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Extraction and identification of phytosterols and Phytostanols from different organs of some Iranian *Echium* plants

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Abstract

Introduction: Seeds, leaves, and stems from six populations of two *Echium* species (Boraginaceae family) were collected from their natural habitats in Iran and were assessed for new natural sources of phytosterol.

Material & Methods: Phytosterols were extracted from different plant organs and analyzed by GC for total phytosterol content and campesterol, β-sitosterol, stigmasterol, and sitostanol.

Results: The phytosterol content of seeds varied between 91.00 mg/100gr DW in stems of *E. italicum*, Kaleybar population to 399.40 mg/100gr DW in seeds of *E. italicum*, Alamute Qazvin population. The content of campesterol ranged from 26.00 mg/100gr DW in *E. amoenum*, Hezarjarib population leaves to 212.00 mg/100gr DW in *E. italicum,* Alamute Qazvin population. In addition, considerable amounts of β-sitosterol were detected, with the highest values of 209.00 mg/100gr DW in leaves of *E. amoenum* from Behshahr population. Other main phytosterol components were stigmasterol and sitostanol, with the highest amounts of 22.00 and 141.40 mg/100gr DW in *E. amoenum* leaves from Behshahr and *E. italicum* seeds from Alamute Qazvin populations, respectively.

Conclusion: These data allow us to evaluate Iranian members of *the Echium* genus as alternative wild sources of valuable phytosterols for commercial purposes.

Keywords: Boraginaceae; *Echium*; Phytosterol; Saponification; Gas chromatography; Sterols

1.Introduction

Plant sterols, also called phytosterols, have been reported to include over 250 different types and related compounds in various plant and marine materials (Piironen et al., 2000). Phytosterols are widely distributed natural substances (Breinholder et al., 2002) that are the counterparts of cholesterol in animal products (Atif et al., 2000). Phytosterols are triterpenes similar to cholesterol, both in structure, given the four-ring steroid nucleus, the 3bhydroxyl group, and often a 5,6-double bond, as in function, given their role in the stabilization of the phospholipid bilayers in cell

membranes. Cholesterol has a side-chain composed of eight carbon atoms; however, more common phytosterols have a side-chain including 9 or 10 carbon atoms out of a total of 28 or 29 carbon atoms (Fig. 1) (Fernandes and Cabral, 2007, Atif et al., 2000).

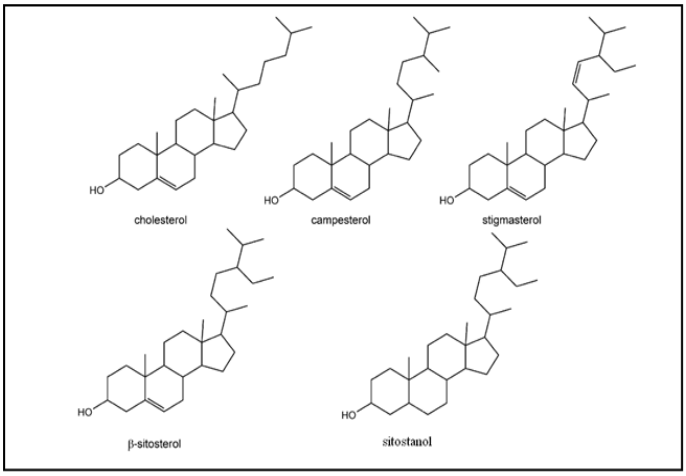

Fig.1. Chemical structures of cholesterol and main phytosterols and phytostanols. This figure illustrates the structures of cholesterol and representative phytosterols and phytostanols.

Plant materials and especially vegetable oils contain free sterols (FS) and four types of conjugated sterols: steryl fatty acid esters (SE), hydroxycinnamate steryl esters (HSE), steryl glycosides (SG), and acylated steryl glycosides (ASG) and steryl glycosides which could be esterified to acylated steryl glycosides (Fig. 2) (Piironen et al., 2000, Rozenberg et al., 2003, Fernandes and Cabral, 2007 and Verleyen et al., 2002) and their saturated derivatives, the stanols, are less abundant in nature (Rozenberg et al., 2003).Fig.2. Chemical structure of phytosterols in different forms (R varies between different phytosterols). The 3-hydroxyl group of free phytosterols may be esterifed by a fatty acid or a phenolic acid to give steryl esters (SEs), or it may be β-linked to the 1'-position of a carbohydrate to form either steryl glycosides (SGs) or acylated steryl glycosides (ASGs). In acylated steryl glycosides the 6'-position of the carbohydrate is esterifed with a long- chain fatty acid. So there are four existence-forms including FSs, SEs, SGs and ASGs.

Free sterols, and to some extent, steryl glycosides and acylated steryl glycosides, are incorporated into cell membranes. Like cholesterol in mammalian cells, they perform principal roles in the structure and function of cell membranes. Plant steryl esters are located intracellularly and represent mostly a storage form of sterols, analogously to cholesteryl esters in the mammalian organism. Thus vegetable oils are rich in plant steryl esters. In addition to their crucial roles in maintaining adequate function of plant cell membranes, plant sterols are precursors of a group of plant growth factors (Yu et al., 2021; Rogowska and Szakiel, 2020; Valitova et al., 2016; Piironen et al., 2000).

Phytosterols are principal components of the unsaponifiable matter of vegetable oils and fats. Due to their structural similarities, phytosterols form oxidation products similar to cholesterol using analogous oxidative pathways (Apprich and Ulberth, 2004). Together with phospholipids and other glycolipids, phytosterols and their derivatives are essential components of plant biomembranes. They are biogenetic precursors of numerous metabolites, including plant steroid hormones (Breinholder et al., 2002).

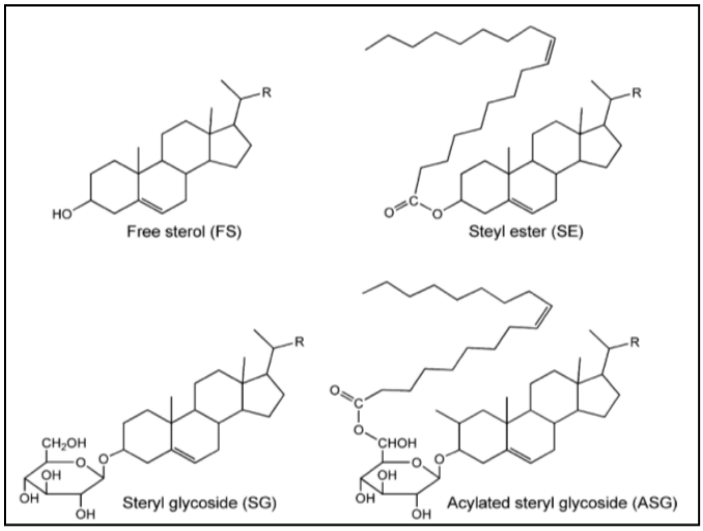

Fig.2. Chemical structure of phytosterols in different forms (R varies between different phytosterols). The 3-hydroxyl group of free phytosterols may be esterifed by a fatty acid or a phenolic acid to give steryl esters (SEs), or it may be β-linked to the 1'-position of a carbohydrate to form either steryl glycosides (SGs) or acylated steryl glycosides (ASGs). In acylated steryl glycosides the 6'-position of the carbohydrate is esterifed with a long- chain fatty acid. So there are four existence-forms including FSs, SEs, SGs and ASGs.

Phytosterols are frequently classified according to double bond positions in the ring skeleton (Breinholder et al., 2002). The most abundant sterols in these natural sources are 4-desmethylsterols, such as sitosterol, campesterol, stigmasterol, Δ5-avenasterol, and Δ7-avenasterol; sitosterol was the predominant sterol (Piironen et al., 2002, Bouic, 2001, Fernandes and Cabral, 2007 and Marangoni and Poli, 2010). Other relevant phytosterols that could be found in plants in minor amounts are brassicasterol, D5-avenasterol, sitostanol and campestanol (Fernandes and Cabral, 2007). Heart diseases are increasing day by day, and heart attack is the principal cause of death worldwide. Heart disease is the number one killer of both men and women in America. More than 90 million American adults have elevated blood cholesterol levels (Sabir et al. 2003). A decrease in blood cholesterol due to the administration of the drugs or by the modification of diet decreases the risk of coronary artery disease. Thus, it is healthy to identify and limit the consumption of foods are rich in cholesterol (Barkas et al., 2023: Sabir et al., 2003). Phytosterols and their saturated derivatives, stanols, play major roles in several areas, namely in pharmaceuticals (production of therapeutic steroids), nutrition (anti-cholesterol additives in functional foods, anti-cancer properties), and cosmetics (creams, lipstick) (Fernandes and Cabral, 2007, Zangenberg et al., 2001, John et al., 2007 and Harrabi et al., 2008). In the last decades, purified plant sterols or stanols have been added to various food items to obtain functional foods with remarkable hypocholesterolemic activity. A daily intake of plant sterols or stanols of 1.6–2 g/day, incorporated in these foods, can reduce cholesterol absorption from the gut by about 30% and plasma LDL cholesterol levels by 8 –10%. Phytosterols, up to 3 g/day, are safe and effective cholesterol-lowering agents (Marangoni and Poli, 2010). Epidemiologic and experimental studies suggest that dietary Phytosterols may offer protection from the most common cancers in Western societies, such as colon, breast, and prostate cancer (Atif et al., 2000), and reduced incidence of various cardiovascular disease, diabetes, and other chronic conditions in populations consuming diets rich in vegetables and fruits (Bouic, 2001). As phytosterols endogenously are not synthesized by humans, their circulating levels are only dependent upon diet and absorption efficiency (Marangoni and Poli, 2010), and sterols produced by higher plants are varied from those of lower plants (Masni et al., 2009). The primary sources of phytosterols are vegetables, nuts, fruits, seeds (Rozenberg et al., 2003), grains, and grain-derived products, as well as sprouts, cabbages, cauliflowers, green and black olives contain plant sterols and stanols incorporation (Marangoni and Poli, 2010; Valitova et al., 2016). The Boraginaceae, to which *Echium* belongs, is a large plant family with about 2500 species in 100 genera (Tsevegsuren & Aitzetmuller, 1996). The present study aimed to determine the total plant sterol contents (the sum of free and conjugated sterols) of the seed, leaf, and stem oils from two frutescent *Echium* species growing in six different locations in Iran.

2. Methods and Materials

2.1. Materials

2.1.1. Chemical materials

Cholesterol (90%), Campesterol (98%, GC), Stigmasterol (95%), β-sitosterol (97%), Sitostanol and bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) were purchased from Sigma (St. Louis, MO, USA). Absolute ethanol, hexane, dry pyridine, dichloromethane, potassium hydroxide, hydrochloric acid (37%), potassium chloride, anhydrous potassium sulfate and other reagent were all analytical grade and obtained from Shanghai No.3 Chemical Reagents Company, China. Deionized water was used throughout the analyses.

2.1.2. Plant materials

Mature seeds of six populations from two *Echium* species were collected from different regions of Iran in their natural habitats during August-September 2009. Leaves and stems of all the mentioned plants were also harvested in June 2009. Table 1 shows the collection sites and date for each plant. The voucher specimens were kept in Tehran University Herbarium (TUH).

Table 1. The collected plant materials, collection sites and date of collection.

Species	Locality	Climate	Latitude [N]	Longitude [E]	Altitude [m]	Date	TUH No.
Echium italicum	Tehran province-Boumehen	Cold and semi-humid	35° 44′	51° 52′	1500-2000	June and September 2009	38891
Echium italicum	East Azerbaijan province-Kaleybar	Temperate and semi-humid	38° 52′	47° 02′	1500-2000	June and August 2009	38892
Echium italicum	Qazvin province-Alamute Qazvin	Cold and semi-humid	36° 27′	50° 23′	1000-1500	June and September 2009	38893
Echium amoenum	Mazandaran province-Hezar Jarib	Cold and semi-humid	36° 40′	53° 20′	2500-3000	June and August 2009	38894
Echium amoenum	Mazandaran province-Behshahr	Temperate and humid	36° 41′	53° 32′	50-200	June and August 2009	38895
Echium amoenum	Mazandaran province-Ramsar	Temperate and humid	36° 53′	49° 40′	1500-2000	June and August 2009	38896

2.2. Analytical procedures

2.2.1. Saponification

Plant organs were freeze-dried and ground to a powder in a mortar. Around 0.5 g of each sample (0.5 g by adding 200µg cholesterol) was placed into a 50-mL round-bottom flask. After adding 5mL 4 mol L^{-1} ethanolic HCl solutions, the samples were vigorously shaken and refluxed for one hour at 80◦C, and then the mixture was cooled to room temperature. After acid hydrolysis, 10mL 4.0 mol L^{-1} ethanolic KOH solution was added into the mixtures (or 0.5 g sample containing 200µg Cholesterol), and the samples were vigorously shaken and refluxed at 70◦C for one hour (Liu et al., 2007).

2.2.2. Solvent extractionAfter the hydrolysates were cooled to room temperature, 10mL hexane, 5mL deionized water, and a little solid potassium chloride were added. The flask was recapped and sonicated for 5 min. Then, the mixtures were transferred to a 125-mL separatory funnel. The mixture was extracted three times with hexane, and the hexane phase was collected. The extracts were washed three times with 3mL 0.25 mol L^{-1} KOH aqueous solution and then adjusted to pH 6 with deionized water. After that, the extracts were transferred to a flat-bottom flask, and anhydrous potassium sulfate was added. Finally, the volume of hexane as solvent was reduced to 1mL using a vacuum rotatory evaporator, and then the extract was transferred into a septum-capped vial (Liu et al., 2007).To determine free phytosterols content, the ground organs (1.0 g sample and 200µg cholesterol) were placed into a conical flask, and after adding 20 mL dichloromethane, the flask was placed on a vibrator and was mechanically vibrated for one hour at 150 rpm. After standing for 30 min, the dichloromethane extract was filtered through a 45 µm nylon membrane filter. The obtained extracts were evaporated according to the method described above (Liu et al., 2007).

2.2.3. Derivatization

The concentrates were dried under a nitrogen stream. Fifty microliters of redistilled dry pyridine and 50 µL of BSTFA (containing 1% TMCS) were added, and the tube was securely capped and left standing overnight at room temperature. The mixture was then diluted to 1mL with dichloromethane, and 1 µL of the solution was analyzed by GC (in 1 week) (Liu et al., 2007*)*.

2.2.4. Equipment and conditions

GC chromatography of samples was performed with a CP-3800 (VARIAN-Australia) provided with FID. The separation was carried out in a siloxane capillary column wall-coated open tubular (WCOT) (*15 m × 0.25 mm i.d. × 0.25 µm film thickness; Teknokroma* Co*, Barcelona, Spain*). The flow of the carrier gas (He) was 1ml min^{-1}. Injector and detector temperature was 300 °C. Split ratio in the injector was 20:1. The oven starting temperature was 150 °C (2 min), and it was increased at a rate of 5 °C min^{-1} until 300 °C (15 min). Injection volume was 1µL, and a blank was run after every ten analyses. Peaks were identified by comparison with known prepared standards from Sigma Co. (campesterol, stigmasterol, β-sitosterol and sitostanol). Cholesterol was used as internal standard for quantitative analyses.

3. Results

Phytosterols as free and conjugated forms in different organs from six populations of two *Echium* species were extracted and were identified by GC-FID methods (Table 2, Table 3). All three organs (seeds, leaves, and stems) were rich in phytosterols. The highest value for total phytosterol content was recorded in seeds, and the lowest one was obtained in stems. Phytosterols were identified by their relative retention times in GC chromatographs. Figure 3 shows typical GC–FID chromatograms of the standard solution. Standard solutions were analyzed from the lowest concentration, and then revered analysis sequence from the highest concentration solution.

Table 2. Sterol Content (mg 100 g^{-1}) Analyzed by the Conjugated/Free Sterol Analysis and Total Sterol Analysis in organs of *Echium*[a]

Species(Population)	Orga	Conjugated/Free sterols analysis										Total sterol analysis				
		Conjugated sterols (mg/100gr)					Free sterols (mg/100gr)					C+F[b]	Total sterol (mg/100gr)				
		Camp	Stigm	B-Sito	Sito	Total	Camp	Stigm	B-Sito	Sito	Total		Camp	Stigm	B-Sito	Sito	Total
E. italicum (Alamute Qazvin)	Seed	190±1.72	-	36±0.89	105.4±0.13	331.4	22±0.16	-	10±0.29	36±0.16	68	399.4	212	-	46	141.4	399.4
E. italicum (Bumehen)		38±1.31	-	62±1.09	12±0.33	112	38±1.58	-	68±0.96	16±0.09	122	234	76	-	130	28	234
E. italicum (Kaleybar)		34±2.90	2±0.02	56±0.91	18±0.12	110	14±0.29	2±0.08	25±0.83	5±0.02	46	156	48	4	81	23	156
E. amoenum (Hezarjarib)		16±0.60	-	32±1.23	14±0.19	62	14±0.18	-	27±0.17	9±0.07	50	112	30	-	59	23	112
E. amoenum (Behshahr)		33±1.00	2±0.11	71±2.01	33±0.09	139	23±0.13	-	70±0.87	18±1.15	111	250	56	2	141	51	250
E. amoenum (Ramsar)		262±1.23	-	72±1.76	3±0.05	101	20±0.41	-	37±0.91	16±1.27	73	174	46	-	109	19	174
E. italicum (Alamute Qazvin)	Leaf	20±0.93	10±0.25	62±0.12	14±0.10	106	10±0.89	3±0.10	27±0.81	8±0.09	48	154	30	13	89	22	154
E. italicum (Bumehen)		17±0.79	6±0.13	53±0.73	14±0.04	90	11±0.28	3±0.05	38±0.37	18±0.05	70	160	28	9	91	32	160
E. amoenum (Hezarjarib)		16±1.34	8±0.04	56±0.35	4±0.07	84	9±0.71	-	27±0.42	2±0.01	38	122	25	8	83	6	122
E. amoenum (Behshahr)		32±1.48	12±0.16	122±1.61	5±0.08	171	31±0.96	10±1.34	87±1.52	6±0.07	134	275	63	22	209	11	305
E. amoenum (Ramsar)		34±0.59	8±0.09	103±1.00	2±0.12	147	20±0.04	6±0.89	60±0.43	4±0.06	90	237	54	14	163	6	237
E. italicum (Alamute Qazvin)	Stem	40±1.37	9±0.33	59±0.39	4±0.07	112	30±1.68	3±0.04	38±0.17	8±0..09	79	191	70	12	97	12	191
E. italicum (Bumehen)		42±2.10	6±0.05	60±0.34	7±0.30	115	32±1.48	7±0.58	36±0.49	-	75	190	74	13	96	7	190
E. italicum (Kaleybar)		18±0.46	10±0.30	26±0.21	8±0.19	62	8±0.92	7±0.39	8±0.09	6±1.00	29	91	26	17	34	14	91
E. amoenum (Hezarjarib)		22.8±1.10	6±0.09	40±0.18	4±0.06	72.8	10±0.87	4±0.09	20±0.87	2±0.04	36	108.8	32.8	10	60	6	108.8
E. amoenum (Behshahr)		26±0.83	10±0.18	68±0.47	1±0.02	105	15±1.02	7±0.07	32±0.62	6±1.02	60	165	41	17	100	7	165
E. amoenum (Ramsar)		26±0.71	4±0.08	38±0.14	6±0.12	74	15±1.14	3±0.03	20±0.17	3±0.03	41	115	41	7	58	9	115

[a]Abbreviations: Camp, campesterol; Stigm, stigmasterol; ß-Sito, ß-sitosterol; Sito, Sitostanol.

[b]Sum of conjugated and free sterols.

Table 3. The percentages of conjugated/free sterols and the content of total sterols (mg gr^{-1} DW) in different organs of the examined *Echium* species.

Species(Population)	Organ	Total sterol (mg/gr)	Conjugated/Free sterols analysis (%)										Total sterol analysis (%)			
			Conjugated sterols (%)					Free sterols (%)					Total sterol (%)			
			Camp	Stigm	B-Sito	Sito	Total	Camp	Stigm	B-Sito	Sito	Total	Camp	Stigm	B-Sito	Sito
E. italicum (Alamute Qazvin)	Seed	3.99	5.51	-	2.51	9.02	17.04	47.62	-	9.02	26.42	83.06	53.13	-	11.53	20.54
E. italicum (Bumehen)		2.34	16.24	-	29.10	6.84	52.18	16.24	-	26.50	5.13	47.87	32.48	-	55.60	11.97
E. italicum (Kaleybar)		1.56	8.97	1.28	16.03	3.21	29.49	21.79	1.28	35.90	11.54	70.51	30.76	2.56	51.93	14.75
E. amoenum (Hezarjarib)		1.12	14.29	-	28.57	12.50	55.36	12.50	-	24.11	8.04	44.65	26.79	-	52.68	20.54
E. amoenum (Behshahr)		2.50	9.20	-	28.00	7.20	44.40	13.20	0.80	28.40	13.20	55.60	22.40	-	56.40	20.40
E. amoenum (Ramsar)		2.01	9.95	-	18.41	7.96	36.32	12.94	-	35.82	14.93	63.69	22.89	-	54.23	22.89
E. italicum (Alamute Qazvin)	Leaf	1.54	6.49	1.95	17.53	5.19	31.16	12.98	6.49	40.26	9.09	68.82	19.47	8.44	57.79	14.28
E. italicum (Bumehen)		1.60	6.88	1.88	23.75	11.25	43.76	10.63	3.75	33.13	8.75	56.26	17.51	5.63	56.88	20.00
E. amoenum (Hezarjarib)		1.22	4.38	-	22.13	1.64	28.15	13.11	6.57	45.90	3.28	68.86	17.49	6.57	68.03	4.92
E. amoenum (Behshahr)		3.05	10.16	3.28	28.52	1.97	43.93	10.49	3.93	40.00	1.64	56.06	20.65	7.21	68.52	3.61
E. amoenum (Ramsar)		2.37	8.44	2.53	25.32	1.69	37.98	14.35	3.38	43.46	0.84	62.03	22.79	5.91	68.78	2.53
E. italicum (Alamute Qazvin)	Stem	1.91	20.49	4.71	30.89	2.09	58.18	15.71	1.57	19.90	4.19	41.37	36.20	5.74	50.79	6.28
E. italicum (Bumehen)		1.90	16.84	3.68	18.95	-	39.47	22.10	3.16	31.58	3.68	60.52	38.94	6.84	50.53	3.68
E. italicum (Kaleybar)		0.91	8.79	7.69	8.79	6.59	31.86	19.78	10.98	28.58	8.79	68.13	28.57	18.67	37.37	15.38
E. amoenum (Hezarjarib)		1.09	9.17	3.67	18.34	1.83	33.01	21.10	5.50	36.70	3.67	66.97	30.27	9.17	55.04	5.50
E. amoenum (Behshahr)		1.65	9.09	4.24	19.39	3.64	36.36	15.76	6.06	41.21	0.60	63.63	24.85	10.30	60.60	4.24
E. amoenum (Ramsar)		1.15	13.04	2.61	17.40	2.61	35.66	22.61	3.48	33.04	5.22	64.35	35.65	6.09	50.44	7.83

Phytosterols were identified by their relative retention times in GC chromatographs. Figure 3 shows typical GC–FID chromatograms of the standard solution. Standard solutions were analyzed from the lowest concentration, and then revered analysis sequence from the highest concentration solution. A calibration curve was performed between the averages of the components/ IS area ratio and the components/IS concentration ratio. LODs were defined as the concentration of phytosterol solutions at a signal/noise ratio of 3 [1] (shown in Table 4).

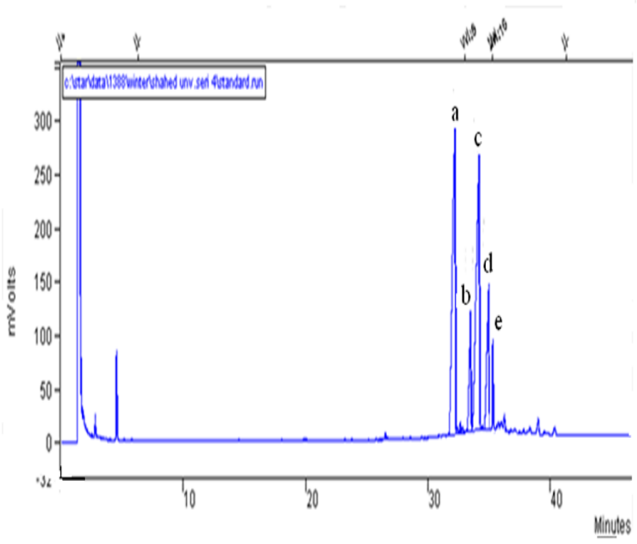

Fig.3. GC–FID chromatograms of TMS derivatives of standard sterols. Peaks: cholesterol (a) (internal standard), campesterol (b), stigmasterol (c), β-sitosterol (d), sitostanol (e).

Table 4. Linear correlation coefficients for the phytosterol contents analyzed by the conjugated /free sterol analytical method and the total sterol analysis

Phytosterol	Linear range ($\mu g\ mL^{-1}$)	Calibration curve	Correlation coefficient	LOD ($\mu g\ mL^{-1}$)
Campesterol	16-256	Y=1.5087X+0.0110	0.9989	**0.43**
Stigmasterol	40-640	Y=1.2032X + 0.0214	0.9997	**0.27**
β-Sitosterol	40-640	Y=1.0914X + 0.0127	0.9992	**0.32**
Sitostanol	40-640	Y= 1.1107X + 0.0173	0.9987	**0.39**

The total phytosterol in seeds of analyzed *Echium* plants ranged from 174.00 (mg$100g^{-1}$) in *E. amoenum* (Ramsar population) to 399.40 (mg $100g^{-1}$) in *E. italicum* (Alamute Qazvin population). Total β-sitosterol (free+conjugated) was present in relatively high amounts in seeds, with values varying from 46.00 (mg $100g^{-1}$) to 141.00 (mg$100g^{-1}$) in *E. italicum* (Alamute Qazvin population) *and E. amoenum* from Behshahr population, respectively. The main phytosterol in seeds was campesterol, ranging from 46.00 (mg $100g^{-1}$) in *E. amoenum* from the Ramsar population to 212.00 (mg $100g^{-1}$) in *E. italicum* from the Alamute Qazvin population. Total sitostanol was also present in all seed samples, with values varying from 19.00 (mg $100g^{-1}$) in *E. amoenum* (Ramsar population) to 141.40 (mg $100g^{-1}$) in *E. italicum*, (Alamute Qazvin population). Stigmasterol did not exist in the seeds of most species; only in the seeds of two populations, its content was recorded as 2.00 (mg $100g^{-1}$) and 4.00 (mg $100g^{-1}$). The lowest and the highest leaf total phytosterol contents of *Echium* plants were obtained at 122.00 (mg $100g^{-1}$) and 305.00 (mg $100g^{-1}$) in *E. amoenum* from populations of Hezarjarib and Behshahr, respectively. The highest amount of total campstreol was measured at 63.00 (mg $100g^{-1}$) in leaves of *E. amoenum* (Behshahr population). The total stigmasterol in leaves varied from 8.00 (mg$100g^{-1}$) in *E. amoenum* in the population of Hezarjarib to 22.00 (mg $100g^{-1}$) in *E. amoenum* from the Behshahr population.

The main component of leaf phytosterol was β-sitosterol, ranging from 83.00 (mg $100g^{-1}$) in *E. amoenum* of the Hezarjarib population to 209.00 (mg $100g^{-1}$) in *E. amoenum*, Behshahr population. Total sitostanol content ranged from 6.00 (mg $100g^{-1}$) in *E. amoenum* (Hezarjarib and Ramsar population) to 32.00 (mg$100g^{-1}$) in *E. italicum* (Bumehen population). Total phytosterol content in stem ranged from 91.00 (mg $100g^{-1}$) in *E. italicum* (Kaleybar population) to 190.00 (mg $100g^{-1}$) in *E. italicum* (Bumehen population). Total campesterol content ranged between 26.00 (mg $100g^{-1}$) in the stem of *E. italicum* (Kaleybar population) to 74.00 (mg $100g^{-1}$) in *E. italicum* (Bumehen population). The total stigmasterol value ranged from 7.00 (mg $100g^{-1}$) in *E. amoenum* (Ramsar population) to 17.00 (mg $100g^{-1}$) in *E. italicum* (Kaleybar population) and *E. amoenum* (Behshahr population). Among the studied plants, the stem of *E. italicum* from the Kaleybar population had the lowest β-sitosterol content 34.00 (mg $100g^{-1}$), while *E. amoenum* from the Behshahr population had the highest value of 100.00 (mg $100g^{-1}$). The amount of total sitostanol in the stem ranged from 6.00 (mg $100g^{-1}$) in *E. amoenum* from the Hezarjarib population to 14.00 (mg $100g^{-1}$) in *E. italicum* from the Kaleybar population.

4. Discussion

The semi-synthesis of steroidal drugs has mostly taken advantage of the ability of microbial cells to make bioconversions to 17-ketosteroids in sterol metabolism. This strategy avoids using diosgenin and solasodine as traditional raw materials for manufacturing steroidal drugs. For instance, phytosterols may be properly bioconverted in 4-androstene-dione (AD) and 1, 4 androsta-diene-3, 17-dione (ADD) by mycobacterial mutants. Several high-value steroid drugs used as progestational, adrenocortical, estrogenic, diuretic, anabolic, and contraceptive agents are mainly produced from these principal compounds by chemical synthesis (Perez et al., 2006).

Another very important source of phytosterols used for incorporation into commercial products is soybean, which constitutes a highly remarkable food additive in Asia and all over the World (Marangoni and Poli, 2010; Perez et al., 2006). In the case of soybean phytosterols, a composition of about 25% stigmasterol, about 70% β-sitosterol, and about 5% miscellaneous phytosterols have been found (Perez et al., 2006). Furthermore, according to a report from the Unilever Research Laboratories, the sterol composition in soybean oil was β-sitosterol (48%), stigmasterol (21%), campesterol (27%), and other sterols (5.9%) (Matvienko et al., 2002). In the case of soybean phytosterols, a composition of about 25% stigmasterol, about 70% b-sitosterol, and about 5% miscellaneous phytosterols have been found (Perez et al., 2006). Furthermore, according to a report from the Unilever Research Laboratories, the sterol composition in soybean oil was β-sitosterol (48%), stigmasterol (21%), campesterol (27%), and other sterols (5.9%) (Matvienko et al., 2002). Compared to soybean, campesterol and β-sitosterol values for Iranian *Echium* plants exhibit high percentages, with the highest one of campesterol 53.13% obtained for the seed of *E. italicum* (Alamute Qazvin population) and the highest one of β-sitosterol (68.78%) being for the leaf of *E. amoenum,* Ramsar population. The noticeable presence of high levels of campesterol recorded in the leaves was 22.79% for *E. amoenum,* Ramsar population, and in the stems 38.94% for *E. italicum,* Bumehen, population and noticeable rate of β -sitosterol determined in the stems and the seeds 60.60% and 56.40% in *E. amoenum,* Behshar population of Iranian *Echium* species can be considered as a good advantage for these species. The cholesterol and β-sitosterol contents of the three species tested in this research were equivalent, or more than sterols contents reported for sugarcane and soybean plants. Total phytosterol concentrations in these organs ranged between 91 and 399.40 (mg 100 gr^{-1}). Total phytosterol concentrations in seed ranged between 112 and 399.40 (mg 100 gr^{-1}) (Table 2). The leaves amount of β-sitosterol in all populations *of Echium* was above 55%, and for three of them was higher than 68%. Campesterol contents in all populations of the examined *Echium* was higher than 15%. Since sterols are lipophilic membrane components essential for diverse cellular functions (Boutte and Grebe, 2009). Meanwhile the *Echium* species are rich in phytosterols and recommended for dietary intake by the plants, they can be a good substitute for cholesterol, as noted. The number of studies on the Boraginaceae family about phytosterols is limited to one case on the stem of *Borago officinalis*. The results showed that sitosterol (23%) and campesterol (33%) were dominant components of sterols in the stems of *B. officinalis* (Tanaka *et al*., 2006). As compared to *B. officinalis*, our results obtained from various populations of *Echium* showed the existence of higher amounts of phytosterols in the stems of all studied populations. Since so far, the *Echium* genus, this is the first report on comparative phytosterols in *Echium*. Due to the high value of phytosterols, including β-sitosterol and stigmasterol, in the pharmacy industry, *Echium* species could be a valuable source for the pharmaceutical industry. Also, *Echium* species examined in this research had high contents of sitostanol. Sitostanol in some plants can be found in minor amounts, which is a rare phytosterol component in plants (Fernandes and Cabral, 2007). Sitostanol amounts in *Echium* organs ranged from 2.53% in the leaf of *E. amoenum* from the Ramsar population to 35.44% in the seed of the *E. italicum* Alamute Qazvin population.

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