



OPEN Phytochemical composition and antidiabetic, anti-obesity, antioxidant, and cytotoxic activities of *Carthamus tinctorius* seed oil

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Carthamus tinctorius L. (Safflower) is widely used in traditional Japanese, Korean, Chinese, Arabian, and Persian herbal medicine to treat metabolic diseases. This study aimed to characterize *C. tinctorius* seed oil components and estimate its inhibitory effects on free radicals, porcine pancreatic lipase, α -amylase, and cytotoxic. To describe the phytochemical components of *C. tinctorius* seed oil, the Gas Chromatography-Mass Spectrometry (GC-MS) technique was performed, while reference biochemical analytical assays were utilized for biological testing. The results showed that seven fatty acids accounting for 100% of the total oil were identified, and the major fatty acid was linoleic acid ($79.98 \pm 0.79\%$), followed by oleic ($11.20 \pm 0.21\%$) and palmitic ($5.71 \pm 0.12\%$) acids. The biological tests revealed that *C. tinctorius* seed oil has potent inhibitory effects on free radicals, porcine pancreatic lipase, and α -amylase, with IC_{50} values of 13.18 ± 0.07 , 43.6 ± 0.09 and 31.62 ± 0.65 $\mu\text{g/ml}$, respectively, in comparison with positive controls commercial drugs Trolox, Orlistat, and Acarbose, which have IC_{50} values of 4.1 ± 0.57 , 12.88 ± 0.94 , and 28.18 ± 1.22 $\mu\text{g/ml}$, respectively. *C. tinctorius* oil showed potential cytotoxic effects against tested cancer cells lines with a concentration-dependent effect on cancer cell viability. Given these findings, it is clear that *C. tinctorius* oil exhibits potent DPPH free radicals, antilipase, porcine pancreatic α -amylase inhibitory, and cytotoxic properties in comparison to the positive controls. Future in vivo research on *C. tinctorius* seed oil is warranted to elucidate the oil's mechanism of action and to decipher the molecular pathways involved in its anti-obesity, antidiabetic, antioxidant, and cytotoxic activities.

Keywords Antioxidant, α -Amylase, Antilipase, *Carthamus tinctorius*, Cytotoxicity, Seeds oil

Abbreviations

GC-MS	Gas chromatography-mass spectrometry
DPPH	2,2-Diphenyl-1-picrylhydrazyl
DM	Diabetes mellitus
HSYA	Hydroxysafflor Yellow A
DMSO	Dimethyl sulfoxide
IC_{50}	50% Inhibition concentration
PNPB	<i>P</i> -nitrophenyl butyrate
DNSA	3,5-Dinitro salicylic acid
Cis	Confidence intervals

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Oxidative stress refers to disturbances in the availability and action of antioxidants and reactive oxygen species¹. This balance is disturbed by increased free radical generation or reduced antioxidant activity. Free radicals are produced from both exogenous and endogenous sources. Exogenous free radicals can be generated from exposure to environmental pollutants, heavy metals, specific medications (including bleomycin, gentamycin, tacrolimus, and cyclosporine), chemical solvents, cooking practices (such as smoked meat, used fat, and oil), cigarette smoke, alcohol consumption, and radiation. Endogenous free radical production is caused by aging, mental stress, excessive exercise, cancer, infection, ischemia, inflammation, and immune cell activation².

Obesity is a metabolic disorder resulting in excessive and abnormal fat accumulation in various organs and tissues³. Individuals with a 30 kg/m² or greater body mass index are classified as obese. Genetic, socioeconomic, and cultural factors interact complexly to induce obesity. Obesity elevates the risk of developing severe chronic and fatal conditions, including but not limited to cardiovascular diseases, hypertension, malignancies, and diabetes mellitus (DM)⁴.

DM is a metabolic disorder characterized by chronic elevated blood glucose levels. Long-term high blood sugar levels may result in severe and fatal health consequences, especially in the cardiovascular, renal, and nervous systems⁵.

Obesity is intricately connected to several health issues, including DM, oxidative stress, and cancer. Excessive body weight often leads to insulin resistance and the development of type 2 diabetes, creating a metabolic environment conducive to chronic inflammation⁶. This inflammatory state, in turn, contributes to oxidative stress, a condition characterized by an imbalance between free radicals and antioxidants in the body⁷.

Oxidative stress plays a pivotal role in the progression of diabetes and obesity-related complications. Furthermore, both diabetes and obesity are established risk factors for various types of cancer⁸. The interplay among these factors involves complex mechanisms, with chronic inflammation and oxidative stress serving as common denominators. Elevated levels of oxidative stress not only contribute to the development of diabetes but also play a significant role in promoting cancer initiation and progression^{9–11}.

Carthamus tinctorius L. (Asteraceae family) is known as Safflower, a thistle-like annual herb, and a highly branched flowering plant. It originated in Iran, Middle Eastern regions, Afghanistan, and Ethiopia. During the 5th to fourteenth centuries, *C. tinctorius* was introduced in Spain, France, and Italy¹². The *C. tinctorius* seed's oil, entire seeds, and florets are used in traditional medicine for various ailments, including joint pain, trauma, amenorrhea, dysmenorrhea, and postpartum abdominal pain¹³. It is one of the oldest known medicinal herbs, dating back to the Egyptian Furon empire¹⁴. Recent investigations showed that hydroxysafflor yellow A (Safflomin A, HSYA), a bioactive compound in *C. tinctorius*, has promising positive effects for treating infertility, cancer, inflammation, thrombosis, and myocardial ischemia¹⁵.

Hence, the present study attempts to identify the chemical constituents of *C. tinctorius* seed oil and estimate its cytotoxic, antioxidant, porcine pancreatic lipase, and α -amylase inhibitory properties.

Material and methods

Processing of plant material

In June 2021, the seeds of the *C. tinctorius* plant were harvested from the Nablus governorate of Palestine. The cultivated plant seeds were collected following the institutional, national, and international legislation and guidelines. Professor Nidal Jaradat, a pharmacognosist, identified the seed samples, which were then lodged at An-Najah National University's Herbal Products Laboratory with a voucher specimen code of (Pharm-PCT-2789). The seeds were then dried in the shade for about three weeks and stored in a glass jar for further use.

Oil extraction

The cold pressing method was used to extract oil from the seeds of the *C. tinctorius* plant for testing. It is a method of extracting (draining) oil only through mechanical processes. Cold pressing is a method used with a low-temperature continuous screw press to press raw, dried seeds. A screw press with a capacity of 4 kg/h of seeds and a 4.0 KW (Kern Kraft, Germany) engine was employed in this study to extract seed oil.

Gas Chromatography-Mass Spectrometry (GC-MS) assessment

The GC and MS techniques were used to identify constituents of *C. tinctorius* Oil, conducted by a Perkin Elmer Clarus 500 gas chromatograph with a Perkin Elmer Clarus 560 mass spectrometer. SLBTM-5 ms fused-silica capillary column (30 m × 0.25 mm, film thickness 0.25 μ m) was utilized to perform the separation. The oven temperature, including the column, was set to rise by 3 °C every minute, with the oven temperature beginning at 100 °C and ending at 240 °C. Helium 99.99% was used as a carrier gas at a constant 1 ml/min flow rate during the chromatographic run. Injector temperature 290 °C, 1 μ l of the methylated seed oil injected in split mode with a split ratio 1:50. Solvent delay 0–8 min, source temperature 250 °C, MS Scan Mass 50.0 to 500 EI+ form 8 min to 65.67 min. The National Institute of Standards and Technology's MS Data Center reference spectra were compared to the mass spectra of the chemical components, and their Kovats retention indices were compared to values given in the literature. The Kovats Retention Index for each compound was calculated using the retention time value from the hydrocarbon alkane standard (C₁₀-C₄₀) (Sigma-Aldrich, Germany)¹⁶.

Free radical scavenging activity

The same procedure stated in¹⁷ was used to conduct the DPPH free radical scavenging activity of *C. tinctorius* seeds oil, and a positive control consisting of Trolox (Sigma-Aldrich, Søborg, Denmark) was included. In contrast, solutions of tested substances (1 mg/ml) were prepared by dissolving 100 mg of each sample in 100 ml of methanol (Loba Chemie, Mumbai, India) before further diluting the solution with methanol to reach the desired concentrations of 5, 10, 20, 30, 50, and 100 μ g/ml. At 517 nm, the absorbance was measured using a

UV-Vis spectrophotometer (Jenway® 7135, Staffordshire, UK). The following formula was used to determine the DPPH-inhibiting activity of each sample.

$$I(\%) = [ABS_{\text{blank}} - ABS_{\text{test}}] / [ABS_{\text{blank}}] * 100\%$$

where I (%) is the percentage of antioxidant activity.

The antioxidant half-maximal inhibitory concentration (IC₅₀) of the used samples and Trolox were assessed by using an online tool, “Quest Graph™ IC₅₀ Calculator.” AAT Bioquest, Inc., 01 May. 2023, <https://www.aatbio.com/tools/ic50-calculator>¹⁸.

Porcine pancreatic lipase inhibition assay

This study used the porcine pancreatic lipase inhibitory method¹⁹. Five different solutions were prepared from a 700 µg/ml stock solution from the plant oil in 10% Dimethyl sulfoxide (DMSO) (Riedel De Haen, Teningen, Germany). The concentrations of these solutions were 10, 50, 100, 500, and 700 µg/ml respectively. Before use, a freshly stocked solution was prepared from 1 mg/ml of porcine pancreatic lipase enzyme (Sigma-Aldrich, St. Louis, USA) in Tris-HCl buffer (Bio Basic, Ontario, Canada). *P*-nitrophenyl butyrate (PNPB) (Merck KGaA, Darmstadt, Germany) was prepared by dissolving 20.9 mg in 2 ml of acetonitrile (Loba Chemie, Mumbai, India). 0.1 ml of porcine pancreatic lipase and 0.2 ml of each diluted solution series for the plant oil were mixed. Then, a Tri-HCl solution was added to the resulting mixture to get a total volume of 1 ml. After that, the final mixture was incubated at 37 °C for 15 min. The next step was the addition of 0.1 ml of PNPB solution to each test tube and incubated for 30 min at 37 °C. The hydrolysis of the PNPB compound into *p*-nitrophenolate ions was measured at 410 nm using a UV-Vis spectrophotometer (Jenway® 7135, Staffordshire, UK) to determine the pancreatic lipase activity. Orlistat (Sigma, St. Louis, USA) was used as a positive control compound by using the same procedure. The following equation was used in this analytical study.

$$\% \text{ lipase inhibition} = (AB - AE) / AB * 100\%$$

where AB is the blank solution's recorded absorbance, AE is the *C. tinctorius* seeds oil sample solution.

α-Amylase inhibitory method

The α-amylase inhibitory experiment was conducted as described in (20), with the antidiabetic medication Acarbose (Sigma, St. Louis, USA) as a positive control. On the other hand, the concentrations of the examined substances were 10, 50, 70, 100, and 500 µg/ml. The plant extract working solution (1 mg/ml) was created by dissolving 25 mg of each plant fraction in 10% DMSO and adding a buffer solution of up to 25 ml. Using a UV-Vis Spectrophotometer, the absorbance of the tested samples was estimated to be 540 nm. The α-amylase inhibitory potential was calculated using the formula below.

$$I(\%) = [ABS_{\text{blank}} - ABS_{\text{test}}] / [ABS_{\text{blank}}] * 100\%$$

where I (%) is the α-amylase inhibitory percentage.

Cell culture and cytotoxicity assay

Cervical cancer (HeLa), human hepatic stellate (LX-2), and breast cancer (MCF-7) cells (ATCC, Rockville, MD, USA) were grown in RPMI-1640 media and supplemented with 1% Streptomycin/Penicillin, 1% l-glutamine (Sigma, G7513, France), and 10% fetal bovine serum (Sigma, 10733056001, Germany). Cultures were maintained at 37 °C in humidified 95% air and 5% CO₂. Cells were seeded at a density of 2.5 × 10⁴ cells per well in 96 well plates. After 24 of culture, the oil concentrations (2, 4, 8, and 16 mg/ml) were screened for their cytotoxic effects. The manufacturer's guidelines (Promega Corporation, Madison, WI) were followed to assess the viability of the cells examined using the CellTiter 96® Aqueous One Solution Cell Proliferation (MTS) Assay. The procedure involved adding 20 µL of MTS solution per 100 µL of media and incubating the medium at 37 °C for two h. At 490 nm, the absorbance was measured²⁰.

$$\% \text{ of Cell Viability} = \left(\frac{Abs \text{ sample} - Abs \text{ blank}}{Abs \text{ negative control} - Abs \text{ blank}} \right) * 100\%$$

$$\% \text{ of Cell inhibition} = 100 - \text{cell Viability}\%$$

Statistical analysis

In our study, all experiments were conducted in triplicates, and the results are expressed as mean values ± standard deviation (SD). To assess the statistical significance of our findings, we applied a regression analysis, where results with *p*-values less than 0.05 were considered significant. The confidence intervals for key findings in Tables 1 and 2, were reported as 95% confidence intervals (CIs) to provide a clearer picture of the variability and reliability of our mean values, especially for IC₅₀ values and the DPPH inhibitory effects. We calculated effect

Peak	Name	Retention time	C.K.R.I	R.K.R.I	Area	% Area
1	Lauric acid	19.79	1522	1521	286,256	0.35 ± 0.01
2	Myristic acid	27.2	1723	1723	109,707	0.14 ± 0.01
3	Palmitic acid	34.03	1924	1924	4,618,589	5.71 ± 0.12
4	Linoleic acid	39.28	2092	2092	64,641,724	79.98 ± 0.79
5	Oleic Acid	39.45	2098	2098	9,052,905	11.20 ± 0.21
6	Elaidic Acid	39.61	2103	2101	344,268	0.43 ± 0.01
7	Stearic Acid	40.28	2125	2125	1,771,900	2.19 ± 0.01
	Total				80,825,349	100

Table 1. The fatty acids constituents of *C. tinctorius* seeds oil identified by GC-MS. Data are reported as mean ± SD ($n = 3$). C.K.R.I: Calculated Kovats Retention Index; R.K.R.I: Reference Kovats Retention Index.

Concentrations	Trolox	<i>C. tinctorius</i> seed oil
0	0 ± 0.0	0 ± 0.0
5	75 ± 0.0	42.26 ± 0.2
10	82.4 ± 0.1	54.3 ± 0.0
20	84.6 ± 1.7	54.4 ± 0.0
30	85.59 ± 1.5	62.8 ± 0.2
50	91.54 ± 0.7	72.4 ± 0.01
100	92.65 ± 0.0	72.4 ± 0.01
IC₅₀ values (µg/ml)	4.1 ± 0.57	13.18 ± 0.07

Table 2. *C. tinctorius* seeds oil and Trolox DPPH inhibitory effect and IC₅₀ values. Data are expressed as means ± SD ($n = 3$). p -value < 0.05. Significant values are in bold.

sizes for the primary outcomes, including comparing the DPPH inhibitory effects of *C. tinctorius* seed oil and Trolox, to provide insight into the practical significance of our results beyond p -values alone. Additionally, we revised the tables to clarify the statistical metrics used, which should further improve the transparency of our data interpretation.

Results

Phytochemical profiling

The GC-MS technique characterized qualitatively and quantitatively the phytochemical components of *C. tinctorius* seeds oil. Seven fatty acids accounting for 100% of the total oil were characterized (Table 1). The major fatty acid was linoleic acid (79.98 ± 0.79%), followed by oleic acid (11.20 ± 0.21%) and palmitic acid (5.71 ± 0.12%).

The quantification of the identified components was investigated using a percent relative peak area. A tentative identification of the compounds was performed based on comparing their relative retention time and mass spectra with those of the National Institute of Standards and Technology's data of the GC-MS system. In addition, each compound's Kovats Retention Index was calculated using the retention time value from the hydrocarbon alkane standard (C₁₀-C₄₀) (Sigma-Aldrich, Germany)¹⁶.

DPPH inhibitory effect by *C. tinctorius* seeds oil and Trolox

Table 2 shows the free radical scavenging activity of the *C. tinctorius* seeds oil at several concentrations (100, 50, 30, 20, 10, and 5 µg/mL). Increasing the oil concentration was directly proportional to the percentage of DDPH inhibition. However, this correlation was variable with different levels of inhibition. According to the regression test, the p -value for *C. tinctorius* seeds oil in this experiment was 0.018, which is considered statistically significant. The calculated free radicals scavenging IC₅₀ values revealed that the *C. tinctorius* seed oil has potent antioxidant activity compared with the pharmaceutical potential antioxidant drug Trolox with IC₅₀ doses of 13.18 ± 0.07 and 4.1 ± 0.57 µg/ml, respectively.

Porcine pancreatic lipase suppressant effect

The calculated antilipase activity IC₅₀ values revealed that the *C. tinctorius* seeds oil has potent antilipase activity compared with the potential anti-obesity pharmaceutical Orlistat with IC₅₀ doses of 43.6 ± 0.09 and 12.88 ± 0.94 µg/ml, respectively (Fig. 1).

α-Amylase inhibitory activity

Regarding α-amylase suppressant activity (Fig. 2), Acarbose was used as a positive control. The calculated α-amylase suppressant activity IC₅₀ values revealed that the *C. tinctorius* seeds oil has potent α-amylase

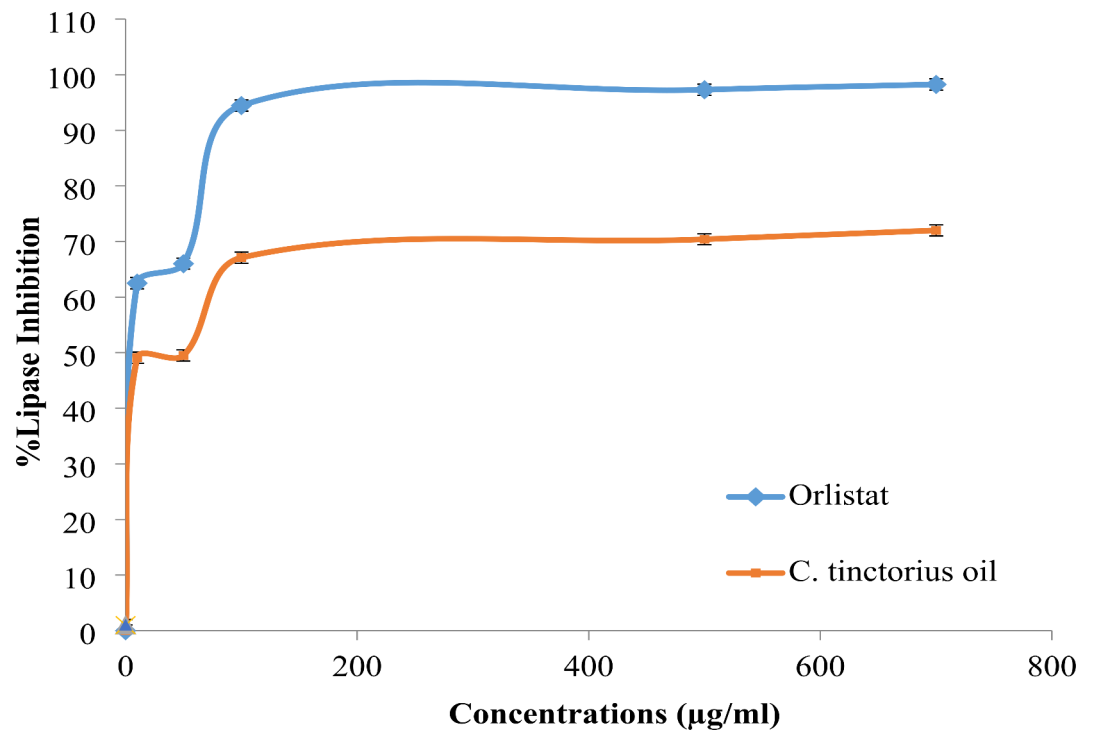


Fig. 1. Porcine pancreatic lipase inhibitory effect by *C. tinctorius* seeds oil and Orlistat. Data are reported as mean \pm SD ($n=3$). p -value < 0.05 .

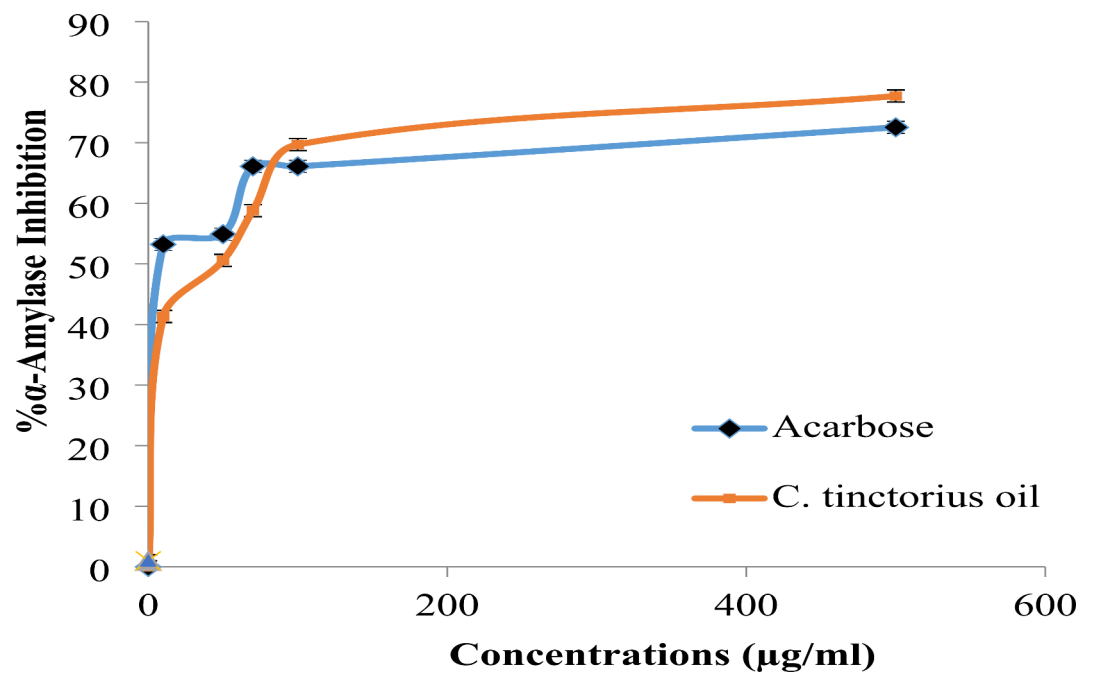


Fig. 2. Porcine pancreatic α -amylase inhibitory effect by *C. tinctorius* seed oil and Acarbose. Data are reported as mean \pm SD ($n=3$). p -value < 0.05 .

suppressant activity compared with the potential antidiabetic drug Acarbose with IC_{50} doses of 31.62 ± 0.65 and 28.18 ± 1.22 $\mu\text{g/ml}$, respectively.

Cytotoxic effect

The cytotoxic effect of *C. tinctorius* seeds oil on LX-2, HeLa, and MCF-7 cells is shown in Fig. 3. The cytotoxic effect of *C. tinctorius* seeds oil was more potent against LX-2 cells (IC_{50} : 7.03 ± 5.91 mg/ml), followed by MCF-7 (IC_{50} : 11.40 ± 6.77 mg/ml), and HeLa cells (IC_{50} more than 16 mg/ml), in comparison with positive control doxorubicin IC_{50} values of 114.14 ± 2.78 , 28.80 ± 1.02 and 5.42 ± 0.91 ng/ml respectively. The effect of *C. tinctorius* oil on cell viability was then evaluated. The cell viability was significantly inhibited at the concentration of 16 mg/ml compared to 4 mg/ml concentration, as shown in Fig. 4. According to the regression test, the p -values for *C. tinctorius* seed oil in this experiment were 0.041, 0.0006, and 0.02 for HeLa, MCF-7, and LX-2 cell lines, which were considered statistically significant, especially for LX-2 cell lines. At the concentration of 16 mg/ml, the viability of the MCF-7, LX-2, and HeLa cells decreased by 44.10, 15.46, 99.55, and 55.90%, respectively. The negative control was DMSO, which showed cell viability around 100%.

Discussion

Since ancient times, natural products and their derivatives have been extensively used in traditional medicine to treat many diseases, and recently, they have played a significant role in the discovery of drugs^{21–23}.

Based on gas chromatography and mass spectrometry analysis techniques in a previous study, *C. tinctorius* extract contained several biologically active compounds, including myristic acid, lauric acid, palmitic acid, linoleic acid, stearic acid, and oleic acid, as well as tetrapentacontane which have several therapeutic potentials²⁴.

In addition, a study by Yilmaz et al. identified the chemical compositions of the oils obtained from the *C. tinctorius* seeds from Turkey harvested in different periods, from flowering to maturation. They found that palmitic and stearic acids were the major components of saturated fatty acids, while oleic and linoleic acids were the abundant molecules of unsaturated fatty acids²⁵.

Çamaş et al. investigated the oil yields and fatty acid compositions of the *C. tinctorius* seeds from five locations in Northern Turkey. They stated that linoleic and oleic acid were the main fatty acids in the oil composition for all cultivars, while fatty acid percentages varied considerably among locations and cultivars²⁶.

In addition, Abuova et al. found that *C. tinctorius* seeds oil's major components from Kazakhstan were linoleic acid (63.7%), stearic acid (15.3%), and oleic acid (10.1%)²⁷. The current study's GC-MS analyses agreed with previous studies as well as the major constituents revealed in this analytical screening that the major compounds were linoleic acid ($79.98 \pm 0.79\%$), oleic acid ($11.20 \pm 0.21\%$), and palmitic acid ($5.71 \pm 0.12\%$).

However, the composition of fixed vegetable oils varies depending on several factors, including the plant's geographical location and agricultural practices. Environmental conditions such as rainfall, sunlight, and temperature affect the fatty acid profile and the presence of bioactive compounds. In addition, the availability of nutrients and soil type affect the quality and nutritional content of the oil. Harvest time and post-harvest processing methods, such as refining and extraction techniques, further change the oil's properties²⁸.

Actually, *C. tinctorius* seed oil contains several other bioactive lipophilic and hydrophilic components, such as flavonoids and alkaloids²⁹.

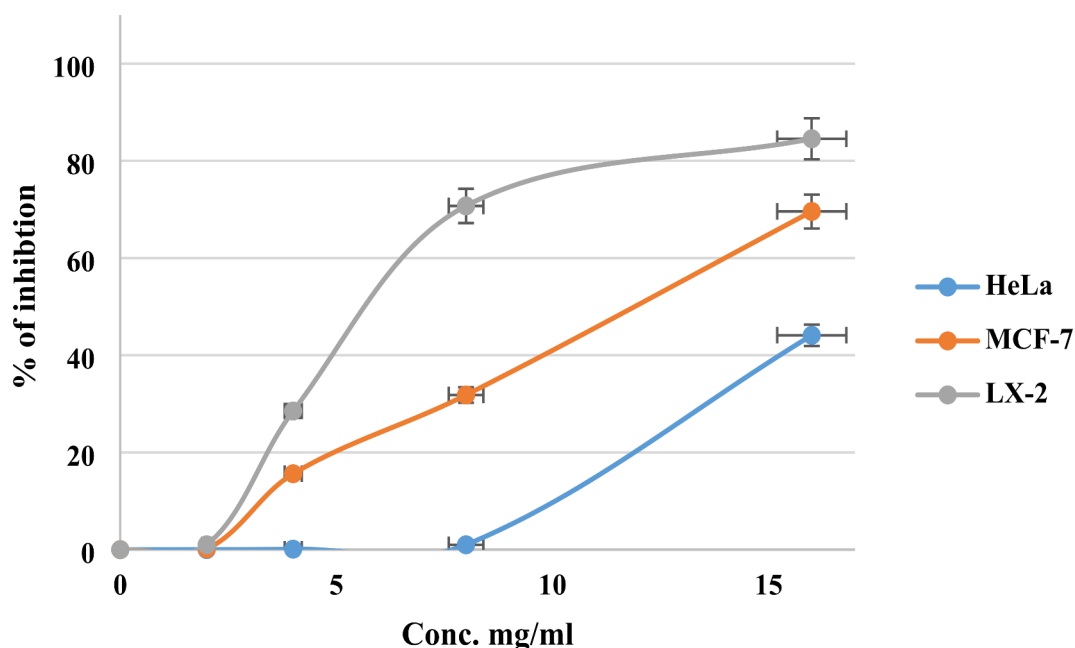


Fig. 3. The cytotoxicity inhibition percentage cervical cancer (HeLa), human hepatic stellate (LX-2), and breast cancer (MCF-7) cells by *C. tinctorius* oil. Data are reported as mean \pm SD ($n = 3$). p -value < 0.05 .

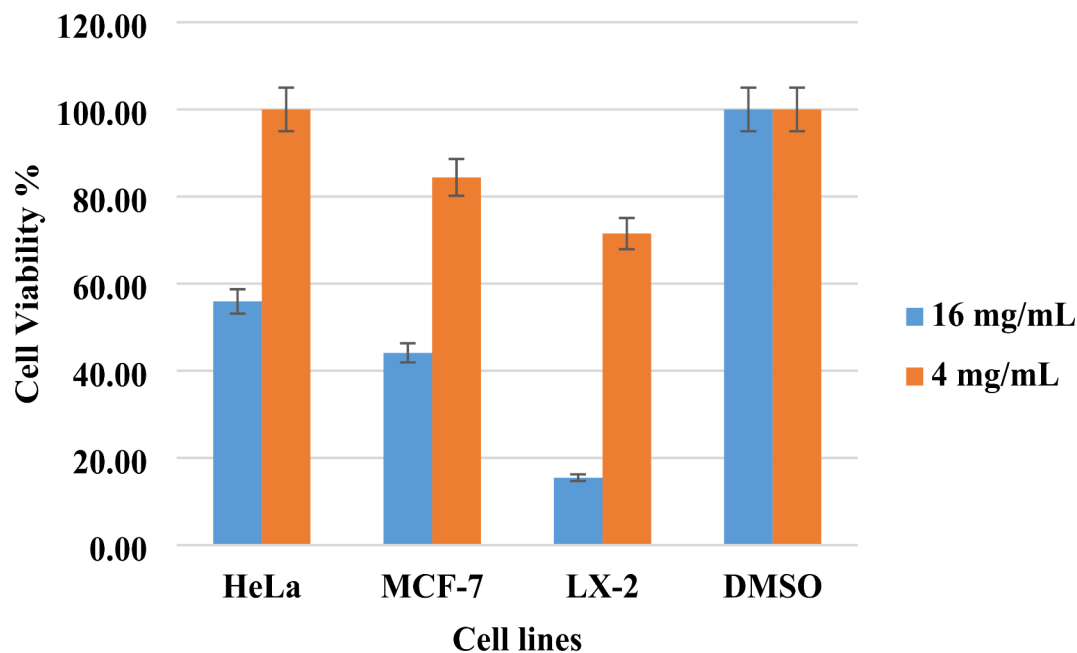


Fig. 4. Compared to the negative control Dimethylsulfoxide (DMSO), the cell viability effect (%) of *C. tinctorius* seed oil on cervical cancer (HeLa), human hepatic stellate (LX-2), and breast cancer (MCF-7) cells. Data are reported as mean \pm SD ($n=3$). p -value < 0.05 .

Our findings revealed that *C. tinctorius* seed oil has potent antioxidant activity as well as at a concentration of 100 $\mu\text{g/ml}$; the tested seed oil inhibited the DPPH action by $72.4 \pm 0.01\%$, while Trolox inhibited the effect by $92.65 \pm 0.0\%$. These outcomes agreed with the Khemiri et al. study, which investigated the DPPH free radical scavenging activity of *C. tinctorius* seed oil from Tunisia and found that the oil had a notable antioxidant capacity with $89.41 \pm 0.38\%$ scavenging activity against DPPH radical³⁰.

In fact, previous investigations revealed that natural oils with high percentages of linoleic acid had potential therapeutic benefits, including antihyperglycemic, antioxidant and preventing Alzheimer's dementia and cardiovascular diseases^{31,32}. To further elucidate the therapeutic potential of *C. tinctorius* seed oil, a deeper insight into the molecular mechanisms of its major bioactive compounds, particularly linoleic acid and hydroxysafflor yellow A (HSYA), are warranted. Linoleic acid, a predominant component, has demonstrated effects on lipid metabolism and inflammation through modulation of the PPAR γ (peroxisome proliferator-activated receptor gamma) signaling pathway, which plays a critical role in glucose and lipid homeostasis^{33,34}. Activation of PPAR γ by linoleic acid may enhance insulin sensitivity and reduce lipid accumulation³⁴, suggesting a pathway by which *C. tinctorius* oil may exert antidiabetic and anti-obesity effects. *C. tinctorius* aqueous methanol extract containing phenolics and flavonoids reduced symptoms of pancreas dysfunction in rats with diabetes³⁵. Much information on *C. tinctorius* came from studies concerning the effect of *C. tinctorius* HSYA in pharmacological research studies. HSYA has significant biological activity in the treatment of coronary heart disease, myocardial infarction, ischaemic encephalopathy, cerebral thrombosis, and stroke. Moreover, some flavonoids in *C. tinctorius* are natural dyes widely used in food, cosmetics, and industrial products³⁶.

Figure 1 demonstrates the antilipase activity of *C. tinctorius* seed oil compared with the anti-obesity drug Orlistat, which was used as a positive control. The results of lipase inhibition caused by *C. tinctorius* seed oil were directly proportional to the increased concentrations (10, 50, 100, 500, and 700 $\mu\text{g/ml}$). However, the inhibition proportion was variable: 49.1 ± 0.15 , 49.5 ± 0.00 , 67.1 ± 0.15 , 70.4 ± 0.05 , and $72 \pm 0.00\%$, respectively. In contrast, Orlistat showed a more potent effect at the same concentrations (62.5 ± 0.8 , 66 ± 0.58 , 94.45 ± 1.5 , 97.3 ± 0.58 , $98.25 \pm 0.94\%$, respectively). According to the regression test, the p -value for *C. tinctorius* seeds oil in this experiment was 0.037, which is considered statistically significant.

An investigation conducted by Conforti et al. found that the *Foeniculum vulgare* seed hydroethanolic extract had an antilipase effect with IC_{50} more than 10 mg/ml ³⁷.

Besides, Gholamhoseinian et al. found that the *Nigella sativa* seeds methanol extract inhibited the action of porcine pancreatic lipase by 25–50%³⁸.

In addition, Moreno et al. estimated the porcine pancreatic lipase suppressant effect of *Vitis vinifera* seeds ethanol extract and found that it had a 30% inhibition³⁹.

The comparison between *C. tinctorius* seeds oil and Orlistat regarding antilipase activity suggests that while the natural oil has significant activity, the pharmaceutical Orlistat exhibits a higher potency in inhibiting lipase.

In fact, J. Liu et al. demonstrated that the isolated flower petals containing HSYA reduce obesity in mice and rats where oral administration of flower petals altered the composition of the diet-dependent intestinal microflora, which resulted in decreased fat accumulation, restored glucose homeostasis, alleviation of insulin resistance, and reduced inflammation in the organism⁴⁰. Moreover, HSYA increased the synthesis of hormone-

sensitive lipase (HSL), altered its activity, and inhibited adipocyte proliferation⁴¹. Furthermore, Studies have shown that Hydroxysafflor can ameliorate diabetes by improving the expression of PPAR γ 2 and insulin sensitivity⁴². In addition, HSYA can protect pancreatic β -cells via the JNK signaling pathway⁴³.

The results of α -amylase inhibition by applying *C. tinctorius* seeds oil were directly proportional to the increased concentrations (10, 50, 70, 100, 500 μ g/ml). The potent effect was 41.32 ± 0.05 , 50.58 ± 0.88 , 58.8 ± 0.3 , 69.7 ± 0.59 , and $77.7 \pm 1.31\%$, respectively, which was higher than the powerful effect of Acarbose at the same concentrations. However, Acarbose demonstrated the percentage of amylase inhibition at the same concentrations as the following: 53.22 ± 1.2 , 54.91 ± 0.58 , 66.1 ± 1.34 , 66.1 ± 1.62 , and $72.54 \pm 1.37\%$, respectively. According to the regression test, the *p*-value for *C. tinctorius* seeds oil in this experiment was 0.032, which is considered statistically significant.

Daoudi et al. found that the *Argania spinosa* seeds oils at 9 mg/ml concentration showed α -amylase inhibitory activities of 62.05% for roasted and 69.28% for unroasted seeds⁴⁴. Another study established by Eid et al. found that Coriandrum sativum seed oil showed a potential anti-amylase effect with an IC₅₀ equal to 79.43 ± 1.50 μ g/ml compared to acarbose, which had an IC₅₀ equal to 28.1 ± 1.13 μ g/ml⁴⁵.

Our results confirm that *C. tinctorius* seed oil possesses potent α -amylase suppressant activity comparable to the pharmaceutical Acarbose. Meanwhile, Acarbose has a slightly lower IC₅₀, contributing to the possible role in managing blood sugar levels. This comparison underscores the potential role of *C. tinctorius* seed oil in providing bioactive compounds with antidiabetic properties.

To our knowledge, the *C. tinctorius* seed oil porcine pancreatic lipase and amylase effects had not previously been elucidated.

HSYA, another bioactive component extensively studied in *C. tinctorius* extracts, had influenced a range of cellular pathways with potential anticancer benefits⁴⁶. HSYA inhibited cancer cell proliferation and induced apoptosis, partly through modulating the JNK (c-Jun N-terminal kinase) and MAPK (mitogen-activated protein kinase) signaling pathways, which regulate cell survival and death⁴⁷. Additionally, HSYA demonstrated the ability to alter the activity of hormone-sensitive lipase (HSL), impacting adipocyte function and inflammatory responses. These mechanisms indicated that HSYA might contribute to the oil's anticancer effects and support its anti-inflammatory and metabolic benefits.

The current findings demonstrated that *C. tinctorius* seed oil has a concentration-dependent effect on cell viability, and the degree of inhibition varies between cancer cell types. HeLa cells were susceptible to treatment with *C. tinctorius* oil at 16 mg/ml, with cell viability almost completely reduced. MCF-7 cells also experienced a significant decrease, while LX-2 cells had a lower impact. Recent research showed that HSYA inhibited proliferation and stimulated apoptosis of liver cancer cells by blocking the autophagic flux⁴⁸. In addition to reducing liver cancer, HSYA inhibited glioma growth⁴⁹, colorectal cancer⁵⁰, and lung cancer⁵¹. Cytotoxic activity of *C. tinctorius* seed oil against cancer cells was previously evaluated using MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl2H-tetrazolium bromide) assay and gene expression profiling. It was revealed that *C. tinctorius* seed oil inhibits cell proliferation, promotes apoptosis, and upregulates the expressions of many pro-apoptotic genes by different mechanisms of action against various cancer cells in vitro⁵². *C. tinctorius* seed oil inhibited the proliferation and migration by significantly increasing caspase-3 activation in hepatocellular (HepG2), colorectal (HCT-116), and gastric carcinoma (AGS) cell lines⁵³.

Further research is warranted to substantiate the promising in-vitro findings of *C. tinctorius* seed oil and translate its therapeutic potential into clinical applications. We recommend initiating in-vivo studies using animal models to evaluate the anti-obesity, antidiabetic, antioxidant, and anticancer properties of *C. tinctorius* seed oil. These studies should focus on assessing the oil's ability to modulate enzymes such as lipase and α -amylase and explore its effects on glucose homeostasis, insulin sensitivity, and lipid metabolism pathways, which are relevant to managing metabolic diseases. Additionally, investigating the oil's impact on cancer cell apoptosis, tumor growth, and molecular pathways related to tumor suppression, such as caspase activation and autophagic flux modulation, would provide valuable insights into its potential as an anticancer agent.

Clinical trials involving human subjects with metabolic disorders are also recommended to confirm the efficacy, optimal dosing, and safety profiles of *C. tinctorius* seed oil. These trials could explore outcomes such as reductions in blood glucose and lipid levels, improved antioxidant markers, and effects on body weight and inflammatory markers.

Conclusion

The findings of this study underscore the therapeutic potential of *C. tinctorius* seed oil as a natural source of bioactive compounds, including high levels of linoleic, oleic, and palmitic acids, which contribute to its antioxidant, antilipase, anti- α -amylase, and anticancer activities. The oil's potential against free radicals, lipase, and α -amylase, alongside its cytotoxic effects against cancer cells, positions *C. tinctorius* seed oil as a promising candidate for managing metabolic diseases and certain cancers. For researchers and practitioners, these insights provide a foundation for exploring *C. tinctorius* seed oil's applications in functional foods, nutraceuticals, and pharmaceuticals. Future studies should focus on the oil's mechanisms of action and molecular pathways, particularly PPAR γ , JNK, and MAPK signaling, to better understand its effects on obesity, diabetes, and cancer. Additionally, research into developing stable, bioavailable formulations and conducting in-vivo and clinical trials will be essential for translating these findings into safe and effective therapeutic options. This study adds valuable knowledge to the field and encourages further exploration of *C. tinctorius* seed oil as a multifunctional natural product with potential health benefits.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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N.J.: Methodology, Supervision, Writing—original draft, Writing—review and editing, Investigation. M.H.: Formal analysis and data curation. M.G.: Writing—review and editing. M.R.: Writing—review and editing. M.D.: Writing—review and editing. L.I.: Investigation. F.H.: Investigation. L.A.: Investigation. L.Y.: Investigation. H.R.: Investigation. A.G.: Investigation. The manuscript was drafted by all authors. All authors read and approved the final manuscript.

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