Determination of Conjugated Linolenic Acid Content of Selected Oil Seeds Grown in Turkey

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ABSTRACT: Seeds originating from some Turkish sources were analyzed with respect to their characteristics and FA compositions. These seeds represented pomegranate (Punica granatum L.), bitter gourd (Momordica charantia L.), pot marigold (Calendula officinalis L.), catalpa (Catalpa bignonoides), bourdaine (Rhamnus frangula L.), Oregon grape (Mahonia aquifolium), sarsaparilla (Smilax aspera), mahaleb (Prunus mahaleb L.), blackthorn (*Prunus spinosa* L.), cherry laurel (*Prunus laurocerasus* L.), and firethorn (Pyracantha coccinea). Bitter gourd and bourdaine seeds contain more than 20% oil. Catalpa, bourdaine, Oregon grape, blackthorn, and cherry laurel seed oil contents ranged from 15 to 20%. In the seeds from plants belonging to the Rosacea family, oil content ranged from 4.5 to 18.5%. Among the seed oils analyzed, pot marigold had one of the lowest oil contents (5.9%). Pomegranate contained the highest amount of total conjugated linolenic acid (CLNA) (86.0%). Seed oils of bitter gourd, pot marigold, mahaleb, and catalpa were rich in CLNA: 60.0, 29.5, 27.6, and 27.5%, respectively. Bourdaine, Oregon grape, and sarsaparilla seeds contained low amounts of CLNA. On the other hand, mahaleb, bourdaine, catalpa, Oregon grape, sarsaparilla, cherry laurel, blackthorn, and firethorn seed oils are basically oleic and linoleic acid-rich oils and therefore have little drying ability (semidrying oil). The results show a potential for the use of endogenous Turkish seeds as a source of CLNA.

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KEY WORDS: Conjugated linolenic acids, fatty acid composition, oil content, seed oils, Turkish flora.

Conjugated linolenic acid (CLNA) is a collective term for the positional and geometric isomers of octadecatrienoic FA. The three double bonds in CLNA are primarily in positions $\Delta 9,11,13$ and $\Delta 8,10,12$. There can also be geometric isomers (cis or trans configuration). Seven different CLNA isomers already have been identified (1). They are not very common in animal fats but are found in various seed oils (1–3). For example, α-eleostearic acid (9cis, 11trans, 13trans-18:3) is found in tung seed oil (Aleurites fordi) (68%) and in bitter gourd oil (Momordica charantia L.) (56.2%) (2). Mahaleb oil obtained from mahaleb seed (Prunus mahaleb L.), which is virtually unique to Turkey, contains about 35% α -eleostearic acid (3). Pomegranate (Punica granatum L.) seed contains about 83% punicic acid (9c,11t,13c-18:3), and catalpa seed (Catalpa bignonoides) oil contains about 42.3% catalpic acid (9t,11t,13c-18:3) (1). Oil from the seeds of pot marigold (Calendula officinalis L.) contains approximately 62% calendic acid (8t,10t,12c-18:3) (2). Jacaric acid (8c,10t,12c-18:3) is found in the seeds of *Jacaranda mimosifolia* (1).

The oils containing these FA are very important raw materials in the manufacture of organic coatings and polymers, as the conjugated unsaturation facilitates good polymerization and imparts adhesive properties when properly treated (4). More recently, the use of calendula oil as a reactive diluent in paint formulation has become very popular (5,6). In this application, it can decrease solvent emissions to reduce adverse effects on human health and in the environment with its very reactive conjugated C18-triene.

In addition, CLNA potentially have beneficial health and biological effects. Suzuki et al. (7) reported a strong cytotoxic effect of CLNA from catalpa, tung, and pomegranate on human monocytic leukemia cells. They also found that the cytotoxicities of punicic acid, α -eleostearic acid, and catalpic acid were much stronger than that of calendic acid. Igarashi and Miyazawa (8) reported that conjugated all-trans trienoic FA have the strongest growth-inhibitory effect among the conjugated trienoic acids and conjugated dienoic acids produced by alkaline treatment of α-linolenic acids. The anticarcinogenic effect of purified α -eleostearic acid (9c,11t,13t-18:3) and of seed oil from bitter gourd, which is a rich source of αeleostearic acid, on human cells is known (9,10). The dietary effects of CLNA from bitter gourd on blood (2,11) and liver lipids (2) in rats have been investigated, and the antioxidant property of α-eleostearic acid from bitter gourd also has been reported (11).

Among the seeds of plants already mentioned as containing CLNA, only bitter gourd, pomegranate, and mahaleb cherry produce materials that are edible by humans. Bitter gourd and pomegranate are consumed for their fruits, whereas mahaleb cherry is used for its seeds. Mahaleb seeds, separated from their dried fruits, are ground to form a spice. This Turkish spice has a pleasant smell and is used as a flavoring in pastry products, sweets and cream (3).

The first objective of this study was to determine the conjugated FA content of oil-containing foods from Turkey that are known to contain CLNA, such as mahaleb seed or bitter gourd oil. The second objective was to investigate the seed oils of some plants that are used in folk medicine and that might contain CLNA, such as Oregon grape, bourdaine, sarsaparilla, cherry laurel, blackthorn, and firethorn for purposes of determining both their industrial and pharmaceutical potential.

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MATERIALS AND METHODS

Seeds and chemicals. Berries of sarsaparilla, Oregon grape, bourdaine, firethorn, blackthorn, and cherry laurel and catalpa fruits were collected from gardens and fields in Istanbul between 2003 and 2004. Pomegranate fruit and bitter gourd, mahaleb, and pot marigold seeds were purchased from local markets in Istanbul. The fruits and berries were kept for no more than 30 d at 4°C until analyzed. All solvents were of chromatographic grade (Merck, Darmstadt, Germany)

Analysis of seeds. The berries were cleaned to remove decayed fruits, stems, and leaves. One hundred (100) berries were selected randomly and weighed. Their seeds were removed manually from their pulps, dried for 12 h at room temperature, and weighed. The percentage of seeds in the berries was calculated based on the fresh weight of the berries. To determine the oil content of seeds, air-dried seeds were ground in a coffee mill and extracted for 6 h with hexane by Soxhlet apparatus. The solvent was removed by rotary evaporation, and the remaining oil was weighed. The oil content of seeds was calculated on a dry weight basis.

Oil extraction for FA analysis. About 5 g of the seeds was ground in a coffee mill and immediately extracted, in duplicate, with 150 mL hexane at room temperature for 4 h. The hexane was removed with a rotary evaporator at 40°C in vacuo and then the residue was stored at –18°C in the dark until use. During extraction and evaporation steps, the seed and oil were not exposed to the light.

Determination of FA composition. The FA compositions of the seeds were determined by GLC. The oils were converted to their corresponding methyl esters according to the AOCS method (12). BF₃/methanol was used for methylation. GC analysis of the FAME was performed using a Hewlett-Packard HP-5890 Series II gas chromatograph equipped with an Ultra 2 (25 m × 0.32 mm × 0.5 μ m film thickness of 5% biphenyl and 95% dimethyl polysiloxane; Hewlett-Packard, Waldron, Germany) capillary column, a split injector (split ratio 88:1), and an FID. The column temperature program was 5 min at 150°C, 10°C/min to 275°C, and 10 min at 275°C. The injector and detector temperatures were 250 and 280°C, respectively. The carrier gas was nitrogen at a flow rate of 1.6 mL/min. Air and hy-

drogen flow rates were 460 and 33 mL/min, respectively. The peaks of CLNA were identified by comparing the retention times with those of a mixture of isomers of standard linolenic acid methyl esters (Sigma Chemicals Co., Deisenhofen, Germany). Each FAME sample was analyzed in duplicate.

RESULTS AND DISCUSSION

Characterization of seeds. Some physical characteristics (seed weight, seed oil content, seed content of berries) of seeds are given in Table 1. The seed content of berries from the four Rosaceae species varied from 7.3 to 19.1%. Johansson *et al.* (13) studied five different Rosaceae species and found that seed percentages of these berries ranged between 4.4 and 20.2%. The seed content in pomegranate fruit was 8.4%. This value falls within the range described by Melgarejo and Artes (14), who reported the seed content was between 37 and 143 g/kg of fruit. Of the four berry species, sarsaparilla and Oregon grape have relatively high seed contents, 43.4 and 23.5%, respectively. Bourdaine has the highest seed content (63.4%) among the investigated plants.

Weight of 100 seeds. One hundred-seed weights of pomegranate, sarsaparilla, bitter gourd, and bourdaine were 2.6, 6.0, 7.2, and 8.3 g, respectively. The lowest seed weights were recorded for Oregon grape (0.9 g), pot marigold (0.4 g), and firethorn (0.2 g). In the literature, the variability in the weight of 1000 seeds of Calendula officinalis has been reported as 5–15 g for 1993 crops in Italy (15). In Rosacea berries, the 100-seed weights of cherry laurel and blackthorn were very similar to each other. Our result for cherry laurel seed weight is in good agreement with the result of Erciyes et al. (16). Mahaleb seed weight was less than those of the two berries just cited. Variations in the weight of 100 seeds of Rosaceae berries has been reported previously to vary from 180 to 2950 mg (13).

Oil content of seeds. The oil content of seeds is presented in Table 1. In bitter gourd and bourdaine seeds it is more than 20%. Bitter gourd oil content is given as 25.4% in literature (17). Catalpa, Oregon grape, blackthorn, and cherry laurel seed oil contents ranged between 15 and 20%. In the seeds belonging to the Rosacea family, oil content ranged from 4.7 to 18.5%. The range for Rosacea oil content in this study was wider than that

TABLE 1 Characteristics of Some Turkish Seeds

Botanical name	Family	Trivial names	Oil content of seeds ^a (wt%)	100-seed weight (g)	Seeds in berries (%)
Catalpa bignonoides	Bignoniaceae	Catalpa	_	_	_
Momordica charantia L.	Cucurbitacae	Bitter gourd	21.0	8.3	_
Calendula officinalis L.	Asteraceae	Pot marigold	5.9	0.4	_
Punica granatum L.	Punicaceae	Pomegranate	18.1	2.6	8.4
Rhamnus frangula L.	Rhamnaceae	Bourdaine	41.9	7.2	63.4
Mahonia aquifolium	Berberidaceae	Oregon grape	15.8	0.9	23.5
Smilax aspera	Liliaceae	Sarsaparilla	10.8	6.0	43.4
Prunus mahaleb L.	Rosaceae	Mahaleb cherry	18.5	3.7	_
Prunus spinosa L.	Rosaceae	Blackthorn	16.5	15.7	19.1
Prunus laurocerasus L.	Rosaceae	Cherry laurel	18.3	15.2	9.2
Pyracantha coccinea	Rosaceae	Firethorn	4.7	0.2	7.3

^aPercentage on a dry weight basis.

TABLE 2
FA Composition of Some Seed Oils from Turkish Flora

		Bitter	Pot			Oregon				Cherry	
FA	Pomegranate	gourd	marigold	Catalpa	Bourdaine	grape	Sarsaparilla	Mahaleb	Firethorn	laureĺ	Blackthorr
12:0			1.4	0.9				Trace	0.3	0.1	
12:1				1.4					0.7		
14:0		Trace	0.6	0.3	Trace		0.1		0.2	0.1	Trace
15:0								0.1			
16:0	2.0	1.7	10.2	5.3	21.6	7.1	5.4	4.6	11.3	13.1	6.2
16:1n-7			0.1	0.2	0.9	0.2	0.1	0.3	0.2	4.0	1.1
18:0	1.6	21.1	3.8	2.8	3.8	3.9	2.9	1.8	3.0	2.5	1.3
18:1n-9	3.7	9.2	8.0	13.7	28.3	31.9	35.7	35.4	10.5	53.7	57.6
18:2n-6	3.3	5.8	43.5	41.7	38.0	51.7	51.4	28.5	70.1	25.5	33.5
18:3n-3	0.1	0.2	1.1	2.1	0.2		0.1	0.1		0.1	Trace
20:0	3.0	0.5	0.7	1.1	0.7	0.2	0.7	0.7	0.8	0.5	0.1
20:1n-9	0.1	1.2	0.2	1.6	0.3	0.4	1.9	0.3	0.1	0.2	0.1
20:1n-11		0.3		0.3							
22:0		Trace	0.9	1.1			0.1	0.1	1.8	0.1	Trace
22:1n-9							0.1				
24:0		Trace		0.1	0.1	0.3	0.1	0.1		0.1	Trace
CLNA (Total)	86.0	60.0	29.5	27.5	6.2	4.4	1.5	27.6	1.0	0.2	
CLNA (9 <i>c</i> ,11 <i>t</i> ,13 <i>t</i>)											
(α-eleostearic acid)	49.9		4.1	3.1	2.2	1.0	21.0			
CLNA (9 <i>c</i> ,11 <i>t</i> ,13 <i>c</i>)											
(punicic acid)	57.3				0.8						
CLNA (9 <i>t</i> ,11 <i>t</i> ,13 <i>c</i>)											
(catalpic acid)	7.6	2.2		14.9	0.9	0.8	0.3	1.3			
CLNA (9 <i>t</i> ,11 <i>t</i> ,13 <i>t</i>)											
(β-eleostearic acid) 21.1	7.9		8.5	1.4	1.4	0.2	5.2			
CLNA (8 <i>t</i> ,10 <i>t</i> ,12 <i>c</i>)											
(calendic acid)			18.3								
CLNA (8t,10t,12t)			11.2								

found in the literature. Johansson *et al.* (13) mentioned that seed oil contents varied from 12 to 16%. Pot marigold seeds had one of the lowest oil contents among the seed oils analyzed here; in our study, the oil content was 5.9%, similar to that reported by Angelini *et al.* (15). In other studies conducted by Janssens and Vernooij (6) and Cromack and Smith (5) for the same seed, the oil contents ranged from 14 to 18% and 13 to 21%, respectively.

FA compositions of seed oils. The FA compositions of seed oils are summarized in Table 2. Pomegranate contained the highest amount of total CLNA (86.0%). Isomers of punicic, β-eleostearic, and catalpic acids accounted for 57.3, 21.1, and 7.6% of total FA, respectively. Pomegranate oil also contained oleic acid (3.7%) and linoleic acid (3.3%). Lesser amounts of palmitic (2.0%) and stearic (1.6%) acid were found. FA compositions reported in the literature for pomegranate (7), bitter gourd (17,18), pot marigold (6,7), and catalpa (7) oils are included in Table 3. As seen in Table 2, the FA composition of Turkish pomegranate seed oil was similar to that of Japanese pomegranate oil (7), especially with respect to the amount of CLNA.

The total CLNA content of bitter gourd seed oil was 60.0% (Table 2). It also contained a considerable amount of β -eleostearic acid (49.9%). b-Eleostearic and catalpic acids were detected in bitter gourd seed oil at 7.9 and 2.2% of total FA, re-

spectively. Notable quantities of stearic (21.1%), oleic (9.2%), and linoleic (5.8%) acids were found in bitter gourd, but only modest levels of palmitic (1.7%) and gadoleic (1.2%) acids were detected. The FA composition of Turkish bitter gourd seed oil was similar to that of Vietnamese and Japanese bitter gourd samples (Table 3).

In pot marigold seed oil, linoleic (43.5%) acid, CLNA (29.5%), palmitic (10.2%), oleic (8.0%) and stearic (3.8%) acids were the major FA. Two CLNA isomers were detected in pot marigold seed oil. Calendic acid (8t,10t,12c-18:3) was the main CLNA isomer, accounting for 18.3% of total FA. Another CLNA isomer (8t,10t,12t-18:3) was also found in pot marigold seed oil. Wide ranges of calendic acid are published in the literature. For example, Cromack and Smith (5) reported that the calendic acid level ranged between 42 and 52% among the nine different kinds of calendula seeds grown in England. Similarly, Angelini et al. (15) found the calendic acid percentage varied from 16 to 46 in the seed oil of Italian pot marigold harvested in 1993 (15). European seed oil (northwest Europe) contained mostly conjugated FA (59.1%) (6), whereas Turkish seed oil contained linoleic acid (43.5%) as the predominant FA.

The FA compositions of catalpa, bourdaine, Oregon grape, and sarsaparilla seed oils are also included in Table 2. The total amount of CLNA of these seed oils varied from 1.5 to 27.5%. The main isomer of CLNA for catalpa was catalpic acid

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TABLE 3 FA Composition for Bitter Gourd (Momordica charantia L.), Pot Marigold (Calendula officinalis L.), Pomegranate (Punica granatum L.), and Catalpa (Catalpa bignonoides)

		Bitter gourd			Pot marigold		Pome	Pomegranate	Catalpa	ılpa
FA	Turkish	Vietnamese (18)	Japanese (19)	Turkish	European (6)	European (6) Japanese (7)	Turkish	Japanese (7)	Turkish	Japanese (7)
12:0				1.4					6.0	
12:1									4.1	
14:0	Trace		0.1–0.2	9.0					0.3	
16:0	1.7	2.1	1.3-8.6	10.2	2.0	4.1	2.0	3.1	5.3	2.8
16:1n-7				0.1					0.2	
18:0	21.1	26.2	17.7–27.7	3.8	1.6	2.1	1.6	2.0	2.8	1.9
18:1n-9	9.2	3.9	2.2-7.7	8.0	3.7	7.4	3.7	4.5	13.7	7.8
18:2n-6	5.8	4.9	3.8-18.6	43.5	28.5	42.5	3.3	5.1	41.7	32.3
18:3n-3	0.2	0.1	9.9–0.0	1.1		1.0	0.1		2.1	1.3
20:0	0.5	9.0	0.2-0.3	0.7	0.4		3.0		1.1	
20:1n-9	1.2	0.3		0.2	0.4		0.1		1.6	
20:1n-11	1.2									
22:0	Trace			6.0					1.1	
24:0	Trace								0.1	
CLNA (Total)	0.09	9.09	44.6–63.5	29.5	59.1	38.1	86.0	81.2	27.5	37.0
CLNA (9 <i>c</i> ,11 <i>t</i> ,13 <i>t</i>) α -eleostearic acid	49.9		43.1–62.6					2.8	4.1	
CLNA $(9c,11t,13c)$ punicic acid			0.5-0.6				57.3	71.7		
CLNA $(9t,11t,13c)$ catalpic acid	2.2		0.0-0.1				7.6	5.1	14.9	31.3
CLNA $(9t,11t,13t)$ β -eleostearic acid	7.9		0.3-0.9				21.1	1.6	8.5	5.7
CLNA (8t,10t,12c) calendic acid				18.3		33.4				
CLNA (8t,10t,12t)				11.2		4.7				

(54.3%). The other CLNA isomers found in catalpa were α-and β-eleostearic acids, 14.9 and 30.9% of total CLNA, respectively. The content of the main FA in Turkish catalpa seed oil resembled that from the literature (Table 3). In bourdaine, Oregon grape, and sarsaparilla seed oils, the proportion of α-eleostearic acid to the total conjugated acid was 50%. Table 2 shows that all of these seeds contained considerable amounts of oleic and linoleic acids. Bourdaine also contained a high amount of palmitic acid (21.6%).

The FA profiles of seed oils from four representatives of the Rosacea family—mahaleb, firethorn, cherry laurel, and blackthorn—are also shown in Table 2. Mahaleb seed oil contains a considerable amount of CLNA, 27.6% of the total FA. Conjugated 18:3 isomers of mahaleb are rich in α-eleostearic acid, which accounted for 76.1% of the total CLNA. Other conjugated isomers detected were catalpic and β-eleostearic acids, 4.9 and 19.0%, respectively. Contrary to expectations, two other seed oils from the same family analyzed in this study contained only minor amounts of CLNA. The level of total CLNA found in firethorn and cherry laurel seed oils was 1.0 and 0.2%, respectively. CLNA was not found in blackthorn seed oil. Our results are in agreement with earlier reports regarding the content of αeleostearic acid in the *Prunus* varieties of mahaleb and cherry laurel (16). Mahaleb, firethorn, cherry laurel, and blackthorn contained high proportions of oleic and linoleic acids.

In this study, seeds of 11 plants originating from Turkish sources were analyzed with respect to seed characteristics and their FA composition. To our knowledge, FA data for bourdaine, Oregon grape, sarsaparilla, blackthorn, and firethorn originating from other countries are not available in the literature, nor is information on the exact composition of the Turkish species investigated in this study. Only one report was encountered in the literature for cherry laurel FA composition; in it, conjugated FA were not detected (16). Pomegranate, bitter gourd, pot marigold, mahaleb, and catalpa seed oils analyzed in this study were found to be very rich in CLNA as indicated in literature (3,6,7). Bourdaine, Oregon grape, and sarsaparilla seeds contained low amounts of CLNA. On the other hand, mahaleb, bourdaine, catalpa, Oregon grape, sarsaparilla, cherry laurel, blackthorn, and firethorn seed oils are basically oleic and linoleic acid-rich oils and therefore have little drying ability (semidrying oil).

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