

Physico-chemical properties and fatty acid composition of *Chrozophora tinctoria* seeds as a new oil source

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SUMMARY: *Chrozophora tinctoria* L., usually known as dyer's Croton, Turnsole or Giradol, has been used in various medicinal and food products for many years. However, no comprehensive research has been undertaken to assess its potential as a new seed oil crop. Therefore, the current study examined the fatty acid composition, physico-chemical properties and antioxidant activity of *C. tinctoria* seeds, grown in the southwest of Iran. The seed oil content was found to be 26.40%. The extracted oil was analyzed for fatty acid composition using gas chromatography (GC). The results showed that unsaturated fatty acids accounted for almost 91% of the total fatty acids. Linoleic acid was the dominant fatty acid (76.68%), followed by oleic acid (13.99%) and palmitic acid (5.32%). δ -tocopherol was the major tocopherol in the oil, representing 70 mg/100 g oil. The total phenolic content (151.70 mg GAE per 100 g oil) and total flavonoid content (1.17 mg QE oil) were also determined in the extracted oil. The antioxidant activity was measured by a DPPH assay and expressed as 45% of the seed oil. Due to its high oil yield and high unsaturated fatty acid content, *C. tinctoria* could be regarded as a new source of edible oil.

KEYWORDS: Antioxidant activity; *Chrozophora tinctoria*; Fatty acids; Seed oil

RESUMEN: *Propiedades físico-químicas y composición de ácidos grasos de las semillas de Chrozophora tinctoria como nueva fuente de aceite.* *Chrozophora tinctoria* L., generalmente conocida como Cencila, o Tornasol, se ha usado en diferentes productos medicinales y alimenticios durante muchos años. Sin embargo, no se ha realizado una investigación exhaustiva para evaluar su potencial como un nuevo cultivo de semillas oleaginosas. Por tanto, en el presente estudio se evaluó la composición en ácidos grasos, las propiedades físico-químicas y la actividad antioxidante de las semillas de *C. tinctoria* del suroeste de Irán. Se encontró que el contenido de aceite de las semillas era del 26,40%. El aceite extraído se analizó para determinar la composición en ácidos grasos mediante cromatografía de gases (GC). Los resultados mostraron que los ácidos grasos insaturados representaron casi el 91% del total de ácidos grasos. El ácido linoleico fue el mayoritario (76,68%), seguido del oleico (13,99%) y palmítico (5,32%). El δ -tocoferol fue el principal tocoferol siendo su valor de 70 mg/100 g de aceite de semillas de *C. tinctoria* silvestre. También fueron determinados en el aceite su contenido fenólico total (151,70 mg de GAE por 100 g de aceite) y el total de flavonoides (1,17 mg QE por 100g de aceite). La actividad antioxidante se midió mediante DPPH y fue de 45% de aceite de semilla. Debido al alto rendimiento de aceite y al alto contenido de ácidos grasos insaturados, *C. tinctoria* podría considerarse como una nueva fuente de aceite comestible.

PALABRAS CLAVE: Aceite de semilla; Ácidos grasos; Actividad antioxidante; *Chrozophora tinctoria*

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1. INTRODUCTION

Chrozophora belongs to the Euphorbiaceae family and consists of nine species (Marzouk *et al.*, 2015). *C. tinctoria* has been used as therapeutic and emetic treatments as well as for fever and warts in traditional medicine (Delazar *et al.*, 2006). The leaves, stems, and seeds of *C. tinctoria* have been used in both food and industrial products. The most conspicuous organ of the plant is its fruit. The fruit is a strange-looking capsule in the shape of 3 spherical bodies fused in a rather rounded-triangular structure. There are three obviate angular seeds per fruit, enclosed by a thin, carunculated apically, with a flat embryo and copious endosperm. They are 0.4 cm in size and gray-to-light brown in color (Van Welzen 1999).

Seed oils contain high amounts of tocopherols, which have been associated with polyunsaturated fatty acids which protect against oxidation and make them important bioactive compounds (Górnaś and Soliven, 2015). They are also members of the vitamin E family. Vitamin E protects humans from the oxidative stress interceded by reactive oxygen species (ROS). The main role of tocopherols in the food industry is to inhibit lipid oxidation and to prevent lipid oxidation in foodstuff during storage, which results in improving shelf-life (Sattler *et al.*, 2004; Azzi and Vitamin, 2006). Many phyto-chemical constituents have been found in different *Chrozophora* species, including alkaloids, coumarins (Abdel-Sattar, 1985), diterpenoids (Tabussum *et al.*, 2013), phenylpropanoid glycosides (Mohamed 2001), phenolic acids, tannins, anthraquinones, saponins and xanthenes (Usman *et al.*, 2007) and flavonoids (Hawas 2007). In addition, it has been reported that *C. tinctoria* has antioxidant (Oke-Altuntas *et al.*, 2017), antimicrobial (Usman *et al.*, 2007) and anticancer (Jamil *et al.*, 2012) characteristics as well as anti-inflammatory (Abdallah *et al.*, 2015), anti-proliferative (Oke-Altuntas *et al.*, 2017) and wound-healing (Maurya and Semwal, 2016) properties. Due to its unique geographical position and climate situation, Iran possesses a rich flora. Studies on economically important crops are of major importance for understanding development conditions and current use of these plants. Therefore, *C. tinctoria* was selected for this study. There has been a great increase in the number of oilseed plant studies regarding the major role of vegetable oils as energy sources in the human diet. To date, several studies have investigated the potential of some new plants in terms of oil quality (Mirghani *et al.*, 1996; Mariod and Mirghani, 2017). To the best of our knowledge, there is no comprehensive study on the oil content and fatty acid composition of *C. tinctoria*, and accordingly, the current study was aimed to examine the oil content, fatty acid composition and antioxidant properties of *C. tinctoria*.

2. MATERIAL AND METHODS

2.1. Plant sampling

Mature, wild *C. tinctoria* were collected from Sepidan, Iran (Latitude 30° 2' 42.21" N and longitude 52° 16' 34.44" E, at an altitude of 551 m above sea level), in 2017. The locations were marked by a Global Positioning System (GPS). The climate is cool and moist-to-semi-arid, with a mean-annual rainfall of 758 mm and a mean-annual temperature of 10 °C. The seeds were washed with sterile water, and then dried at ambient temperature for seven days before storing in a sealed container at 4 °C until oil extraction (for a maximum of 14 days).

2.2. Oil extraction

The seeds were removed from the fruits. The seeds were milled using a grinder to obtain a fine powder, and then they were subjected to Soxhlet extraction using n-hexane for 8 h. After extraction, the solvent was evaporated under reduced pressure at 60 °C.

2.3. Physico-chemical analysis

The refractive index (at 25 °C) and free fatty acids (percentage in oleic acid), were determined according to the standard AOCS method (2009). Unsaponifiable matter was calculated according to the method defined by Sbihi *et al.*, (2013). The theoretical iodine value was calculated according to the method described by Miyake and Yokomizo, (1998). The total protein content was determined according to the official method of the AOAC (2005) by the macro- Kjeldahl method using a sulphate-sodium sulphate-copper catalyst in digestion.

2.4. Fatty acid profile

The Fatty acids were transformed into their corresponding fatty acid methyl esters (FAME) as conferring to the Metcalf and Shmitz, (1966) method, and identified using a gas chromatography (Unicam 4600) equipped with a FID detector. Capillary silica column BPX70 (30 m × 0.22 mm i.d, 0.25 µm film thickness (SGE)) was used as the stationary phase and 0.2 µl of the FAME sample were injected into the chromatograph using a micro syringe. The carrier gas was helium with a head pressure of 18 psi. The injector and detector temperatures were adjusted to 250 and 300 °C, respectively. The oven temperature was programmed to 160 °C for 5 min and subsequently increased by 2.0 °C/min to 200 °C and held for 40 minutes. The FAME samples were identified through comparison of standards (Aldrich or Sigma) and the closest match to the retention time data and mass spectra. The fatty acid patterns were calculated and evaluated from the total identified fatty acids.

2.5. Tocopherol profile

The tocopherol composition was identified according to the AOCS method Ce 8-89 (2009) using high performance liquid chromatography (HPLC). Before to the HPLC analysis, the seed oil was diluted with hexane (0.4:10 w/w) and 20 μ L samples were injected. The samples were analyzed with a (Waters, USA), equipped with a fluorescence detector. The operating conditions of the fluorescence detector were λ excitation 295 nm and λ emission 330 nm. A normal phase column 5 Micron column (4.6 \times 250 mm) was used with hexane/isopropanol (99.5/0.5 v/v) as mobile phase. The system was operated isocratically at a flow rate of 1.3 mL/min. The identification of tocopherols (α , β , γ and δ -tocopherol) was conducted by comparing the HPLC retention time with those of standard compounds (Supelco, Bellefonte, USA) under the same operating conditions. The quantification of tocopherols was based on external standard curves of standard solutions of α , β , γ and δ -tocopherol.

2.6. Total phenolic content

The total phenolic content was determined using Folin-Ciocalteu according to the method described by Kaur and Kapoor, 2002. The results of total phenolic content were estimated using a calibration curve with Gallic acid (Sigma-Aldrich, St Louis, EUA) and were expressed as mg of Gallic acid equivalents per gram of extract.

2.7. Total flavonoid content

The total flavonoid content was determined using aluminum chloride (AlCl_3) with quercetin as standard (Ordonez *et al.*, 2006). In brief, 400 μ L of crude extract were added to 0.3 ml distilled water followed by 5% NaNO_2 (0.03 ml). After 5 min of incubation at 25 $^\circ\text{C}$, AlCl_3 (0.03 ml, 10%) was added. Then, 0.2 ml of 1 mM NaOH was added to the reaction mixture. Finally, the reaction mixture was diluted to 1 ml with water and the absorbance was measured at 510 nm. The total flavonoid content was calculated from a calibration curve and the results were expressed as mg quercetin QE/g of extract.

2.8. Antioxidant activity

2.8.1. DPPH assay

The antioxidant activity was measured in accordance with the DPPH (2,2'-diphenyl-1-picrylhydrazyl) free radical scavenging method (Akroum *et al.*, 2010). Radical scavenging activity was evaluated based on the following equation:

$$\text{Percent radical scavenging activity} = (\text{Abs control} - \text{Abs sample}) / \text{Abs control} \times 100$$

2.8.2. β -carotene-linoleic acid assay

The antioxidant activity of the extract was determined using the β -carotene linoleic acid model system (Jayaprakasha and Singh, 2001). The carotene solution was prepared by dissolving 0.2 mg / mL in chloroform. All the samples were assayed in triplicate. The antioxidant activity (AA) was calculated in terms of the percentage inhibition relative to the control, using the following equation:

$$\text{AA} = (\text{R}_{\text{control}} - \text{R}_{\text{sample}}) / \text{R}_{\text{control}} \times 100$$

2.9. Chlorophyll and total carotenoids

The chlorophyll and carotenoid contents were identified according to the methods described by Allalout *et al.*, (2009) with some modifications.

3. RESULTS

3.1. Seed and oil physico-chemical characteristics

The seed and oil physico-chemical characteristics are given in Table 1. The seeds contain 26.40% yellow oil. The results indicated that *C. tinctoria* was also rich in protein with 14% protein content. The unsaponifiable matter and the free fatty acid percentages were found to be 0.32 and 2.41%, respectively. In addition, the refractive index was equal to 1.47. The iodine value was found to be 170.10 g/100 g oil.

3.2. Total phenolic, flavonoid, pigment contents and antioxidant activity

As shown in Table 2, the total phenolic content was 151.70 mg Gallic acid per 100 g oil. The total flavonoid content was found to be 1.17 mg/g oil.

TABLE 1. Physico-chemical properties of oils extracted from *Chrozophora tinctoria*

Physico-chemical parameters	Unit	Content
Weight of 1000 seeds	g	15 \pm 0.98
Oil extraction yield	%	26.40 \pm 0.29
Protein	%	14 \pm 0.25
Color		yellow
State at ambient temperature		liquid
Refractive index (25 $^\circ\text{C}$)		1.47 \pm 0.04
Unsaponifiable matter	(%, w/w)	0.32 \pm 0.02
Free fatty acid	as oleic %	2.41 \pm 0.49
Iodine value	g/100 g oil	170.10 \pm 2.60

Mean value \pm standard error (n = 3).

TABLE 2. Total phenolic, chlorophyll, and carotenoid contents and antioxidant activity of *Chrozophora tinctoria* seed oil.

OIL PROPERTIES					
Cl (mg/100 g oil)	β -Carotene linoleic acid Inhibition (%)	TCC (mg/100 g oil)	TPC (mg GAE/100 g oil)	TFC (mg QE/100 g oil)	AA (%) (DPPH)
0.92±0.13	42±0.57	29.45±2.97	151.70±0.61	1.70±0.02	45±3.11

Cl: Chlorophylls; TCC: Total carotenoid content; TPC: Total phenol content; TFC: Total flavonoid content; AA: Antioxidant activity. Mean value \pm standard error (n = 3).

The DPPH percentage after 30 min was found to be 45%, which suggests high activity in neutralizing free radicals. The β -carotene/linoleate model system has been used as an important assay to assess the efficacy of anti-oxidative constituents for the inhibition of linoleic-acid-induced oxidation of β -carotene. *C. tinctoria* oil was capable of retarding the oxidation of β -carotene in the emulsion system (Table 2). The inhibition percentage of β -carotene degradation as expressed by percentage of discoloration of *C. tinctoria* oil extract was 42%.

The results indicated that chlorophyll and carotenoid concentrations were 0.92 and 29.45 mg/100 g oil, respectively (Table 2).

3.3. Fatty acid composition

The fatty acid composition of *C. tinctoria* seed oil is shown in Table 3. According to the obtained results, the main fatty acids were unsaturated fatty acids, accounting for 91.45% of the total oil, including monounsaturated (oleic and palmitoleic acid) and polyunsaturated fatty acids (linolenic and linoleic acid). Hence, *C. tinctoria* seed oil contains high amounts of polyunsaturated fatty acids, such

TABLE 3. Fatty acid composition of oil extracted from *Chrozophora tinctoria* seeds.

Fatty acid	Content (%)
Palmitic acid (C16:0)	5.32±0.047
Palmitoleic acid (C16:1)	0.11±0.50
Stearic acid (C18:0)	3.15±0.19
Oleic acid (C18:1)	13.99±0.29
Linoleic acid (C18:2)	76.68±0.16
Linolenic acid (C18:3)	0.67±0.13
Σ UFA	91.45
Σ SFA	8.43
Σ MUFA	14.10
Σ PUFA	77.35
Σ UFA : S SFA	10.85
Σ MUFA: SPUFA	0.18

Note: UFA, unsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids. Mean value \pm standard error (n = 3).

as linoleic acid, as the main fatty acid. The main fatty acids were identified as linoleic (76.68%), oleic acid (13.99%), palmitic acid (5.32%) and stearic acid (3.15%), (Table 3).

3.4. Tocopherol composition

The tocopherol contents of the *C. tinctoria* seed oils are presented in Table 4. The major tocopherol found in the seed oils were α , δ - and γ -tocopherols and their amounts were found to be 4.20, 70 and 12.30, respectively. *C. tinctoria* seed oil showed high concentrations of total tocopherols (87.35 mg/100g).

4. DISCUSSION

Seed oil content is an important indicator to measure industrial production efficiency. The high amount of oil in the seeds of this plant, as compared to other oilseeds, can be considered as an advantage for this plant to be used in different industrial applications. In terms of protein percentage, the current consequences are in agreement with those found by Mirghani *et al.*, 1996 and Hussein and Mirghani, 2006). Hence *C. tinctoria* seeds have potential as a suitable source for animal feed due to fits high protein content.

The free fatty acid content in the extracted oil was observed to be less than that in *C. brocchiana* seed oil, which was reported by Hussein and Mirghani, (2006). Lower free fatty acid is associated with longer shelf-life in oils. Consequently, the oil can be classified among liquid oils and can be used extensively in the food industry. The refractive index of the *C. tinctoria* oil in our study was similar to *C. brocchiana* as reported by Ahmed (2015).

TABLE 4. Vitamin E (tocopherols) composition of oil from *Chrozophora tinctoria* seeds.

Compounds	Concentration (mg/100 g oil)
α -tocopherol	4.2±0.45
β -tocopherol	nd
γ -tocopherol	12.3±0.91
δ -tocopherol	70.0±1.11
Total Tocopherols	87.5±1.37

Mean value \pm standard error (n = 3).

Phenolic compounds play a significant role in flavor and extend the shelf-life of oil (Jorge and da Silva, 2015). The phenolic content obtained in the present experiment was higher than that of many other oilseeds. In a study done by Kozłowska *et al.*, (2016), on the phenolic content in anise, nutmeg, white mustard, coriander and caraway determined 2.52, 3.21 and 1.50 and 0.20 and 0.78 mg g⁻¹ oil, respectively. In addition, it has been described that higher phenolic content can protect oil against oxidation (Kozłowska *et al.*, 2016). Flavonoids are polyphenol compounds that possess antioxidant properties. Carotenoids and chlorophylls are the key pigments in plants. In addition, these pigments are essential elements in auto-oxidation and photo-oxidation processes (Mínguez-Mosquera *et al.*, 1991).

These pigments act as antioxidants in dark conditions (Psoimiadou and Tsimidou 2002) and have medicinal and biological properties (Ranalli *et al.*, 2000). Furthermore, *C. tinctoria* oil is yellow in color due to its high carotene, apocarotenal and annato contents, which are used in oil industries (Oomah *et al.*, 2000).

These results recommend that the plant can be used as a good source of edible oil. Stearic and palmitic acid as saturated fatty acids accounted for 8.43% of the total oil. Similar results have been reported by other researchers who indicated that linoleic, oleic, stearic, and palmitic acids were the main fatty acids in *C. brocchiana* oil (Ahmed 2015). Similar outcomes have been obtained for other plant species such as *Caryodendron orinocense* with 75.13% linoleic acid (Alfaro 1994) and *C. plicata* with 59.3% linoleic acid (EL Bassam 2013). The reported linoleic level in *C. tinctoria* oil was significantly higher in comparison to other oilseeds. MUFA: PUFA, an indicator of oil autoxidation, was found to be 0.18, much less than other oils (Asnaashari *et al.*, 2015; Hashemi *et al.*, 2018).

The results showed that *C. tinctoria* seed oil has high levels of tocopherols, and this content is higher than what has been obtained in oils such as sunflower (44.0 mg/100g), sesame (33.0 mg/100g), groundnut oil (17.0 mg/100g) grape (24–41 mg/100g), palm (26 mg/100g) and saffron (24–67 mg/100g), according to the Codex Alimentarius Commission (1999; 2009). Tocopherols are produced in plants in variable levels, and antioxidant activity varies between individual compounds. Tocopherols possess a protective effect against the oxidative stress related to metabolic syndrome and they are also essential for regular neurological function (Dias, 2012).

5. CONCLUSION

One of the most important findings in this study is the introduction of *C. tinctoria* as a new source of plant oil with a high yield (26.40%). The

results also indicated that *C. tinctoria* oil is rich in unsaturated fatty acids (91.45%). In addition, the oil has a high level of polyunsaturated fatty acids (77.35%), which is important in terms of health and medicine. The main fatty acids were C18:2 (76.68%) and C18:1 (13.99%). *C. tinctoria* seeds are a rich source of vitamin E (tocopherol) (87.50 mg/100 g oil). Furthermore, its high total phenolic and flavonoid contents suggest that this oil is beneficial for resistance against oxidation, which leads to higher storage time. Overall, *C. tinctoria* oil can be used as a raw material oil in several industries in the near future.

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