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Original Article

Analysis of the Chemical Composition and Evaluation of the Antioxidant, Anti-inflammatory and Hemolytic Activities of the Essential Oil and its Oxygenated Fraction of *Chrysanthemum coronarium*

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Abstract

Introduction: The main objective of this research was to study the chemical composition, as well as the antioxidant, anti-inflammatory, and hemolytic activities of the aerial part of the essential oil of *Chrysanthemum coronarium* and its isolated oxygenated fraction.

Materials and Methods: The oxygenated fraction was isolated from the oil by column chromatography. Analyses by GC coupled with mass spectrometry allowed for the characterization of these components. The antioxidant properties of the essential oil and its oxygenated fraction were evaluated using the DPPH and FRAP methods. The anti-inflammatory effect was characterized by the denaturation of proteins. In addition, the hemolytic impact was studied through a suspension of erythrocytes in human blood.

Results: The essential oil studied was characterized by a composition dominated by oxygenated monoterpenes (39.3%), followed by hydrocarbon sesquiterpenes (21.1%) and oxygenated sesquiterpenes (16.5%). The oxygenated fraction consisted mainly of perillyl alcohol (14.3%) and lyratyl acetate (14.5%). The results of the DPPH and FRAP tests showed significant antioxidant activity of the essential oil (IC_{50} = 3 ml/L) and the oxygenated fraction (IC_{50} = 4.4 ml/L), surpassing that of the synthetic antioxidant BHT. Additionally, the oxygenated fraction exhibited perfect anti-inflammatory activity, compared to essential oil and diclofenac sodium. The toxicity assessment on human erythrocytes shows that both essential oil and oxygenated fraction of *Chrysanthemum coronarium* have a very low hemolysis rate even at high concentrations.

Conclusions: Tests on the essential oil and its oxygenated fraction have revealed promising properties. These results suggest promising opportunities for the development of new agents in the pharmaceutical field.

Keywords: Oxygenated Fraction, Antioxidant Activity, Anti-inflammatory Activity, Hemolytic Effect, Egg Albumin

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Introduction

A therapeutic approach focused on the reduction of chronic inflammation and oxidative stress can provide benefits in the treatment of various diseases, including cardiovascular diseases, autoimmune diseases, type 2 diabetes, and cancer. These conditions are often associated with these two physiological processes.[1,2] The use of synthetic drugs has been shown to carry side effects and health risks, which vary according to the disease treated, dosage, and individual reaction of each patient.[3] In contrast, herbal medicine, an ancient approach adopted in various world cultures, relies on the use of plant extracts. These extracts contain a range of bioactive compounds, such as flavonoids, terpenes, and alkaloids, which are likely to have positive health effects. These compounds exhibit various biological activities, positioning them as a promising source in the search for new therapeutic agents.[3] Essential oils, rich in terpenoid compounds, are of significant importance in the pharmaceutical, cosmetic and food sectors, with diversified applications.[4] Their use includes the formulation of fragrances and aromas, as well as applications in the field of physical and mental health.[5] These oils also play a crucial role in food preservation[6] and are incorporated into aromatherapy practices.[7] In the context of cardiac rehabilitation, various essential oils are deployed to optimize the quality of sleep of patients.[8] At the same time, they are used as insecticides, antimicrobial agents, and anti-inflammatory agents.[5,9]

C. coronarium, commonly known as *chrysanthemum* garland, daisy crown or edible *chrysanthemum*, is a species of flowering plant in the Asteraceae family. It is native to the Mediterranean region, but has been naturalized in various

parts of the world. The plant is cultivated not only for its ornamental value, but also for its edible leaves and flowers.[10] In traditional medicine, *C. coronarium* has been used for its potential medicinal properties. Some cultures believe it possesses anti-inflammatory and antioxidant properties. Various studies have been conducted to characterize the chemical composition of the essential oil and extracts of *C. coronarium*, as well as to evaluate their biological properties. The essential oil has shown antiproliferative effects *in-vitro* on various lines of human cancer cells, with increased sensitivity especially in the context of colon cancer.[11] Additionally, its anti-inflammatory potential has been shown to be comparable to that of Diclofenac, a well-established non-steroidal anti-inflammatory.[12] To our knowledge, no chemical and pharmacological studies have yet been undertaken to characterize the chemical composition of the isolated oxygenated fraction of *C. coronarium* essential oil. Thus, the present study was conducted in order to determine the chemical composition of the essential oil of *C. coronarium* as well as its oxygenated fraction. The ultimate goal was to evaluate their antioxidant and anti-inflammatory activities to discover new biologically active agents. Additionally, the hemolytic potential of these two extracts was evaluated using appropriate tests. These investigations aim to enrich the understanding of the pharmacological properties of the essential oil of *C. coronarium*, particularly regarding its oxygenated fraction, and to identify potential benefits in the context of antioxidant activity, anti-inflammatory effects and hemolytic potential.

Materials and Methods

Plant Materials and Extraction

The plant material used in this study originates from the aerial part of *C. coronarium*. Harvesting took place between late April and early May 2022 in Tlemcen (Algeria). Botanical identification of the plants was carried out at the Department of Ecology and Ecosystem Management of the University of Tlemcen. The essential oils were extracted using a hydrodistillation process employing a Clevenger-type apparatus. The extraction process was conducted for an average of 5 hours to ensure efficient oil extraction.

Isolation of the Oxygenated Fraction

The essential oil was divided into two distinct parts, an apolar fraction and an oxygenated fraction, by separation by column chromatography. The apolar fraction was isolated using a mobile phase composed of 100% pentane, while the oxygenated fraction was obtained using diethyl ether as the mobile phase. We are particularly interested in studying the oxygenated fraction.[13]

Identification of Components

Gas chromatography (GC) analysis was performed using a Perkin Elmer Auto system XL GC apparatus equipped with two capillary columns (60 m x 0.22 mm i.d. 0.25 µm film thickness). The oven temperature was regulated for an increase from 60 °C to 220 °C at 2 °C/min and then kept isothermally for 35 min at 230 °C. The temperatures of the detector and injector continued at 280 °C. The injection volume was 0.1 µL. Gas chromatography-mass spectrometry (GC/MS) was carried out using a Perkin Elmer Turbo mass detector coupled to a Perkin Elmer Autosynstem XL equipped with dual fused silica capillary columns, which functioned with the same Gas chromatography mentioned above except for the split was 1/80. The ion source temperature was 150 °C, the ionization energy (IE) was 70 eV, and the mass spectra were acquired with a mass range of 35 to 350 Da. The identification of components was conducted using two main approaches. Firstly, it involved comparing their GC retention indices (RI) on nonpolar and polar columns, which were determined in relation to the retention time of a series of n-alkanes through linear interpolation. This comparison was made with reference to authentic compounds or literature data.[14,15] Secondly, computer matching was employed, utilizing commercial mass spectral libraries[16,17] and spectra were compared with those stored in our in-house laboratory library.

Determination of Antioxidant Activity

2,2-diphényl 1-picrylhydrazyle (DPPH) is a relatively stable free radical that absorbs in the UV-Visible range at 515-520 nm. In order to measure or verify the free radical scavenging capacity of molecules known antioxidants, we use DPPH, which turns yellow when it is reduced. According to the protocol described by Que et al[18], 1 ml of various concentrations of samples ranging from (1 mg/ml to 50 mg/ml)) was mixed with 1 ml of the ethanolic solution of DPPH (0.1 mM). After 30 minutes of incubation at room temperature and in the dark, the antioxidant activity was measured at 517 nm against blank and the standard antioxidant (BHT). The percentage of anti-radical activity was calculated by the following equation:

$(\%) = [(Ab_{control} - Ab_{sample})/Ab_{control}]*100$

Where; Ab: absorbance

Iron Reduction Test (Reducing Ferric Antioxidant Strength: FRAP)

The method used for the samples was based on the one described by Ainseba[19] with some modifications. One milliliter of the extract at different concentrations (0.5 to 30 ml/L) was mixed with 2.5 ml of phosphate buffer (0.2 M, pH = 6.6) and 2.5 ml of a 1% $K_3Fe(CN)_6$ potassium ferricyanide solution. The mixture was then centrifuged at 3000 rpm for 10 min. At the end, 2.5 ml of supernatant from each concentration was mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (0.1%). After 30 min of incubation,

absorbances were measured at 700 nm using a visible dual beam spectrophotometer against ethanol (80%) as a vacuum. BHT was used as a positive control. Analyses were performed in triplicate.[20]

In-vitro Anti-inflammatory Activity

According to the method of protein denaturation, the *in-vitro* activity was assessed. A mixture of 0.2 ml of fresh egg albumin, 2.8 ml of phosphate-buffered saline pbs (pH = 6.4) and 2 ml of different concentrations of samples was incubated for 15 minutes at 37 °C and then heated in a hot water bath at 70 °C for 5 minutes. After cooling, the absorbance was measured at 660 nm.[20] while diclofenac sodium was used as the reference drug.[21] The percentage inhibition of protein denaturation was determined by using the following formula:

inhibition (%) = $[(A_{bt} / A_{bc}) - 1)]*100$

Where: A_{bt}: the absorbance of the sample, A_{bc}: the absorbance of control

Evaluation of the Hemolytic Activity

Hemolytic activity was evaluated as previously described by Andra et al.[22] with a slight modification. Red blood cells (erythrocytes) were centrifuged and washed with phosphate buffered saline solution (PBS: 1.5 mM KH_2PO_4, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 135 mM NaCl, pH-7.4) to remove residual plasma. The suspended red blood cells were washed in saline PBS to obtain a concentration of 2%. A series of concentration of the extract were prepped. 150 µl of extract mixed with PBS were incubated with 150 µl suspension of human erythrocytes for 60 minutes at 37 °C. After 10 minutes of incubation the solution was centrifuged to separate the lytic red blood cells from the plasma. The release of hemoglobin in plasma was measured by spectrophotometry at a wavelength of 540 nm. Hemolytic activity was determined using the following formula.

$$\text{Hemolysis (\%)} = \frac{A_{(\text{extract})} - A_{(\text{Negative control})}}{A_{(\text{Positive control})}} \times 100$$

Statistical Analysis

All experiments were performed in triplicate (n = 3). Data were expressed as means ± standard deviation (SD). Statistically significant differences between experimental groups were considered when p <0.05. The concentration that achieved 50% inhibition (IC_{50}) was calculated by nonlinear regression with the use of Microsoft Office Excel.

Results

Chemical Composition of Essential Oil

The essential oil, characterized by its bluish hue and narcotic aroma, was extracted by hydrodistillation of the dry matter, demonstrating a yield of 0.1%. Analysis of this oil allowed the identification of 56 compounds, representing 91.3% of its total composition. The characterization of the compounds was performed by comparing the mass spectra (EI-MS) and the retention indices (IR) with the mass spectral library. The predominant composition of the essential oil studied consists mainly of oxygenated monoterpenes, representing 39.3% of the total. Hydrocarbon sesquiterpenes contribute 21.1%, while oxygenated sesquiterpenes account for 16.5% of the overall composition (Table 1).

Table1. Chemical Composition of Essential Oil and Oxygenated Fraction of *C. coronarium*

N°[a]	Compounds	IR[b]	IR[c]	% EO[d]	% OF[e]	Identification[f]
1	(E)-2-Hexenal	829	1206	-	2.3	RI. MS
2	α-Pinene	934	1016	0.2	-	RI. MS
3	Myrcene	982	1153	0.6	-	RI. MS
4	Yomogi alcohol	985	1381	0.4	-	RI. MS
5	Limonene	1021	1196	0.7	-	RI. MS
6	Artemisia ketone	1041	1349	tr	5.1	RI. MS
7	Nonanal	1081	1396	-	0.6	RI. MS
8	Linalool	1081	1545	0.8	tr	RI. MS
9	Chrysanthenone	1096	1480	-	0.9	RI. MS
10	Camphor	1120	1516	12.5	3.1	RI. MS
11	trans-Chrysanthenol	1130	1268	-	1.7	RI. MS
12	Citronellal	1133	1495	0.2	tr	RI. MS
13	Pinacarvone	1139	1644	5.5	tr	RI. MS
14	Isomenthone	1140	1495	1.9	tr	RI. MS
15	cis-Chrysanthenol	1146	1253	-	3.6	RI. MS
16	Borneol	1148	1776	0.9	7.1	RI. MS
17	Lyratol	1159	1780	0.2	0.9	RI. MS
18	Terpinen-4-ol	1163	1589	0.2	Tr	RI. MS
19	Cymene-8-ol	1169	1833	0.4	tr	RI. MS
20	Terpineol	1177	1714	0.2	-	RI. MS
21	Decanal	1180	1499	-	0.3	RI. MS
22	Geranyle isovalerate	1202	1663	0.1	tr	RI. MS
23	Pulegone	1212	1620	0.1	tr	RI. MS
24	trans-Chrysanthenyl acetate	1240	1699	0.3	0.1	RI. MS
25	cis-Chrysanthenyl acetate	1247	157	4.8	0.8	RI. MS
26	Lyratyl acetate	1259	1630	3.5	14.5	RI. MS
27	Bornyl acetate	1264	1572	1.1	6.3	RI. MS
28	Lavandulol acetate	1270	1597	0.2	tr	RI. MS

29	Perillyl alcohol	1275	2006	2.8	14.3	RI. MS
30	Myrtenyl acetate	1326	1729	0.8	3.6	RI. MS
31	Verbenone	1358	1604	0.7	-	RI. MS
32	Caryophyllene	1380	1593	3.1	-	RI. MS
33	6.8-Nonadien-2-one. 6-methyl-5-(1-methylethylidene)	1404	1750	1.1	-	RI. MS
34	Bergamotene	1414	1581	0.5	-	RI. MS
35	Farnescene	1446	1771	6.2	-	RI. MS
36	α-Farnesene	1459	1687	1.5	-	RI. MS
37	Lyratyl isovalerate	1472	1801	4.4	-	RI. MS
38	Germacrene-D	1476	1782	5.1	-	RI. MS
39	Murolene	1490	1740	0.4	-	RI. MS
40	β-Ssquiphallandrene	1516	1768	3.1	-	RI. MS
41	α-Cadinene	1525	1715	0.6	-	RI. MS
42	(E)-α-Bisabolene	1532	1750	0.4	-	RI. MS
43	cis. trans Farnesene	1548	1734	0.2	-	RI. MS
44	Nerolidol	1552	1843	0.3	-	RI. MS
45	cis-3-Hexenyl benzoate	1559	1971	5.3	-	RI. MS
46	Spathulenol	1566	2107	1.1	-	RI. MS
47	Pinocarveol	1571	1683	0.7	-	RI. MS
48	Caryophyllene oxide	1579	1905	2.9	0.6	RI. MS
49	Shyobunol	1586	2262	0.2	-	RI. MS
50	Isoaromadendrene	1590	1616	0.2	-	RI. MS
51	Viridiflorol	1598	2064	0.8	-	RI. MS
52	Acorenol	1610	2123	0.3	-	RI. MS
53	Di-epi-1.10-cubenol	1612	2056	-	3.6	RI. MS
54	Epi-cis-sesquisabinene hydrate	1622	2156	2.2	-	RI. MS
55	τ-Cadinol	1636	2253	1.3	3.6	RI. MS
56	α-Cadinol	1643	2259	0.6	3.5	RI. MS
57	Neryl tiglate	1650	2055	-	1.9	RI. MS
58	Bisabolol	1670	2218	2.1	6.2	RI. MS
59	Tetradecanoic acid	1756	2650	-	1.6	RI. MS
60	Hexadecanoic acid	1954	2820	-	2.8	RI. MS
61	Geranyl-α-terpinene	1957	2218	0.8	-	RI. MS
62	Methyl linoleate	2106	2596	0.3	-	RI. MS
63	Linoleic acid methyl ester	2108	2596	-	0.9	RI. MS
64	Phytol	2131	2675	2.5	1.2	RI. MS
65	Tricosan	2302	2316	0.3	-	RI. MS
66	Heptacosan	2504	2500	0.5	-	RI. MS
67	Pentacosan	2500	2500	3.2	-	RI. MS
	Total			**91.3**	**91.1**	
Hydrocarbon monoterpenes %				1.5	-	
Oxygenated monoterpenes %				39.3	62.0	
Hydrocarbon sesquiterpenes %				21.1	-	
Oxygenated sesquiterpenes %				16.5	19.4	
Diterpenes %				3.3	1.2	
Non-terpenic compounds %				9.6	8.5	
Total oxygenated compounds %				55.8	81.4	

[a]Order of elution is given on apolar column (Rtx-1); [b]Retention indices of literature on the apolar column (lR); [c]Retention indices on the polar Rtx-Wax column (RIp); [d]EO: Essential oil roots; [e]OF: Oxygenated fraction; [f]RI: Retention indices; MS: Mass spectrometry in electronic impact mode

Table 2. Inhibition (%) of DPPH of *C. coronarium* Essential Oil and the Oxygenated Fraction

Samples		**Antioxydant activity**				
BHT	Concentration (ml/L)	2.5	5	10	25	50
	I%	18.6±0,8	30.4±1,6	35.9±0,8	55.9±0,3	84.7±0,9
	IC_{50} (ml/L)			**22.3**		
HE CR	Concentration (ml/L)	2.5	5	10	25	50
	I%	34.7±0,6	59.8±0,7	78.7±0,6	83.9±0,1	89.3±1,1
	IC_{50} (ml/L)			**4.4**		
FOXCR	Concentration (ml/L)	1.5	3	6	12.5	25
	I%	39±0,2	50±0,1	82.2±0,4	87.4±0,3	90.4±1,4
	IC_{50} (ml/L)			**3.0**		

FOXCR: Oxygenated fraction of *C. coronarium*; HECR: Essential oil of *C. coronarium*

The main components of oxygenated monoterpenes in this essential oil mainly include camphor (12.5%), followed by pinacarvone (5.5%), cis-chrysantheenyl acetate (4.8%), lyratyl acetate (3.5%) and trans-chrysanthenyl acetate (3.1%). For hydrocarbon sesquiterpenes, the second predominant class, farnescene (6.2%), germacrene-D (5.1%) and caryophyllene (3.1%) were identified. The oxygenated sesquiterpenes were present in smaller quantities, representing about 12.5% of the total composition of the oil. They consisted mainly of caryophyllene oxide (2.9%), epi-cis-sesquisabinene hydrate

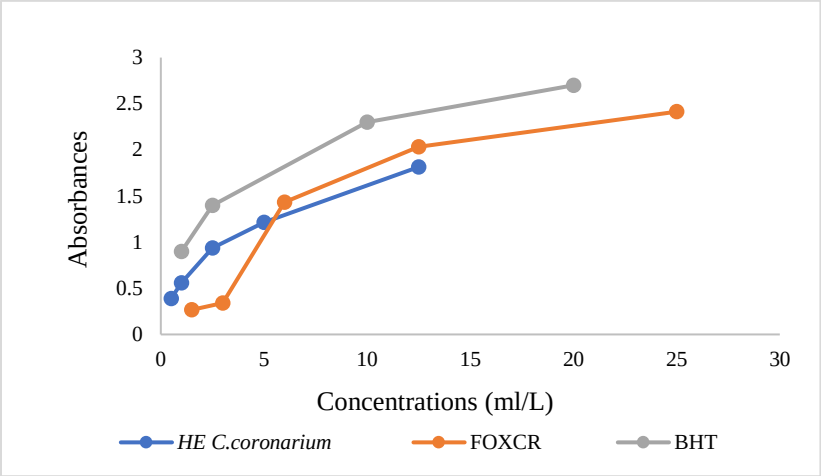

Figure 1. Reducing Power of the Essential Oil (HE) and Oxygenated Fraction (FOX) of *C. coronarium.*

(2.2%) and bisabolol (2.1%). Additionally, the essential oil included a diterpene, phytol, which made up about 3.3% of its composition.

Chemical Composition of the Oxygenated Fraction Isolated from the Essential Oil of C. coronarium

The essential oil fractionation process revealed the identification of 11 additional oxygenated compounds. These include three monoterpene alcohols, namely cis-chrysanthenol (3.6%), trans-chrysanthenol (1.7%), and di-epi-1,10-cubenol (3.6%). The oxygenated fraction also includes a ketone, chrysanthenone (0.9%), two aldehydes, namely (E)-2-hexenal (2.3%) and decane (0.9%), as well as an ester, neryl tiglate (1.9%). Additionally, it contains two carboxylic acids: hexadecanoic acid (2.8%) and tetradecanoic acid (1.6%). The majority composition of the oxygenated fraction was characterized by the notable presence of perillyl alcohol (14.3%), lyratyl acetate (14.5%), borneol (7.1%), bornyl acetate (6.3%), bisabolol (6.2%), artemisia ketone (5.1%), cis-chrysanthenyl acetate (4.8%), τ-cadinol (3.6%), myrtenyl acetate (3.6%), and α-cadinol (3.5%) (Table 1).

Evaluation of the Antioxidant Activity of C. coronarium Essential Oil and the Oxygenated Fractions

The results of the study of the free radical trapping activity DPPH of the essential oil of *C. coronarium* and the oxygenated fraction when compared to that of the synthetic compound BHT, revealed a very good antioxidant activity in comparison to the latter. The results of the free radical trapping activity revealed that the oxygenated fraction (90.4%) and the essential oil (89.2%) exhibited excellent antioxidant activity at a concentration of 50 ml/L. The oxygenated fraction demonstrated the highest activity in neutralizing the DPPH radical with an IC_{50} of 3 ml/L, followed by *C. coronarium* essential oil (4.4 ml/L). These values were about 7 and 3 times lower than those of the reference synthetic antioxidant BHT, with an IC_{50} of 22.3 mL/L, respectively (Table 2). The FRAP method test demonstrated an increase in antioxidant activity relative to concentration (Figure 1). In comparison to BHT, the oxygenated fraction exhibited intriguing activity, albeit lower than that of BHT. At higher concentrations, the *C. coronarium* essential oil displayed less activity compared to its oxygenated fraction (Figure 1).

Evaluation of the Anti-inflammatory Activity

The anti-inflammatory effect of the essential oil and the oxygenated fraction, along with that of the standard anti-inflammatory drug (Diclofenac sodium), was assessed using the egg albumin denaturation method. The results show an inhibition of protein denaturation, including albumin, which is depending on the concentration of the samples. The observations reveal that both extracts have a significant inhibitory effect, with inhibition rates of 90.4% for the oxygenated fraction and 89.3% for the essential oil at a concentration of 50 ml/ L. These performances were comparable to those of diclofenac, which reaches an inhibition rate of 84.7% at the same concentration (Table 3). The concentration at which the sample or diclofenac achieves 50% inhibition (IC_{50}) was determined by estimating the percentage inhibition relative to the treatment concentration. A comparison of the results of anti-inflammatory activity revealed that the oxygenated fraction (IC_{50} = 8.6 ml/L) exhibited better activity than the reference diclofenac (IC_{50} = 13.3 ml/L), while the essential oil showed the lowest activity with an IC_{50} of 23.1 ml/L (Table 3).

Hemolytic Activity

According to the data in Table 4, it is observed that essential oil and oxygenated fraction of *C. coronarium* induce dose-dependent hemolysis. After 60 minutes of incubation, the hemolysis rate for both extracts varies between 1.2% and 8.2%. This level remains relatively low compared to gallic

acid, whose maximum concentration of 2000 µg/mL causes a very powerful hemolytic effect, reaching about 80%. Notably, at the maximum concentration of 2000 µg/mL, the oxygenated fraction has the lowest hemolytic effect, with a percentage of 6.1%, followed by the essential oil, which has a percentage of 8.2% (Table 4).

Table 3. Chemical Composition of Essential Oil and Oxygenated Fraction of *C. coronarium*

Samples	Anti-inflammatory activity						IC_{50} (ml/L)
Essential oil	Concentration (ml/L)	2	5	10	20	30	40	
	Inhibition of denaturation (%)	3.4±0.4	18.4±1.2	28.1±0.4	43.2±1.4	62.3±1.4	85.6±2.3	23.1
Oxygenated fraction	Concentration (ml/L)	2	5	10	20	30	40	
	Inhibition of denaturation (%)	30.7±1.2	46.1±0.8	57.1±0.4	75.2±2.2	82.1±2.1	94.9±2.6	8.6
Diclofenac sodic	Concentration (µg/ml)	5	10	15	20	25	30	
	Inhibition of dĭnaturation (%)	29.9±0.8	40.6±1.6	55.7±0.9	65.7±0.6	80.4±1.2	86.1±1.4	13.3

Samples and positive control were done in triplicates (n = 3). Values expressed are means ± S.D

Table 4. Results of the Hemolytic Activity of Essential Oil and Oxygenated Fraction of *C. coronarium*

Concentration (µg/ml)	% Hemolysis of essential oil	% Hemolysis of oxygenated fraction	% Hemolysis of gallic acid
300	1.2±0.6	1.4±0.3	38.9±0.6
600	1.6±0.6	1.9±0.2	48.9±0.5
900	2.8±0.4	1.7±0.2	58.9±0.6
1000	6.9±0.5	1.7±0.1	78.9±0.5
2000	8.2±0.2	6.1±0.3	80.1±0.8

Samples and positive control were done in triplicates (n = 3). Values expressed are means±S.D

Discussion

Many studies have examined the chemical composition of *C. coronarium*. However, it is important to note that the composition of the essential oil of *C. coronarium* can vary significantly, both in quantity and quality, depending on the genotype, geographical origin and environmental conditions. For example, in samples from Spain, the main constituents of the essential oil were camphor (29.2%), α-pinene (14.8%), lyratyl acetate (9.8%), and β-pinene (8.5%).[23] On the other hand, the capitula of *C. coronarium* of Tunisian origin had a different essential oil composition, dominated by cis-chrysanthényle acetate (21.82%), trans-chrysanthényle acetate (12.78%), (E)-β-farnésène (8.97%), germacrene-D (8.92%), and camphor (6.03%).[24] In Jordan, camphor (17.5%) was the main component of *C. coronarium* capitula, while santolina triene (4.3%), neoiso-3-thujanol (5.6%), cis-chrysantheyl acetate (10.8%), perillary aldehyde (11.7%), isoitalicene (4.7%), and phenylpropyl butanoate (4.9%) were among the main constituents of the essential oil.[25] Indeed, several studies have demonstrated that essential oils can prevent and ameliorate oxidative stress under both physiological and pathological conditions. Additionally, research has indicated that essential oils rich in oxygenated compounds possess protective effects against oxidative stress, inflammation, diabetes, and cancer.[26,27] GC-MS analysis of the essential oil and oxygenated fraction identified a high concentration of oxygenated compounds, including oxygenated monoterpenes and oxygenated sesquiterpenes. According to various reports, oxygenated compounds have already demonstrated a more effective antioxidant potential or equivalent to those of synthetic antioxidants such as butylated hydroxyanisole (BHA) or butylated hydroxytoluene (BHT).[28,29] These properties are probably related to their chemical profile, especially the relatively high percentage of compounds such as perillyl alcohol, lyratyl acetate and camphor.[30,31] Due to their redox properties, these compounds play a role as reducing agents, suppliers of hydrogen and monoxygene. Moreover, the intensity of the antioxidant activity could be associated with the effect generated by the interaction of all the components present in the hydrosol extract, whether they are present in significant proportions or in more limited quantities. The antioxidant properties demonstrated by oxygenated fraction and essential oil represent a very promising prospect for food preservation, since the adverse effects of synthetic antioxidants, such as liver damage or carcinogenesis, are widely recognized. The suppression of inflammation can represent a crucial strategy for the prevention and treatment of cancer. The results of the *in-vitro* evaluation of the anti-inflammatory properties of the essential oil and its oxygenated fraction, as presented in this study, indicate that the oxygenated fraction exhibits a more pronounced anti-inflammatory activity than the essential oil. This activity appears to be linked to the chemical structure, highlighting the significant role of oxygenated terpenes.[32] Regarding the chemical composition of our oxygenated fraction, it consists of more than 81% oxygenated terpenes, while the essential oil consists of a lower percentage (55.8%), confirming the high activity of the oxygenated fraction. Previous research by Mezza et al.[33] has shown that the fraction of oxygenated monoterpenes presents in rosemary essential oil exhibits more significant antioxidant activity than other isolated fractions of this same essential oil. In addition, the oxygenated fraction extracted from the essential oil of *Anacyclus valentinus* revealed an antioxidant

activity even more pronounced than that of the essential oil as a whole.[34] The essential oil and terpenoid fraction of *Helichrysum italicum* had a higher antimicrobial activity compared to the terpene fraction. Antimicrobial activities of the terpenoid fraction were more pronounced against *Staphylococcus aureus* and *Candida albicans*.[35] On the other hand, these results demonstrate very limited toxicity of essential oil and oxygenated fraction, even at higher concentrations and after an incubation period of 60 minutes, with respect to isolated human erythrocytes (red blood cells).

Conclusion

The study focuses on the analysis of the chemical composition and the evaluation of the antioxidant, anti-inflammatory and hemolytic activities of the essential oil and its oxygenated fraction from the *C. coronarium*. The results of the analysis revealed the predominance of compounds such as perillyl alcohol, lyratyl acetate, borneol, bornyl acetate and bisabolol in the oxygenated fraction. The conclusions of the study indicate that these compounds confer notable properties to the essential oil and its oxygenated fraction in terms of antioxidant and anti-inflammatory activities. Moreover, it has been observed that, even at higher concentrations, these extracts exhibit limited toxicity to red blood cells. These findings suggest that *C. coronarium* could be considered as a potential natural source for the food and pharmaceutical industries, particularly in the treatment of diseases associated with oxidative stress and inflammation. Nevertheless, it is crucial to emphasize the need for extensive research, clinical trials and safety assessments before considering the practical application of these extracts.

Authors' Contributions

BA and DMA design of activity tests and experiments, RIM and DMA performed the experiments, RIM. and BA analyzed and interpreted the data, AN and DMA wrote and edited the article.

Conflict of Interest Disclosures

The authors declare that they have no conflicts of interest.

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