Original Article
DOI: 10.4274/tjps.galenos.2021.84484

Assessment of Commercially Safflower Oils (Carthami Oleum Raffinatum) In Terms of European Pharmacopoeia Criteria and Their Weight Control Potentials

Ticari Olarak Satılan Aspir Yağlarının (Carthami Oleum Raffinatum) Avrupa Farmakopesi Kriterleri ve Kilo Kontrol Potansiyelleri Açısından Değerlendirilmesi

Didem Deliorman Orhan[1], Sultan Pekacar[1], Onur Kenan Ulutaş[2], Burçin Özüpek[1], Demet Sümmeoğlu[1], aysel Berkkan[3]
[1]Department Of Pharmacognosy, Faculty Of Pharmacy, Gazi University, Ankara, Turkey
[2]Department Of Toxicology, Faculty Of Pharmacy, Gazi University, Ankara, Turkey
[3]Department Of Analytical Chemistry, Faculty Of Pharmacy, Gazi University, Ankara, Turkey

Corresponding Author Information
Prof. Dr. Didem Deliorman Orhan
didemdeliorman@gmail.com
05337459668
https://orcid.org/0000-0003-3916-4048
04.07.2021
24.08.2021
24.08.2021

ABSTRACT

INTRODUCTION: Safflower oils, which are sold commercially, are in demand with food, cosmetics and health-promoting claims. In this study, safflower oil samples belonging to 11 different brands were evaluated in terms of European Pharmacopoeia criteria 7.0. In addition, in vitro weight control potential of all the samples was investigated.
METHODS: Samples to be analyzed were purchased from pharmacies, herbal, online and cosmetics stores. Acid and peroxide values of 11 safflower samples and analysis of their fatty acids by Gas Chromatography–Mass Spectrometry were carried out according to the "Carthami oleum raffinatum" monograph registered in the European Pharmacopeia 7.0. In order to test the effects of all samples on weight control, their inhibitory effects on carbohydrate digesting enzymes (α-glucosidase and α-amylase) were evaluated by spectrophotometric methods.
RESULTS: Out of eleven oil samples, only two of them have acid and peroxide values below the reference value. According to the gas chromatography analysis safflower oils samples are rich in monounsaturated fatty acids (oleic acid) and polyunsaturated fatty acids (linoleic acid) (67.10-99.53%) of the total fatty acids in its content are oleic, linoleic, palmitic and stearic acids. Saturated fatty acids are 0.58 to 12.18% of total FAMEs in oils. When evaluated in terms of inhibition of α-amylase and α-glucosidase enzymes that hydrolyze carbohydrates, the results showed that safflower oil samples had no activity on these enzymes.

DISCUSSION AND CONCLUSION: It has been determined that many safflower oil samples on the market do not meet the quality criteria recommended in the European Pharmacopoeia 7.0 in this report. It was observed that safflower oil did not show any inhibitory effect on these two enzymes, which is considered a rational approach for weight control.

Keywords: Fatty acids, Gas Chromatography-Mass Spectrometry, Quality control, Safflower oil
İngilizce Kısa Başlık: Assessment of Market Safflower Oils

ÖZ

GİRİŞ ve AMAÇ: Ticari olarak satılan aspir yağları gıda, kozmetik ve sağlığı geliştirici iddiaları ile rağbet görmektedir. Bu çalışmada 11 farklı markaya ait aspir yağı örnekleri Avrupa Farmakopesi kriterleri 7.0 açısından değerlendirilmiştir. Ayrıca tüm örneklerin in vitro kilo kontrol potansiyeli araştırılmıştır.
YÖNTEM ve GEREÇLER: Analiz edilecek numuneler eczanelerden, bitkisel, çevrimiçi ve kozmetik mağazalarından satın alınmıştır. 11 aspir örneğinin asit ve peroksit değerleri ve yağ asitlerinin Gaz Kromatografi-Kütle Spektrometrisi ile analizleri Avrupa Farmakopesi 7.0'da kayıtlı "Carthami oleum raffinatum" monografına göre yapılmıştır. Tüm örneklerin ağırlık kontrolü üzerindeki etkilerini test etmek için karbonhidrat sindirici enzimler (α-glukosidaz ve α-amilaz) üzerindeki inhibitör etkileri spektrofotometrik yöntemlerle değerlendirilmiştir.
BULGULAR: 11 yağ örneğinden 2 tanesi referans değerin altında asit ve peroksit değerlerine sahiptir. Gaz kromatografisi analizine göre aspir yağı örnekleri tekli doymamış yağ asitleri (oleik asit) ve çoklu doymamış yağ asitleri (linoleik asit) bakımından zengindir (%67.10-99.53), içeriğindeki toplam yağ asitleri oleik, linoleik, palmitik ve stearik asitlerdir. Doymuş yağ asitleri, yağlardaki toplam yağ asitlerinin %0.58 ila %12.18'idir. Karbonhidratları hidrolize eden α-amilaz ve α-glukosidaz enzimlerinin inhibisyonu açısından değerlendirildiğinde, sonuçlar aspir yağı örneklerinin bu enzimler üzerinde aktivite göstermediği yönündedir.
TARTIŞMA ve SONUÇ: Bu çalışmada piyasadaki birçok aspir yağı örneğinin Avrupa Farmakopesi 7.0'da önerilen kalite kriterlerini karşılamadığı tespit edilmiş, kilo kontrolünde akılcı bir yaklaşım olarak kabul edilen bu iki enzim üzerinde aspir yağının herhangi bir inhibitör etki göstermediği gözlemlenmiştir.

Anahtar Kelimeler: Yağ asitleri, Gaz Kromatografisi Kütle Spektrometrisi, Kalite kontrol, Aspir yağı
Türkçe Kısa Başlık: Piyasa Aspir Yağlarının Değerlendirilmesi

INTRODUCTION

Due to the oil, protein, carbohydrate, mineral and vitamins it contains, some plant seeds have a very important place in human and animal nutrition and biodiesel production. Most of the oils needed in human nutrition are met from vegetable oils. 92% of the world's oil production is obtained from vegetable sources and 8% from animal sources (1). Seeds are important sources of vegetable oil. The fatty acid content and biological activity of seeds are factors that determine the use of a vegetable oil in nutrition, pharmaceutical or industrial areas (1,2). Vegetable oil production in the world is mainly met with palm oil, soybean, rapeseed, olive, safflower, corn, sunflower, peanut, sesame, castor oil, poppy, flax, hemp and jojoba. Most of the vegetable oil production in Turkey is based mainly on sunflower; sunflowers constitute 69% of vegetable oil production, 32% of total oil consumption. Therefore, safflower, whose production is low, may be a hope to close the vegetable oil gap in the market. The 80% of

world safflower production is carried out by Kazakhstan (24.6%), Russia (19.2%), Mexico (16.1%), India (11.9%), and Turkey (8.2%)(1).

Carthamus tinctorious L. is a bushy, herbaceous plant from the Asteraceae family and is grown in arid or semi-arid regions or where moderately salty water is used. Safflower Oil (Carthami oleum raffinatum) is a fixed oil obtained by squeezing or extracting the seeds of *C. tinctorius* and then refined (3-5).

Except using the oils for dietary purposes, many vegetable oils are sold with health-promoting claims or statements that they are beneficial against diseases while safflower oils are one of them, as they are sold in “natural”, “organic products”, “local products” shops, cosmetics store chains and pharmacies. Safflower oil, which is marketed for health benefits and cosmetics, must meet the European pharmacopoeia criteria. Currently these safflower oils are marketed as fixed oils with the producer’s own marketing and quality criteria, while a pharmacopoeia’s core mission is to protect public health by creating and making available public standards to help ensure the quality of products while the user or procurer can make an independent judgement regarding quality, thus safeguarding the health of the public.

In European Pharmacopoeia 7.0 “Safflower oil, refined; Carthami oleum raffinatum” is registered under the name "Refined safflower oil" is defined as oil obtained from the seeds of *C. tinctorius* (Type I) or hybrid *C. tinctorius* seeds by extraction and/or extraction followed by refining (6). The type II oil is rich in oleic acid and contains antioxidant (7). The fatty acid content is one of the main factor that determine the use of a vegetable oil in nutrition, pharmaceutical or industrial areas (2).

Safflower seeds contain high levels of polyunsaturated fatty acids and is used for dietetic, medical and industrial purposes (8,9). 96-99% of the total fatty acids in its content are oleic, linoleic, palmitic and stearic acids, 9.7-10.8% of saturated fatty acids(2). Fatty acid compositions in safflower seeds usually consist of 71 to 75% linoleic acid (C18:2), 16 to 20% oleic acid (C18:1), 6 to 8% palmitic acid (C16:0), and 2 to 3% stearic acid (C18:0) (2,10-12).

There are many news about the use of safflower oil in weight control in the media and on the internet. Pharmacists also state that especially women often demand safflower oil for this purpose. Literature surveys have shown us that there is no scientific study on the effect of this oil on weight control. Therefore, in this study, it is aimed to test the effects of safflower oil on enzymes that digest carbohydrates, which is a rational approach to weight control. On the other hand, quality control evaluations of 11 safflower oil samples obtained from pharmacies, Akhtar and cosmetics shops were made in terms of the criteria in the relevant monograph in the European Pharmacopoeia 7.0. For this purpose, acid and peroxide values of oil samples by volumetric method and fatty acid analysis were performed using Gas Chromatography-Mass Spectrometry (GC-MS) technique.

MATERIAL AND METHODS

Chemicals and instruments

All chemicals used were analytical reagent grade. Heptane (99%), potassium hydroxide (KOH), methylene chloride (CH_2Cl_2) for Gas Chromatography MS Supra Solv®, sodium chloride (NaCl), sodium sulphate (Na_2SO_4), Supelco 37 component FAME mix (FAME37, C4-24), chloroform ($CHCl_3$), glacial acetic acid, acetone, phosphomolybdic acid, ether, phenolphthalein, 3,5-dinitrosalicylic acid, sodium potassium tartrate were purchased from Sigma Aldrich Co. and Riedel-de Haën (Seelze, Germany). Methanol (MeOH) containing not more than 0.5% (w/w) water, starch, potassium iodide (KI), sodium thiosulfate, and peroxide-free ether were purchased from Merck, Germany. Oksan Co., Ltd. (Ankara, Turkey) provided helium, hydrogen, and dried air gases for Gas Chromatography with 99.99% purity. Deionized water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Gas Chromatography (7890A GC System, Agilent Technologies Inc, US), a capillary column Rt-2560 (100 m, 0.25 mm ID, 0.2 µm) (Restek Corporation Bellefonte, US), vial insert, 250 µL, glass with polymer feet, vial, screw top, 2 mL, amber and cap, screw, blue, PTFE/red silicone septa (Agilent Technologies Inc., US), and Elisa (Versamax Tunable Microplate Reader) were used in GC analysis and enzyme activity studies, respectively.

Safflower oil samples

Eleven different brands of safflower oils were purchased from pharmacies, akhtars, online, and cosmetics stores in Ankara/Turkey in 2019. The samples were stored at 4°C until used in studies.

Acid value (V_A)

About 10 g. of the oil (m) was dissolved in 50 ml of 96% methanol and ether mixture (1:1, v/v). 0.1 M KOH was used as a titrant in the presence of phenolphthalein indicator until the pink color remained stable for at least 15 s (n ml of 0.1 M KOH). Acid values of samples were calculated from the equation of V_A= (5.610 x n)/m and compared the value of maximum 2.0 in 5.0 g oil sample (13).

Peroxide value (V_P)

About 5 g of oil (m) was placed in a 250 mL conical flask fitted with a ground-glass stopper. 30 ml of a mixture of $CHCl_3$ and glacial acetic acid (2:3, v/v) was added. After the oil dissolved, 0.5 ml of saturated KI solution was added and shaken exactly 1 min, then 30 ml of water was added. It was titrated with 0.01 M sodium thiosulfate until the yellow color is almost discharged. 5 mL of starch solution was added and continued the titration, until the color is discharged (n_1 ml of 0.01 M sodium thiosulfate). It was carried out a blank test under the same conditions (n_2 ml of 0.01 M sodium thiosulfate). The volume of 0.01 M sodium thiosulfate used in the blank titration did not exceed 0.1 ml. Peroxide index was calculated from the equation of V_P= 10(n_1-n_2)/m, and compared the value of max 15.0 in 5 g oil (14,15).

Fatty acids analysis by GC-MS

The standard mixture of 37 fatty acids methyl esters (Supelco™ 37 Component FAME Mix, FAME37, C4-24) used for the gas-chromatographic analyses. 100 mg of FAME37 was stored at -20°C and all standard solutions were prepared in an ice bath. To prepare 400 mg/ml FAME 37 standard solution, 250 µl of CH_2Cl_2 solution was added to 100 mg of FAME37 standard, and vortexed. 75 µl of this solution was taken into a gas chromatography vial and 925 µl CH_2Cl_2 was added, and then vortexed by closing the mouth. 100 µl 30 mg/ml FAME37 standard solution was placed in a 250 µl polymer-footed glass tube placed in 2 ml amber colored vial and sealed, then analyzed by GC-MS under the following chromatographic conditions (Table1).

Fatty acid methyl esters (FAME) were prepared by trans esterification of oils with methanol, using KOH as a catalyst before the GC analysis. 2 ml of safflower oil sample was placed in a flat bottom, approximately 50 mm diameter and 30 mm long container and dried in the oven at 100-105°C. It was allowed to cool in a desiccator with silica gel. 1 g of oil was weighed into a 25 ml round-bottomed flask with a ground-glass neck fitted with a reflux condenser and a gas port into the flask. 10 ml of anhydrous methanol, and 0.2 ml of 60 g/l KOH in MeOH were added. Then flux condenser was attached, passed nitrogen through the mixture at a flow rate of about 50 ml/min, mixed and heated to boiling. When the solution is clear (usually after about 10 min), it was continued heating for a further 5 min and cooled the flask and transferred the contents to a separating funnel. The flask was rinsed with 5 ml of heptane, transferred the rinsing to the separating funnel and shacked. 10 ml of a 200 g/l NaCl solution was added and shacked vigorously. It was allowed to separate two phases and transferred the upper organic layer to a vial containing anhydrous Na_2SO_4, allowed to stand, then filtered and FAMEs compositions were determined with GC-MS under the chromatographic conditions (Table 1) (16). Results are expressed as % (w/w) with respect to all fatty acids detected.

Enzyme inhibition methods

α-Amylase enzyme inhibition

The effects of safflower oil samples on α-amylase enzyme were determined by modifying the method of Ali, Houghton and Soumyanath (2006)(17). α-Amylase enzyme (EC 3.2.1.1, type I-A, Sigma) was prepared in water at a concentration of 4 U/ml. 0.5% potato starch solution prepared in 20 mM phosphate buffer was used as substrate.

Safflower oil samples was prepared in 100% methanol at concentrations of 1, 0.5 and 0.25 mg/ml. The samples were treated with α-amylase enzyme (4 U/ml) at 37°C. They were incubated at 37°C, and starch solution was added. Further 50 µl of DNS color solution (96 mM 3,5-dinitrosalicylic acid, 5.31 M sodium potassium tartrate in 2 M NaOH) was added and kept at 85°C for 15 min. Later, the mixtures were diluted with water and the tubes were allowed to cool in order to complete the reaction. The absorbance of the mixture was measured at 540 nm with an Elisa (Versamax Tunable Microplate Reader) plate reader. Acarbose was used as reference substance. The change in absorbance resulting from the amount of maltose formed was calculated using the equation 1.

The amount of maltose formation was measured using the maltose standard calibration curve (between 0.0 and 0.1%, w/v) maltose concentration versus net absorbance value, y = 0.4428x+ 0.0264, r^2=0.9926. Percentages of inhibition were calculated using the equation 1.

[A (control solution) / A (oil sample)] = [A (oil sample) / A (blank)] (Equation 1)

A: Absorbance

α-Glucosidase enzyme inhibition

The effects of safflower oil samples on α-glucosidase enzyme were determined using the method of Lam, Chen, Kang, Chen and Lee (2008) (18). The α-glucosidase type IV enzyme obtained from *Bacillus stearothermophilus* was prepared in 0.5 M phosphate buffer (pH 6.5). Safflower oil samples were prepared by dissolving in 100% methanol at concentrations of 1, 0.5 and 0.25 mg/ml. The solutions were incubated with α-glucosidase enzyme in a 96-well microplate reader at 37°C. Subsequently, substrate solution (10 µl, 20 mM p-nitrophenyl-α-D-glucopyranoside) was added and the reaction was allowed to take place for at 37°C. At the end of the period, color intensity was measured with an Elisa (Versamax Tunable Microplate Reader) plate reader at a wavelength of 405 nm. Acarbose was used as reference substance. Percentages of inhibition were calculated using the equation 2.

Inhibition (%) = [1 - (A (oil sample) / A (control)] × 100(Equation 2)

A: Absorbance

Statistical analysis

While evaluating the test results, standard deviations calculated in the MS Excel program on Windows XP operating system. All the results are given for at least triplicates and values are given as mean ± SD (standard deviation).

RESULTS

Acid and Peroxide values of the oil samples

Acid and peroxide values of safflower oil samples were given in Table 2. According to the European Pharmacopoeia 7.0, the acid value of safflower oils should be at most 0.5 and the peroxide value should be 10. Except for n1 and n5 oils, the acid value of 9 other oils is above the reference value of 0.5. On the other hand, only 2 (n1 and n4) out of 11 oil samples have the peroxide value below the reference value.

Fatty acid composition of the oil samples

The pharmacopoeia mentions two types of safflower oil. The oil obtained from the seeds of *C. tinctorius* by expression and/or extraction is called Type I, and the oil obtained from the seeds of the hybrids of this plant is called Type II fixed oil. Since it is not stated on the packages of the purchased oil samples whether the plant from which the oil is obtained is hybrid or not,

the results of the fatty acid analysis were evaluated according to the results of the Type I oil (Table 3).

The quantitative analysis of the fatty acids contained in the samples was carried out over the peak areas by comparing the retention times of the standard fatty acids and the results are given in Table 4. According to this, safflower oils samples are rich in monounsaturated (MUFA) (oleic acid) and polyunsaturated (PUFA) (linoleic acid) fatty acids (67.10-99.53%) of the total fatty acids in its content are oleic, linoleic, palmitic and stearic acids. Saturated fatty acids are 0.58 to12.18% of total FAMEs in oils. Fatty acid compositions in safflower oils consist of 47.04 to 92.59% linoleic acid (C18:2), 4.09 to 40.34% oleic acid (C18:1), 0.54 to 12.01% palmitic acid (C16:0), and 0.05 to1.35% stearic acid (C18:0). Considering all these results and the values in the monograph, it was seen that none of the oil samples were suitable for the pharmacopeia.

α-Amylase and α-glucosidase enzyme inhibitory activities of the oil samples

All oil samples were tested at concentrations of 0.25, 0.5, and 1.0 mg/ml to evaluate the inhibitory effects on both enzymes (Table 5). Acarbose was used as the reference compound. The results showed that safflower oil samples had no activity on α-glucosidase enzyme, which is one of the enzymes that digest carbohydrates. A very weak α-glucosidase enzyme inhibitory effect was detected only in sample number 11. The inhibition values of this sample were calculated as 6.70 ± 2.55% at a concentration of 0.25 mg/ml and 4.58 ± 1.03% at a concentration of 1 mg/ml. Inhibition values of acarbose used as reference material were determined as 98.19 ± 0.53% (0.25 mg/ml), 99.53 ± 0.04% (0.5 mg/ml), and 99.57 ± 0.04% (1 mg/ml).

In general, it was determined that the oil samples did not show a significant inhibitory effect on the α-amylase enzyme. Only sample number 1 showed moderate inhibitory activity (52.13 ± 2.87%) at a concentration of 1 mg/ml. At the same concentration, acarbose showed 100% inhibitory activity.

DISCUSSION

Acid value is used as a shelf-life monitoring parameter in the quality control of oils. The high free fatty acidity is one of the rancidity indicators of the oil and an increase in the oxidation potential of the oil and causes a decrease in stability. Except for oil samples 1 and 5, all samples were found to be rancid and oxidized. Peroxide value is a measure of the amount of active oxygen in oils, and the amount of peroxide in 1 kg of oil in milliequivalent grams of oxygen. Oxygen causes to be formed smaller molecule fatty acids. Oils can deteriorate due to various factors (storage conditions, metal ions, temperature, light, etc.). The peroxide value shows the oxidation state of the oil. It also shows whether the deodorization process has been done effectively (17, 18). Again, it was determined that all samples were highly oxidized except for oil samples 1 and 4.

Palmitic, stearic, oleic, and linoleic acids were detected in all oil samples. It was determined that oleic acid (8.0-21.0%) and linoleic acid (68.0-83.0%), which were reported as the main fatty acids in the European Pharmacopoeia 7.0, were not among the desired amounts in the samples. The contradictory situation we detected in the samples for these two fatty acids led us to predict that oleic acid is oxidized into linoleic acid.

There are many *in vitro* and *in vivo* methods to evaluate the effects of pure compounds or plant extracts on weight control. In this study, the effects of safflower oils, which have been in high demand from pharmacies for weight control in recent years, on α-amylase and α-glucosidase enzymes that help carbohydrate digestion were evaluated and the oil samples did not show any inhibitory effect. However, the fact that none of the oil samples met the quality standards may have caused unpredictable interactions in the activities on enzymes. Previously, Takahashi and Miyazawa (2012) studied the effects of methanol extracts of safflower seeds on α-glucosidase enzyme. As a result, compounds with stronger inhibitory

effects than acarbose (N-*p*-coumaroyl serotonin and N-feruloyl serotonin) were isolated from the extract (19). However, it is clear that the secondary metabolite contents of seed oils will be different from seed methanol extracts. In a study examining the activities of 10 fatty acids on enzymes known to be associated with diabetes, Su et al. (2013) displayed that oleic and linoleic acids have potent glucosidase enzyme inhibitory activity. In short, the fact that the oils used in the study are highly oxidized and the fatty acid content is not between the desired values should be one of the factors to be considered in determining the effectiveness (20). Safflower oils are among the commonly used oils because they have many activities (antioxidant, antiulcer, cardioprotective, antinociceptive, anti-inflammatory, hepatoprotective, anti-cancer, anti-diabetic, weight control activities (21-26) as well as their use in food and cosmetics. While most of these health benefits are associated with the oil content and the fatty acid composition of the respected oils. These oils, which contain significant amounts of oxygen radicals, will cause serious health problems after chronic exposure, especially considering that they are used daily by people who buy them for weight control. In the meantime, evaluating the effectiveness of safflower oils on weight control with other methods can be considered as another research topic. In conclusion, the high oxidation rates and significant variability in fatty acid content (especially for unsaturated fatty acids) detected in the 11 purchased samples indicated that more attention should be paid to the quality control of these oils, which led to the conclusion that they would be beneficial in terms of the health benefits expected from safflower oils.

CONCLUSION

Our literature survey has shown that the quality control analysis of safflower oils in the Turkish market and their effectiveness on two enzymes (α-amylase and α-glucosidase) were evaluated with this study for the first time. With this study we conducted on eleven safflower oil samples we bought from the market; it was concluded that these oil samples did not meet the European Pharmacopoeia quality criteria. Interestingly, it was observed that the amount of oleic acid was very low, while the amount of linoleic acid was high. This result suggested that oleic acid in oils was oxidized to linoleic acid due to production methods and storage conditions. The results of enzyme inhibitory activities of safflower oil samples showed that these oils could not have any effect on weight control by inhibiting the enzymes that provide carbohydrate digestion.

Safflower, whose market cap and production are low, may be the hope to close the vegetable oil gap for the present/future of the growing world's population demand. In addition to increasing the production, it is also important that the end product meets the expectation in terms of quality. And also, if the oil is used or at least marketed with health claims (with or without pharmaceutical usage), to establish the necessary quality criteria whereas show no harm to user, the safflower oil (if for human use) needs to be encouraged to be European Pharmacopoeia compliance.

Conflicts of interest: No conflict of interest was declared by the authors. The authors alone are responsible for the content and writing of the paper.

REFERENCES

1. Kıllı F, Beycioğlu T. Türkiye'de ve dünyada yağlı tohum ve ham yağ üretim durumu türkiye yağlı tohum üretimine İlişkin önemli sorunlar. UAZIMDER IJAAES 2019;SI1:17-33.
2. Zhou X, Tang L, Xu Y, Zhou G, Wang Z. Towards a better understanding of medicinal uses of carthamus tinctorius l. In traditional chinese medicine: A phytochemical and pharmacological review. J Ethnopharmacol 2014;151:27-43. Epub 2013/11/12
3. Carthamus tinctorius taxon page, Turkish Plants Data Service, 2021.
4. Farmasötik botanik ders kitabi. Baytop A. Istanbul, Istanbul Universitesi, 1996.

5. Classification for kingdom plantae down to species carthamus tinctorius l., United States Department of Agriculture Natural Resources Conservation Service, 2021.
6. Mendes-da-Silva RF, Ferreira DJS, Lopes-de-Morais AAC, de Macedo PFC, Lagranha CJ, Batista-de-Oliveira-Hornsby M. Safflower (carthamus tinctorius l.) oil during pregnancy and lactation influences brain excitability and cortex oxidative status in the rat offspring. Nutr Neurosci 2018;21:753-60. Epub 2017/08/09
7. Nidhi Srivastava SG, Sameer S. Bhagyawant, Nidhi Srivastava. Nidhi Srivastava et al. Detailed study on therapeutic properties, uses and pharmacological applications of safflower (carthamus tinctorius l.). International Journal of Ayurveda and Pharma Research 2014;2:5-16.
8. Crampon C, Nikitine C, Zaier M, Lepine O, Tanzi CD, Vian MA, Chemat F, Badens E. Oil extraction from enriched spirulina platensis microalgae using supercritical carbon dioxide. Journal of Supercritical Fluids 2017;119:289-96.
9. Scientific opinion on the substantiation of health claims related to carthamus tinctorius l. And maintenance of skin (id 2748) and maintenance of hair (id 4242) pursuant to article 13(1) of regulation (ec) no 1924/2006, Authority EFS, 2009.
10. Wang Y, Chen P, Tang C, Wang Y, Li Y, Zhang H. Antinociceptive and anti-inflammatory activities of extract and two isolated flavonoids of carthamus tinctorius l. J Ethnopharmacol 2014;151:944-50. Epub 2013/12/18
11. Konopka I, Roszkowska B, Czaplicki S, Tanska M. Optimization of pumpkin oil recovery by using aqueous enzymatic extraction and comparison of the quality of the obtained oil with the quality of cold-pressed oil. Food Technol Biotechnol 2016;54:413-20. Epub 2017/01/25
12. Emongor V. Safflower (carthamus tinctorius l.) the underutilized and neglected crop: A review. Asian Journal of Plant Sciences 2010;9:299-306.
13. Composition of fatty acids by gas chromatography. Commission EP. 8th ed. Strasbourg, France, European Pharmacopeia 8.0, 2013; 136-137.
14. EU. European pharmacopeia 7th edition. In: Healthcare EDftQoMa (ed). Vol 1. Germany, Druckerei C.H. Beck Press, 2011;1346.
15. EU. European pharmacopeia 8th edition. In: Healthcare EDftQoMa (ed). Vol a. Germany, Druckerei C.H. Beck Press, 2014;155.
16. Nutritional labeling fames on rt-2560 by aoac method 996.06,
17. Ali H, Houghton PJ, Soumyanath A. Alpha-amylase inhibitory activity of some malaysian plants used to treat diabetes; with particular reference to phyllanthus amarus. J Ethnopharmacol 2006;107:449-55. Epub 2006/05/09
18. Lam SH, Chen JM, Kang CJ, Chen CH, Lee SS. Alpha-glucosidase inhibitors from the seeds of syagrus romanzoffiana. Phytochemistry 2008;69:1173-8. Epub 2008/01/29
19. Takahashi T, Miyazawa M. Potent alpha-glucosidase inhibitors from safflower (carthamus tinctorius l.) seed. Phytother Res 2012;26:722-6. Epub 2011/10/25
20. Su CH, Hsu CH, Ng LT. Inhibitory potential of fatty acids on key enzymes related to type 2 diabetes. Biofactors 2013;39:415-21. Epub 2013/01/29
21. Khalid N, Khan RS, Hussain MI, Farooq M, Ahmad A, Ahmed I. A comprehensive characterisation of safflower oil for its potential applications as a bioactive food ingredient - a review. Trends in Food Science & Technology 2017;66:176-86.
22. Han XJ, Cheng LM, Zhang R, Bi JC. Extraction of safflower seed oil by supercritical co2. Journal of Food Engineering 2009;92:370-76.
23. Flora of turkey and the east aegean islands. Vol. 5 vol. 5. Davis PH. 2008.
24. Rashmi Dehariya AKD. A review on potential pharmacological uses of carthamus tinctorius l. World J Pharm Sci 2015;3:1741-7.
25. Safflower. Smith JR. Taylor & Francis, 1996.

26. Arslan Y, Katar D, Güneylioğlu H, Subaşı İ, Şahin B, Bülbül AS. Tarla bitkileri merkez araştırma enstitüsü dergisi türkiye florasındaki yabani carthamus l. Türleri ve aspir (c. Tinctorius l.) islahında değerlendirme olanakları. Tarla Bitkileri Merkez Araştırma Enstitüsü Dergisi 2010;19:36-43.

Table 1. GC-MS condition

GC condition	
Component	**Condition**
Device	Agilent Technologies 7890A GC System, Agilent Technologies Inc, Santa Clara, MS detector
Column	Restek-2560, bissyano propyl polysiloxane (100 m x 0.25 µm ID x 0.20 µm)
Oven temperature	100 °C (hold 4 min), increased to 24 °C by 3°C min (hold 15 min)
Injection temperature	225 °C
Detector temperature	250 °C
Carrier gas, flow rate	He, 1.0 ml/ min
Injection volume	2 µL
Split ratio	100: 1
MS condition	
Component	**Condition**
Device	Agilent Technologies 5977E MS
Mode	Scan
Solvent Delay	11.5 min
Mass range	20-300
Step size	0.1 m/z
Scan speed	3.125 (u/s)
Frequency	9.0 scan/sec
Cycle time	110.96 ms

Table 2. Acid and peroxide values of the oil samples

Samples	**n1**	**n2**	**n3**	**n4**	**n5**	**n6**	**n7**	**n8**	**n9**	**n10**	**n11**
V_A[a]±	0.2±0.	1.4±0.1	1.3±0.	1.2±0.	0.4±0.	0.7±	0.8±0.2	1.3	1.9±0.	0.8±0.1	1.1±0.1
SD[c]	1		2	0	0	0.1		±0.1	1		
V_P[b]±	7.5±1.	28.6±0.	14.6±	8.2±0.	12.8±	14.8±	157.7±	25.4±3.	18.4±	87.8±0.	30.4±1.
SD[c]	6	2	0.3	4	2.1	1.5	6.1	4	1.9	0	5

[a]acid value [b]peroxide value [c]Standard deviation, n= 3

Table 3. Fatty acid composition of safflower oil (Type I) according to the European pharmacopoeia monograph 7.0 (14)

FAMEs	% (w/w)
C16:0	4-10
C18:0	1-5
∑saturated FAMEs (C16:0 and C18:0)	5-15
C18:1	8-21
C18:2	68-83
∑unsaturated FAMEs (C18:1 and C18:2)	76-105

Table 4. Fatty acid compositions of the oil samples with GC-MS

FAMEs	Samples (% w/w)										
	n1	**n2**	**n3**	**n4**	**n5**	**n6**	**n7**	**n8**	**n9**	**n10**	**n11**
C16:0	3.27	4.08	12.01	5.32	3.57	6.16	0.54	2.24	2.21	2.43	2.73
C18:0	0.05	0.43	0.17	1.35	1.28	0.52	0.04	0.23	0.09	0.46	0.58
∑saturated FAMEs (C16:0 and C18:0)	3.32	4.51	12.18	6.67	4.85	6.68	0.58	2.47	2.30	2.89	3.31
C18:1	4.09	6.32	32.06	40.34	24.51	23.17	9.71	9.61	6.25	12.29	9.75
C18:2	92.59	87.73	47.04	49.69	68.20	69.52	89.64	89.92	91.45	84.81	86.90
∑unsaturated FAMEs (C18:1 and C18:2)	96.68	94.05	79.10	90.03	92.71	92.69	99.35	99.53	97.70	67.10	96.65

Table 5. α-Amylase enzyme inhibition results of the oil samples

Samples	**İnhibition % ± SD**		
	0.25 mg/ml	**0.5 mg/ml**	**1 mg/ml**
n1	-	5.37 ± 2.87	52.13 ± 2.87
n2	13.81± 3.57	-	7.73 ±0.86
n3	-	15.53± 3.26	6.32 ±1.89
n4	13.96± 3.50	3.42 ±1.05	13.81 ±1.06
n5	8.59 ± 3.50	4.91 ± 1.77	-
n6	-	11.52± 1.07	-
n7	8.11 ± 2.86	5.38 ± 1.18	21.08 ± 7.68
n8	16.09 ± 3.73	17.23 ± 1.05	24.45 ± 5.06
n9	28.37 ± 4.90	20.93 ± 2.40	24.66 ± 2.81
n10	40.38± 3.25	18.15 ± 4.14	16.98 ± 2.98
n11	-	7.54 ± 1.83	4.01 ± 5.50
Acarbose	92.70 ± 5.80	98.45 ± 6.66	100.00 ± 0.04

-: no inhibition, SD: Standard deviation, n= 3