Investigation of the Potential Use, Phytochemical and Element Contents of Acacia Plant Seeds Grown in Wild Form, Considered as Environmental Waste

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Abstract: In this study, the effect of altitude on oil amounts, antioxidant activity, polyphenol content and mineral contents of Acacia seeds collected from two different locations (up to 1100 m above sea level) was investigated. Total carotenoid and flavonoid contents of Acacia seeds were detected as 0.76 (Konya) and 1.06 µg/g (Taşucu-Mersin) to 1343.60 (Konya) and 184.53 mg/100 g (Taşucu-Mersin), respectively. Total phenol contents and antioxidant activity values of Acacia seeds were identified as 255.11 (Konya) and 190.00 mgGAE/Taşucu-Mersin) to 64.18% (Konya) and 75.21% (Taşucu-Mersin), respectively. The oils extracted from Acacia seeds in Konya and Mersin province contained 62.70% and 70.39% linoleic, 23.41% and 16.03% oleic, 6.45% and 6.04% palmitic and 2.93% and 4.94% stearic acids, respectively. While 3,4-dihydroxybenzoic acid amounts of seeds are determined as 3.89 (Konya) and 4.83 mg/100 g (Taşucu-Mersin), (+)-catechin contents of Acacia seeds were identified as 3.42 (Konya) and 9.51 mg/100 g (Taşucu-Mersin). Also, rutinhydrate and ferulic contents of Acacia seeds were found as 23.37 (Konya) and 11.87 mg/100 g (Taşucu-Mersin) to 14.74 mg/100 g (Konya) and 1.12 mg/100 g (Taşucu-Mersin), respectively. Acacia seeds collected from Konya and Mersin contained 4003.75 and 3540.89 mg/kg P, 9819.12 and 16175.69 mg/kg K, 4347.47 and 5078.81 mg/kg S and 187.53 and 905.52 mg/kg Na, respectively.

Key words: Acacia seed, location, antioxidant activity, bioactive compounds, fatty acids, phenolic compounds, minerals, GC, HPLC, ICP-OES

1 Introduction

Acacia sp, which belongs to the Leguminosae family, usually grows in tropical regions, show strong resistance to drought and salinity, grow on pure stands or in environments with deciduous trees1. In recent years, interest in vegetable oil woody species has increased significantly due to its therapeutic properties. For thousands of years, plant seeds have been used for a wide variety of purposes, and due to the rapid growth of the world population, it has been reported that the demand for vegetable oils is increasing2-4. Only a small part of the plant material is used for direct human consumption. The vast majority of these, such as fruit seeds, are disposed of as waste every year, causing destruction problems5. As industrial waste problems become increasingly difficult to solve, much effort will be required to improve the nutritional and inductive potential of by-products, waste and underused agricultural products6. Recently, plant seeds have been used as a food source, nutritional components and supplements for the nutritional needs of people in daily life due to their protein contents, carbohydrates, a large number of secondary metabolites7-9. In addition, the interest in oil is increasing due to the physiological effects of unsaturated fatty acids. Therefore, new plant seeds as an oil source should be investigated10, 11. It has been reported that similar plant seeds have been used for this purpose recently12. The characteristics of oils change depending on the fatty acid composition, which is of great importance due to their beneficial effects13. Vegetable oils have been found to consist of a complex mixture contain-
ing a wide variety of compounds. Because, the composition of triacylglycerol is known as a measurement criterion for the quality of vegetable oils. Recently, the continuous increase in the number of industries using vegetable oil as the raw material has led to a gradual increase in the demand for domestic vegetable oils. As a result of various studies, it has been determined that Acacia species are rich in terpenes, flavonoids and condensed tannins. Shrubs and legumes such as Acacia species are widely used as a feed source for livestock in tropical and subtropical regions of the World. Plant seeds, especially underused plant seeds such as Acacia are now creating new oil sources. Limited information is known about the composition, quantitative values and quality characteristics of phenolic components in Acacia seeds. Also, it is not clear how the phenolic constituents, bioactive properties, fatty acids and mineral profiles of Acacia seeds may vary depending on locations. The objective of the present work was to investigate the effect of altitude on oil amounts, antioxidant activity, polyphenol content and mineral content of Acacia seeds collected from two different locations (up to 1100 m above sea level).

2 Material and Methods

2.1 Material

Acacia (Robinia pseudoacacia) and gladiça (Gleditsia triacanthos) seeds were provided from Konya (1100 m high level) and Taşcu-Mersin (sea level) provinces in Turkey. Seed samples were harvested in October 2020. During the day and night in Konya and Mersin, the temperatures varied between about 20-24°C and 10-12°C to 24-28°C and 18-22°C in October, respectively. Seeds brought to the laboratory in paper bags were immediately dried at normal room temperature for one week, and then they were stored up to analysis.

2.2 Methods

2.2.1 Moisture content

Moisture contents of Acacia seeds were detected at 105°C using an oven (Nüve FN055 Ankara, Turkey) up to constant weight.

2.2.2 Oil content

The oil yields of seeds belong to each color type were established according to AOAC. About 10 g of seed powder was placed into a Soxhlet section and extracted using petroleum ether at 50°C for 5 hours, and then it was evaporated by the evaporator at 50°C.

2.2.3 Carotenoid content

After the 2 g Acacia seed powder was mixed with 25 mL of acetone, it was vortexed for 10 min. Then, after it was filtrated by filter paper (Whatman No. 1), their phases were separated in a separation funnel. The filtrate was mixed with 20 mL of petroleum ether and washed triplicate with 100 mL of distilled water. The absorbance value of seed extract dissolved in 25 mL petroleum ether was measured at 450 nm.

2.2.4 Extraction procedure

For extraction of seed powder, after about 2.5 g ground Acacia seed was mixed with 25 mL methanol:water solution (80:20, v/v), it was shaken in rinsing water-bath for 72 h at 25°C. Then, it was filtrated with Whatman No:1. The extract was evaporated at 40°C in a rotary evaporator with vacuum, followed by dissolved in 10 mL methanol: water mixture (80:20, v/v). Before analyses, the extract was filtered through a 0.45 μm filter.

2.2.5 Total phenolic content

The Folin-Ciocalteu was used for analysis of total phenol contents of Acacia seed extracts. After 1 mL Folin-Ciocalteu and 10 mL 7.5% Na₂CO₃ were added on seed extract, it was stirred by a vortex. After the final volume of the sample solution was completed to 25 mL with deionized water, the mixture was left in the dark for 1 hour. The absorbances of seed extracts were recorded at 750 nm in a spectrophotometer. The results obtained were shown as mg gallic acid equivalent/100 g (fw).

2.2.6 Total flavonoid content

After 0.3 mL 7.5% NaNO₂, 0.3 mL of AlCl₃ and 2 mL of NaOH, respectively, were added onto 1 mL extract, it was mixed by vortex for 1 min. Then, it was left in dark for 15 min. The absorbance of sample was determined at 510 nm. The results are given as mg catechin (CA)/100 g of fresh weight.

2.2.7 Antioxidant activity

The DPPH (1,1-diphenyl-2-picrylhydrazyl) was applied for free radical scavenging activity of Acacia seed extracts. After 2 mL of DPPH in methanol was added onto 0.2 mL extract, it was stirred by vortex. Then, it was stored 30 min incubation in the dark at room. Later, the absorbances of extract samples were recorded at 517 nm. Following relation was used for calculation of antioxidant activity (% samples):

\[
\text{Inhibition} &= \frac{\Delta \text{Control } 517 - \Delta \text{Extract } 517}{\Delta \text{Control } 517} \times 100
\]

2.2.8 Fatty acid composition

The fatty acid methyl esters (FAMEs) of Acacia seed oils were determined using a gas chromatography (Shimadzu GC-2010) consisting of flame-ionization detector (FID) and capillary column. The nitrogen with 1.51 mL/min flow rate and 80 mL/min were applied for the carrier gas and total flow rate, respectively (Aljuhaimi et al., 2019). The temperature of injection block and detector was 260°C and column temperature was programmed 120°C for 5 minutes and increased 240°C at 4°C/min and held 25 minutes at 240°C.

2.2.9 Determination of phenolic compounds

A Shimadzu-HPLC equipped with a PDA detector and an
Inertsil ODS-3 column was applied for the qualification and quantification of phenolic compounds were performed. 20 μL of the extract was injected and run at 1 mL/min at 30°C for a total running time of 60 min. The peaks were measured at 280 and 330 nm using a PDA detector. The total running time per sample was 60 min.

2.2.10 Determination of mineral

Acacia seeds were dried in a drying cabinet at 70°C up to constant weight. Then, 0.5 g Acacia seed powder digested by using 5 mL of 65% HNO₃ and 2 mL of 35% H₂O₂ in a closed microwave system (Cem-MARS Xpress) were brought to 20 mL with ultra-deionized water till the volumes. ICP OES (Varian-Vista, Australia) was applied for mineral contents of Acacia seed sample.

2.3 Statistical Analyses

All analysis were done three times and data of triplicate analyses were averaged and subjected to analysis of student t-test. Results were described as mean ± standard deviation of two different Acacia seeds.

3 Results and Discussion

3.1 The physico-chemical and bioactive properties of Acacia seeds

The physico-chemical and bioactive properties of Acacia seeds provided from two different locations are shown in Table 1. Results showed differences depending on altitude and locations. While moisture contents of seeds are measured as 7.41% (Konya) and 7.50% (Taşucu-Mersin), the oil contents of Acacia seeds were determined as 4.30% (Konya) and 3.00% (Taşucu-Mersin). Also, while total carotenoid amounts of Acacia seeds are detected as 0.76% (Konya) and 1.06 μg/g (Taşucu-Mersin), total flavonoid contents of seed samples were recorded as 1343.60 mg/100 g (Konya) and 184.53 mg/100 g (Taşucu-Mersin), while antioxidant activity values of Acacia seeds are found as 64.18% (Konya) and 75.21% (Taşucu-Mersin). In general, oil contents, bioactive compounds of Acacia seeds provided from Konya distinct were determined higher than those of values of Acacia seeds growing in Mersin province. The seeds of 12 species of Acacia contained 2.5-10.2% oil. The oil contents of Acacia cyclops, Acacia cyanophylla and Acacia mollissima seeds were 8.85%, 11.13% and 7.16%, respectively. Acacia arabica and A. radiata seeds contained 10.06 and 5.14% oils, respectively. The oil contents of Acacia species changed between 6.83 (A. ligulata) and 12.18% (A. salicina). Youzbachi et al. determined that A. cyanophylla seed in Tunisia contained about 10% oil. Dry matter, total phenolic, and total flavonoid contents of Acacia victoriae seeds roasted at different times changed between 13.96 and 15.49%, 1.19 and 12.19 mg GAE/g and 0.23 and 1.93 mgCE/g, respectively. The total phenol, total flavonoid and total carotenoid contents of Acacia seed extracts changed between 17.5 and 18.2 mg GAE/g, 4.9 and 6.3 mgCAE/g and 14.2 and 15.5 mg/g, respectively. Wild Acacia seeds contained 10.2% oil and 180.44 mg GAE/100 g total phenol. The antioxidant activity values of the methanol extracts of Acacia nilotica seeds varied between 73 and 78%. Some variations in this study were observed compared to previous results. These differences among results can be probably due to climatic conditions, altitude, some analytical conditions and genetic factors. There was established statistically significant variations among physico-chemical and bioactive properties of Acacia seeds at 0.05. The content of total phenolic substances constantly changes depending on various factors such as plant species, genetics, location, and climate.

3.2 The fatty acid compositions of Acacia seed oils

The fatty acids of Acacia seed oils are presented in Table 2. Acacia seed oil extracted from Acacia seeds grown in Konya province contained 62.70% linoleic, 23.41% oleic, 6.45% palmitic and 2.93% stearic acids. The oil obtained

| Table 1 Chemical and bioactive properties of wild Acacia (Konya) and gladiçya (Taşucu-Mersin) seed samples. |
|-----------------------------------------------|
| Samples          | Moisture content (%) | Oil content (%) | Carotenoid content (μg/g) |
|------------------|----------------------|----------------|--------------------------|
| Konya            | 7.41 ± 0.11b         | 4.30 ± 0.10a   | 0.76 ± 0.09b             |
| Taşucu-Mersin    | 7.50 ± 0.04a         | 3.00 ± 0.20b   | 1.06 ± 0.04a             |
|                  | Total phenolic content (mg GAE/100 g) | Total flavonoid content (mg/100 g) | Antioxidant activity (%) |
| Konya            | 255.11 ± 11.06a      | 1343.60 ± 32.53a | 64.18 ± 0.67b           |
| Taşucu-Mersin    | 190.00 ± 6.07b       | 184.53 ± 17.11b | 75.21 ± 0.42a           |

*standard deviation; **values within each column followed by different letters are significantly different at p < 0.05.
from Acacia seeds grown in Mersin location contained 70.39% linoleic, 16.04% oleic, 6.04% palmitic and 4.94% stearic acids, 2.70% linolenic, 1.44% behenic and 1.28% arachidonic acids. In general, stearic, oleic, arachidic, linolenic, behenic and arachidonic acid contents of Acacia seed oil provided from Mersin province were higher compared to results of Acacia seed oil provided from Konya distinct. The fatty acid profiles of both Acacia samples were different as quantity. Acacia seed oil provided from Mersin province were higher compared to results of Acacia seed oil provided from Konya distinct. The fact that Mersin location is warmer than Konya may have caused the palmitic acid content to be high. Adewusia et al. \(^\text{[4]}\) reported that Acacia colei and Acacia tumida seed oils contained 9.4-14.6% palmitic, 3.5-7.0% stearic, 18.0-23.5% oleic, 31.7-55.9% linoleic, 1.2-4.1% arachidonic and 1.0-1.8% lignoceric acids. The oil of Acacia species grown in Tunisia contained 9.63-15.10% palmitic, 0.8-2.10% stearic, 15.06-27.13% oleic, 52.39-68.1% linoleic, 0.47-1.08% arachidic acids\(^\text{[5]}\). The oil of wild Acacia seeds contained 0.14% myristic, 9.97% palmitic, 0.39% palmitoleic, 1.82% stearic, 22.71% oleic, 61.29% linoleic and 0.52% arachidic acids\(^\text{[5]}\). The phenolic compounds and their quantity values of Acacia seeds collected from two different locations

### Table 3

| Phenolic content | Konya          | (Taşucu-Mersin) |
|------------------|----------------|-----------------|
| Gallic acid      | 2.91 ± 0.23*a  | 1.03 ± 0.49b    |
| 3,4-Dihydroxybenzoic acid | 3.89 ± 0.73b** | 4.83 ± 0.81a    |
| Catechin         | 3.42 ± 0.59b   | 9.51 ± 0.46a    |
| Caffeic acid     | 5.20 ± 0.28a   | 1.23 ± 0.15b    |
| Syringic acid    | 5.29 ± 0.40a   | 2.65 ± 0.28b    |
| Rutin            | 23.37 ± 1.62a  | 11.87 ± 1.96b   |
| p-Coumaric acid  | 2.44 ± 0.78a   | 0.76 ± 0.20b    |
| Ferulic acid     | 14.74 ± 0.42a  | 1.12 ± 0.06b    |
| Resveratrol      | 1.33 ± 0.28a   | 0.50 ± 0.09b    |
| Quercetin        | 3.64 ± 0.57b   | 4.60 ± 0.50a    |
| Cinnamic acid    | 0.36 ± 0.10a   | 0.25 ± 0.08ab   |
| Kaempferol       | 2.76 ± 0.94a   | 0.66 ± 0.30b    |

*standard deviation;** values within each row followed by different letters are significantly different at \(p < 0.05\).
and 4.83 mg/100 g (Taşcuu-Mersin), (+)-catechin contents of Acacia seeds were identified as 3.42 mg/100 g (Konya) and 9.51 mg/100 g (Taşcuu-Mersin). Also, rutinhydrate contents of Acacia seeds were found as 23.37 mg/100 g (Konya) and 11.87 mg/100 g (Taşcuu-Mersin) while ferulic acid amounts of Acacia seed samples are detected as 14.74 mg/100 g (Konya) and 1.12 mg/100 g (Taşcuu-Mersin). In addition, quercetin amounts of seed samples were measured as 3.64 mg/100 g (Konya) and 4.60 mg/100 g (Taşcuu-Mersin). Also, Acacia seeds provided from Konya province contained 2.91 gallic acid, 5.29 syringic acid, 2.44 p-coumaric acid and 2.76 mg/100 g kaempherol. In general, the amounts of phenolic constituents of Acacia seeds provided from Konya were higher (except 3,4-dihydroxybenzoic acid, caffeic acid, and quercetin) compared to results of Acacia seeds provided from Mersin distinct. There were established statistically significant variations among the amounts of phenolics of both Acacia seeds (p < 0.05). Climatic and soil factors had affected on phenolic constituents of Acacia seeds provided from Konya and Mersin provinces. In addition, it is thought that phenolic components in Konya Acacia seeds are partially high due to the fact that the temperature is partially lower than Mersin during the growing period of the Acacia plant in Konya location and the weather is cool. Jelessi et al. 33 determined 1.19-1.94% phenylacetic acid, 0.67-1.17% trans-cinnamic acid, 0.11-0.18% 3-hydroxybenzoic acid, 0.01-0.33% tyrosol, 0.10-0.15% 4-hydroxybenzoic acid, 2.85-6.28% syringic aldehyde, 0.57-0.65% vanillic acid, 6.55-6.67% protocatechuic acid, 0.62-10.04% o-coumaric acid, 35.4-38.97% syringic acid, 2.7-6.6% p-coumaric acid, 0.21-0.26% gallic acid, 30.72-34.43% ferulic acid and 0.50-0.87% caffeic acid in Acacia seeds. Ee et al. 25 reported that succinic and gallic acid contents of acetone extract of wattle seeds (Acacia victoriae Bentham) changed between 0.06 and 0.32 mg/mL to 0.01 and 0.11 mg/mL, respectively. These values are similar or high to those obtained with the seeds of other plants commonly used, such as peanut, corn and soybean. The differences in the results may have resulted from the genetic structure of the plant, environmental factors such as temperature, rainy, location and altitude.

3.4 The mineral contents of Acacia seeds collected from two different locations

The mineral contents of Acacia seeds provided from Konya and Mersin (Taşcuu-Silifke) locations are presented in Table 4. Locations had affected on mineral profiles of Acacia seeds. In general, mineral contents of Acacia seeds collected from Mersin district were found higher than that of collected from Konya province. Acacia seeds collected from Konya and Mersin locations contained 4003.75 and 3540.89 mg/kg P, 9819.12 and 16175.69 mg/kg K, 4347.47 and 5078.81 mg/kg P, 2195.77 and 2317.90 mg/kg Mg, 1015.75 and 2665.60 mg/kg S and 187.53 and 905.52 mg/kg Na, respectively. In addition, Acacia seeds collected from the same locations (Konya and Mersin) contained 58.18 and 112.86 mg/kg Fe, 22.40 and 24.43 mg/kg Cu, 19.39 and 14.62 mg/kg Mn, 0.88 and 2.77 mg/kg Ni, 29.70 and 29.79 mg/kg Zn and 10.58 and 19.67 mg/kg B, respectively. Acacia nilotica seeds collected from Palwal and Hisar provinces in India contained 198 and 203 mg/100 g Ca, 110 and 100 mg/100 g K, 51.4 and 50.7 mg/100 g P, 25 and 27 mg/100 g Na, 2.5 and 2.4 mg/100 g Mg, 18 and 20 mg/100 g Fe, 2.4 and 2.5 mg/100 g Zn, 1.9 and 1.4 mg/100 g Co, 3.0 and 3.2 mg/100 g Mn, and 0.3 and 0.2 mg/100 g Cu, respectively. Gebeeyew et al. 48 determined 850-936 g (kg Ca, 41-64 g/kg Mg, 131-141 g/kg P, 195-481 g/kg S, 169-372 g/kg Mn, 42-121 g/kg Mo, 2.6-4.7 g/kg Zn, 1.6-2.4 g/kg Co, 30.0-88.4 g/kg Cu, 24.7-74.4 g/kg Fe and 2.4-11.2 g/kg Se in some selected Acacia species seeds. Acacia turnda, A. colei seed oils contained 308 and 597 mg/kg Mg, 1424 and 2146 mg/kg Ca, 17 and 48 mg/kg Zn, 194 and 91 mg/kg Fe and 12 and 11 mg/kg Cu, respectively. When the results were compared with results of previous studies, it was observed that some minerals increase and decrease. It has been observed that Acacia seeds obtained from both locations are rich in minerals. Therefore, it is thought that these seeds can be added to feeds as mineral additives. There were observed statistically significant differences among mineral contents of both Acacia seeds depending on locations (p < 0.05). These differences in the results obtained may be due to altitude, maturation, climatic factors, precipitation, genetic, locations, and soil structure.

| Minerals | Konya | (Taşcuu-Mersin) |
|----------|-------|----------------|
| P        | 4003.75 ± 17.29**a | 3640.89 ± 19.27b |
| K        | 9819.12 ± 11.54b** | 16175.69 ± 209.21a |
| Ca       | 4347.47 ± 9.89b    | 5078.81 ± 35.29a |
| Mg       | 2195.77 ± 12.47b   | 2317.90 ± 19.32a |
| S        | 1015.75 ± 8.65b    | 2665.60 ± 29.56a |
| Na       | 187.53 ± 5.67b     | 905.52 ± 12.81a |
| Fe       | 58.28 ± 2.38b      | 112.86 ± 13.58a |
| Cu       | 22.40 ± 1.27b      | 24.43 ± 2.18a |
| Mn       | 19.39 ± 2.76a      | 14.62 ± 1.69b |
| Ni       | 0.88 ± 0.13b       | 2.75 ± 0.87a |
| Zn       | 29.70 ± 6.89a      | 29.79 ± 3.25a |
| B        | 10.58 ± 1.38b      | 19.67 ± 1.52a |

*standard deviation;** values within each row followed by different letters are significantly different at p < 0.05.
Conclusion

In general, oil contents, total phenolic and total flavonoid amounts of Acacia seeds provided from Konya distinct were determined higher than those of values of Acacia seeds growing in Mersin province. As a result of this study, Acacia seed oil can be a new source of oil due to its high linoleic-oleic acid contents and has economic importance that can meet the edible oil needs of the population living in the areas where it is grown. In addition, oleic, arachidic, linolenic, and behenic acid contents of Acacia seed oil provided from Mersin province were higher compared to results of Acacia seed oil provided from Konya distinct. The amounts of phenolic constituents of Acacia seeds provided from Konya were higher except 3,4-dihydroxybenzoic acid, caffeic acid, and quercetin compared to results of Acacia seeds provided from Mersin distinct. Locations had affected on mineral profiles of Acacia seeds. But, it has been observed that Acacia seeds obtained from both locations are rich in minerals.

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