Supporting Information

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Evaluation of Fatty Acid Composition, Antioxidant and Antimicrobial Activity, Mineral Composition and Calorie Values of Nuts and Seeds in Turkey

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S1: Storing samples: The samples were kept in their shells at 4 °C and 60-65% relative humidity. Just before analyses, the deshelled nuts were ground in a blender, then sieved through a 0.5 mm sieve. The samples were extracted with hexane (HPLC grade) by using Soxhlet apparatus at 80 °C for 8 h. The extracts of nut and seed samples was filtered and concentrated under vacuum at 50 °C by using a rotary evaporator (Heidolph, Laborota 4000, Germany) [1]. An aliquot of the extracts was lyophilized (Christ Alpha 1-2 LD Plus, Germany) for antimicrobial activity and stored in the dark at 4 °C until used within a maximum period of one week.

S2: Fatty Acid Composition Test: For this purpose, samples $(1 \ \mu L)$ were injected into a Supelcowax 10 column (60m x 0.25 mm i.d., 0.25 μ m film thickness; Supelco, Bellefonte, PA) coated with polyethylene glycol. The column was connected to a Hewlett Packard 5890 Series II (Little Falls, Willmington, DE) GC equipped with a FID detector. The oven temperature was programmed as follows 180 °C for 2 min, increased to 200 °C at 2 °C/min, held at 200 °C for a further 10 min, and then increased to 215 °C at 2 °C/min and kept there for 10 min. The injector and detector temperatures were 210 and 250 °C, respectively. Helium was used as the carrier gas at a flow rate of 1.5 mL/min. FAME identification was based on retention times compared with those of standard FAME. The percentage composition of the oils was calculated from GC peak areas.

S3: Radical-Scavenging Activity (Antioxidant Activity) Test: Extract concentration providing IC_{50} inhibition values were calculated from graph plotting using nonlinear regression and expressed in mg dried material equivalents/mL for sample extracts or in mM for pure compounds. Butylated hydroxytoluen (BHT) and Vitamin C (Ascorbic acid) were used as a positive control. A lower value of IC_{50} (defined as the concentration of the compounds that was able to inhibit 50% of the total DPPH radicals) indicates a higher antioxidant activity.

S4: Vitamin A (Retinol) and Vitamin E (α -Tocopherol) Content Test: The chromatography was carried out using a Shimahdzu system composed of gradient LC-20AD Prominence pumps, SIL-20A Prominence autosampler, CTO-10ASvp column oven, SPD-20A Prominence UV detector and SCL-20A Prominence controller. The data was acquired by LC Solution software. The separation was achieved an Inertsil ODS-III C18 column (46x150 ID, 5 µm particle size).

S5: The Antimicrobial Activity Test: Disk diffusion method: Sterilized antibiotic discs (6 mm) were used. The discs were impregnated with 20 μ L of these solutions. All the bacteria were incubated and activated at 30 °C for 24 h inoculation into Nutrient Broth (OXOID), and the yeasts were incubated in Malt Extract Broth (OXOID) for 48 h. Inoculums containing 10⁶ bacterial cells or 10⁸ yeasts cells per cm³ were spread on Mueller-Hinton Agar (OXOID) plates (1 cm³ inoculums for each plate). The discs injected with solutions were placed on the inoculated agar by pressing slightly and incubated at 35 °C (24 h) and at 25 °C (72 h) for bacteria and yeast, respectively. On each plate an appropriate reference antibiotic disc was applied depending on the test microorganisms. In each case triplicate tests were performed and the average was taken as the final reading.

S6: Mineral Content Test: The mineral contents of samples were determined by the wet ashing method. Each sample (2g wet weight) was weighed in a Kjeldahl flask. Twenty milliliters of concentrated nitric acid was added to each sample and the flask was left to stand overnight. Five milliliters of concentrated perchloric acid and 0.5 mL of concentrated sulfuric acid were added, and the flask was then heated until no white smoke was emitted. The samples were dissolved in 2% of hydrochloric acid and transferred into a volumetric flask. After this, solution was put into the ICP-AES apparatus' samples tubes and spectroscopic measurement was made under optimum instrumental parameters. The minerals were measured by ICP-AES and concentrations were calculated in micrograms and milligrams per gram wet weight [2].

Parameter	Description
Rf power	1.20 kW
Plasma gas flow rate	15.0 L/min
Auxiliary gas flow rate	1.50 L/min
Nebulizer gas flow rate	0.70 L/min
Sample uptake rate	1.8 mL/min
Argon gas (high purity)	99.99%
Torch type High solids	Axial (1.8 mm, quartz)
Nebulizer type	Concentric glass; cyclonic spray chamber
Nebulizer pressure	200 kPa
Pump rate	20 rpm
Replicates	3
Sample uptake delay	30 s

S7: Table 1. Instrument operating parameters for ICP-AES

S8: Table 2. n-3 and n-6 Fatty acid composition (%) of nut and seed oils

	n-3 Fatty acid		n-6 Fatty	acid					
-	α-	Eicosa	Linoleic	γ-	Arachidonic	Docosa	Eicosa	Total	Total
	Linolenic	pentaenoic	18:2	Linolenic	20:4	dienoic	trienoic	n-3	n-6
	18:3	20:5		18:3		22:2	20:3		
Hazelnut	12.14	-	13.64	0.03	-	0.03	0.04	12.14	13.74
Peanut	0.06	-	26.51	1.46	-	0.13	2.00	0.06	30.10
Pistachio	0.31	-	20.53	0.18	-	0.08	0.11	0.31	20.9
Almond	0.04	-	20.37	0.06	-	0.04	-	0.04	20.47
Walnut	12.22	-	63.42	0.09	-	0.03	0.03	12.22	63.57
Chestnut	2.00	1.15	28.86	0.57	0.22	0.40	0.27	3.15	30.32
Pumpkin seed	0.11	-	70.17	0.27	-	0.04	0.75	0.11	71.23
Sunflower seed	0.18	0.43	48.58	0.31	-	0.04	0.12	0.61	49.32



S9: Figure 1. % 50 Inhibition values of nut and seed samples

S10: Table 3.	Antimicrobial	activity data	of Turkish nu	ut and seed	samples (I	nhibition Zo	ne,
mm)							

	Inhibition zone (mm)										
Microorganisms/Compounds	Hazelnut	Peanut	Pistachio	Almond	Walnut	Chestnut	Pumpkin	Sunflower			
							seed	seed			
Escherichia coli	12	13	17	11	11	24	12	15			
Staphylococcus aureus	14	12	16	12	13	15	15	14			
Klebsiella pneumoniae	11	14	15	13	12	16	12	15			
Bacillus cereus	12	11	15	10	12	25	14	14			
Micrococcus luteus	14	13	14	11	13	14	15	12			
Proteus vulgaris	16	14	15	14	18	16	14	17			
Mycobacterium smegmatis	10	12	16	10	12	18	12	13			
Listeria monocytogenes	11	13	17	12	13	14	13	18			
Pseudomonas aeruginosa	12	13	11	14	12	17	11	10			
Kluyveromyces fragilis	14	13	14	11	11	12	15	18			
Rhodotorula rubra	13	11	17	11	12	15	14	14			
Candida albicans	12	14	13	12	10	19	13	14			
Hanseniaspora guilliermondii	14	14	13	11	13	16	12	14			
Debaryomyces hansenii	13	15	15	11	13	15	14	13			

S11: Table 4. Antimicrobial activities of some standard antibiotics and antifungals (Inhibition Zone, mm)

	Inhibition zone (mm)									
Microorganisms/Antibiotics	P10	SAM20	CTX30	VA30	OFX5	TE30	NY100	KETO20	CLT10	
Escherichia coli	18	12	10	22	30	28	-	-	-	
Staphylococcus aureus	13	16	12	13	24	26	-	-	-	
Klebsiella pneumoniae	18	14	13	22	28	30	-	-	-	
Pseudomonas aeruginosa	8	10	54	10	44	34	-	-	-	
Proteus vulgaris	10	16	18	20	28	26	-	-	-	
Bacillus cereus	14	12	14	18	30	25	-	-	-	
Mycobacterium smegmatis	15	21	11	20	32	24	-	-	-	
Listeria monocytogenes	10	12	16	26	30	28	-	-	-	

Micrococcus luteus	36	32	32	34	28	22	-	-	-
Candida albicans	-	-	-	-	-	-	20	21	15
Kluyveromyces fragilis	-	-	-	-	-	-	18	16	18
Rhodotorula rubra	-	-	-	-	-	-	18	22	16
H. guilliermondii	-	-	-	-	-	-	21	24	22
Debaryomyces hansenii	-	-	-	-	-	-	16	14	18

P10 : Penicillin G (10 Units), SAM20 : Ampicillin 10 μg, CTX30 : Cefotaxime 30 μg, VA30 : Vancomycin 30 μg, OFX5 : Oflaxacin 5 μg, TE30 : Tetracyclin 30 μg, NY100 : Nystatin 100 μg, KETO20 : Ketaconazole 20 μg : CLT10 : Clotrimazole 10 μg

S12: Table 5. Mineral compositions of nut and seed samples (mg/kg)

Material	Ca	Mg	K	Na	Fe	Cu	Mn	Se	Zn	Cr	Al
Hazelnut	806.23	427.38	2566.32	1414.78	8.79	2.42	5.82	2.2	8.02	-	1.43
Peanut	633.46	427.17	2166.83	1416.12	17.94	4.19	6.95	2.11	15.77	0.25	11.03
Pistachio	1221.74	427.83	2779.56	1416.16	10.8	1.868	4.24	2.54	9.02	0.34	2.25
Almond	1075.25	426.14	2457.67	1414.11	23.95	2.85	10.16	3.08	18.23	-	10.04
Walnut	977.5	426.72	2291.88	1417.94	24.9	8.14	35.13	3.05	20.87	0.19	6.15
Chestnut	476.5	423.08	4739.15	403.91	16.9	5.39	20.81	-	5.33	-	8.74
Pumpkin seed	599.10	420.90	4050.61	1411.83	40.98	3.66	35.30	1.79	53.14	0.35	2.27
Sunflower seed	713.78	424.39	3094.09	1413.89	28.30	10.13	17.45	2.65	38.65	0.245	2.89

S13: References

[1] AOAC. (1990). Official Methods of Analysis. 15th ed. Washington, DC: Association of Official Analytical Chemists.

[2] W. Mertz (1987). Trace Elements in Human and Animal Nutrition (Vols 1 and 2), Academic Press, San Diego.