

Purslane (*Portulaca oleracea* L.) seed oil as a valuable source of important phytonutrients: effect of the extraction method on the lipid composition

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Research Article

Keywords: phytonutrients, purslane *Portulaca oleracea*, lipid classes, fatty acids, phytosterols, extraction, oxidative stability

Posted Date: August 20th, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-4743339/v1>

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Additional Declarations: No competing interests reported.

Abstract

Purslane (*Portulaca oleracea* L.) is a widespread weed plant used since ancient times as remedy and as food. Its seed oil possesses significant bioactive potential due to the high content of important phytonutrients, mainly essential fatty acids and phytosterols. Although the total fatty acids composition of oil is well documented, till now there is no data published about individual lipid classes. The information on sterols is scarce as well. Therefore, the aim of this work was to characterize in details for the first time the individual lipid classes and their fatty acids composition, sterols, as well as the oil oxidative stability, depending on the extraction method by either hexane, chloroform-methanol mixtures or super-critical CO₂. The results revealed no significant effect of extraction method on the individual lipid classes (monoacylglycerols, diacylglycerols, free fatty acids, free sterols, triacylglycerols, sterol esters, wax esters and hydrocarbons), including the fatty acids composition of saponifiables, and quite weak effect on the oil oxidative stability regarding super-critical CO₂ vs. organic solvents. Detailed analyses of lipid composition confirmed the potential of purslane seed oil as a cheap and highly valuable source of phytonutrients as essential fatty acids and phytosterols, for application in food, pharmaceutical and cosmetic industries.

1 Introduction

Purslane (*Portulaca oleracea* L.) is a succulent annual plant widespread all over the world, mostly in tropical and subtropical zones [1]. Although considered a weed it has been used in traditional medicine since ancient times as a remedy for skin inflammations, liver, kidney, stomach, respiratory and other problems [1–3]. Nowadays there are scientific evidences also for its beneficial effects on people with diabetes and high blood cholesterol levels, even as an anti-cancer remedy [4–7]. Besides as a medicinal plant, purslane is very popular and consumed as fresh salad or cooking vegetable [1, 8]. Along with the leaves and stems, its seeds attract more and more attention as well because of the specific fatty acids composition of seed oil, namely quite high (> 30%) content of essential omega-3 18:3 (alpha-linolenic) fatty acid [9]. Thus, the purslane seed oil reveals its potential for use as a food supplement rich in valuable phytonutrients. In addition to essential fatty acids, phy-tosterols are highly desired components of the healthy diet, too [5]. However, while the fatty acids composition of purslane seed oil has been studied by many researchers, infor-mation in literature on sterols is scarce. In addition, there is no data about individual lipid classes of seed oil in order to assess comprehensively its value for food, pharmaceutical and cosmetic industries.

So, the aim of this work was to characterize purslane seed oil regarding its individual lipid classes and their fatty acids composition, as well as to estimate the oil oxidative stability, depending on the extraction method. For the purpose, four of the most used methods of extraction were applied: three classical procedures with organic solvents (*n*-hexane and two chloroform-methanol mixtures) and extraction with super-critical carbon dioxide (SC-CO₂) which is increasingly preferred in recent years as non-toxic, non-corrosive, non-flammable and cheap solvent. Yields of oil from purslane seeds extracted

by these methods were compared as well in addition to estimation of their effect on the purslane oil lipid composition.

2 Materials and Methods

2.1 Samples and reagents

The purslane (*Portulaca oleracea* L.) was collected in June-August 2018 in full flowering stage in Novo Zhelezare village (42°25'09.2"N/24°39'53.8"E), Plovdiv region, Bulgaria. The plant material was identified by Dr. Ina Aneva (Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences). Voucher specimen CO 1437 has been deposited at the Herbarium of the Institute of Biodiversity and Ecosystem Research (SOM), Bulgarian Academy of Sciences, Bulgaria. The purslane seeds were dried at room temperature in shadow and then portions of them were subjected to respective extraction method. Solvents of HPLC grade were used for extraction and preparative thin layer chromatography (TLC), whereas the reagents for hydrolysis and methylation were of analytical grade (Merck, Sigma-Aldrich). Carbon dioxide for super-critical extraction was delivered by Messer, Sofia, Bulgaria. Reference fatty acids methyl esters (FAME) mixture, individual lipid classes (monoacylglycerols, diacylglycerols, triacylglycerols, sterols, free fatty acid, sterol ester) and a reference mixture of hydrocarbons (C7-C40) were from Sigma-Aldrich Co. (St. Louis, MO, USA).

2.2 Extraction of oil and determination of fat content

2.2.1 Hexane extraction

Portions of about 30 g (precisely weighed) milled purslane seeds were extracted with n-hexane in Soxhlet apparatus for 8 h [25] and then the solvent was distilled in rotary evaporator. The residue was weighted to calculate the fat content by the Eq. (1):

$$\text{Fat \%} = (m_{\text{oil}} / m_{\text{seeds}}) \times 100 \text{ (Eq. 1)}$$

where m was the mass [g], respectively of the residue (oil) and the initial sample (purslane seeds). Five extractions were performed to collect oil for further investigations. The oil samples were kept at -20°C until analyses.

2.2.2 Chloroform-methanol (2:1) extraction

The procedure of Folch et al. [26] was used as follows: 10 g (precisely weighed) purslane seeds were milled and homogenized with 50 ml methanol in an Erlenmeyer flask with magnetic stirrer for 30 min. Then 100 ml chloroform were added for subsequent one hour homogenization, followed by addition of 50 ml chloroform-methanol mixture (2:1, v/v) for another 90 minutes homogenization. Finally, the sample was filtered through a Buechner funnel and the precipitate was washed with another portion of 50 ml chloroform-methanol mixture (2:1, v/v). The filtrates were combined and transferred to a separatory funnel after measuring of their volume by a graduated glass cylinder. One fourth of the total volume of

0.88% aqueous KCl solution was added, the mixture was vigorously shaken and allowed to settle. The upper (aqueous) layer was discarded and the bottom (lipid) layer was washed again with one fourth of its volume of mixture of 0.88% aqueous KCl solution-methanol (1:1, v/v). After separation in a funnel the bottom layer was filtered and the solvent was evaporated on a rotary vacuum evaporator. The yield of the extract was determined gravimetrically and calculated by Eq. (1) as explained in Section 2.2.1. Five extractions were performed to collect oil for further investigations. The oil samples were kept at -20°C until analyses.

2.2.3 Chloroform-methanol (1:2) extraction

The procedure of Bligh & Dyer [27] was used as follows: portion of 10 g (precisely weighed) milled purslane seeds were homogenized with 200 ml chloroform-methanol mixture (1:2, v/v) in an Erlenmeyer flask with magnetic stirrer for 3 hours. Then the sample was filtered and the precipitate was washed with 50 ml chloroform. The filtrates were combined, their total volume was measured by a graduated glass cylinder and were transferred to a separatory funnel. One fourth of the total volume of 0.88% aqueous KCl solution was added, the mixture was vigorously shaken and allowed to settle. Then the bottom (lipid) layer was collected and the solvent was evaporated on a rotary vacuum evaporator. The yield of the extract was determined gravimetrically and calculated by Eq. (1) as explained in Section 2.2.1. Five extractions were performed to collect oil for further investigations. The oil samples were kept at -20°C until analyses.

2.2.4 Super-critical CO₂ extraction

About 5 g (accurately weighed) milled purslane seeds were subjected to SC-CO₂ extraction using a SFT-110-XW apparatus (Supercritical Fluid Technologies Inc., Newark, DE, USA) with two 50 ml extractors equipped with a temperature controller allowed temperature up to 200°C. The SFT Nex10 SCF piston pump and HYAC50-25 compressor (Hyundai, Seoul, Republic of Korea) provided pressure of maximum 60 MPa. The carbon dioxide flow rate at the extractor outlet was measured with an Alicat Scientific (Tucson, AZ, USA) flow meter, model M-5SLPM-D/5M [28]. The extractions were carried out with pure CO₂ at 50°C and pressure of 35 MPa [14] with an initial 20 min static and then 90 min dynamic time (continuous passage of SC-CO₂ through the extractor). The resulting extract was collected at atmospheric pressure in a vial placed in ice. The oil yield was calculated according to Eq. (1). Five SC-CO₂ extractions were performed to collect oil for further investigations and it was kept at -20°C until analyses.

2.3 Peroxide value, conjugated Dienes and Trienes, and Oxidative stability index

Peroxide value, expressed as meq active O₂/kg oil, was determined by the modified iodometric method of Yanishlieva et al. [29] based on titration with Na₂S₂O₃. The contents of conjugated dienes and trienes were measured in 1 cm cuvettes by their absorbances at 232 nm and 270 nm, respectively, of 1% oil solutions in iso-octane using Cecil Series 8000 UV/VIS spectrophotometer with reference of pure solvent

[30]. The results were presented as K_{232} and K_{270} , respectively. Oxidative stability index as a measure of oxidative stability of oils was determined by Metrohm Professional Rancimat apparatus (Model 892, Switzerland) on 3 g oil samples at 80°C, 100°C, 110°C and 120°C, with air flow at 20 L/h.

2.4 Lipid classes

About 100 mg (accurately weighed) purslane seed oil were applied as hexane solution on a silica gel preparative TLC glass plate (20 x 20 cm). After development with a mobile phase of hexane-acetone (100:8, v/v) the separated zones of individual lipid classes were visualized under UV light (366 nm) by spraying with 2,7-dichlorofluoresceine (ethanolic solution). Identification of each zone was according to reference mixture of lipid classes applied alongside on the plate. After this development each separated zone of wax esters + hydrocarbons, sterol esters, triacylglycerols and the band below them (called zone X) was scraped from the plate, transferred to small glass column and eluted with diethyl ether. After its evaporation under gentle stream of nitrogen, the residue was weighed for determination of lipid class yield (equivalently to Eq. (1)). Then sterol esters were subjected to hydrolysis and methylation [31] for subsequent determination, respectively, of sterols and fatty acids composition. Triacylglycerols, unidentified lipid zone and a part of zone wax esters + hydrocarbons were transmethylated [31] for analysis of their fatty acids composition. On the other hand, the whole combined zone of free sterols, free fatty acids, diacylglycerols, monoacylglycerols and polar lipids was scraped from the plate, eluted with diethyl ether and subjected to second development with a mobile phase of hexane-diethyl ether-acetic acid (70:30:1, v/v/v) for better separation of these lipid classes. Except for triacylglycerols the other classes were in minor quantities (below 5%) therefore 10–12 preparative TLC plates were used to collect sufficient amounts for their further analysis including yield evaluation. Free fatty acids, mono- and di-acylglycerols were methylated for further analysis of their fatty acids composition whereas free sterols were analyzed for determination of sterol profile.

2.5 Fatty acids composition

Gas chromatography (GC) on methyl esters was used for determination of fatty acids composition. Prior to analysis, the sample (oil or respective individual lipid class as explained in Section 2.4.) was (trans)methylated by 1% H_2SO_4 in methanol to fatty acids methyl esters [31]. After their purification by preparative TLC on silica gel plate with a mobile phase of hexane-acetone (100:6 v/v), FAME were injected as hexane solution in Shimadzu Nexis GC 2030 chromatograph equipped with a flame ionization detector and Simplicity Wax capillary column (30 m × 0.32 mm × 0.25 μ m, Supelco). A temperature program from 170°C to 260°C at 2°C/min and 5 min hold at final temperature was applied. The injector and detector temperatures were 260°C and 280°C, respectively. Nitrogen was used as a carrier gas at a flow rate of 1.0 mL/min; split 1:50. The peaks identification was according to retention times compared to that of FAME in a reference mixture. Amounts of fatty acids were calculated by corresponding peak areas and presented as rel. %. Measurements were done in triplicate.

2.6 Sterols composition

Composition of sterols in both classes Free sterols and Sterol esters (after hydrolysis as explained in section 2.4) was determined by Shimadzu GC Nexis 2030 chromatograph equipped with a flame-ionization detector and TRB-5 capillary column (30 m × 0.25 mm × 0.25 µm, Teknokroma). Identification of sterols was carried out using GC-MS equipment Shimadzu QP 2020 with Sapiens-5MS column (30 m x 0.25 mm x 0.25 µm) operated from 85°C to 150°C at 15°C/min, then to 320°C at 4°C/min and hold at this temperature for 20 min; split 1:10; injector and detector temperature 280°C and 320°C, respectively; carrier gas helium with a flow rate of 0.8 ml/min. Mass spectral library NIST 2017 was used to identify individual peaks.

2.7 Hydrocarbons and wax esters

Hydrocarbons and wax esters were isolated by preparative TLC as one zone as described in Section 2.4. A part of this zone was analyzed by GC for determination of hydrocarbons and wax esters, and the other part was used for analysis of fatty acids composition of wax esters as described in Section 2.5. Identification of hydrocarbons and wax esters was done by GC-MS equipment Shimadzu QP 2020 with Sapiens-5MS column (30 m x 0.25 mm x 0.25 µm) operated from 65°C to 320°C at 5°C/min and hold at this temperature for 20 min; split 1:10; injector and detector temperature 280°C and 320°C, respectively; carrier gas helium with a flow rate of 0.8 ml/min. NIST 2017 was used as Mass spectral library, additionally to a standard mixture of hydrocarbons (C7-C40). Quantification was carried out on Shimadzu GC Nexis 2030 chromatograph equipped with a flame-ionization detector and TRB-5 capillary column (30 m × 0.25 mm × 0.25 µm, Teknokroma) under the same chromatographic conditions.

2.8 Statistical analysis

Measurements were performed in triplicate. Results are presented as a mean value of 3 parallel determinations ± standard deviation and have been compared by t-test (MS Excel 2010 software).

3 Results and Discussion

3.1 Fat content and oxidative stability of oil

Extraction of purslane seeds with different solvents produced dark brownish green oil without obvious difference in appearance (Fig. 1S). Data about oil yield depending on the extraction method are presented in Table 1. As could be seen the lowest value for fat content was obtained by SC-CO₂ extraction (15%) while both chloroform-methanol mixtures ensured about 22% and the procedure with hexane in Soxhlet apparatus provided 17% oil. Predominant literature data about fat content of purslane seeds concern Soxhlet extraction by hexane resulting in oil yield from 8–17% [10–15]. A single publication reports oil yield after SC-CO₂ extraction [14] but much lower (7.5%) than the present result (14.8%, Table 1). No information about yield by chloroform-methanol mixtures was found to compare with results in Table 1.

Table 1

Fat content and initial oxidation quality (peroxide value, conjugated dienes and trienes) of purslane seed oil obtained by different extraction methods

Purslane seeds	Hexane	SC-CO ₂	Chloroform-Methanol (2:1)	Chloroform-Methanol (1:2)
Fat content (w/w %)	17.1 ± 1.2 ^a	14.8 ± 0.4 ^b	21.0 ± 1.0 ^c	23.0 ± 1.1 ^c
Peroxide value (meqO ₂ /kg)	0.8 ± 0.2 ^a	0.9 ± 0.2 ^a	0.7 ± 0.2 ^a	0.7 ± 0.1 ^a
conj. Dienes (K ₂₃₂)	2.2 ± 0.1 ^a	2.7 ± 0.2 ^b	2.1 ± 0.1 ^a	2.3 ± 0.1 ^a
conj. Trienes (K ₂₇₀)	0.60 ± 0.01 ^a	0.64 ± 0.01 ^b	0.59 ± 0.01 ^a	0.61 ± 0.01 ^a

Values are mean of three determinations ± standard deviation. Different letters within each row indicate statistically significant difference (p < 0.05).

Freshly extracted oil samples were subjected to evaluation of their quality concerning the oxidative stability expressed by the peroxide value (PV) and amount of conjugated dienes and trienes (presented by K₂₃₂ and K₂₇₀) as measures of initial products of lipid oxidation (Table 1). The results revealed that peroxide value was not affected by the extraction method and was within the limits of freshly produced seed oil. Very similar values (PV of 0.91–0.98 meqO₂/kg) were published by other authors for hexane- and SC-CO₂ purslane seed extractions [14, 16]. As for conjugated dienes and trienes, the results in Table 1 showed slightly higher values for K₂₃₂ and K₂₇₀ of oil extracted by SC-CO₂ compared to the procedures with organic solvents. Data in literature about that is controversial: K₂₃₂ and K₂₇₀ of hexane- and SC-CO₂ extracted oils has got practically the same but at levels of 0.2 [14] or 2.1–2.5 [16] for K₂₃₂ and of 0.03–0.04 [14] vs. 0.6–0.7 [16] for K₂₇₀, the latter values [16] being very similar to present. In addition to PV and conjugated dienes and trienes, oxidative stability index (OSI) was estimated as a measure of secondary and subsequent oxidation products, and OSI was determined at four temperatures for hexane extracted oil (Table 2). As could be seen, the oxidative stability gradually decreased with increasing of temperature, and the OSI values were common for highly unsaturated oil. Only two papers were found in literature with OSI data for purslane oil [15, 16]. In the first work [15] two extraction methods were applied: by hexane at room temperature for 36 h, and mechanical pressing with and without preliminary microwave irradiation of the seeds, resulting in OSI values (110°C, 20 L/h air flow), respectively, of 5.12h, 9.7h and 4.6h. The second work [16] used SC-CO₂ extraction (at 50°C, pressure of 35 MPa, 210 min dynamic time), and hexane extraction at room temperature for 36 h. The OSI determined at 110°C and 20 L/h air flow were, respectively, 12.5h and 11.5h. The differences in oxidative stability could be explained by the effect of antioxidants which amount depended on the extraction method. On the other hand, fatty acids composition of oil had a major effect on oxidative stability as well. For that reasons comparison of OSI values of oils produced at different conditions with

initially different fatty acids composition and antioxidants is only relative and if could be concluded, the results in Table 2 are generally similar to data presented by Delfan-Hosseini et al. [15].

Table 2
Oxidative stability index at different temperatures of purslane seed oil obtained by hexane extraction

Purslane seed oil	80°C	100°C	110°C	120°C
Oxidative Stability Index (h)	42.7 ± 0.3 ^a	8.3 ± 0.3 ^b	4.1 ± 0.2 ^c	2.3 ± 0.1 ^d

Values are mean of three determinations ± standard deviation. Different letters within each row indicate statistically significant difference ($p < 0.05$).

3.2 Lipid classes

Only one publication has been found in literature to demonstrate some separation of lipid groups during fractionation of oil from purslane leaves and seeds to neutral lipids, glycolipids and phospholipids [17]. To the best of our knowledge the present work reports for the first time detailed results about the individual neutral lipid classes and their amounts, namely: monoacylglycerols (MAG), diacylglycerols (DAG), free fatty acids (FFA), free sterols (St), some unidentified compounds with slightly lower polarity than that of sterols, triacylglycerols (TAG), sterol esters (StE), hydrocarbons and wax esters (Fig. 1). As in typical seed oil TAGs were the main component (> 90%) of the oil whereas the other classes were far less (Table 3). Traces of polar lipids were observed faintly on plate start but impossible for quantitation. No effect of extraction procedure on lipid classes proportions was observed.

Table 3
Lipid classes in purslane seed oil obtained by different extraction methods

Lipid class	Hexane	SC-CO ₂	Chloroform-methanol (2:1)	Chloroform-methanol (1:2)
MAG	0.2 ± 0.05	0.2 ± 0.05	0.3 ± 0.05	0.3 ± 0.05
FFA	0.3 ± 0.05	0.3 ± 0.05	0.2 ± 0.05	0.2 ± 0.05
DAG	3.1 ± 0.3	2.8 ± 0.3	3.4 ± 0.4	3.0 ± 0.2
St	0.8 ± 0.2	1.2 ± 0.3	1.2 ± 0.2	0.9 ± 0.2
X	1.0 ± 0.2	1.1 ± 0.1	0.8 ± 0.2	1.1 ± 0.2
TAG	90.6 ± 0.8	90.3 ± 0.7	90.1 ± 0.8	90.2 ± 0.5
StE	2.4 ± 0.3	2.5 ± 0.3	2.8 ± 0.2	2.9 ± 0.3
Waxes	1.6 ± 0.3	1.6 ± 0.3	1.2 ± 0.2	1.4 ± 0.2

Values are mean of three determinations ± standard deviation. No statistically significant difference ($p < 0.05$) was found within each row. Abbreviations of lipid classes: MAG – monoacylglycerols, FFA – free

fatty acids, DAG – diacylglycerols, St – free sterols, X – zone with unidentified compounds, TAG – triacylglycerols, StE – sterol esters, Waxes - hydrocarbons and wax esters.

3.3 Fatty acids composition of lipid classes

Initially, the total fatty acids composition of purslane oil was determined and revealed quite high amount of the essential alpha-linolenic (18:3 n -3) and linoleic (18:2 n -6) fatty acids, respectively almost 40% and 33% (Table 4). Except for palmitic (16:0), oleic (9–18:1) and stearic (18:0) fatty acids about 12%, 10% and 4% respectively, the other fatty acids were near to or below 1%. Having in mind the similar amounts of linolenic and lin-oleic acids in purslane oil it could be expected that in some cases their proportion would be opposite, i.e. linoleic acid to be more than linolenic acid. Indeed half of the publications report predomination of linolenic acid [16–22] in contrast to the others [11–15, 23, 24]. In fact purslane oil with predominant alpha-linolenic acid possesses better bioactive potential and is preferable as healthier phytonutrient.

No effect of extraction method on the total fatty acids composition was detected.

Table 4
Total fatty acids composition of purslane seed oil obtained by different extraction methods

Fatty acid, rel. %	Hexane	SC-CO ₂	Chloroform-methanol (2:1)	Chloroform-methanol (1:2)
14:0	0.1 ± 0.05	0.1 ± 0.05	0.1 ± 0.05	0.1 ± 0.05
16:0	11.7 ± 0.3	12.3 ± 0.6	12.1 ± 0.2	11.8 ± 0.3
16:1	0.1 ± 0.05	0.1 ± 0.05	0.1 ± 0.05	0.1 ± 0.05
18:0	3.6 ± 0.2	3.7 ± 0.2	3.8 ± 0.2	3.7 ± 0.1
9–18:1	9.9 ± 0.3	9.7 ± 0.2	9.5 ± 0.2	9.5 ± 0.1
11–18:1	1.1 ± 0.1	1.0 ± 0.1	1.1 ± 0.2	1.1 ± 0.1
18:2	33.2 ± 0.1	32.7 ± 0.4	32.8 ± 0.4	32.9 ± 0.3
18:3	38.2 ± 0.2	38.5 ± 0.3	38.1 ± 0.3	38.6 ± 0.4
20:0	0.9 ± 0.1	0.9 ± 0.2	0.9 ± 0.1	0.9 ± 0.1
20:1	0.1 ± 0.05	0.1 ± 0.05	0.2 ± 0.05	0.2 ± 0.05
22:0	0.6 ± 0.1	0.5 ± 0.1	0.7 ± 0.1	0.6 ± 0.1
22:1	0.1 ± 0.05	0.1 ± 0.05	0.2 ± 0.05	0.1 ± 0.05
24:0	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1

Values are mean of three determinations ± standard deviation. No statistically significant difference ($p < 0.05$) was found within each row.

After estimation of total fatty acids composition, the fatty acids composition of each lipid class was determined. Results for the main four acids (alpha-linolenic, linoleic, palmitic and oleic acids) are presented on Fig. 2, and for the others twelve (12:0, 14:0, 16:1, 18:0, 11–18:1, 20:0, 20:1, 22:0, 22:1, 24:0, 24:1 and 26:0) on Figure S2. It can be seen that distribution of fatty acids among classes is different and specific but does not depend on the extraction method. Thus, alpha-linolenic acid (Fig. 2a) predominates in triacylglycerols and the next X zone with amounts above 36%. The other lipid classes are relatively rich in it, too (> 23%), excepting wax esters (6.5%). Linoleic acid (Fig. 2b) is at similar levels (> 24%), excluding wax esters (9.3%), reaching almost 42% and 48% in diacylglycerols and sterol esters, respectively. Palmitic acid (Fig. 2c) has the opposite distribution with the highest amount in wax esters (42%) and levels in the range 11–22% in the other lipid classes. Oleic acid (Fig. 2d) gradually increases from 6–

12% with increasing of lipid class polarity. It should be noted that seven of the eight saturated fatty acids measured in purslane oil, namely 12:0, 14:0, 16:0, 18:0, 22:0, 24:0 and 26:0 are concentrated in wax esters in amounts quite higher than in the other lipid classes, at levels of 42% for 16:0 (Fig. 2c), 15% for 18:0 (Fig. 2S a), in the range of 2–7% for 22:0 (Fig. 2S b), 24:0 (Fig. 2S c), 26:0 (Fig. 2S d) and 14:0 (Fig. 2S f), and 0.7% 12:0 (Fig. 2S e). Similar predomination in wax esters can be seen also of 20:1 (Fig. 2S j). On the other hand, 20:0 (Fig. 2S i) and 11–18:1 (Fig. 2S h) do not predominate in a single lipid class. The other fatty acids 16:1 (Fig. 2S g), 22:1 (Fig. 2S k) and 24:1 (Fig. 2S l) have specific distribution as well despite their minor to traces amounts. Figure S3 contains diagrams with the same results for fatty acids composition of individual lipid classes but in different presentation to facilitate the comparison between lipid classes regarding their fatty acids composition. Thus, it is obvious that total fatty acids composition of the oil (Fig. 3S a) is almost equal to that of triacylglycerols (Fig. 3S b). This result is expected and confirms the content above 90% of triacylglycerols (Table 3). Monoacylglycerols (Fig. 3S c) and free fatty acids (Fig. 3S d) have similar fatty acids composition but different from that of diacylglycerols (Fig. 3S e), unidentified lipid zone (Fig. 3S f) and sterol esters (Fig. 3S g). Wax esters (Fig. 3S h) differ the most from the other lipid classes in the high content of saturated fatty acids as discussed above.

To the best of our knowledge such detailed fatty acids composition of individual lipid classes in purslane oil is published for the first time. This information could be necessary and useful as base for further biochemical and physiological investigations and conclusions, and, on the other hand, for practical purposes in production of food supplements, pharmaceutical and cosmetic goods.

3.4 Sterols composition

Sterols in both fractions of free and esterified sterols in purslane oil were determined. In the first fraction nine sterols were detected (Fig. 3) and sitosterol was much more (about 77%) than the others (below 10%). In the fraction of sterol esters thirteen sterols were measured (Fig. 4). However, in contrast to the free sterols, sterol esters contained far less sitosterol (about 48%) but much more campesterol and delta-5 avenasterol. In the single publication yet in literature with results about sterol composition of purslane oil (15 identified sterols but without data about sterol esters) beta-sitosterol predominated (53%) followed by campesterol (17%) and delta-5-avenasterol (11%). The other sterols were below 3% [12].

Similarly to the fatty acids composition, the present results revealed no effect of extraction method on sterol composition of purslane oil concerning both free sterols and sterol esters.

3.5 Waxes

The most unpolar compounds in purslane seed oil were separated and isolated as lipid zone Waxes (Fig. 1, Table 3) containing mainly hydrocarbons and minor amounts of wax esters. Concerning the former, twenty-five hydrocarbons with chain length from 14 to 37, as well as squalene, were identified and quantified (Fig. 5). Among them only two (C33 and C31) and squalene were more than 10%. As for

wax esters, traces peaks were detected but with ambiguous identification. For that reason they are not discussed here and require additional investigation.

No data from other authors have been found in literature for comparison. As with the other lipid classes no effect of extraction method on hydrocarbons was observed.

4 Conclusions

Detailed analysis of purslane seed oil regarding its lipid composition including separation and quantitation of seven individual lipid classes (monoacylglycerols, diacylglycerols, free fatty acids, free sterols, triacylglycerols, sterol esters, wax esters + hydrocarbons) and determination of fatty acids composition of saponifiables was done for the first time, in order to study the effect of extraction method on lipids and oxidative stability of purslane seed oil extracted by three procedures with organic solvents (hexane, chloroform-methanol mixtures) vs. extraction with super-critical CO₂. The results revealed no significant effect of extraction method on the individual lipid classes and their fatty acids composition, and quite weak effect on the oil oxidative stability regarding super-critical CO₂ vs. organic solvents. Analyses of lipid composition confirmed the potential of purslane seed oil as a cheap and highly valuable source of phytonutrients as essential ω -3 fatty acids and phytosterols, for application in food, pharmaceutical and cosmetic industries.

Declarations

Acknowledgments The support by the Operational Program “Science and Education for Smart Growth”, co-financed by the European Union through the European Structural and Investment Funds, grant BG05M2OP001-1.002-0012 Center of Competence “Sustainable utilization of bio-resources and waste of medicinal and aromatic plants for innovative bioactive products”, is gratefully acknowledged.

Funding This research was funded by the Operational Program “Science and Education for Smart Growth”, co-financed by the European Union through the European Structural and Investment Funds, grant BG05M2OP001-1.002-0012 Center of Competence “Sustainable utilization of bio-resources and waste of medicinal and aromatic plants for innovative bioactive products”.

Conflicts of interest The authors declare no conflicts of interest.

Data availability All data supporting the findings of this study are available within the paper. Any raw data files are available from the corresponding author upon reasonable request.

Code availability Not applicable.

Authors' contributions Sabina Taneva: Investigation, Chemical Analyses, Acquisition of Data, Data Analysis and Interpretation; Svetlana Momchilova: Methodology, Data Analysis and Interpretation, Drafting of article and Editing.

Ethics approval Experiments with the plant material adhere to international/national guidelines.

Consent for publication Not applicable.

References

1. Srivastava R, Srivastava, V. Multipurpose Benefits of an Underexplored Species Purslane (*Portulaca oleracea* L.): A Critical Review. *Environm Manag* 2021; <https://doi.org/10.1007/s00267-021-01456-z>
2. Montoya-García CO, García-Mateos R. Bioactive compounds of purslane (*Portulaca oleracea* L.) according to the production system: A review. *Sci Hortic* 2023; <https://doi.org/10.1016/j.scienta.2022.111584>
3. Uddin K, Juraimi A. Purslane Weed (*Portulaca oleracea*): A Prospective Plant Source of Nutrition, Omega-3 Fatty Acid, and Antioxidant Attributes. *Sci World J* 2014; <https://doi.org/10.1155/2014/951019>
4. Arshad Z, Rezapour-Firouzi S. The Sources of Essential Fatty Acids for Allergic and Cancer Patients; a Connection with Insight into Mammalian Target of Rapamycin: A Narrative Review. *Asian Pac J Cancer Prev* 2018; <https://doi.org/10.22034/APJCP.2018.19.9.2391>
5. Li K, Xia T. A review on ethnopharmacology, phytochemistry, pharmacology and potential uses of *Portulaca oleracea* L. *J Ethnopharm* 2024; <https://doi.org/10.1016/j.jep.2023.117211>
6. Saad B, Kmail A, Haq SZH. Anti-Diabetes Middle Eastern Medicinal Plants and Their Action Mechanisms. *Evid-Based Complem Altern Med* 2022; <https://doi.org/10.1155/2022/2276094>
7. Naeem F, Khan S. Purslane (*Portulaca oleracea* L.) as Phytogenic Substance - A Review. *J Herbs Spices Med Plants* 2013; <https://doi.org/10.1080/10496475.2013.782381>
8. Petropoulos S, Karkanis A. Phytochemical composition and bioactive compounds of common purslane (*Portulaca oleracea* L.) as affected by crop management practices. *Trends Food Sci Technol* 2016; <https://doi.org/10.1016/j.tifs.2016.06.010>
9. Zhou Y-X, Xin H-L, Rahman K, Wang S-J, Peng C, Zhang H. *Portulaca oleracea* L.: A Review of Phytochemistry and Pharmacological Effects. *BioMed Res Intern* 2015; <https://doi.org/10.1155/2015/925631>
10. Desta M, Molla A, Yusuf Z. Characterization of physico-chemical properties and antioxidant activity of oil from seed, leaf and stem of purslane (*Portulaca oleracea* L.). *Biotech Rep* 2020; <https://doi.org/10.1016/j.btre.2020.e00512>
11. Gunenc A, Rowland O, Xu H, Marangoni A, Hosseinian F. *Portulaca oleracea* seeds as a novel source of alkylresorcinols and its phenolic profiles during germination. *LWT-Food Sci Technol* 2019; <https://doi.org/10.1016/j.lwt.2018.10.075>
12. Matthäus B, Babiker EE, Özcan MM, Al-Juhaimi FY, Ahmed IAM, Ghafoor K. Changes in Fatty Acid, Tocopherol and Sterol Contents of Oils Extracted from Several Vegetable Seeds. *J Oleo Sci* 2021;

<http://doi.org/10.5650/jos.ess21225>

13. Srivastava M, Banerji R, Rawat AKS, Mehrotra S. Fatty Acid Composition of Some Medicinally Useful Seeds. *J Herb Pharmacother* 2006; http://doi.org/10.1300/J157v06n01_04
14. Zadeh JH, Özdikicierler O, Pazi F. A Comparative Study on Response Surface Optimization of Supercritical Fluid Extraction Parameters to Obtain *Portulaca Oleracea* Seed Oil with Higher Bioactive Content and Antioxidant Activity Than Solvent Extraction. *Eur J Lipid Sci Technol* 2022; <http://doi.org/10.1002/ejlt.202200136>
15. Delfan-Hosseini S, Nayebzadeh K, Mirmoghtadaie L, Kavosi M, Hosseini SM. Effect of extraction process on composition, oxidative stability and rheological properties of purslane seed oil. *Food Chem* 2017; <http://dx.doi.org/10.1016/j.foodchem.2016.11.150>
16. Sodeifian G, Ardestani NS, Sajadian SA, Moghadamian K. Properties of *Portulaca oleracea* seed oil via supercritical fluid extraction: Experimental and optimization. *J Supercr Fl* 2018; <https://doi.org/10.1016/j.supflu.2017.12.026>
17. Venkateshwari V, Vijayakumar A, Vijayakumar AK, Prasanna Anjaneya Reddy L, Srinivasan M, Rajasekharan R. Leaf lipidome and transcriptome profiling of *Portulaca oleracea*: characterization of lysophosphatidylcholine acyltransferase. *Planta* 2018; <https://doi.org/10.1007/s00425-018-2908-8>
18. Abozed SS, Elaraby GM, Zahran HA. Application of Spray-dried Microcapsules of Purslane (*Portulaca oleracea* L.) Seed Oil Enhances Quality of Mango Juice. *Open Agric J* 2021; <https://doi.org/10.2174/1874331502115010001>
19. El-Sayed M-I K. Effects of *Portulaca oleracea* L. seeds in treatment of type-2 diabetes mellitus patients as adjunctive and alternative therapy. *J Ethnopharmacol* 2011; <https://doi.org/10.1016/j.jep.2011.06.020>
20. Liu L, Howe P, Zhou Y-F, Xu Z-Q, Hocart C, Zhang R. Fatty acids and beta-carotene in Australian purslane (*Portulaca oleracea*) varieties. *J Chromatogr A* 2000; [https://doi.org/10.1016/S0021-9673\(00\)00747-0](https://doi.org/10.1016/S0021-9673(00)00747-0)
21. Petropoulos SA, Fernandes A, Arampatzis DA, Tsiropoulos NG, Petrović J, Soković M, Barros L, Ferreira ICFR. Seed oil and seed oil byproducts of common purslane (*Portulaca oleracea* L.): A new insight to plant-based sources rich in omega-3 fatty acids. *LWT-Food Sci Technol* 2020; <https://doi.org/10.1016/j.lwt.2020.109099>
22. Petropoulos SA, Fernandes A, Calhelha RC, Rouphael Y, Petrović J, Soković M, Ferreira ICFR, Barros L. Antimicrobial Properties, Cytotoxic Effects, and Fatty Acids Composition of Vegetable Oils from Purslane, Linseed, Luffa, and Pumpkin Seeds. *Appl Sci* 2021; <https://doi.org/10.3390/app11125738>
23. Mousavi SRJ, Niazmand R. Fatty Acids Composition and Oxidation Kinetic Parameters of Purslane (*Portulaca oleracea*) Seed Oil. *Agric Res* 2017; <https://doi.org/10.1007/s40003-017-0271-9>
24. Stroescu M, Stoica-Guzun A, Ghergu S, Chira N, Jipa I. Optimization of fatty acids extraction from *Portulaca oleracea* seed using response surface methodology. *Ind Crops Prod* 2013; <https://doi.org/10.1016/j.indcrop.2012.07.051>
25. ISO 659:2009, Oilseeds – Determination of oil content (Reference method), p. 12 (2009).

26. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 1957; [https://doi.org/10.1016/S0021-9258\(18\)64849-5](https://doi.org/10.1016/S0021-9258(18)64849-5)
27. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959; <https://doi.org/10.1139/o59-099>
28. Boyadzhieva S, Coelho JAP, Errico M, Reynel-Avilla HE, Yankov DS, Bonilla-Petriciolet A, Stateva RP. Assessment of *Gnaphalium viscosum* (Kunth) valorization prospects: Sustainable recovery of antioxidants by different tech-niques. Antioxidants 2022; <https://doi.org/10.3390/antiox11122495>
29. Yanishlieva N, Popov A, Marinova E. Eine Modifizierte Jodometrische Methode zur Bestimmung der Peroxidzahl in kleinen Lipidproben. Copt Rend Acad Bulg Sci 1978;31:869-871.
30. AOCS Official method Ch 5-91. Specific Extinction of Oils and Fats, Ultraviolet Absorption. In: Official methods and rec-ommended practices of the AOCS. Urbana, IL: AOCS Press.
31. Christie WW. Lipid analysis: isolation, separation, identification, and structural analysis of lipids. 3rd ed. The Oily Press: Bridgwater, England, 2003; pp. 205-224.

Figures

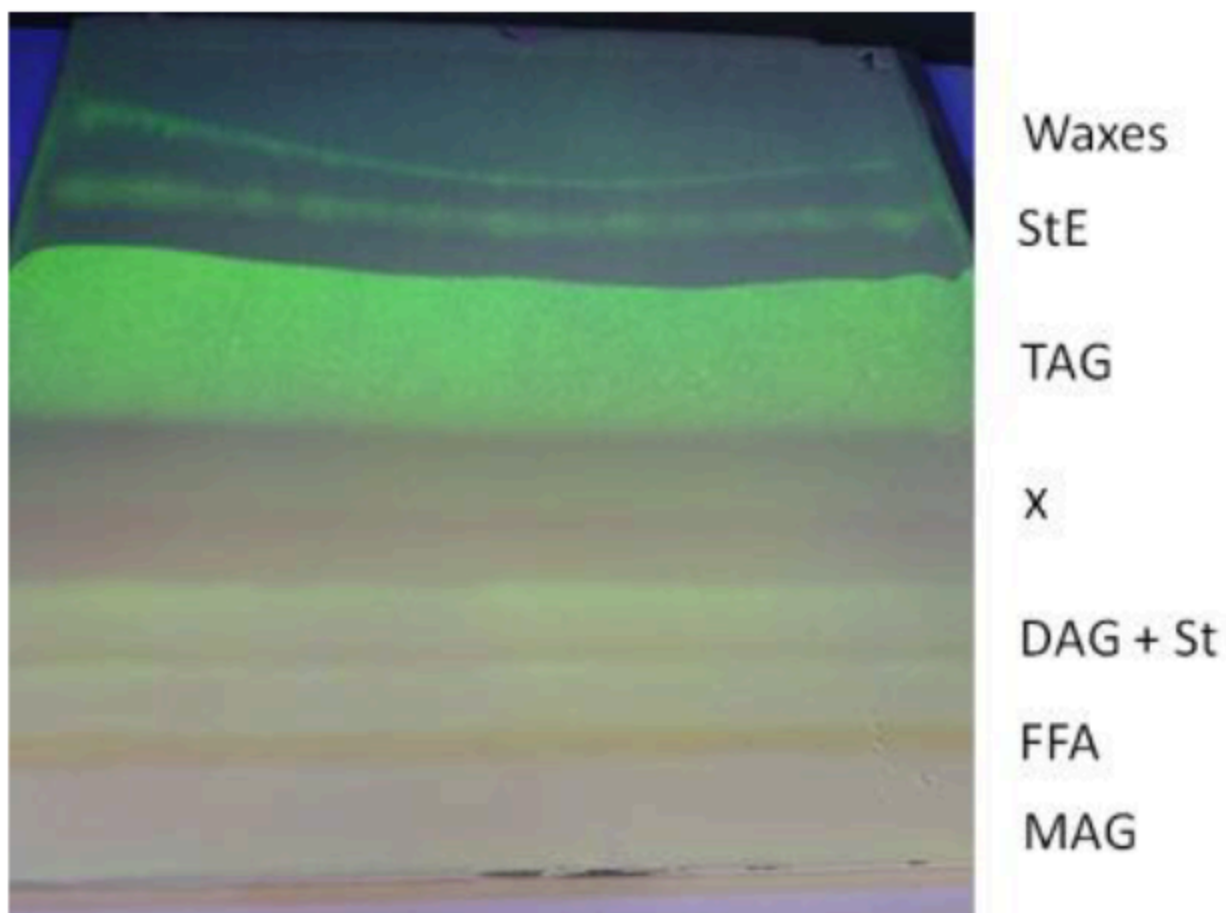


Figure 1

Lipid classes in purslane seed oil separated on preparative silica gel TLC plate by mobile phase of hexane-acetone (100:8, v/v); UV visualization at 366 nm after spraying with 2,7-dichlorofluoresceine ethanolic solution. Abbreviations: MAG – monoacylglycerols, FFA – free fatty acids, DAG – diacylglycerols, St – free sterols, X – zone with unidentified compounds, TAG – triacylglycerols, StE – sterol esters, Waxes - hydrocarbons and wax esters.

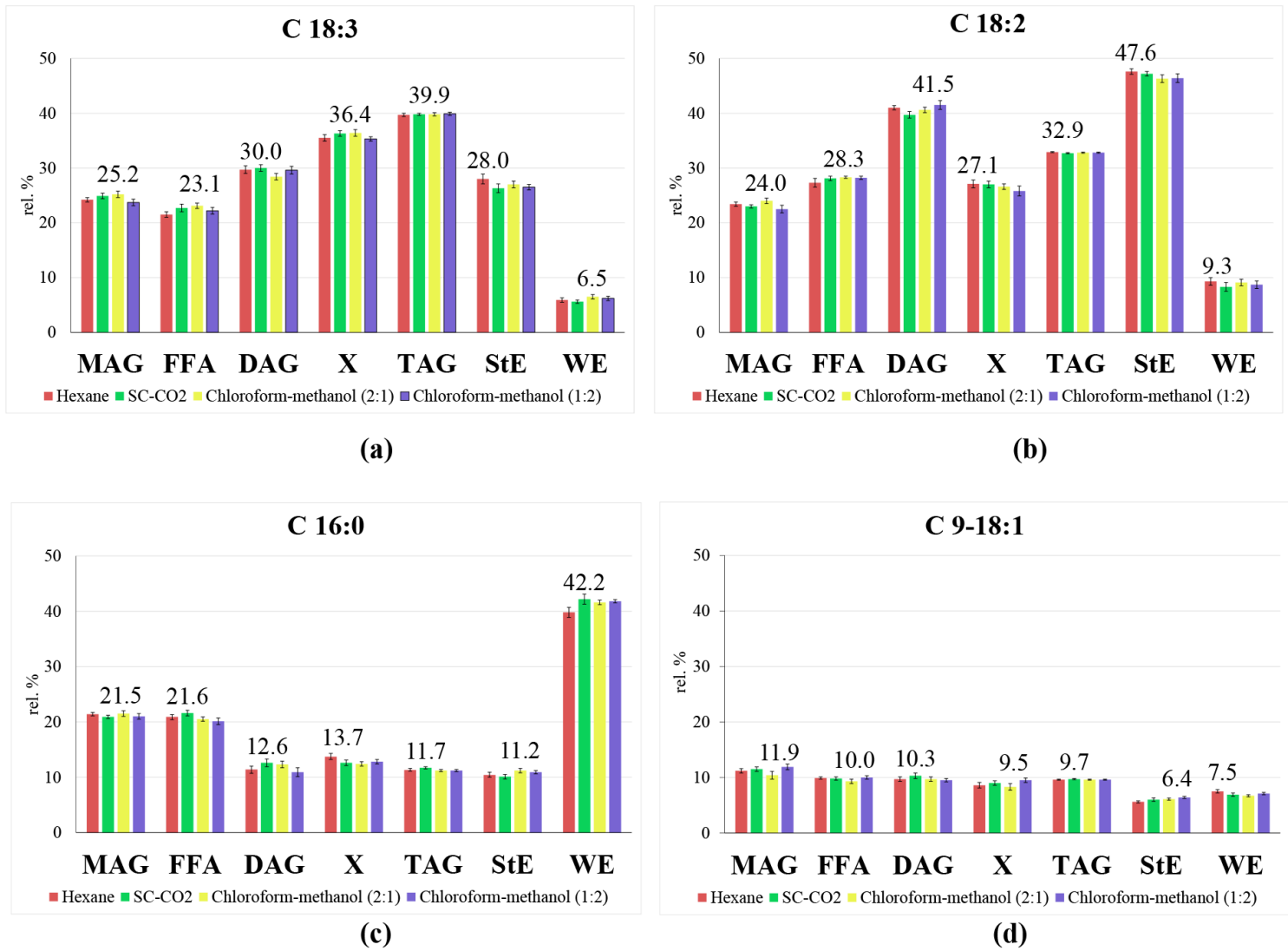


Figure 2

Content of the main fatty acids in individual lipid classes in purslane seed oil obtained by different extraction methods: (a) Linolenic acid (9,12,15-18:3); (b) Linoleic acid (9,12-18:2); (c) Palmitic acid (16:0); (d) Oleic acid (9-18:1). Content of the other fatty acids is given in Figure S2 (a-l). Abbreviations: MAG – monoacylglycerols, FFA – free fatty acids, DAG – diacylglycerols, X – minor zone with unidentified compounds, TAG – triacylglycerols, StE – sterol esters, WE - wax esters.

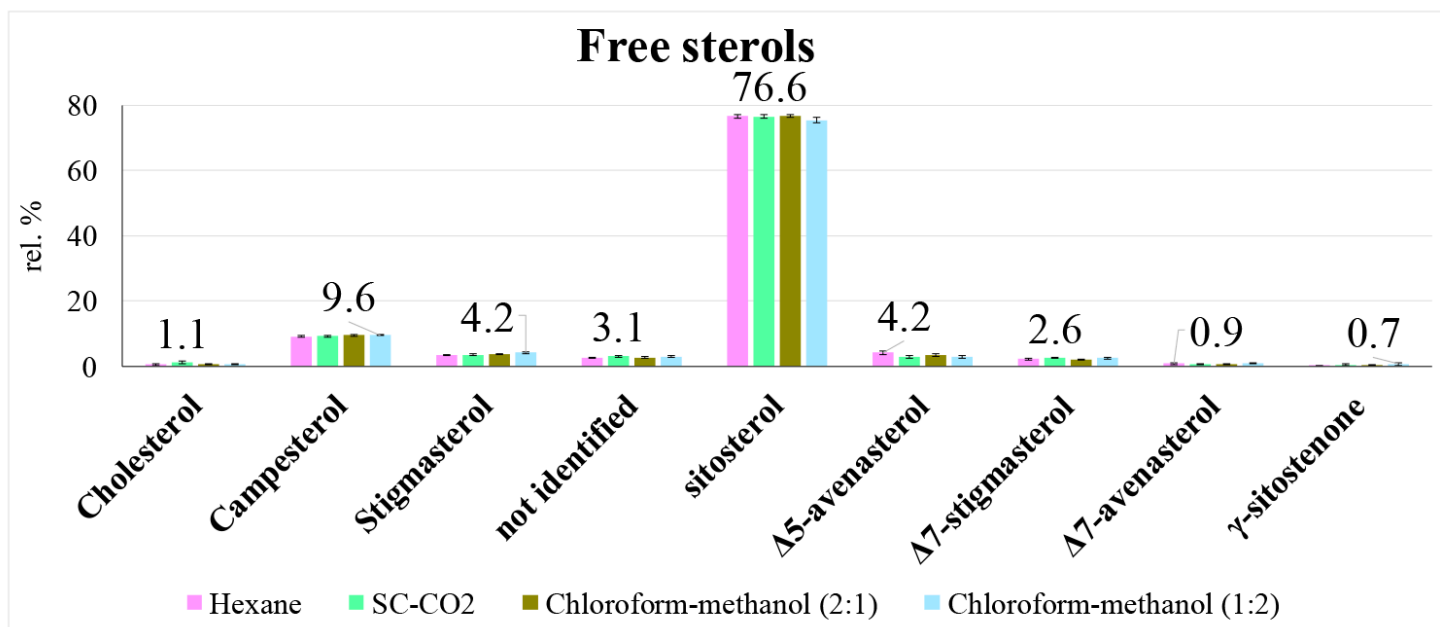


Figure 3

Free sterols in purslane seed oil obtained by different extraction methods

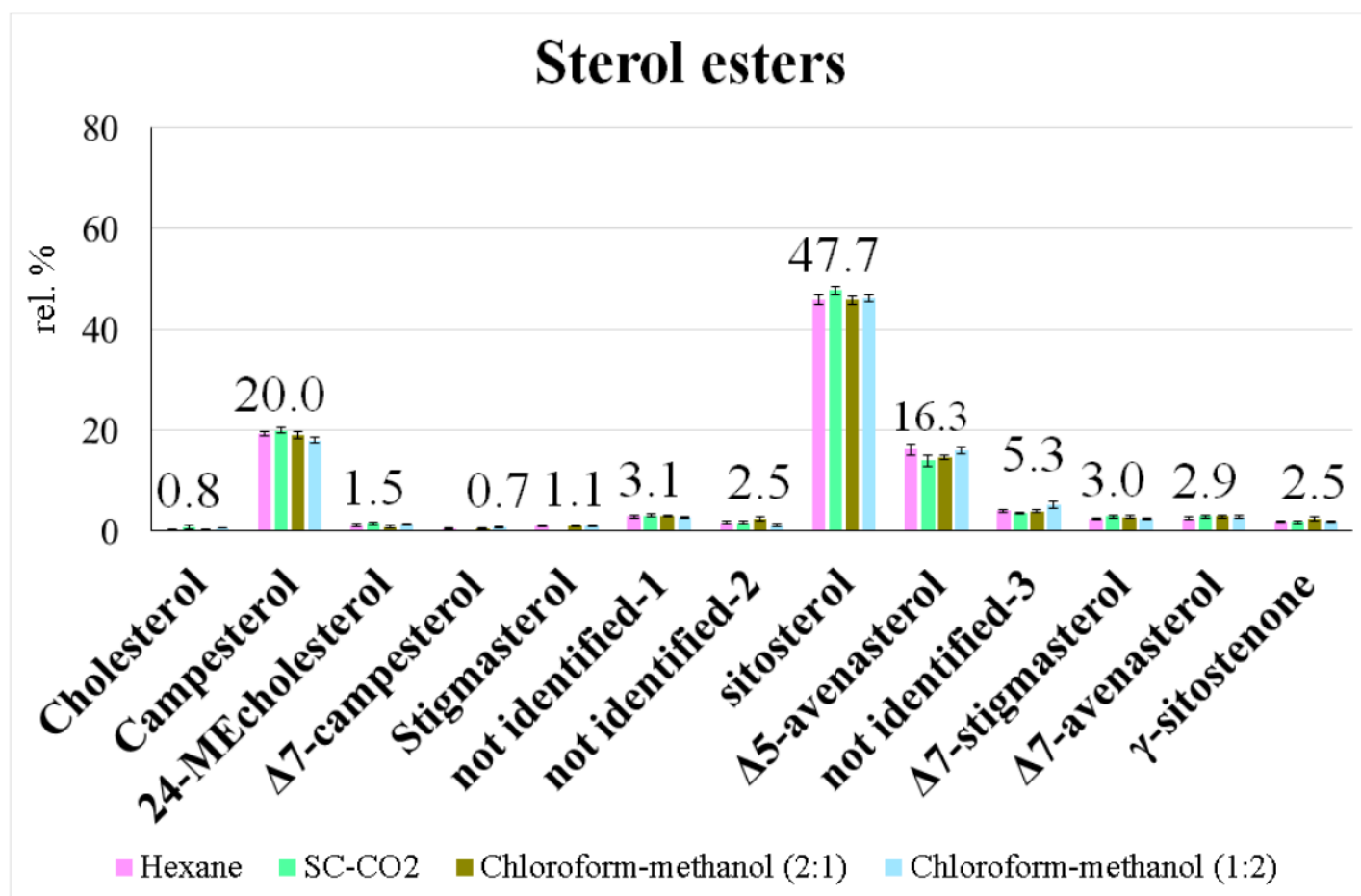


Figure 4

Sterols of sterol esters in purslane seed oil obtained by different extraction methods

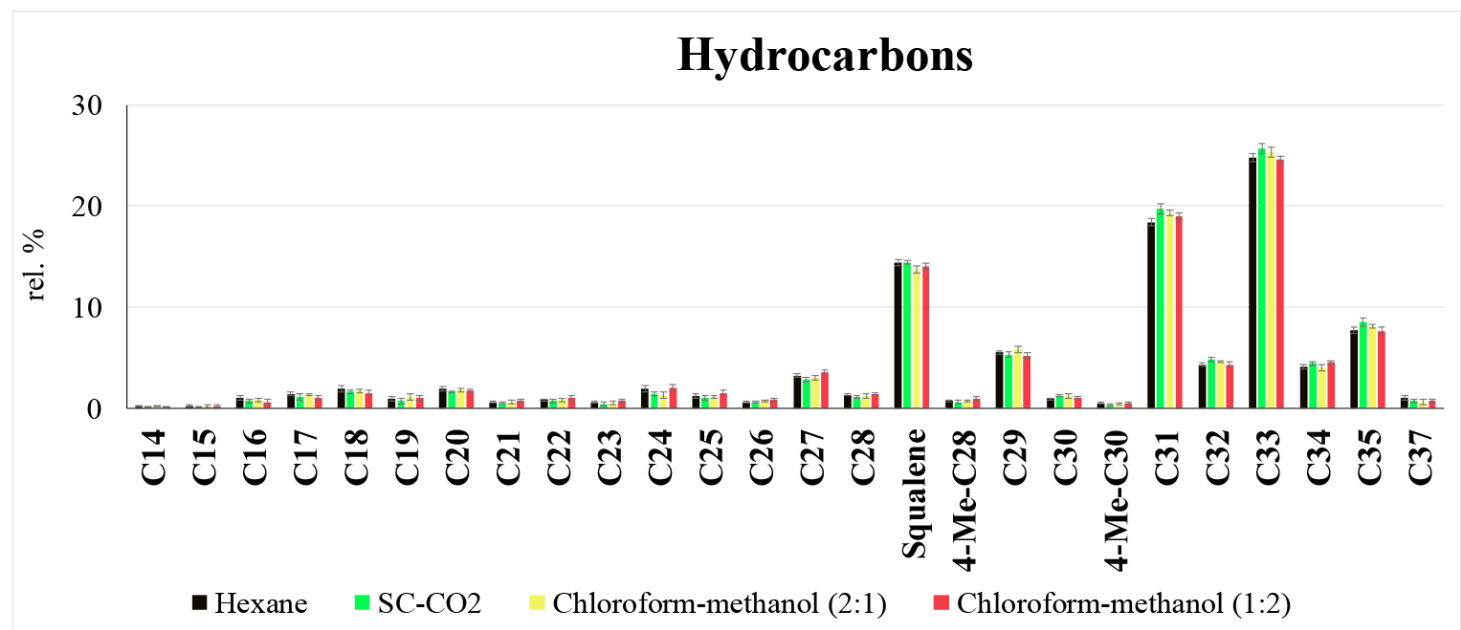


Figure 5

Hydrocarbons in purslane seed oil obtained by different extraction methods

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