

GC-MS Analysis, phytochemical characterization, antioxidant, antifungal, and antimicrobial activities of *Carthamus tinctorius* oil

Análise GC-MS, caracterização fitoquímica, atividades antioxidante, antifúngica e antimicrobiana do óleo de *Carthamus tinctorius*

Análisis GC-MS, caracterización fitoquímica, actividades antioxidante, antifúngica y antimicrobiana del aceite de *Carthamus tinctorius*

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Abdelkader Gadoum

Lecturer in Genetics, Molecular Physiology, and Plant Microbiology at the University of Tissemsilt, Algeria. Agronomy and Environment Laboratory, Department of Natural and Life Sciences, Faculty of Science and Technology, Tissemsilt University
Laboratory of Biology and Physiology of Organisms, University of Science and Technology Houari Boumediene
Algeria

E-mail: abdelkadergadoum@yahoo.fr, gadoum.abdelkader@univ-tissemsilt.dz

Dyhia Boukirat

Lecturer in Ecosystem Protection at the University of Tissemsilt, Algeria
Agronomy and Environment Laboratory, Department of Natural and Life Sciences, Faculty of Science and Technology, Tissemsilt University
Tissemsilt City. Algeria

Henni Asma

PhD Student in Animal Production at the University of Tissemsilt, Algeria
Agronomy and Environment Laboratory, Department of Natural and Life Sciences, Faculty of Science and Technology, Tissemsilt University
Tissemsilt City. Algeria

E-mail: asmahenni37@gmail.com, asma.henni@univ-tissemsilt.dz

Mohamed Chahbar

Lecturer in Animal Production at the University of Tissemsilt, Algeria
Agronomy and Environment Laboratory, Department of Natural and Life Sciences, Faculty of Science and Technology, Tissemsilt University
Tissemsilt City. Algeria

E-mail: chahbar_mohamed@yahoo.com

Ahmed Adda

Professor in Plant Breeding and Biotechnology at the University of Tiaret, Algeria
Laboratory of Plant Biotechnology and Genetic Improvement, Ibn Khaldoun University, Faculty of Natural and Life Sciences, Department of Agronomic Sciences, Tiaret. Algeria

E-mail: adda2ahmed@yahoo.fr

ABSTRACT

The current work aimed to determine the antioxidant and antimicrobial activities of *Carthamus tinctorius* seed oil, also called safflower, from the Tiaret region (Algeria). The primary goals of the present study were to identify the chemical composition of the oil by gas chromatography coupled with mass spectrometry analysis, as well as the biological activities, especially antioxidant and antimicrobial activities. Seeds were carefully harvested, identified, and then subjected to Soxhlet apparatus extraction. From the GC-MS profile, it was observed that oil was rich in bioactive principles dominated by such compounds as benzyl chloride (22.01%), hexanal (7.61%), and 2,4-decadienal (15.74%), known to possess antioxidant activity besides providing sensory quality to oils. Antioxidant activity was assayed by using DPPH and ABTS assays, which presented the maximum inhibition of 75.82% at 500 µg/mL concentration, with an IC₅₀ calculated at 351.98 µg/mL, indicating a good capacity for neutralizing free radicals. Therefore, according to the results, safflower oil is a beneficial natural antioxidant agent in the food and cosmetic industries. However, antimicrobial action tested against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* did not have any essential effect, evidenced by the absence of inhibiting zones; hence, it is of low efficiency against such strains. This may be attributed to the low concentration of volatile antibacterial compounds in safflower oil. In conclusion, while *Carthamus tinctorius* oil has a high potential in terms of antioxidant action, antimicrobial efficiency remains low. More focused studies are probably required to consider improved extraction methods or the supplementation of synergistic compounds to enhance the overall effectiveness.

Keywords: *Carthamus tinctorius*, antioxidants, GC-MS, antimicrobial activity, DPPH, essential oil.

RESUMO

O presente trabalho teve como objetivo determinar as atividades antioxidantes e antimicrobianas do óleo de semente de *Carthamus tinctorius*, também chamado de cártamo, da região de Tiaret (Argélia). Os principais objetivos do presente estudo foram identificar a composição química do óleo por cromatografia gasosa acoplada à análise de espectrometria de massa, bem como as atividades biológicas, especialmente as atividades antioxidante e antimicrobiana. As sementes foram cuidadosamente colhidas, identificadas e, em seguida, submetidas à extração em aparelho Soxhlet. A partir do perfil de GC-MS, observou-se que o óleo era rico em princípios bioativos dominados por compostos como cloreto de benzila (22,01%), hexanal (7,61%) e 2,4-decadienal (15,74%), conhecidos por possuírem atividade antioxidante, além de proporcionar qualidade sensorial aos óleos. A atividade antioxidante foi avaliada por meio dos ensaios DPPH e ABTS, que apresentaram a inibição máxima de 75,82% na concentração de 500 µg/mL, com um IC₅₀ calculado em 351,98 µg/mL, indicando uma boa capacidade de neutralizar os radicais livres. Portanto, de acordo com os resultados, o óleo de cártamo é um agente antioxidante natural benéfico para os setores de alimentos e cosméticos. No entanto, a ação antimicrobiana testada contra *Staphylococcus aureus*, *Escherichia coli* e *Candida albicans* não teve nenhum efeito essencial, evidenciado pela ausência de zonas de inibição; portanto, é de baixa eficiência contra essas cepas. Isso pode ser atribuído à baixa concentração de compostos antibacterianos voláteis no óleo de cártamo. Em conclusão, embora o óleo de *Carthamus tinctorius* tenha um alto potencial em termos de ação antioxidante, a eficiência antimicrobiana permanece baixa. Provavelmente, são necessários estudos mais focados para considerar métodos de extração aprimorados ou a suplementação de compostos sinérgicos para aumentar a eficácia geral.

Palavras-chave: *Carthamus tinctorius*, antioxidantes, GC-MS, actividad antimicrobiana, DPPH, aceite esencial.

RESUMEN

El presente trabajo tenía como objetivo determinar las actividades antioxidante y antimicrobiana del aceite de semilla de *Carthamus tinctorius*, también llamado cártamo, procedente de la región de Tiaret (Argelia). Los objetivos principales del presente estudio eran identificar la composición química del aceite mediante cromatografía de gases acoplada a análisis de espectrometría de masas, así como las actividades biológicas, especialmente las actividades antioxidante y antimicrobiana. Las semillas se recolectaron cuidadosamente, se identificaron y se sometieron a extracción con el aparato Soxhlet. A partir del perfil GC-MS, se observó que el aceite era rico en principios bioactivos dominados por compuestos como el cloruro de bencilo (22,01%), el hexanal (7,61%) y el 2,4-decadienal (15,74%), conocidos por poseer actividad antioxidante además de aportar calidad sensorial a los aceites. La actividad antioxidante se evaluó mediante los ensayos DPPH y ABTS, que presentaron una inhibición máxima del 75,82% a una concentración de 500 µg/mL, con un IC₅₀ calculado en 351,98 µg/mL, lo que indica una buena capacidad para neutralizar los radicales libres. Por lo tanto, según los resultados, el aceite de cártamo es un agente antioxidante natural beneficioso en las industrias alimentaria y cosmética. Sin embargo, la acción antimicrobiana probada contra *Staphylococcus aureus*, *Escherichia coli* y *Candida albicans* no tuvo ningún efecto esencial, evidenciado por la ausencia de zonas inhibitorias; por lo tanto, es de baja eficacia contra dichas cepas. Esto puede atribuirse a la baja concentración de compuestos antibacterianos volátiles en el aceite de cártamo. En conclusión, mientras que el aceite de *Carthamus tinctorius* tiene un alto potencial en términos de acción antioxidante, la eficacia antimicrobiana sigue siendo baja. Probablemente se requieran estudios más específicos para considerar la mejora de los métodos de extracción o la suplementación de compuestos sinérgicos para aumentar la eficacia global.

Palabras clave: *Carthamus tinctorius*, antioxidantes, GC-MS, actividad antimicrobiana, DPPH, aceite esencial.

1 INTRODUCTION

Various medicinal plants have been of prime importance in traditional pharmacopoeia for ages in treating various ailments (Gadoum *et al.*, 2019). *Carthamus tinctorius* stands out as one such medicinal plant that has acquired uses from being used as food and medicine in other sectors. Native to the Mediterranean and Asian countries, this plant is currently being cultivated in many parts of the world, including Algeria (Gadoum *et al.*, 2024), mainly for its oil-bearing seeds. This oil, widely used in the agri-food and cosmetic industries, is made up of polyunsaturated fatty acids, among which linoleic acid has particular beneficial effects on cardiovascular and metabolic health. It was documented by such authors as Boukhenoufa *et al.* (2020) and Amine *et al.* (2022).

Increasingly, greater attention has been devoted to the bioactive properties of safflower extracts, mainly their antioxidant, antimicrobial, and anti-inflammatory potentials. A series of studies indicates the potentiality of the bioactive compounds in safflower, unsaturated fatty acids, phenols, and flavonoids to prevent chronic diseases linked to oxidative stress, such as cardiovascular diseases, diabetes, and certain cancers (Bouyahya *et al.*, 2019; Carvalho *et al.*, 2020). These

compounds also contribute to antioxidant activity, which is important for protecting cells from oxidative damage produced by free radicals.

Given that Algeria's climatic conditions and biodiversity are optimal (Gadoum *et al.*, 2021), this country was an ideal destination for cultivating safflower. The zone of Tiaret may be considered one of this plant's central production locations (DJETTI T *et al.*, 2024). In this context, the current research aims to introduce the bioactive potential of *Carthamus tinctorius* seed oil produced in Tiaret: its antioxidant, antifungal, and antibacterial characteristics. New techniques such as GC-MS permit the detailed elucidation of the chemical composition, particularly that of volatile compounds, in this oil, which could be very important for its bioactive properties (Toumi *et al.*, 2011).

Besides this, there is an increased concern about the inappropriate application of artificial additives and antioxidants in food and cosmetic industries (Henni *et al.*, 2024); therefore, there is a need to develop research in this field to find natural alternatives that are less hazardous and sustainable (Muthuvelu *et al.*, 2023). For this reason, vegetable oils, such as safflower, have become one of the most promising alternatives because they are rich in phenolic and antioxidant compounds (Chakradhari *et al.*, 2020). Recent studies have shown that the extracts could be used not only in the conservation of food products but also to enhance health by reducing oxidative stress and inflammation (Carvalho *et al.*, 2020).

Therefore, this work aims to intensely discuss the antioxidant, antifungal, and antibacterial properties of *Carthamus tinctorius* seed oil from Tiaret. This work is part of a broader framework aimed at adding value to plant resources for possible applications in food, cosmetics, and therapeutic industries, bringing scientific contribution to this underexplored plant in the Algerian context.

2 MATERIALS AND METHODS

2.1 PLANT MATERIAL

Carthamus tinctorius, commonly known as Safflower, is a medicinal plant cultivated at the Technical Institute of Field Crops (ITGC, Tiaret), Algeria. The geographical coordinates of this region are 34°11'10.7"N 3°55'26.5"W.

The agronomy and environment laboratory, Department of Natural and Life Sciences, University of Tissemsilt, Algeria, performed the botanical identification of *Carthamus tinctorius* with care. The seed samples were carefully prepared to enable detailed studies concerning their bioactive principles and therapeutic use.

2.2 EXTRACTS PREPARATION

2.2.1 Solvent Extracts

Prepare 25 g of plant material, seeds in this case. This should be ground, placed in porous cartridges suitable for a Soxhlet apparatus, and installed in the Soxhlet setup. Once the solvent reaches boiling point, it is condensed using a balloon condenser. The process is repeated several times through the cartridge until the colour is clear. After collecting the whole extract, which includes both the condensed solvent and the oil—it is sent to a rotary evaporator for oil separation from the recycled solvent. After that, the oil is transferred to hermetically sealed tubes, wrapped in aluminium foil, and left to cool to room temperature (around +4°C).

2.3 PHYTOCHEMICAL ANALYSIS

2.4 GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS) ANALYSIS

Our previous paper (Gadoum *et al.*, 2024) described that we examined samples inside the gas chromatography-mass spectrometry (GC-MS) analytical framework using predetermined operating settings. The injection temperature was maintained at 250°C, while the column temperature was at 55.0°C. The procedure used for injection was "split," with a split ratio of 20:1. With an aggregate flow rate of 17.7 mL/min and a pressure application of 24.4 kPa, flow regulation was maintained by linear velocity. A linear velocity of 35.0 cm/sec, or 0.77 mL/min, was chosen as the column flow rate. An initial purge flow rate of 1.5 mL/min was set. Maintaining temperatures of 200.0°C for the ion source and 250°C for the interface were done separately. We ensured the results were repeatable and reliable by outlining the experimental settings in our previous study.

Our previous research (Gadoum *et al.*, 2024) used GC-MS to analyse samples. We supplemented this method with additional biological tests to increase the scope of our investigation in this study.

2.5 DETERMINATION OF ANTIOXIDANT ACTIVITY

2.5.1 DPPH (2,2-diphenyl-1-picrylhydrazyl) radical assay

The antioxidant activity of safflower oil was assessed using the method developed by Yen *et al.* (1995). During the measurement of antioxidant activity, sample solutions were made at 100, 200, 300, 400, and 500 µg/mL concentrations. For this, 1 mM solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanol was mixed with samples and positive control. After incubation for 30 minutes at 37°C, their absorbance was determined with a UV-Vis spectrophotometer at $\lambda = 517$ nm. The inhibition of the DPPH radical determined the antioxidant activity of the sample. The blank contained distilled water and the solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in

methanol at the concentration of 1 mM. Antioxidant activity of the sample was expressed as % inhibition of DPPH radical absorption, determined using an equation expressed as :

$$\% \text{inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100$$

The IC₅₀ values are used to calculate the standards and test samples required to inhibit DPPH free radicals by 50%. Data obtained on antioxidant activities are expressed as IC₅₀ values calculated from regression analysis of concentration, X-axis, versus % inhibition, Y-axis.

2.5.2 ABTS method's (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid))

Utilising the methods established by Khan *et al.* (2012), the antiradical activity was evaluated through the ABTS cation radical decolorisation assay. Seven nanomoles of ABTS were solubilised in 7 nanolitres of distilled water. An ABTS cation radical solution was prepared by combining an equal volume of ABTS stock solution with a 2.45 mM potassium persulfate solution. After that, the mixture was incubated in the dark at room temperature for 12 to 16 hours. Before use, the ABTS solution was diluted with ethanol until achieving an absorbance of 0.700 ± 0.02 at 734 nm. Different quantities of extract (100 - 200 - 300 - 400 - 500 µg/mL) were mixed with 1.5 mL of ABTS solution to accomplish this objective. Absorbances were recorded at 734 nm following a 10-minute incubation in a dark environment at ambient temperature. The IC₅₀ and Percentages of Inhibition (PI) values convey the findings.

2.6 ANTIFUNGAL ACTIVITY

This experiment observed the development of the *Candida albicans* strain using oat extract. This was done using a method based on the confrontation between wells with an extract added to them and the PDA disc with *Candida albicans* mycelium. First, PDA medium was prepared in Petri dishes. Following its complete solidification, a mycelium disc was placed opposite the wells designated to receive the extract.

2.7 ANTIBACTERIAL ACTIVITY

The minimum inhibitory concentration of the synthesized ionic liquid was determined by the procedure of Feo *et al.* (1998). Three bacteria grown on Mueller-Hinton agar were incubated at 37°C for 18 to 24 hours. Under microaerophilic conditions, they were suspended in a physiological solution and adjusted to the 0.5 McFarland scale. A 0.10 mL aliquot of each suspension was

transferred into a Petri dish containing Mueller-Hinton agar. The described volume was distributed homogeneously on the agar surface with a spreader until the microorganisms were adsorbed. Following this, a well was created using the sterilized tip of a 6 mm diameter Pasteur pipette, and this well was filled with the extract to be tested. All the Petri dishes were incubated at 37°C under microaerophilic conditions for 24 h. Then, after the incubation period, bacterial growth inhibition zones were measured with a calliper. All experiments were done in triplicate.

3 RESULTS AND DISCUSSION

The Oil Yield

The oil yield extracted from the *Carthamus tinctorius* seeds from Tiaret, ITGC, was 14.98 % $\pm$ 2.84 per 100 g of raw material. The yield is in good agreement with data from the literature in general about safflower oil because the yield of oil extraction ranges between 10_% and 20_%, depending on the mode of extraction, and can also be affected by environmental conditions and the quality of seeds (Amine *et al.*, 2022). It is considered satisfactory to obtain an extraction yield of about 15_% in the extraction of oil from safflower seeds, thus inferring a good oil content of the seeds, probably due to the richness of lipid compounds in this variety.

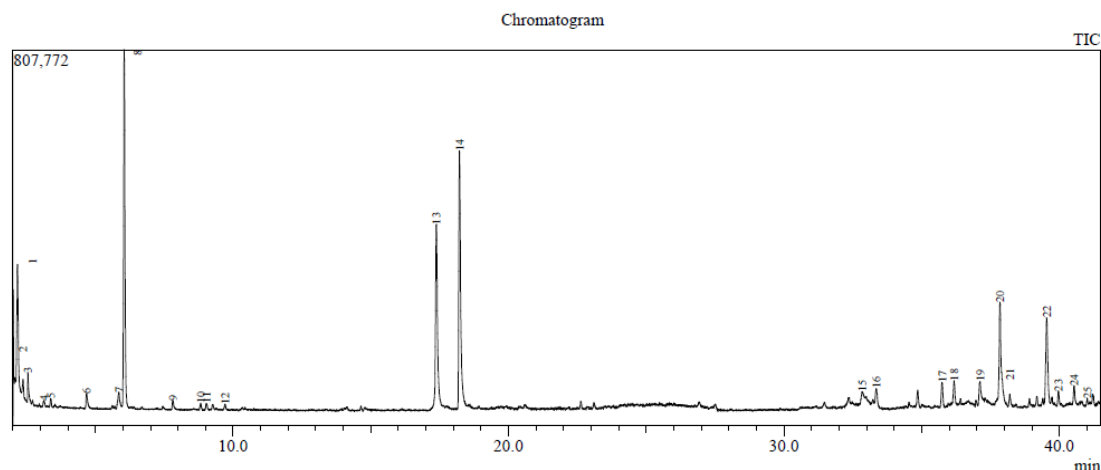
Optimisations may be necessary to maximise oil extraction at an industrial level due to the possibility of modest heterogeneity in seed composition or extraction conditions, as indicated by this standard deviation $\pm$ 2.84.

3.1 GC-MS IDENTIFICATION

The GC-MS analysis of *Carthamus tinctorius* seed oil from Tiaret, as detailed in the study by Gadoum *et al.* (2024), indicates a complex chemical composition characterised by a predominance of aldehydes, hydrocarbons, and volatile alcohols (Fig.1). The gas chromatography-mass spectrometry results for the seed oil from *Carthamus tinctorius* cultivated in Tiaret (ITGC) reveal a profile distinguished by a significant presence of aldehydes, hydrocarbons, and volatile alcohols.

The primary components identified are benzyl chloride (22.01_%), hexanal (7.61_%), and 2,4-decadienal (15.74 % and 20.61%). These compounds are essential to the oil's sensory and antioxidant characteristics.

Figure 1: GCMS spectrum of *Carthamus Tinctorius* seed oil from ITGC Tiaret.



These results further substantiate the conclusions of Bouyahya *et al.* (2019) and Carvalho *et al.* (2020), who examined the effects of chemicals like 2,4-decadienal on oils' stability and sensory attributes. The findings in our paper, Gadoum *et al.* (2024), facilitate additional research to enhance comprehension of this oil's chemical interactions and prospective commercial applications.

The chromatographic peaks and their retention periods, regions, percentages, heights, chemical names, and base mass-to-charge ratios were recorded. The recognised chemical classes include aldehydes, hydrocarbons, acids, ketones, and alcohols. The primary components are hexanal, octane, 2-pentanone, 4-hydroxy-4-methyl-, hexanoic acid, and benzyl chloride (Table 1). These chemicals constitute the principal constituents of *Carthamus tinctorius* seed oil.

Table 1: Analysis of *Carthamus tinctorius* seed oil from Tiaret (ITGC). Source: Prepared by Gadoum (2024).

	Retention Time (min)	Area	Area %	Height	Compound Name	Base m/z
1	2,173	931288	7,61	264505	Hexanal	44
2	6,049	2693131	22,01	768220	Benzyl chloride	91,05
3	17,38	1926165	15,74	391738	2,4-Decadienal, (E,E)-	81
4	18,22	2521523	20,61	550727	2,4-Decadienal, (E,E)-	81
5	37,842	1047399	8,56	211490	1-Heptatriacotanol	43
6	39,537	955075	7,81	186592	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	149,05

Hexanal, which imparts the distinctive aroma of freshly cut grass, and 2,4-decadienal, an aldehyde possessing antioxidant capabilities, substantially enhance the oil's durability and preservation attributes. Our results correspond with recent research (Boukhenoufa *et al.*, 2020; Khlifi *et al.*, 2021), highlighting the significance of volatile aldehydes in the stability and oxidation of vegetable oils. Furthermore, as compared to other oils, Tiaret oil has a lower level of polyunsaturated fatty acids and a higher content of aromatic compounds (Ez-Zoubi *et al.*, 2023).

3.2 ANTIOXIDANT ACTIVITY

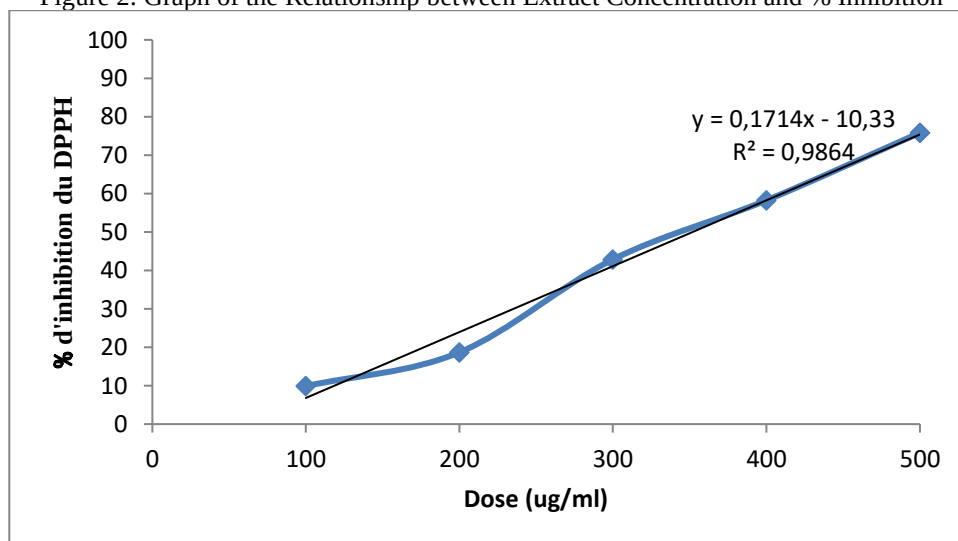
The DPPH method is used to evaluate antioxidant activity. This method depends on the ability of antioxidant chemicals to form a stable pair with the DPPH free radical by hydrogen atom transfer. A UV-Vis spectrophotometer can measure the amount of colour change at 517 nm caused by this reaction. An estimate of the antioxidant capacity can be obtained by using this absorbance value to determine the % suppression of the radical activity using a specific formula (Table 2).

Table 2: DPPH Inhibition Rate (%) Source: Prepared by Gadoum (2024).

Concentration (ug/ml)	<i>Cartamus oil</i> % Inhibition
100	9,89010989
200	18,68131868
300	42,85714286
400	58,24175824
500	75,82417582

This curve represents the percentage inhibition of DPPH with various doses of the extract expressed in µg/mL. The linear regression line was plotted using the equation $y = 0.1714x - 10.33$ and coefficient of determination $R^2 = 0.9864$, reflecting strong positive interrelations between extract concentration and antioxidant activity (Fig.2). This means that with increasing doses of the extract, there is practically a linear increase in the percentage inhibition of DPPH. In this case, the slope of line one is 0.1714, and the inhibition sensitivity is according to their concentrations. This result evidences the antioxidant efficiency of the extract, whose inhibition has reached circa 80 % at the highest dose tested, which suggests a high capacity to neutralize free radicals.

Figure 2: Graph of the Relationship between Extract Concentration and % Inhibition



Source: Prepared by Gadoum (2024).

The IC₅₀ value is the concentration of extract required to inhibit 50% of the DPPH radical. It is determined by an exponential equation modelling the percentage of inhibition as a function of extract concentration. Hence, the IC₅₀ of the safflower extract is 351.98 µg / mL.

Antioxidant activity by the DPPH test of *Carthamus tinctorius* seed oil (safflower oil) shows the linearity between the extract concentration and the inhibition percentage. As seen from the regression equation $y = 0.1714x - 10.33$ and the determination coefficient $R^2 = 0.9864$, the inhibition percentage reaches almost 80% at 500 µg/mL concentration. This is because of the presence of polyunsaturated fatty acids, such as linoleic acid, among other bioactive compounds, that are well-documented for their strong antioxidant action.

Recent research confirms that safflower oil has excellent antioxidant potential, close to all other vegetable oils rich in unsaturated fatty acids and phenolic compounds. These were confirmed by Boukhennoufa *et al.* (2020) and Carvalho *et al.* (2020). Due to its high content of fatty acids and phenolic compounds, safflower oil can reduce free radicals; therefore, it may be helpful in the food and cosmetic industries for preventing lipid oxidation and skin ageing. This would, however, need further studies to compare the antioxidant properties with other vegetable oils and an in-depth analysis of bioactive compounds to optimize their industrial use (Bertella *et al.*, 2021; Bazioli *et al.*, 2019, Walid *et al.*, 2024).

Analysis of the Antioxidant Activity of Safflower Extract Using the ABTS Method

The ABTS test of the antioxidant activity of safflower extract showed that there was highly significant concentration-dependent inhibition of the ABTS⁺ cation radical; thus, the % inhibition ranged between 9.86%, at a concentration of 100 µg/mL to 78.87% at 500 µg/mL, demonstrating an apparent dose-response relationship (Table 3).

Table 3. Percentage Inhibition of ABTS⁺ Cation by Safflower Oil (%)

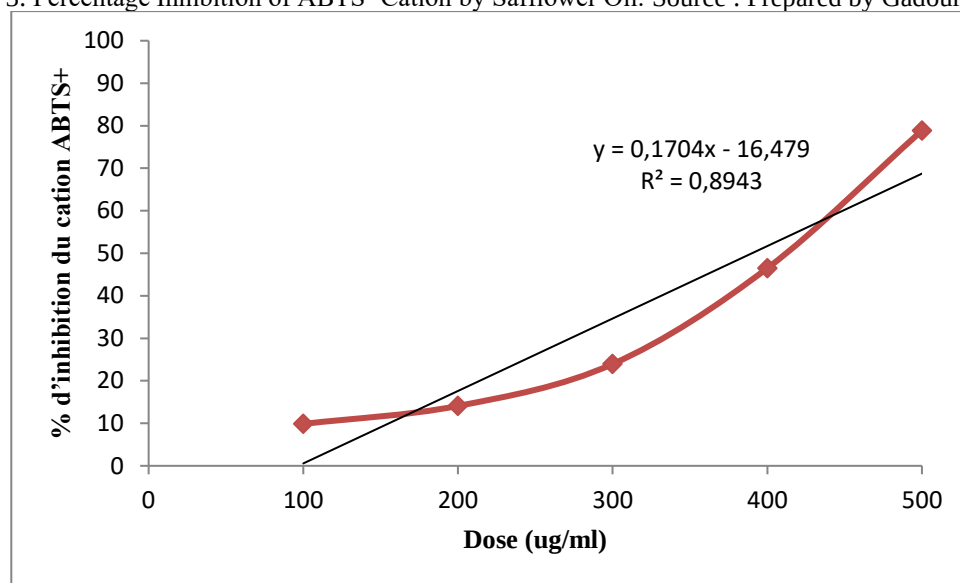
Concentration (ug/ml)	Safflower Oil
	% Inhibition
100	9,85915493
200	14,08450704
300	23,94366197
400	46,47887324
500	78,87323944

Source: Prepared by Gadoum (2024).

This trend suggests that active compounds in the safflower extract are capable of effectively neutralising free radicals. Their activity increases with rising concentration (Fig. 3). In comparison with other plant extracts, which have been researched in recent literature and which also show

antioxidant properties, such as green tea or rosemary extract, the safflower extract has moderate antioxidant activity, represented by the IC₅₀ value of 390.13 µg/mL (Amine *et al.*, 2022). The lower the IC₅₀, the higher the antioxidant efficacy. However, it is particularly remarkable because of its rich composition of phenolic compounds, among which avenanthramides are highly known for their antioxidant and anti-inflammatory action (Khlifi *et al.*, 2021).

Figure 3: Percentage Inhibition of ABTS⁺ Cation by Safflower Oil. Source : Prepared by Gadoum (2024).



The IC₅₀ value is the concentration of the extract that inhibits 50% of the ABTS⁺ cation. This value was calculated using an exponential equation to model the percentage inhibition as a function of oil concentration. Accordingly, the IC₅₀ of safflower oil is 390.13 µg/mL.

Compared to other extracts with a lower IC₅₀ value, such as fruits or plants highly rich in polyphenols, safflower may be an exciting alternative in the food and cosmetic industries in general due to its high tolerance and nutritional value. Moreover, potential optimization studies on the extraction of bioactive compounds could enhance safflower's antioxidant capacity (Boukhennoufa *et al.*, 2020). Thus, while safflower ranks relatively low in a direct comparison with other plant sources of antioxidants, its profile makes it of interest for various applications, ranging from the prevention of food product oxidative stress to integration into natural cosmetic products.

3.3 ANTIFUNGAL ACTIVITY

Our study examined the antifungal activity of safflower extract on *Candida albicans*. Its efficacy was not reported, and the results were negative.

In vitro evaluation of the antifungal efficacy of the safflower extract against *Candida albicans* exhibited no activity. This suggests that the extract of safflower does not contain active

principles capable of inhibiting this pathogenic fungus's growth. This observation is maintained by several studies undertaken of late, which have established that certain plant extracts, while effective as either antioxidants or antimicrobials, may not necessarily exhibit direct antifungal activity against *Candida albicans* (Bouyahya *et al.*, 2019). Safflower is composed mainly of fibres, proteins, and lipids; it could thus lack these secondary antifungal metabolites like highly bioactive terpenes or phenols found in other medicinal plants known to exhibit antifungal activity, such as thyme or oregano (Benbrahim-Tallaa *et al.*, 2020). It would be interesting to explore other fractions or methods of extraction in order to find out if an antifungal activity would become evident by adjusting the concentrations or with other, more labour-intensive means of purification (Bertella *et al.*, 2021).

3.4 ANTIBACTERIAL ACTIVITY

The inhibition zone diameters of bacterial growth, according to the antibacterial activity estimation scale developed by Moreira (Ponce *et al.*, 2005, Soumia *et al.*, 2024)), are classified into resistant ($D < 8$ mm), sensitive ($9 \text{ mm} < D < 14$ mm), susceptible ($15 \text{ mm} < D < 19$ mm), and extremely sensitive ($D > 20$ mm). The following table represents the results obtained for the oat extract against antibacterial activities on the solid medium by the well diffusion method (table 4).

Table 4: The inhibition zone diameters of bacterial growth.

Strains	Safflower Oil			
	Diameter (mm)	Inhibition Zone (%)	Germ Sensitivity	Transcription
<i>Staphylocoque Blanc</i> Gram+ (S1)	0±0	0	-	Résistant
<i>Proteus mirabilis</i> Gram- (S2)	0±0	0	-	Résistant
<i>Citrobacter Koseri</i> Gram- (S3)	0±0	0	-	Résistant

Source: Prepared by Gadoum (2024).

The results obtained from the evaluation of safflower oil's antibacterial activity, using the excellent diffusion method on a solid medium, reveal a total absence of inhibitory activity against the three bacterial strains tested: *Staphylococcus albus* (S1), *Proteus mirabilis* (S2), and *Citrobacter koseri* (S3). No zone of inhibition was observed, 0 mm, which makes, by the scale of Moreira (Ponce *et al.*, 2005) safflower oil "resistant" since $D < 8$ mm. Such results highlight that, under the conditions of the present study, safflower oil is devoid of crucial antibacterial activity against the Gram-positive bacteria under study. These study results indicate no detectable antibacterial action

of safflower oil was expressed against the bacterial strains studied. Because safflower oil is characterized mainly by a high content of polyunsaturated fatty acids, the bioactive presence of other responsible compounds for antibacterial properties and terpenes or volatile compounds may be deficient. In contrast with most plant oils, which are rich in monoterpenes and sesquiterpenes, where most antimicrobial effects have been widely established, oil appears less effective based on the results obtained.

In general, the antimicrobial activity of safflower oil is fragile compared to results obtained in studies concerning other vegetable oils. For instance, very effective inhibition of the growth of bacteria was detected in some authors concerning thyme and oregano oils, which contain thymol and carvacrol in high proportions (Bouyahya *et al.*, 2019, Rachida *et al.*, 2024). Furthermore, it has been presented that terpene-rich essential oils exhibit antibacterial solid effects against six strains of Gram-positive and Gram-negative bacteria (Ouchelli *et al.*, 2020). That may also be because safflower oil has no antibacterial activity due to some volatile or phenolic compounds in a concentration too low to induce an antibacterial effect.

Finally, while vegetable oils like coconut or extra virgin olive oil achieve great renown for the antimicrobial properties of their significant contents, lauric acid or polyphenols, respectively, there is no notable observed efficacy in safflower oil. Further research could thus be directed toward the extraction and processing necessary to concentrate the bioactive compounds and, possibly this way, to enhance the antibacterial properties of this oil (Bertella *et al.*, 2021).

4 CONCLUSION

This work has emphasized that the seed oil from *Carthamus tinctorius* cultivated in Tiaret, Algeria, is very promising as an antioxidant, mainly because of its high content of polyunsaturated fatty acids and other volatile bioactive compounds. Hexanal, benzyl chloride, and 2,4-decadienal were identified among the significant functional compounds with the help of GC-MS. In vitro antioxidant assays showed a remarkable ability for free radical scavenging with an IC₅₀ value similar to those of other vegetable oils; it could, therefore, be of interest to health and cosmetic industries.

However, the oil's antifungal and antibacterial activities were limited, which generally indicates that, though highly effective in antioxidant action, safflower oil, per se, is less active against microbial infection. Further studies may develop an advanced extraction technique to enhance the extraction yield and assess the possibility of incorporating safflower oil with other antimicrobial agents for extended therapeutic applications.

Carthamus tinctorius oil has emerged as a potential natural product against an antioxidant drug; hence, it is supposed to find its place in industries that are looking for purely natural products rather than synthetic compounds. Further research should be directed toward optimizing extraction techniques and studying synergistic effects with other bioactive ingredients to maximize therapeutic benefit.

This study provides significant new insights into the GC-MS analysis, phytochemical characterisation, and bioactivities of *Carthamus tinctorius* oil. However, it is essential to recognise specific limitations. The study was performed in controlled laboratory environments, which may not accurately represent real-world conditions. A restricted range of strains was employed to evaluate antibacterial and antifungal activities, thereby omitting a wider array of diseases. Conventional in vitro techniques, which fail to account for the complex interactions present in vivo, were utilised to evaluate antioxidant activity. Furthermore, despite their significant importance for pharmacological and therapeutic applications, the potential cytotoxicity of the oil remain unexamined.

To help to overcome these limitations, future studies should focus on several important areas. Initially, the spectrum of bacterial and fungal strains assessed should be expanded to verify the effectiveness of the oil against many different diseases. Second, in vivo studies have to be conducted to provide a better knowledge of the biological effects of the oil inside complex living systems. Thirdly, to assess the medicinal efficacy of *Carthamus tinctorius* oil, it is essential to examine its cytotoxicity and safety profile. Furthermore, research on the stability and bioavailability of the oil in pharmaceutical formulations would enhance its utility. Ultimately, examining the synergistic effects of *Carthamus tinctorius* oil with additional natural constituents may enhance its overall efficacy.

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