

## Research Article

# Vitamin, Trace Element, and Fatty Acid Levels of *Vitex agnus-castus* L., *Juniperus oxycedrus* L., and *Papaver somniferum* L. Plant Seeds

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The levels of fat-soluble vitamin, trace element and fatty acid of *Vitex agnus-castus* L., *Juniperus oxycedrus* L., and *Papaver somniferum* L. seeds in Turkey were determined by using HPLC, ICP-OES, and GC, respectively. In the *Vitex agnus-castus* L., *Juniperus oxycedrus* L., and *Papaver somniferum* L. seeds, linoleic acid (18:2) was determined with the highest level rates (%54.11, %28.03, and %72.14, resp.). In the *Vitex agnus-castus* L. seeds, R-tocopherol,  $\alpha$ -tocopherol, and K<sub>1</sub> levels were determined as 9.70  $\mu$ g/g, 18.20  $\mu$ g/g, and 24.79  $\mu$ g/g, respectively; In the *Juniperus oxycedrus* L. seeds, R-tocopherol,  $\alpha$ -tocopherol, and K<sub>1</sub> were determined as 18.50  $\mu$ g/g, 0.84  $\mu$ g/g, and 5.00  $\mu$ g/g, respectively, and in the *Papaver somniferum* L. seeds, R-tocopherol,  $\alpha$ -tocopherol, K<sub>1</sub>, and D<sub>2</sub> levels were determined as 43.25  $\mu$ g/g, 122.05  $\mu$ g/g, 12.01  $\mu$ g/g, and 0.62  $\mu$ g/g, respectively. In the *Vitex agnus-castus* L., *Juniperus oxycedrus* L., and *Papaver somniferum* L. seeds, nickel (Ni), zinc (Zn), and iron (Fe) were determined with the trace element level rates (4.42 mg/kg, 10.43 mg/kg, 3.71 mg/kg for Ni, 7.00 mg/kg, 7.70 mg/kg, and 24 mg/kg for Zn and 93.73 mg/kg, 187.95 mg/kg, and 149.64 mg/kg for Fe, resp.). These parameters in seeds are very important for human life.

## 1. Introduction

Plant seeds are important food sources for the people. Therefore, many studies are devoted to quality and nutritional values of plant seeds [1]. Fatty acids, vitamins, and trace elements are very important for human life [2, 3]. *Vitex agnus-castus* L. is a shrub widely distributed in the Middle East and southern Europe [4]. This plant has been proven to have a wide range of biological activities. Among them, it is commonly used in the treatment of menstrual disorders resulting from corpus luteum deficiency, including premenstrual symptoms and spasmodic dysmenorrhea, for certain menopausal conditions, for insufficient lactation, and for the treatment of acne [5, 6]. The fruits, flowers, and leaves of *Vitex*

*agnus-castus* L. are reported to contain phenolic acids and their derivatives, flavonoids, tannins, iridoids, diterpenoids, and essential oil composition [7–10]. *Juniperus oxycedrus* L. is one of the most widespread species of the genus, with a range extending from Africa and the Iberian Peninsula, throughout Mediterranean Europe and Anatolia to the Caucasus and Iran. In Turkey, juniper tar, leaves, and fruits are widely used to heal wounds, common cold, cough, bronchitis, abdominal pain and stomachic disorders, hemorrhoids, calcinosis in joints, kidney inflammation, against fungal infections, and gynecological diseases [11–15]. *Papaver somniferum* L. is cultivated as an annual crop in countries such as China, India, Czechoslovakia, and Turkey. It is an annual herb native to Turkey and adjacent countries, having grayish-green leaves

and variously colored (white, pink, red, or purple) flowers. *Papaver somniferum* L. seeds also range over a wide variety of colors, from white, yellow, and brown to gray and blue. It is generally rich in polyunsaturated fatty acids [16].

This study is intended to assess levels of metals, vitamins, and fatty acids in seeds of the *Vitex agnus-castus* L., *Juniperus oxycedrus* L. and *Papaver somniferum* L. plants grown in Turkey's area. The study is expected to deliver preliminary data on chemical composition of these plants grown in Turkey and provide useful information for future studies which will be conducted on agronomy and physiology of the these plants and nutritional, medical, and toxicological effects in relation to the these plants.

## 2. Experimental

**2.1. Chemical Agents.** All chemicals and reagents were purchased from Sigma-Aldrich.

**2.2. Plant Material.** Seeds were purchased as grinded fresh from closed bazaar in Elazig in August 2010. The material was transported in polypropylene bags, held at room temperature, and analyzed in two days. Seeds have been identified by Z. Tel from the Section of Botany, Department of Biology, Faculty of Science, Adiyaman University.

**2.3. Fatty Acid and Vitamin Analyses.** One g of plant seed materials for fatty acid and vitamin analyses were finely ground in a mill and were then extracted with hexane/isopropanol (3:2 v/v) [17]. The lipid extracts were centrifuged at 10.000 g for 5 minutes and filtered. The solvent was then removed on a rotary evaporator at 40°C. The extracted lipids were stored at -25°C until further analysis. The experiment was repeated three times. Fatty acids in the lipid extracts were converted into methyl esters by means of 2% sulphuric acid (v/v) in methanol [18]. The fatty acid methyl esters were extracted with n-hexane. The methyl esters were then separated and quantified by gas chromatography and flame-ionization detection (Shimadzu GC 17 Ver.3) coupled to Glass GC 10 computer software. Chromatography was performed with a capillary column (25 m in length and 0.25 mm in diameter) (PermaBound 25, Macherey-Nagel, Germany) using nitrogen as a carrier gas (flow rate 0.8 mL/min.). The temperatures of the column, detector, and injection valve were 130–220, 240, and 280°C, respectively. Identification of the individual methyl esters was performed by frequent comparison with authentic standard mixtures that were analyzed under the same conditions.

Lipid-soluble vitamins were extracted from the lipid fraction according to the method of Emre [19]. The extracted lipids of seed material were dissolved in acetonitrile/methanol (75/25 v/v) and 50 mL were injected into the HPLC instrument (Shimadzu, Kyoto, Japan). The column used was a Supelcosil TM LC18 (250 × 4.6 mm, 5 mm, Sigma, USA). The mobile phase was acetonitrile/methanol (75/25, v/v) and the elution was performed at a flow rate of 1 mL/min. The temperature of the analytical column was kept at 40°C. The results of the analyses were expressed as µg/g for samples.

TABLE 1: Fatty acid composition (percentage levels) of seed.

Fatty acids	Seeds		
	<i>Vitex agnus-castus</i> L.	<i>Juniperus oxycedrus</i> L.	<i>Papaver somniferum</i> L.
8:0	—	2.9951	—
10:0	—	—	0.5250
11:0	—	5.1812	—
14:1	—	1.3856	—
15:0	6.3029	—	1.7175
16:0	8.6646	10.7426	7.9749
18:0	3.8324	1.7992	1.4935
18:1	16.8548	28.6401	13.8195
18:2	54.1187	28.0318	72.1454
18:3	6.8649	13.2545	2.3243
20:3	—	6.1682	—
22:0	—	—	—
22:1	3.3617	—	—
22:2	—	1.8017	—
Σsat.	18.7999	20.7181	11.7109
Σunsat.	81.2001	79.2819	88.2892

**2.4. Determination of Trace Elements.** Perkin-Elmer 3100 inductively coupled plasma optical emission spectrometer (ICP-OES) (Norwalk, USA) with a Gem Cone nebulizer on a cyclonic spray chamber and an autosampler AS 91 (Perkin-Elmer) was used in the current study. Microwave-assisted acid digestions were done using a Premier microwave system. Seeds were digested using a microwave system. A 2.00 g portion of each sample was dried at 80°C accurately and 0.50 g directly weighed into PTFE (polytetrafluorethylen) bombs, and 4 mL of HNO<sub>3</sub> (65%, w/w) and 1 mL of HClO<sub>4</sub> (60%, w/w) were added. In a tightly closed system, the six-step microwave digestion program was applied. PTFE bomb was left to cool for an hour and then carefully opened. Colorless solution was transferred into a beaker and evaporated to dryness with a hot plate. Afterwards final volume was diluted to 20 mL with 0.1 M HNO<sub>3</sub>. The blank samples were digested in the same way. Sample solutions were analyzed by ICP-OES [20].

## 3. Results and Discussion

The fatty acid levels of plant seeds were presented in Table 1. The palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), 18:2, and linolenic acid (18:3) fatty acids were commonly determined in all of the three plant seeds. The maximal rate (%72) for 18:2 was obtained in the *Papaver somniferum* L. On the other hand, the rates were obtained as %28 (for 18:1 and 18:2) in *Juniperus oxycedrus* L., %54 (for 18:2) and %16 (for 18:1) in *Vitex agnus-castus* L. Similarly, in another published research, the level of 18:2 in *Papaver somniferum* L. seeds was obtained as maximal ratio [21]. And also in several studies the fatty acids in seeds were determined in different levels and diversities [22–24].

The vitamin levels of plant seeds were presented in Table 2. In *Papaver somniferum* L. seeds,  $\alpha$ -tocopherol and R-tocopherol were determined as 122.05  $\mu\text{g/g}$  and 43.25  $\mu\text{g/g}$ , respectively. In the literature, there are some studies that contain the determination of  $\alpha$ -tocopherol and  $\gamma$ -tocopherol in *Papaver somniferum* L. seeds [21–24]. However, in the present study, we also observed D<sub>2</sub> and K<sub>1</sub> vitamins as 0.62  $\mu\text{g/g}$  and 12.01  $\mu\text{g/g}$ , respectively. The literature does not contain any study about the determination of trace elements and vitamin levels of *Vitex agnus-castus* L. and *Juniperus oxycedrus* L. In this study, the levels of trace elements and vitamins in *Vitex agnus castus* L. and *Juniperus oxycedrus* L. have been firstly reported and represented in Tables 2 and 3, respectively.

The trace element levels of plant seeds were presented in Table 3. The trace element levels of *Papaver somniferum* L. seeds in Turkey were determined as 6 mg/kg for copper (Cu), 3.71 mg/kg for Ni, 24 mg/kg for Zn, and 149.64 mg/kg for Fe. Most of these results show parallelism with the results of Ozcan [22]. However, we observed Fe level higher than Ozcan's results. On the other hand, Azcan and coworkers determined the trace element levels of *Papaver somniferum* L. seeds in Turkey as 1.6 mg/kg for Cu, 44.8 mg/kg for Fe, and 74.5 mg/kg for Zn [25]. We think that these disparities result from the different ground and climatic effects.

The trace element levels of *Vitex agnus-castus* L. seeds in Turkey were determined as 3.00 mg/kg for Cu, 4.42 mg/kg for Ni, 7.00 mg/kg for Zn, 93.73 mg/kg for Fe, and 0.26 mg/kg for cobalt (Co). Also, The trace element levels of *Juniperus oxycedrus* L. seeds in Turkey were determined as 7.10 mg/kg for Cu, 27.79 mg/kg manganese (Mn), 2.87 mg/kg chrome (Cr), 10.43 mg/kg for Ni, 7.70 mg/kg for Zn, and 187.95 mg/kg for Fe.

Elements are required for proper functioning of the human system and play a vital role in the biological system through their interaction with biomolecules, the deficiency of which causes serious metabolic abnormalities and the increase of which leads to toxicity [26]. The levels of trace elements in some diseases such as chronic kidney, liver, and lung have been studied, and important results have also been obtained. Fe, Cu, Mn, and Zn are essential elements in enzyme metabolism. They have immunomodulatory functions and thus influence the susceptibility to the course and the outcome of a variety of viral infections. Co in the form of vitamin B-12 is in its physiologically active form [27–30]. Vitamins are important organic compounds. It has been determined that vitamins are important in the prevention of diseases [31]. Tocopherols are believed to be the protection of polyunsaturated fatty acids against peroxidation [32]. Unsaturated fatty acids which cannot be synthesized by human and animals are known as essential fatty acids. This group includes five fatty acids, unsaturated 18:1, 16:1, 18:2, 18:3, and 20:4 [20]. *Papaver somniferum* L. seed and oil having high nutritive value were recommended for processing as healthy food products. Linoleic acid was established as the dominant fatty acid in all varieties [22]. *Papaver somniferum* L. seed oil appears to be of good quality for human consumption since it is generally rich in polyunsaturated fatty acids [16]. This seed may be a suitable

TABLE 2: Lipid-soluble vitamin contents in the seeds ( $\mu\text{g/g}$ ).

Vitamins	Seeds		
	<i>Vitex agnus-castus</i> L.	<i>Juniperus oxycedrus</i> L.	<i>Papaver somniferum</i> L.
R-Tocopherol	9.70	18.50	43.25
$\alpha$ -Tocopherol	18.20	0.84	122.05
K <sub>1</sub>	24.79	5.00	12.01
D <sub>2</sub>	—	—	0.62

TABLE 3: The levels of metals in seed samples (mg/kg).

Trace element	Seeds		
	<i>Vitex agnus-castus</i> L.	<i>Juniperus oxycedrus</i> L.	<i>Papaver somniferum</i> L.
Cu	3.00	7.10	6
Mn	—	27.79	—
Cr	—	2.87	—
Ni	4.42	10.43	3.71
Zn	7.00	7.70	24
Fe	93.73	187.95	149.64
Co	0.26	—	—

oil seed crop for the food industry due to its very low content of linolenic and high content of linoleic acids [33]. In our study, we determined that these three seeds have high level total unsaturated oil acid. Due to these seeds have high content of total unsaturated oil acid (especially 18:2) and have suitable vitamin and trace elements, these seeds and their oils can be recommended as healthy nutrition.

#### 4. Conclusion

The present study indicates that these seeds are a good natural source of fatty acids. In addition, the findings in this study are important for the nutrition sciences, because fatty acids, vitamins, and trace element, in particular, seem to have considerable effects on health.

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