\pm$ 0.11	63.38 $\pm$ 0.11
<b>ΣEFA<sup>b</sup></b>	33.38 $\pm$ 0.07	41.48 $\pm$ 0.06
$\Sigma n3$	17.12 $\pm$ 0.01	28.68 $\pm$ 0.01
$\Sigma n6$	33.23 $\pm$ 0.04	26.80 $\pm$ 0.05
n3/n6	0.52 $\pm$ 0.01	1.07 $\pm$ 0.01
n6/n3	1.94 $\pm$ 0.01	0.93 $\pm$ 0.01
AI <sup>c</sup>	1.18 $\pm$ 0.01	0.93 $\pm$ 0.01
TI <sup>c</sup>	0.44 $\pm$ 0.01	0.30 $\pm$ 0.01
Oil content	6.71	6.14

a Values reported are means  $\pm$ S.D.

b SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids, UFA: Unsaturated fatty acids, EFA: Essential fatty acids.

c AI: Atherogenic index, TI: Thrombogenicity index.

## DISCUSSION

Phenolics or polyphenols are secondary plant metabolites that are ubiquitously present in plants and plant products. These compounds are reported as highly effective free radical scavengers and antioxidants (Tung et al., 2009). Thus, it is important to determine the amount of phenolic compounds of plant samples. The total phenolic content in *A. complicatus* parts are comparable to the values reported in the literature for other Fabaceae species such as *Trifolium pannonicum* (106.81mgGAE/g), *Oxytropis halleri* (78.84mgGAE/g), *Coronilla emerus* (38mgGAE/g) (Godevac, Zdunic, Savikin, Vajs, & Nebojsa, 2008), *Ceratonia siliqua* (16.4-39.4mgGAE/g in male, female and hermaphrodite) (Custodio et al., 2009), *Glycyrrhiza echinata* (146.30mgGAE/g in aerial parts and 114.33mgGAE/g in roots) (Cakmak, Aktumsek, & Duran 2012). Flavonoids are the major components of phenolic compounds and its levels are correlated with antioxidant capacity. In previously published papers, there is little information about flavonoid content of Fabaceae species. They include *Glycyrrhiza echinata*, *G. uralensis* and *Indigofera* species (Bakasso et al., 2008, Cakmak et al., 2012, Li et al., 2011). In *G. echinata* aerial and root parts, the flavonoid content reached 116.54mgRE/g and 99.64mgRE/g, respectively.

DPPH is a compound that consists of a nitrogen free radical which is easily quenched by free radical scavengers such as phenolic compounds. DPPH scavenging method has been used extensively to predict antioxidant capacity because of the relatively short time required for analysis. The free radical scavenging activity of the extracts increased depending on extract concentration. The reported IC<sub>50</sub> values ranged from 0.027 to 0.118mg/mL in the flower and leaf of three Fabaceae species (*Bauhinia kockiana*, *Caesalpina pulcherrima* and *Cassia surratensis*) (Chew, Goh & Lim, 2009). From our results, we concluded that the scavenging activity of different parts of *A. complicatus* was less effective than that observed for other Fabaceae species. Similarly,

leaves of two Fabaceae species (*Platypodium elegans* (IC<sub>50</sub>=184.92µg/mL) and *Pseudopiptadenia concorta* (IC<sub>50</sub>=13.84µg/mL) have a free radical scavenging capacity stronger than the one obtained from different parts of *A. complicatus* (Mensor et al., 2001). IC<sub>50</sub> values were determined between 13.19 and 156.98µg/mL in nine Fabaceae species (Godevac et al., 2008). Sabudak et al., (2009) reported that five *Trifolium* species have fairly weak radical scavenging capacity as measured by the DPPH assay. In another study, aerial and root parts of *Glycyrrhiza echinata* (IC<sub>50</sub>=453.94 and 184.99µg/mL, respectively) (Cakmak et al., 2012) demonstrated high DPPH radical scavenging activity as compared to our study.

Several reports have demonstrated that the reducing power of plant extracts is related to their antioxidant capacity (Tanaka, Kuie, Nagashima & Taguchi, 1998); nevertheless, literature is scarce about ferric and cupric ion reducing power of Fabaceae species (Godevac et al., 2008). The ferric ion reducing power in the *A. complicatus* parts analysed was lower than ferric reducing power activities obtained from aerial and root parts of *Glycyrrhiza echinata* which were 432.63 and 582.14µg/mL, respectively. Cupric ion reducing power activities of some Fabaceae species, such as *Pterocarpus erinaceus* (Ouedraogo et al., 2011) and *Glycine max* (Gorinstein et al., 2008) were evaluated as trolox equivalents. Transition metal ions in biological systems could catalyze some reactions and result in the generation of some free radicals such as hydroxyl radicals. Thus, the main strategy to avoid ROS generation that is associated with redox active metal catalysis involves chelating of the metal ions. Metal chelating activities of different parts of some Fabaceae species such as *Caesalpina pulcherrima*, *Cassia surratensis* and *Bauhinia kockiana* were found to be ≥80%, 19-56% and ≤40%, respectively (Chew et al., 2009). Five *Trifolium* species were shown very low metal chelating effects (21.35-30.14% at 200µg concentration) (Sabudak, Ozturk, Goren, Kolak & Topcu, 2009).

Linoleic, linolenic and oleic acids were found as main fatty acid components in



Fabaceae species. For example, the results obtained from *A. complicatus* oils were in agreement with the earlier results reported by Bagci (2006). Similarly, Sabudak et al. (2009) reported that linoleic acid was the most abundant fatty acid in five *Trifolium* species growing in Turkey. The level of linoleic acid obtained from *Adenocarpus* oils was lower than some Fabaceae species such as *Arachis hypogaea* (75.2%) and *Vigna unguiculata* subsp. *cylindrical* (35.4%) (Orhan, Ozcelik & Sener 2011; Thangadurai, 2005). When compared to eight *Vicia* species seeds, linoleic acid contents were observed from 14.6% to 35% in theirs fatty acid composition (Akpinar et al., 2001). Moreover,  $\alpha$ -linolenic acid (1.95-9.20%) and arachidonic acid (1.23-6.83%) were found to be lower than the amounts found in different parts of *A. complicatus*. Amounts of EFAs (essential fatty acids) also were observed as higher than some Fabaceae species such as *Glycyrrhiza echinata* (Cakmak et al., 2012), *Astragalus edmondi* and *A. gymnalopecias* (Keskin & Kacar, 2013). The *Adenocarpus* oils indicated higher nutritional quality compared to *G. echinata* oils in terms of AI and TI values (Cakmak et al., 2012)

The results obtained in this study clearly showed that the extract of different parts of *Adenocarpus complicatus* have moderate antioxidant capacity. Because synthetic antioxidants such as BHA and BHT have some adverse effects, the extract of this plant species may be useful for treating oxidative damage in the food and pharmaceutical industries. However, further studies to isolate and identify active compounds, especially phenolics, and *in vivo* studies are needed, to better understand their action mechanisms as pharmacological agents. Moreover, the plant can be considered as a source of essential fatty acids.

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#### RESUMEN

**Capacidad antioxidante y composición de ácidos grasos de diferentes partes de *Adenocarpus complicatus* (Fabaceae) de Turquía.** *Adenocarpus complicatus* se distribuye por toda la península de Anatolia y es ampliamente utilizado para la nutrición humana y animal. El propósito de este trabajo fue estudiar las propiedades antioxidantes y la composición de ácidos grasos de diferentes partes de la planta (frutos y partes mezcladas). Las especies fueron recolectadas en Golyuzu, Seydisehir, cerca de la provincia Konya en Turquía. Para preparar los extractos metanólicos se tomó una muestra de 15g de frutas y partes mezcladas de esta especie. Muestras de plantas en polvo se extrajeron con 100ml de metanol en un agitador mecánico. Los extractos obtenidos se filtraron y se concentraron a sequedad bajo presión reducida y posteriormente se almacenaron a -20°C. Para cada extracto, mediante métodos espectrofotométricos se detectaron los componentes antioxidantes, llamados contenido total de fenoles y flavonoides. La capacidad antioxidante se evaluó mediante diversos ensayos: fosfomolibdeno, capacidad de captación de radicales libres DPPH, actividad quelante de metales y poder reductor de iones ferrosos y cúpricos. También se determinaron los perfiles de ácidos grasos de partes de la planta mediante el uso de cromatografía de gases. El contenido fenólico total de la fruta (36.21mgGAE/g) fue mayor que la de los materiales mezclados (13.79mgGAE/g). El extracto metanólico de material mezclado tenía una mayor cantidad de flavonoides que el extracto de la fruta. La actividad captadora de radicales libres de los extractos se expresó como valor de IC<sub>50</sub> (mg/ml) (cantidad necesaria para inhibir la formación de radicales DPPH en un 50%). El valor bajo de IC<sub>50</sub> refleja mejor acción eliminadora de radicales libres. La actividad captadora de radicales de las muestras se comparó con BHT, se mostró que el material mezclado es casi dos veces más potente que el extracto de la fruta. Sin embargo, BHT es un excelente eliminador de radicales libres con una IC<sub>50</sub> de 34.061 $\mu$ g/mL. El potencial de reducción ferroso y cúprico de los extractos se expresó como valor de CE<sub>50</sub> (la concentración efectiva a la que la absorbancia fue de 0.5). El extracto de la fruta exhibe fuerte poder reductor ferroso con una EC<sub>50</sub> de 871.25 $\mu$ g/mL. La actividad quelante de metales de los extractos aumentó con la concentración. El efecto quelante de extracto de fruta fue de 83.60% en una concentración de 1mg/ml. El contenido de aceite del fruto y partes mixtas fue 6.71 y 6.14%, respectivamente. Un total de 32 ácidos grasos fueron encontrados en el aceite. Los ácidos grasos esenciales (ácido linoleico y  $\alpha$ -linolénico) fueron identificados como los ácidos grasos más abundantes en el aceite. Estos resultados demostraron que esta especie vegetal se

puede considerar como una alternativa a los antioxidantes sintéticos. Del mismo modo, el aceite obtenido de la planta se puede utilizar como una fuente de ácidos grasos esenciales para alimentos y aplicaciones farmacológicas.

**Palabras clave:** *Adenocarpus complicatus*, capacidad anti-oxidante, composición de ácidos grasos, contenido total de flavonoides, contenido total de fenoles.

## REFERENCES

- Akpınar, N., Akpınar, A. M., & Turkoglu, S. (2001). Total lipid content and fatty acid composition of the seeds of some *Vicia* L. species. *Food Chemistry*, 74, 449-453.
- Anderson, D. (1999). Antioxidant defenses against reactive oxygen species causing genetic and other damage. *Mutation Research*, 350, 103-108.
- Apak, R., Guclu, K., Ozyurek, M., Karademir, S. E., & Ercag, E. (2006). The cupric ion reducing antioxidant capacity and polyphenolic content of some herbal teas. *International Journal of Food Science and Nutrition*, 57, 292-304.
- Bagci, E. (2006). Fatty acid composition of some *Astragalus* species from Turkey. *Chemistry of Natural Compound*, 42, 645-648.
- Bakasso, S., Lamien-Meda, A., Lamien, C., Kiendrebeogo, M., Millogo, J., Ouedraogo, A. G., & Nacoulma, O. G. (2008). Polyphenol contents and antioxidant activities of five *Indigofera* species (Fabaceae) from Burkina Faso. *Pakistan Journal of Biological Sciences*, 11, 1429-1435.
- Bouayed, J., Piri, K., Rammal, H., Dicko, A., Desor, F., Younos, C., & Soulaimani, R. (2007). Comparative evaluation of the antioxidant potential of some Iranian medicinal plants. *Food Chemistry*, 104, 364-368.
- Cakmak, Y. S., Aktumsek, A., & Duran, A. (2012). Studies on antioxidant activity, volatile compound and fatty acid composition of different parts of *Glycyrrhiza echinata* L. *Excli Journal*, 11, 178-187.
- Chew, Y., Goh, J., & Lim, Y. (2009). Assessment of in vitro antioxidant capacity and polyphenolic composition of selected medicinal herbs from Leguminosae family in Peninsular Malaysia. *Food Chemistry*, 116, 13-18.
- Custodio, L., Fernandes, E., Escapa, L. A., Lopez-Aviles, S., Fajardo, A., Aligue, R., Alberico, F., & Romano, A. (2009). Antioxidant activity and *in vitro* inhibition of tumor cell growth by leaf extracts from carob tree (*Ceratonia siliqua*). *Pharmaceutical Biology*, 47, 721-728.
- Davis, P. H. (1970). *Flora of Turkey and the East Aegean Islands*. Edinburgh University: Edinburgh Press.
- Dinis, T. C. P., Madeira, V. M. C., & Almeida, L. M. (1994). Action of phenolic derivate (acetaminophen, salicylate and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxy radical scavengers. *Archives of Biochemistry Biophysics*, 315, 161-169.
- Ellnain-Wojtaszek, M., Kruczynski, Z., & Kasprzak, J. (2003). Investigation of the free radical scavenging activity of *Ginkgo biloba* L. leaves. *Fitoterapia*, 74, 1-6.
- Godevac, D., Zdunic, G., Savikin, K., Vajs, V., & Makovic, N. (2008). Antioxidant activity of nine Fabaceae species growing in Serbia and Montenegro. *Fitoterapia*, 79, 185-187.
- Gorinstein, S., Lojek, A., Ciz, M., Pawelzik, E., Delgado-Licon, E., Medina, O. J., Moreno, M., Salas, I. A., & Goshev, I. (2008). Comparison of composition and antioxidant capacity of some cereals and pseudocereals. *International Journal of Food Science and Technology*, 43, 629-637.
- Halliwell, B. (2001). Vitamin C and genomic stability. *Mutation Research*, 45, 29-35.
- Ismail, A., Marjan, M. Z., & Foong, C. W. (2004). Total antioxidant activity and phenolic content in selected vegetables. *Food Chemistry*, 87, 581-586.
- Iupac. (1979). *Standards methods for analysis of oils, fats and derivatives*. C. Paquot (Ed.). England: Oxford Pergamon Press.
- Keskin, C., & Kaçar, S. (2013). Fatty acid composition of root and shoot samples of some *Astragalus* L. (Fabaceae) taxa growing in the east and southeast of Turkey. *Turkish Journal of Biology*, 37, 122-128.
- Li, M., Xu, Y., Yang, W., Li, J., Xu, X., Zhang, X., Chen, F., & Li, D. (2011). In vitro synergistic anti-oxidant activities of solvent-extracted fractions from *Astragalus membranaceus* and *Glycyrrhiza uralensis*. *LWT-Food Science and Technology*, 44, 1745-1751.
- Mensor, L. L., Menezes, S. F., Leitao, G. G., Reis, S. A., Dos Santos, C. T., Coube, S. C., & Leitao, G. S. (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Pharmaceutical Biology*, 15, 127-130.
- Mill, R. R., & Tan, K. (1988). Flora of Turkey and the East Aegean Islands. In P. H. Davis (Ed.), *Flora of Turkey Supplement Vol. 10*. Edinburgh Press: Edinburgh University.
- Namiki, M. (1990). Antioxidants/antimutagens in foods. *Critical Reviews in Food Science and Nutrition*, 29, 273-300.
- Orhan, I., Ozcelik, B., & Sener, B. (2011). Evaluation of antibacterial, antifungal, antiviral and antioxidant potentials of some edible oils and their fatty acid profile. *Turkish Journal of Biology*, 35, 251-258.
- Ouedraogo, N., Tibiri, A., Sawadogo, R. W., Lompo, M., Hay, A. E., Koudou, J., Dijoux, M. G., & Guissou, I. P. (2011). Antioxidant anti-inflammatory and



- analgesic activities of aqueous extract From stem bark of *Pterocarpus erinaceus* Poir. (Fabaceae). *Journal of Medicinal Plants Research*, 5, 2047-2053.
- Oyaizu, M. (1986). Studies on products of browning reactions: antioxidative activities of browning reaction prepared from glucosamine. *Japanese Journal of Nutrition*, 44, 307-315.
- Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphor molybdenum complex: Specific application to the determination of vitamin E. *Analytical Biochemistry*, 269, 337-341.
- Sabudak, T., Ozturk, M., Goren, C. A., Kolak, U., & Topcu, G. (2009). Fatty acids and other lipids composition of five *Trifolium* species with antioxidant activity. *Pharmaceutical Biology*, 47, 137-141.
- Salah, N., Miller, N. J., Paganga, G., Tijburg, L., Bolwell, G. P., & Rice-Evans, C. (1995). Polyphenolic flavonols as scavenger of aqueous phase radicals and as chain-breaking antioxidants. *Archives of Biochemistry Biophysics*, 2, 339-346.
- Sarikurkcu, C., Tepe, B., Daferera, D., Polissiou, M., & Harmandar, M. (2008). Studies on the antioxidant activity of the essential oil and methanol extract of *Marrubium globosum* subsp. *globosum* (Lamiaceae) by three different chemical assays. *Bioresource Technology*, 99, 4239-4246.
- Slinkard, K., & Singleton, V. L. (1977). Total phenol analyses: automation and comparison with manual methods. *American Journal of Enology and Viticulture*, 28, 49-55.
- Tanaka, M., Kuie, C. W., Nagashima, Y., & Taguchi, T. (1998). Applications of antioxidative Maillard reaction products from histidine and glucose to sardine products. *Nippon Suisan Gakkaishi*, 54, 1409-1414.
- Thangadurai, D. (2005). Chemical composition and nutrition potential of *Vigna unguiculata* ssp. *cylindrical* (Fabaceae). *Journal of Food Biochemistry*, 29, 88-98.
- Tung, Y., Wu, J., Hsieh, C., Chen, P., & Chang, S. (2009). Free radical-scavenging phytochemicals of hot water extracts of *Acacia confuse* leaves detected by an on-line screening method. *Food Chemistry*, 115, 1019-1024.
- Ulbricht, T. L. V., & Southgate, D. A. T. (1991). Coronary disease seven dietary factors. *Lancet*, 338, 985-992.
- Van Acker, S.A., Van Den Berg, D.J., Tromp, N.M., Griffioen, H.D., Van Bennekom, P.W., Vandervijgh, J.W., & Bast, A.(1996). Structural aspect of antioxidant activity of flavonoids. *Free Radical Biology & Medicine*, 3, 331-342.
- Zou, Y., Lu, Y., & Wei, D. (2004). Antioxidant activity of a flavonoid-rich extract of *Hypericum perforatum* L. *in vitro*. *Journal of Agricultural and Food Chemistry*, 52, 5032-5039.