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Influence of Extraction Solvent on Phytochemical Constituents, Phenolic and Flavonoid Contents, and Antioxidant, Anti-Lipid-Oxidation, Anti-Hemolytic, and Anti-Lipoxygenase Activities of Two Algerian Medicinal Plants

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ABSTRACT: The present study investigated the phytochemical composition, antioxidant, anti-peroxidative, antihemolytic, and anti-lipoxygenase activities of *Cistus villosus* and *Globularia arabica* extracts obtained from different plant organs using solvents of increasing polarity (hexane, dichloromethane, methanol, and DMSO). Phytochemical screening revealed a solvent- and organ-dependent distribution of secondary metabolites, with methanol showing the highest efficiency for recovering polar compounds, particularly phenolics, flavonoids, tannins, and glycosides. Methanolic extracts exhibited the highest extraction yields, reaching 43.46% in *G. arabica* leaves/flowers and 23.54% in *C. villosus* leaves/flowers. These extracts also showed the greatest total phenolic and flavonoid contents, with maximum values of 46.99 mg GAE/g DR and 8.54 mg RE/g DR, respectively. Antioxidant evaluation using DPPH, ABTS, and CUPRAC assays demonstrated strong activities of methanolic extracts, especially *C. villosus* leaves/flowers, which reached 2.706 mg VEEAC/mg DR in the DPPH assay. Antioxidant activities were strongly correlated with phenolic composition, with significant correlations observed between TPC and DPPH ($r = 0.884$), ABTS ($r = 0.936$), and CUPRAC ($r = 0.949$) ($p < 0.05$). Methanolic extracts also exhibited the highest lipid peroxidation inhibition, while dichloromethane extracts showed remarkable antihemolytic and anti-lipoxygenase activities. The findings demonstrate that solvent polarity and plant organ selection are key factors influencing bioactive compound recovery and biological properties, highlighting *C. villosus* and *G. arabica* as promising sources of natural antioxidant and anti-inflammatory agents.

1. INTRODUCTION

Plants constitute an important source of bioactive secondary metabolites, particularly phenolic compounds, and flavonoids, which have attracted considerable interest because of their antioxidant and health-promoting properties. These compounds can

scavenge reactive oxygen species (ROS), donate electrons or hydrogen atoms, chelate transition metals, and inhibit oxidative enzymes, thereby protecting lipids and other biological macromolecules from oxidative damage (Arzani et al., 2025; Qamar et al., 2026). Their effectiveness has encouraged the exploration of medicinal plants as natural antioxidants for food, cosmetic, and pharmaceutical applications (Bešlo et al., 2022; Jain et al., 2025).

Lipid peroxidation is a free-radical chain reaction that deteriorates lipid-rich foods and contributes to oxidative damage in biological systems (SLIMENI et al., 2026). Because antioxidant activity depends on both the phytochemical composition of plant extracts and the methods used to evaluate their activity, several complementary assays are generally required to assess their capacity to inhibit lipid oxidation (Wu et al., 2024). At the same time, concerns regarding the safety of synthetic antioxidants such as BHA, BHT, TBHQ, PG, and octyl gallate have increased interest in plant-derived phenolic compounds as safer natural alternatives (N. Zhang et al., 2026). Since the recovery of these bioactive compounds is strongly influenced by extraction conditions, particularly solvent polarity, selecting an appropriate extraction solvent is essential for maximizing their biological potential (Esazadeh et al., 2024).

In this context, this research objectives were to study the effect of extraction solvent on the recovery of phytochemical substances, and the contents of total phenolic and flavonoids extracted from two plant parts (stems, and flowers plus leaves) of *Cistus villosus* L. and *Globularia alypum* L. ssp. Arabica, and to determine how solvent polarity influences their antioxidant, anti-lipid peroxidation, anti-hemolytic, and lipoxygenase inhibitory activities. Furthermore, Pearson's correlation analysis was performed to investigate the relationships between phytochemical contents, extraction solvents, and biological responses, and to identify the main factors influencing the activity profiles of the obtained extracts.

2. MATERIALS AND METHODS

2.1 Chemical materials

All the chemical products and solvents utilized in the experiments were of analytical grade and were purchased from Sigma-Aldrich (USA), Analar Normapur (France), VWR Chemicals Prolabo, Riedel de Haën (Germany), Hexane (C₆H₁₄), methanol (CH₃OH), acetone (C₃H₆O), dichloromethane (CH₂Cl₂), acetic acid (CH₃COOH), hydrochloric acid HCl (37%), dimethyl sulfoxide (DMSO), sodium carbonate (Na₂CO₃) sodium sulfate (Na₂SO₄), Folin Ciocalteu reagent (H₃PW₁₂O₄₀-H₃PMo₁₂O₄₀), aluminum trichloride (AlCl₃), gallic acid (C₆H₂(OH)₃COOH), rutin (C₂₇H₃₀O₁₆), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), potassium iodide (KI), 2,2-azinobis-(3-ethyl-6-benzothiazoline-6-sulfonic acid) (ABTS), 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH), copper(II) chloride (CuCl₂), ammonium acetate (CH₃COONH₄), néocuproïne (C₁₄H₁₂N₂), tertibutylhydroquinone (TBHQ), α -tocopherol, peroxidase, hydrogen peroxide (H₂O₂), potassium chloride (KCl), sodium chloride (NaCl) monobasic potassium phosphate (KH₂PO₄), dibasic sodium phosphate (Na₂HPO₄), linoleic acid (C₁₈H₃₂O₂), lipoxygenase (C₂₀H₃₂O₂), dibasic potassium phosphate (K₂HPO₄), sodium sulphite (Na₂SO₃), sodium hydroxide (NaOH), tris Base [NH₂C(CH₂OH)₃], tween 20 (C₅₈H₁₁₄O₂₆).

2.2 Plant material collection

Aerial parts of the four plants were collected from three distinct geographical sites. The botanical specimens were identified by Professors Amar Djeridane and Brahim Guit from Amar Telidji University of Laghouat, Algeria, and Ziane Achour University of Djelfa, Algeria, respectively. *Cistus villosus* L. (Cistaceae) and *Globularia alypum* L. ssp. Arabica (Globulariaceae) were collected in February 2021 from Madna (Oued Morra) (34° 10'N, 2° 19' E). The specimens were subsequently dried in a shaded environment under ambient laboratory conditions. The flowers with leaves (LF) and stems (S) of the studied plants were separated and washed several times with distilled water. The samples were then dried in a dark and dry place. Next, the samples were ground in a mortar, sieved through a 200 μ m mesh, and stored in polyethylene bottles until analysis.

2.3 Preparation of plant extracts

A mass of 20 g was extracted by Soxhlet using three solvent systems of increasing polarity: hexane; dichloromethane, and methanol, until the solids were exhausted. After each extraction, the solvent used is evaporated under vacuum using a rotary evaporator. The residue recovered is then weighed and solubilized in different volumes of methanol. Cold storage of the methanolic extracts resulted in the formation of precipitates with varying physical appearances. Following filtration, the resulting residues were dissolved in DMSO, to obtain another DMSO fractions. The extracts thus obtained are stored at 4 °C and protected from light, to be used for various analyses.

2.4 Qualitative phytochemical screening

Preliminary phytochemical screening was carried out on the different extracts of *Cistus villosus* and *Globularia arabica* using standard qualitative phytochemical tests to detect the presence or absence of major secondary metabolites, including tannins, alkaloids (Dragendorff, Mayer, and Wagner tests), anthocyanins, flavonoids, sterols and triterpenes, coumarins, saponins, anthraquinone derivatives, reducing compounds, leucoanthocyanins, starch, and glycosides (Feriha et al., 2019). The results were expressed semi-quantitatively as absent (-), weak (+), moderate (++), or strong (+++).

2.5 Total phenolic contents (TPC)

Total phenolic content (TPC) was assessed via the Folin-Ciocalteu colorimetric method, as stated previously by Singleton and Rossi (Singleton & Rossi, 1965). For this purpose, 100 μ L of the plant extract diluted with distilled water was mixed with 500 μ L of 10% Folin-Ciocalteu reagent. After incubation for 2 minutes, an aliquot of 2 mL of a 2% Na_2CO_3 solution was added. The mixture was then incubated in the dark at room temperature for 30 minutes. The absorbance was measured using a spectrophotometer at 760 nm. Gallic acid served as the reference standard. The Folin-Ciocalteu reducing capacity was expressed as milligrams of gallic acid equivalents per gram of dry residue (mg GAE/g DR).

2.6 Total Flavonoid contents (TFC)

The total flavonoid content was evaluated via the Lamaison and Carnat (Lamaison & Carnat, 1990) method, which employed an aluminium trichloride reagent. A volume of 100 μ L from each diluted sample was mixed with 1 mL of AlCl_3 solution 2% and then incubated for 20 minutes in the dark at room temperature. The absorbance was measured at 420 nm. Total flavonoid content was quantified as milligrams of quercetin equivalent (QE) per gram of dry residue (mg QE/g DR).

2.7 Evaluation of in vitro antioxidant properties

Radical-scavenging and reducing properties were evaluated using ABTS, DPPH, and CUPRAC assays, and the results were expressed as mg of vitamin E equivalent capacity (VEAAC) per mg of dry residue, or mg of TBHQ equivalent capacity (TBEAC) per mg of dry residue, using the calibration curves of standards.

2.7.1 DPPH assay

On a test tube, 100 μ L of each diluted extract was mixed with 1000 μ L of DPPH methanolic solution (150 μ M). After incubation in the dark for 30 minutes, the absorbance values were measured at 517 nm (Brand-Williams et al., 1995).

2.7.2 ABTS assay

100 μ L of 20 mM ABTS+ aqueous solution was combined with 100 μ L of peroxidase enzyme prepared in phosphate buffer solution at pH 6.9 (0.2 mg/mL), and 15 μ L of 1 mM hydrogen peroxide (H_2O_2). The total volume was increased to 10 mL by adding distilled water. The mixture was then incubated for 24 h in the dark. Subsequently, 40 μ L of each extract dilution was homogenized with 200 μ L of ABTS+ solution. After incubation in the dark for 7 min, the absorbance was obtained at 417 nm using a 96-well microplate reader (Re et al., 1999).

2.7.3 CUPRAC assay

In a test tubes, 500 μ L of diluted extract was mixed with 500 μ L cupric ion (10 mM), 500 μ L of neocuproin (NCu) (7.5 mM), and 100 μ L acetate buffer (1 M, pH 7.0). After 30 min of incubation at room temperature, absorbance was measured at 456 nm against a blank (Apak et al., 2014).

2.8 In vitro inhibition of lipid peroxidation capacities

2.8.1 Inhibition of sunflower oil and ghee peroxidation assays

The inhibition of lipid peroxidation assay measures sunflower oil and cow ghee oxidation by radicals generated by 2,2-azobis(2-amidinopropane) dihydrochloride (AAPH) added to the solution. The peroxides formed from sunflower oil or cow ghee. Test samples were prepared by mixing 1 mL sunflower oil and cow ghee (5 mg/mL) solution with 1 mL of 0.05 M Tris-HCl buffer, pH 7.4, containing 0.3% Tween-20 and 0.1 mL of extracts or standards at 0.5 mg/mL. All samples were incubated at 50°C for 10 min. Then 0.1 mL of 2% AAPH solution was added, and the samples were vortexed and kept in a water bath at 50°C with no light exposure for 90 min. The mixture is then stirred for 3 minutes in an ice bath. 1 mL of 1% potassium iodide containing

3% glacial acetic acid was added, and the absorbance of the solution was measured at 360 nm using a UV-1601 UV-Visible spectrophotometer (Djokhdem et al., 2016). The results were expressed as % and the calibration curves of standards, vitamin E and TBHQ, was also tested using same protocol and used as positive controls.

2.8.2 AAPH-induced hemolysis assay

Human blood samples were obtained from healthy donors by venipuncture and collected into blood bags equipped with a leukoreduction filter. After filtration, the blood samples were immediately centrifuged at 2000 rpm for 10 min to separate erythrocytes from plasma and the buffy coat. The blood bags were stored at 4 °C until use. The erythrocyte suspension was washed twice with 0.9% NaCl solution and finally with phosphate-buffered saline (PBS, pH 7.4) by centrifugation at 2000 rpm for 10 min. After each washing step, the supernatant was carefully removed to obtain packed erythrocytes. The erythrocytes were then resuspended in PBS, and reaction mixtures containing 2 mL of 10% erythrocyte suspension, 100 µL of plant extract or standard solution, and PBS were pre-incubated for 15 min at 37 °C. Subsequently, 200 µL of AAPH solution (100 mM) prepared in PBS was added, and the mixtures were gently shaken and incubated for 3 h at 37 °C. After incubation, the reaction mixtures were centrifuged at 2000 rpm for 10 min, and the absorbance of released hemoglobin in the supernatant was measured at 540 nm to determine the percentage of hemolysis. Each experiment was performed in triplicate (Bey et al., 2024). The protective effect of the tested extracts against AAPH-induced hemolysis was expressed as mg vitamin E equivalent antioxidant capacity (VEAAC) per mg dry residue and mg TBHQ equivalent antioxidant capacity (TBEAC) per mg dry residue, based on the calibration curves established using vitamin E and TBHQ as reference standards.

2.8.3 Lipoygenase (LOX) inhibition assay

The inhibitory activity of type IB lipoygenase (EC 1.13.11.12) was determined using the spectrophotometric method. In brief, 100 µL of enzyme solution (1000 U/mL, prepared in 0.1 M phosphate buffer, pH 8.0), 100 µL of plant extract, and 2 mL of phosphate buffer (0.1 M, pH 8.0) were mixed. After incubation for 5 min at 37 °C, the enzymatic reaction was initiated by adding 100 µL of linoleic acid solution (10 mM in methanol). The increase in absorbance at 235 nm, corresponding to the formation of conjugated hydroperoxydienes, was continuously monitored for 10 min using a Shimadzu UV-1601 spectrophotometer. A reaction mixture without extract served as the blank reference (Guibadj et al., 2026). The anti-lipoygenase effect of the tested extracts samples was expressed as mg of vitamin E equivalent capacity (VEAAC) per mg of dry residue, or mg of vitamin TBHQ equivalent capacity (TBEAC) per mg of dry residue, using the calibration curves of standards.

2.9 Statistical analysis

Data is presented as a mean SD of three replicates from three independent experiments (n = 3). Analysis was conducted using Excel 2018 and XLSTAT 2018.503. Pearson's correlation coefficient was calculated at a 95% confidence interval. Group comparisons were assessed using one-way ANOVA with Tukey's multiple comparisons test.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Variation Between Plant Species and Organs

The phytochemical screening demonstrated that the extraction solvent strongly influenced the presence and distribution of secondary metabolites in *Cistus villosus* and *Globularia arabica*. Methanol showed the highest extraction capacity, allowing the detection of a wide range of polar compounds, particularly tannins, flavonoids, anthocyanins, glycosides, and phenolic derivatives. In contrast, hexane mainly extracted non-polar constituents such as sterols and triterpenes, while dichloromethane exhibited an intermediate extraction ability, favouring compounds of medium polarity. These results confirm the importance of solvent polarity in the selective extraction and separation of different phytochemical families (Table 1).

Comparison between the two species revealed differences in their phytochemical profiles. *Cistus villosus* generally showed a higher diversity of detected compounds, especially in methanolic extracts, whereas *Globularia arabica* displayed a more selective distribution of metabolites. These variations may be related to differences in plant metabolism, ecological adaptation, and the accumulation of specialized metabolites.

The distribution of compounds also varied according to the plant organ. Leaves and flowers exhibited a richer phytochemical composition compared with stems, which contained fewer detected compounds. The higher abundance of flavonoids, tannins,

and anthocyanins in aerial parts can be attributed to their roles in plant protection, pigmentation, and defense mechanisms. Stems, mainly serving structural functions, generally showed lower levels of these metabolites.

Overall, the results indicate that solvent polarity and plant part selection are critical factors affecting the recovery of phytochemical constituents. Methanol was the most efficient solvent for extracting polar bioactive compounds, while hexane and dichloromethane allowed the selective recovery of less polar molecules. The differences observed between *C. villosus* and *G. arabica* highlight the importance of considering species and tissue specificity in phytochemical investigations.

Table 1. Comparative Analysis of Phytochemical Profiles in Leaves, Stems, and Flowers of *Cistus villosus* and *Globularia arabica*

Plants	Extract	Tannin	Alkaloids			Anthocyanins	Flavonoides	Sterols and triterpenes	Coumarines	Saponines	Free anthraquinone derivatives	Combined anthraquinone derivatives			Reducing compounds	Leuco-anthocyanins	Starch
			Dragendorff	Mayer	Wagner							C-heterosides	O-heterosides	O-heterosides with reduced			
<i>Cistus villosus</i>	CV-LF-He	-	++	+	++	-	-	+	-	-	-	+	-	+	-	-	-
	CV-S-He	-	+	+	+	-	-	+	-	-	-	-	-	+	-	-	-
	CV-LF-Di	+	+	+	+	-	+	-	-	-	-	+	-	+	-	-	-
	CV-S-Di	+	+	+	+	-	+	-	-	-	-	+	-	-	-	-	-
	CV-LF-Me	++	+	+	+	+	+++	-	-	+	-	++	-	++	++	-	-
	CV-S-Me	++	-	-	-	+	+++	-	-	+	-	++	-	++	++	-	-
	CV-LF-Dm	+	-	+	+	-	++	-	-	+	-	+	-	+	+	-	-
	CV-S-Dm	-	-	-	-	-	++	-	-	-	-	-	-	+	+	-	-
<i>Globularia arabica</i>	GA-LF-He	-	++	++	++	-	-	+	-	-	-	-	-	+	-	-	-
	GA-S-He	-	++	++	++	-	-	+	-	-	-	-	-	+	-	-	-
	GA-LF-Di	-	+	+	+	-	+	-	-	-	-	+	-	++	-	-	-
	GA-S-Di	-	+	+	+	-	+	-	-	-	-	+	-	++	-	-	-
	GA-LF-Me	+	-	+	+	++	+++	-	-	+	-	++	-	++	++	-	-
	GA-S-Me	+	-	-	-	++	+++	-	-	+	-	++	-	++	++	-	-
	GA-LF-Dm	+	-	-	-	+	+	-	-	+	-	+	-	+	+	-	-
	GA-S-Dm	+	-	-	-	+	+	-	-	+	-	+	-	+	+	-	-

(-): negative reaction; (+): weakly positive reaction; (++): moderately positive reaction; (+++): strongly positive reaction.

3.2 Yields of extracts, total phenolic and flavonoid contents

Table 2 shows the results of the extraction yield and the contents of total phenolics (TPC), and total flavonoids (TFC) obtained from extracts of *Cistus villosus* (CV) and *Globularia arabica* (GA). For each plant, the aerial part is divided into two parts, leaves and flowers (LF) and stems (S) and each one was extracted successively with hexane (He), dichloromethane (Di), methanol (Me), and DMSO (Dm).

First, the results of the yield proved that methanol is the most effective solvent in comparison to hexane, dichloromethane and DMSO which yielded less than 4%. Also, the methanolic extracts of leaves and flowers (LF) yielded more than stems (S) extracts of all test species, where the highest yield obtained in *Globularia arabica* (43.46%), followed by *Cistus villosus* (23.54%). The differences among the used extraction solvents were statistically significant ($p < 0.001$), emphasizing that solvent polarity markedly influenced extraction yield and phytochemical recovery.

Table 2. Extraction yield, TPC and TFC in the 16 plant extracts.

Plant	Extract	Extraction Yield (%)	TPC (mg GAE/g DR)	TFC (mg RE/ g DR)
<i>Cistus villosus</i>	CV-LF-He	2.947	0.031±0.022 ^A	0.064±0.018 ^A
	CV-S-He	1.667	0.015±0.009 ^A	0.034±0.023 ^A
	CV-LF-Di	0.705	0.411±0.031 ^{AB}	0.435±0.050 ^{AB}
	CV-S-Di	0.399	0.090±0.040 ^A	0.141±0.028 ^A
	CV-LF-Me	23.540	46.994±3.608 ^G	6.681±0.559 ^{GH}
	CV-S-Me	13.180	21.228±2.578 ^E	3.372±0.401 ^{DEF}
	CV-LF-Dm	1.519	1.680±0.418 ^{ABC}	1.568±0.512 ^{ABCD}
	CV-S-Dm	0.200	0.660±0.052 ^{AB}	0.282±0.032 ^A
<i>Globularia arabica</i>	GA-LF-He	3.930	0.027±0.009 ^A	1.276±0.285 ^{ABCD}
	GA-S-He	1.420	0.018±0.008 ^A	0.083±0.022 ^A
	GA-LF-Di	1.971	0.885±0.609 ^{AB}	1.486±0.275 ^{ABCD}
	GA-S-Di	1.555	0.497±0.375 ^{AB}	0.147±0.009 ^A
	GA-LF-Me	43.460	31.481±1.390 ^F	8.535±0.479 ^H
	GA-S-Me	20.756	19.110±0.670 ^E	4.500±0.350 ^{EFG}
	GA-LF-Dm	3.690	3.515±0.487 ^{ABCD}	0.190±0.044 ^A
	GA-S-Dm	0.594	0.290±0.095 ^A	0.096±0.012 ^A
Values of TPC, and TFC are presented as mean ± SD of three replicates from three independent experiments (n = 3); Significant differences among all the extracts are indicated by different letters in the same column according to Tukey's test at $P < 0.0001$; DR: Dry Residue; GAE: Gallic Acid Equivalent; RE: Rutin Equivalent; S: stem; LF: Leaves/Flowers; Me: Methanol; DM: DMSO; DI: Dichloromethane; He: Hexane				

Second, methanolic extracts also exhibited the highest significant ($p < 0.001$) concentrations of total phenolics, and the extracts of leaves and flowers marked a higher quantity of phenolics than stems extracts. The methanolic leaves/flowers extract of *Cistus villosus* marked by the highest TPC (46.99 ± 3.61 mg GAE/g DR), followed by *Globularia arabica* (31.48 ± 1.39 mg GAE/g DR),

whereas the lowest phenolic contents were observed in the hexane extracts. Also, the extraction yield was strongly and positively correlated with TPC ($r = 0.860$, $p < 0.05$), indicating that the higher recovery obtained with methanol was associated with an increased extraction of phenolic compounds (Table 5).

Third, total flavonoid content of the methanolic leaves/flowers extract of *Globularia arabica* (8.54 ± 0.48 mg RE/g DR) exhibited the highest value followed by *Cistus villosus* (6.68 ± 0.56 mg RE/g DR), while the dichloromethane extracts contained substantial amounts of flavonoids. DMSO, also found to be suitable for the quantification of flavonoids especially for *Cistus villosus*. Besides, TFC varied significantly according to the extraction solvent ($p < 0.001$), although the most suitable solvent depended on the plant species.

These results are in agreement with the finding of research on *Hammada elegans* Botsch where methanol proved to be more suitable than hexane, dichloromethane, and chloroform for extracting phenolics (Labioth et al., 2019). Besides this research, an investigation of the effect of methanolic and acetonetic extraction solvents on the extraction and quantification of phenolic compounds of *Cleome arabica*, *Atriplex halimus*, and *Hammada elegans*, from the same region of the current study, showed that extraction using methanol gives the highest phenolics in comparison to acetonetic solvent (Feriha et al., 2019).

Additionally, the researchers used polyvinylpyrrolidone, polyphenolics binder reagent, to assure that no other reducing compounds could interfere in the quantification of phenolics, since the Folin-Ciocalteu reagent is not specific for the quantification phenolic compounds (Feriha et al., 2019).

Moreover, the selectivity of extraction solvents toward the flavonoids was also noticed in many studies. For instance, the addition of an amount of water to acetone solvent enhanced the extraction of flavonoids compared to 80% methanol or absolute methanol or absolute acetone (Hachani et al., 2018, 2023). Other research found that methanol was more suitable than ethanol, 80% Methanol, 60% Methanol, and water in quantifying both total phenolic compounds and total flavonoids from *K. wilmsii* plants from Africa (Chigayo et al., 2016). These findings indicate that methanol is more effective toward the recovery of TPC while flavonoid recovery is highly dependent on solvent selectivity and varies according to plant species.

3.4 *In vitro* antioxidant capacity

The antioxidant activity of the different plant extracts was evaluated using three *in vitro* assays, namely DPPH, ABTS, and CUPRAC (Table 3). The results were expressed as Vitamin E Equivalent Antioxidant Capacity (VEEAC) and TBHQ Equivalent Antioxidant Capacity (TBEAC). The use of two different expressions was to facilitate comparison with natural and synthetic antioxidant standards.

The DPPH assay tends to measure the ability of plant extract to stabilize free radical by hydrogen atoms donation. Results showed that the methanolic leaves/flowers extract of *Cistus villosus* exhibited the highest scavenging activity, reaching 2.706 mg VEEAC/mg DR (1.136 mg TBEAC/mg DR). The methanolic extracts of both stem and leaves/flowers of *Globularia arabica* are also recorded by high scavenging activity with 2.007 mg VEEAC/mg DR and 1.612 mg VEEAC/mg DR respectively. The hexane extract of all species marked with low scavenging DPPH activity with values less than 0.20 mg VEEAC/mg DR. The differences among extraction solvents were statistically significant ($p < 0.001$), with methanolic extracts consistently exhibiting the strongest DPPH radical scavenging activity. Furthermore, Pearson's analysis revealed a strong positive correlation between DPPH activity and TPC ($r = 0.884$, $p < 0.05$) (Table 5), also the correlation with TFC was positive and strong ($r = 0.889$), indicating that both flavonoids and phenolic compounds were the main contributors to free-radical scavenging.

A recent study on aerial part of *Cistus villosus* from Algeria showed that methanolic extract was four times stronger than the commercial standard vit E with an $IC_{50} = 8.19$ mg/L while Vit E. marked with $IC_{50} = 36.35$ mg/L (Bouakkaz et al., 2025). For *Globularia arabica* species, little has been known, most studies in literature tend to investigate *Globularia alypum* L., their results proved that the genus of *Globularia* have strong scavenging activity (Djeridane et al., 2010; Es-Safi et al., 2007; Güçlü et al., 2025). Another study on *Globularia alypum* antioxidant found that the aqueous and methanolic extracts exhibited the strongest DPPH activity, with IC_{50} values of 83.48 and 90.53 μ g/mL, respectively, emphasizing on the influence of extraction solvent on antioxidant capacity (EL-Mernissi et al., 2023).

Regarding ABTS assay, significant differences were found among extraction solvents ($p < 0.001$), with methanolic extracts showing the highest activity. The methanolic leaves/flowers extract of *Cistus villosus* exhibited the highest antioxidant capacity (1.372 mg VEEAC/mg DR), followed by the methanolic stem extract (0.913 mg VEEAC/mg DR) with a considerable ABTS activity of stem dichloromethane extract (0.312 mg VEEAC/mg DR). Nevertheless, methanolic and DMSO extracts of the

remaining species generally demonstrated higher ABTS scavenging activity compared to Hexane and dichloromethane extracts (Table 3). Table 5 shows that ABTS values were strongly correlated with DPPH ($r = 0.939$, $p < 0.05$) and TPC ($r = 0.936$, $p < 0.05$), supporting the similar response of both assays toward phenolic antioxidants. Based on literature, the 80% ethanolic extract of *Globularia orientalis*, from Turkey, could stabilize ABTS⁺ radical with IC₅₀ value of 1.35 mg/L which was very close to the tested gallic acid standard (0.39 mg/L) (Güçlü et al., 2025). Besides, in previous study, the methanolic and ethyl acetate extracts of the aerial part of *Cistus villosus* L. marked with ABTS radical scavenging activity equals to 1.41 and 5.10 respectively, which indicates that methanol solvent extract more antioxidants capable to quench ABTS⁺ radicals (Bouakkaz et al., 2025).

Furthermore, the CUPRAC assay was chosen to test the ability of different plant extract to reduce cooper ions by electron transfer. The primary results confirmed that the tested extract possessed this ability, and the extracts varied significantly according to extraction solvent ($p < 0.001$). For instance, the methanolic leaves/flowers extract of *Cistus villosus* exhibited the highest reducing capacity followed by the methanolic stem extract with values equals to 1.732 mg VEEAC/mg DR and 1.287 mg VEEAC/mg DR respectively. Besides, the methanolic extracts of *Globularia arabica* also displayed strong reducing power. Besides, the methanolic extracts of *Globularia arabica* also displayed strong reducing power. Additionally, as presented in Table 4, CUPRAC activity was positively correlated with DPPH ($r = 0.965$), ABTS ($r = 0.977$), TFC ($r = 0.939$), and TPC ($r = 0.949$) ($p < 0.05$), confirming that the reducing power of the extracts is largely associated with their phenolic composition. In the previous research, both methanolic and ethyl acetate extracts displayed strong reducing capacity compared to the tested standards: vit E, BHA, and BHT with A_{0.5} less than 13.28 mg/L. Also, the ethyl acetate extract proved to be best to reduce cooper ions compared to methanolic extract with A_{0.5} = 8.74 mg/L (Bouakkaz et al., 2025).

Moreover, hexane extract consistently exhibited the lowest antioxidant capacities in all three tests of antioxidant activity, confirming that the non-polar fractions contained only limited amounts of bioactive constituents that could possess antioxidant activities. Interestingly, the DMSO extracts retained considerable DPPH, ABTS and CUPRAC antioxidant activities despite being obtained after the sequential extraction process, knowing that this is the first time where such a solvent is investigated. This observation was particularly evident for both *Cistus villosus* and *Globularia arabica*, suggesting that residual antioxidant constituents remained in the extraction residue and were subsequently recovered by DMSO. Many bioactive compounds when bound to protein or sugars or even long polymer chain become less attainable by hexane, methanol or even ethyl acetate, yet more polarity solvents are needed to extract them. Butanol and DMSO becomes widely used to assure better extraction and full compounds recovery (Hachani et al., 2023; Hamdi et al., 2025).

Overall, the antioxidant activity of medicinal plants is influenced by both the extraction solvent and the intrinsic characteristics of the plant. Since solvents differ in their polarity, they extract different groups of bioactive compounds that have polarity similar to the used solvent. Also, the optimization of extraction is needed to maximize the recovery of active compounds in a short time and without excessive use of solvent and energy resulting in a comprehensive and effective phytochemical evaluation (Benarfa et al., 2020; Hachani et al., 2020, 2026).

Table 3. Radical-scavenging (DPPH and ABTS) and cupric-reducing (CUPRAC) of the 16 plant extracts.

Extract	DPPH		ABTS		CUPRAC	
	mg equivalent of standard / mg of dry residue					
	VEEAC	TBEAC	VEEAC	TBEAC	VEEAC	TBEAC
CV-LF-He	0.164±0.002 ^D	0.069±0.001 ^D	0.091±0.004 ^{CDEF}	0.030±0.001 ^{CDEF}	0.034±0.002 ^{ABC}	0.015±0.001 ^{ABC}
CV-S-He	0.105±0.002 ^C	0.044±0.001 ^C	0.058±0.004 ^{ABCD}	0.019±0.001 ^{ABCD}	0.032±0.004 ^{ABC}	0.014±0.002 ^{ABC}
CV-LF-Di	0.933±0.002 ^L	0.392±0.001 ^L	0.078±0.002 ^{BCDE}	0.025±0.001 ^{BCDE}	0.210±0.012 ^H	0.091±0.005 ^H
CV-S-Di	0.917±0.007 ^{JK}	0.385±0.003 ^{JK}	0.312±0.005 ^M	0.101±0.002 ^M	0.405±0.006 ^J	0.176±0.002 ^J
CV-LF-Me	2.706±0.011 ^W	1.136±0.005 ^W	1.372±0.021 ^Q	0.445±0.007 ^Q	1.732±0.009 ^Q	0.754±0.004 ^Q
CV-S-Me	1.354±0.005 ^T	0.568±0.002 ^T	0.913±0.016 ^P	0.296±0.005 ^P	1.287±0.005 ^P	0.560±0.002 ^P
CV-LF-Dm	1.003±0.002 ^N	0.421±0.001 ^N	0.700±0.007 ^{NO}	0.227±0.002 ^{NO}	1.004±0.007 ^N	0.437±0.003 ^N

CV-S-Dm	0.974±0.005 ^M	0.409±0.002 ^M	0.641±0.030 ^N	0.208±0.010 ^N	0.951±0.007 ^M	0.414±0.003 ^M
GA-LF-He	0.051±0.001 ^{AB}	0.021±0.001 ^{AB}	0.008±0.001 ^A	0.003±0.001 ^A	0.002±0.0001 ^A	0.001±0.0001 ^A
GA-S-He	0.039±0.001 ^A	0.016±0.001 ^A	0.081±0.002 ^{CDE}	0.026±0.001 ^{CDE}	0.065±0.004 ^{CD}	0.028±0.002 ^{CD}
GA-LF-Di	0.906±0.003 ^J	0.380±0.001 ^J	0.114±0.005 ^{DEFGH}	0.037±0.001 ^{DEFGH}	0.105±0.003 ^{DE}	0.046±0.001 ^{DE}
GA-S-Di	0.672±0.001 ^E	0.282±0.001 ^E	0.153±0.006 ^{GHIJ}	0.050±0.002 ^{GHIJ}	0.142±0.004 ^{EFG}	0.062±0.002 ^{EFG}
GA-LF-Me	1.612±0.003 ^U	0.677±0.001 ^U	0.680±0.047 ^O	0.221±0.015 ^O	1.034±0.050 ^N	0.450±0.022 ^N
GA-S-Me	2.007±0.013 ^V	0.843±0.005 ^V	0.874±0.072 ^P	0.284±0.023 ^P	1.146±0.019 ^O	0.499±0.008 ^O
GA-LF-Dm	1.021±0.004 ^O	0.429±0.002 ^O	0.733±0.027 ^O	0.238±0.009 ^O	1.040±0.018 ^N	0.452±0.008 ^N
GA-S-Dm	0.920±0.003 ^{KL}	0.387±0.001 ^{KL}	0.677±0.049 ^N	0.220±0.016 ^N	0.999±0.006 ^N	0.435±0.003 ^N

The results of the tests carried out are expressed as mean ± standard deviation, n = 3. Mean values followed by a different lowercase letter are significantly different (p < 0.0001). VEEAC: Vitamin E Equivalent Antioxidant Capacity; TBEAC: TBHQ Equivalent Antioxidant Capacity; S: stem; LF: Leaves/Flowers; Me: Methanol; DM: DMSO; DI: Dichloromethane; He: Hexane.

3.5 In vitro lipid peroxidation inhibition capacity

Lipids belong to primary metabolism i.e. could be synthesized by plants and Man; these molecules play vital role in the living organisms (Domínguez-Avila & González-Aguilar, 2018). However, lipid peroxidation may occur and inhibit their vital role (J. Zhang & Kirkham, 1996). For that reason, the inhibition of lipid peroxidation is very important to preserve tissue and ensure better function in living organisms and improving the oxidative stability of food products (Dinis et al., 1994).

First, the extracts of the two medicinal plants were used as inhibitors of lipid peroxidation where sunflower oil and cow ghee are used as model lipid systems. The inhibitory effects were significantly influenced by the extraction solvent (p < 0.001). The results revealed that the methanolic extracts of leaves/flowers exhibited the highest inhibitory activities in which the extract of *Globularia arabica* inhibit 53.88 % and 40.38 % of lipid peroxidation of sunflower oil and cow ghee respectively. Both extracts inhibit lipid peroxidation better than vit E and TBHQ standard. Overall, polar solvents were more effective than non-polar solvents in extracting bioactive substances responsible for preventing lipid oxidation and only the hexane extracts CV-S-He (7.40%) in the cow ghee system marked with lower inhibitory activities than the tested standards (Table 4). These findings indicate that most of the tested extracts possess promising lipid-protective properties.

Table 4 shows the correlational study between anti-peroxidation activity and other tests. In sunflower oil, the anti-peroxidation activity showed high positive correlations with DPPH (r = 0.798), TFC (r = 0.695), TPC (r = 0.623), and extraction yield (r = 0.627). Similarly, anti-peroxidation in the cow ghee system was positively correlated with DPPH (r = 0.75) and showed a positive moderate relationship with ABTS and CUPRAC (r = 0.678, and r = 0.695 respectively), but, negative correlation was observed with TPC, TFC, and the yield indicating that extracts effective in one lipid model are not necessarily effective in the other. Moreover, and in agreement with present findings, medicinal plants collected from the same Algerian arid region, Sidi Makhlouf, Laghouat, and traditionally used by locals for medicinal purposes also exhibited remarkable antilipoperoxidative activity (Feriha et al., 2019). Specifically, the hydromethanolic extract of *Cleome arabica* showed the strongest inhibition of lipid peroxidation with EC₅₀ = 0.031 mg/mL, emphasizing the high antioxidant potential of these medicinal plants that grow under similar environmental conditions (Feriha et al., 2019).

Table4. Lipid peroxidation inhibition capacity of the 16 plant extracts.

Extract	Inhibition of peroxidation (0.5 mg/mL)		Antihemolytic		Anti-LOX	
	Sunflower oil	Cow ghee	mg equivalent of standard / mg of dry residue			
			VEEAC	TBEAC	VEEAC	TBEAC
CV-LF-He	13.970±0.244 ^{CD}	10.090±0.174 ^{DE}	0.375±0.023 ^A	0.602±0.039 ^A	0.002±0.0001 ^A	0.001±0.0001 ^A
CV-S-He	10.720±0.184 ^{AB}	7.400±0.131 ^C	0.363±0.067 ^A	0.590±0.114 ^A	0.002±0.0001 ^A	0.001±0.0001 ^A
CV-LF-Di	44.210±0.767 ^P	22.800±0.401 ^{JK}	98.627±0.384 ^L	165.596±0.653 ^L	1.525±0.026 ^O	0.742±0.013 ^O
CV-S-Di	28.700±0.497 ^{GH}	15.670±0.271 ^F	86.667±1.488 ^K	145.345±2.530 ^K	1.343±0.026 ^N	0.653±0.013 ^N
CV-LF-Me	47.910±0.837 ^{RST}	38.220±0.663 ^P	20.200±0.988 ^{BCD}	33.570±1.680 ^{BCD}	0.531±0.012 ^J	0.258±0.006 ^J
CV-S-Me	36.450±0.637 ^M	22.010±0.383 ^{IJ}	22.274±0.863 ^{DE}	37.084±1.467 ^{DE}	0.483±0.023 ^I	0.235±0.011 ^I
CV-LF-Dm	31.110±0.540 ^{IJ}	25.130±0.436 ^L	38.971±0.696 ^{HI}	65.153±1.183 ^{HI}	0.255±0.013 ^{EF}	0.124±0.006 ^{EF}
CV-S-Dm	21.370±0.375 ^E	19.770±0.349 ^H	18.551±0.202 ^{BC}	30.941±0.344 ^{BC}	0.109±0.003 ^B	0.053±0.001 ^B
GA-LF-He	15.900±0.279 ^B	11.020±0.192 ^E	0.444±0.018 ^A	0.741±0.031 ^A	0.003±0.001 ^A	0.001±0.0001 ^A
GA-S-He	11.640±0.201 ^{AB}	8.890±0.157 ^D	0.614±0.143 ^A	0.999±0.243 ^A	0.002±0.0001 ^A	0.001±0.0001 ^A
GA-LF-Di	48.760±0.855 ST	34.200±0.593 ^O	158.073±1.057 ^O	265.972±1.797 ^O	2.882±0.030 ^Q	1.401±0.014 ^Q
GA-S-Di	34.860±0.610 ^{LM}	20.210±0.349 ^H	144.052±0.750 ^N	242.270±1.275 ^N	1.543±0.024 ^O	0.750±0.011 ^O
GA-LF-Me	53.880±0.942 ^U	40.380±0.707 ^Q	23.804±0.868 ^E	39.609±1.475 ^E	0.919±0.023 ^L	0.447±0.011 ^L
GA-S-Me	46.890±0.819 ^{QRS}	29.140±0.506 ^M	19.698±1.095 ^{BCD}	32.726±1.863 ^{BCD}	0.412±0.014 ^H	0.200±0.007 ^H
GA-LF-Dm	33.750±0.593 ^{KL}	31.570±0.549 ^N	36.502±0.914 ^{GH}	61.069±1.555 ^{GH}	0.315±0.007 ^G	0.153±0.004 ^G
GA-S-Dm	23.930±0.419 ^F	28.600±0.497 ^M	34.139±0.906 ^G	57.011±1.541 ^G	0.273±0.011 ^{EF} G	0.133±0.006 ^{EF} G
Vitamin E	13.970±0.863 ^T	10.090±0.557 ^N				
TBHQ	10.720±0.715 ^O	7.400±0.401 ^{JK}				
The results of the tests carried out are expressed as mean ± standard deviation, n = 3. Mean values followed by a different lowercase letter are significantly different (p < 0.0001). VEEAC: Vitamin E Equivalent Antioxidant Capacity; TBEAC: TBHQ Equivalent Antioxidant Capacity. LOX: Lipoxygenase. S: stem; LF: Leaves/Flowers; Me: Methanol; DM: DMSO; DI: Dichloromethane; He: Hexane.						

These findings support the traditional use of the medicinal flora from this region as a valuable source of natural bioactive compounds capable of protecting lipids against oxidative damage. Also, many other studies explored the ability of plant extracts to preserve food products through reducing the lipid peroxidation. In a study conducted on twelve Chinese medicinal plants, methanolic extracts and its fractioning extracts inhibiting up to 80% of lipid peroxidation in a linoleic acid model system and effectively protecting lipids from oxidative degradation. This activity was related to phenolics and flavonoid compounds of the crude and purified fractions (Cho et al., 2003).

Similarly, methanolic medicinal plant extracts have demonstrated strong inhibition of lipid peroxidation in ex vivo biological substrates, including cholesterol, LDL, and mouse brain homogenates. The inhibition of lipid peroxidation was up to 45%, reached 97.6%, 72.2%, and 49.6% in cholesterol, LDL, and in mouse brain homogenates respectively (Pai Kotebagilu et al.,

2015). These promising results support the use of plant-derived antioxidants as natural alternatives for preserving lipid stability in both food and biological systems.

Second, antihemolytic activity was tested to measure the ability of medicinal plants extracts to protect the membrane of erythrocyte against oxidative damage usually caused by ROS and results the hemolysis. The erythrocyte membrane is largely composed of lipid; the ROS subsequently initiates the lipid peroxidation. But, with the presence of medicinal plants bioactive compounds that could prevent lipid peroxidation, the hemolysis will be reduced (Sharma & Sharma, 2001).

The results of the tested extract in this study present a variability between plants species, its selected organs, and their corresponding extraction solvents ($p < 0.001$). Among all aerial extracts, the leaves and flowers extracts displayed higher activities than the corresponding stem extracts, whereas hexane extracts showed negligible antihemolytic effects. Also, the leaves and flowers of dichloromethane exhibited the highest Antihemolytic capacity among all solvents, with *Globularia arabica* showing the greatest activity with value 158,073 mg VEEAC /g DR. The hexane extracts showed negligible antihemolytic effects (Table 4), indicating that bioactive compounds have moderate polarity, and the plant organ markedly influenced the recovery of compounds responsible for antihemolytic effects. Unlike the antioxidant assays, anti-hemolytic activity showed negative correlations with TPC ($r = -0.012$) (Table 5), suggesting that membrane stabilization depends on compounds other than the major phenolics alone.

Table 5. Pearson's correlation coefficients (r) for the relationships between antioxidants, anti-peroxidation assays and total phenolic and flavonoid contents of the 16 plant extracts.

	Yield	TPC	TFC	DPPH - VEEAC	ABTS-VEEAC	CUPRA C-VEEAC	Anti-hemolytic	Anti-LOX	Anti-Oil	Anti-Ghee
Yield	1.000									
TPC	0.860	1.000								
TFC	0.966	0.922	1.000							
DPPH-VEEAC	0.667	0.884	0.889	1.000						
ABTS-VEEAC	0.551	0.936	0.924	0.939	1.000					
CUPRA-VEEAC	0.551	0.949	0.939	0.965	0.977	1.000				
Anti-hemolytic	-0.012	-0.037	0.051	0.044	-0.271	-0.269	1.000			
Anti-LOX	-0.012	-0.037	0.051	0.167	-0.212	-0.213	0.930	1.000		
Anti-Oil	0.627	0.623	0.695	0.798	0.498	0.509	0.471	0.619	1.000	
Anti-Ghee	-0.249	-0.238	-0.191	0.815	0.678	0.695	0.272	0.401	0.471	1.000
Anti-Ghee: Cow ghee peroxidation inhibition (VEEAC)										
Anti-Oil: Sunflower oil peroxidation inhibition (VEEAC)										
Bold values are different from 0 at a significance level $\alpha=0.05$										

Our findings are in line with previous studies reporting on the antihemolytic potential of medicinal plants. In a screening of 26 Pakistani medicinal plants, only few numbers of species exhibited significant antihemolytic activity, with *Cedrus deodara* and

Enicostemma hyssopifolium providing up to 72% protection against cobra venom-induced hemolysis, highlighting the influence of plant species on this activity (H B Asad et al., 2014).

Similarly, a study on different solvent extracts of *Eucalyptus camaldulensis* leaf reported an inhibition of over 90%, and with IC_{50} values of 212.9, 163.4, and 188.3 $\mu\text{g/mL}$ corresponding to ethanolic, methanolic, and aqueous leaf extracts respectively, which were even lower than that of ascorbic acid (236.7 $\mu\text{g/mL}$). This protective effect was attributed to the high content of phenolics and flavonoid compounds capable of scavenging ROS and preserving membrane integrity (Chbib et al., 2026).

Third, the results of anti-lipoxygenase activity (anti-LOX) show a similar trend with antihemolytic activity in which the dichloromethane of leaves/flowers extracts were the most active ($p < 0.001$), with *Globularia arabica* exhibiting the highest anti-LOX activity with values of 2.882 mg VEEAC /g DR, while most hexane extracts showed little or no inhibitory effect (Table 4). These findings indicate that the extraction solvent plays a crucial role in recovering bioactive compounds involved in lipoxygenase inhibition, with medium-polarity solvents being particularly effective for this biological activity. Additionally, a very strong positive correlation was observed between anti-LOX and anti-hemolytic activities ($r = 0.930$, $p < 0.05$), suggesting that the same group of bioactive constituents may contribute to both membrane protection and lipoxygenase inhibition (Table 5).

Further a study on medicinal plants collected from the Algerian Saharan region, where all extracts of *Hammada elegans*, *Atriplex halimus*, and *Cleome arabica* plants inhibited lipoxygenase in a dose-dependent manner ($IC_{50} = 0.382\text{--}19.210$ mg/mL) (Feriha et al., 2019). These findings suggest that Saharan medicinal plants are rich in bioactive compounds capable of limiting oxidative degradation of membrane lipids while simultaneously reducing inflammatory processes.

Overall, both methanol and dichloromethane were more effective solvents to extract bioactive compounds marked with valuable activity. Extracted compounds from methanolic extracts were responsible for antioxidant and anti-peroxidation activities, whereas bioactive compounds from dichloromethane extracts marked the strong anti-hemolytic and anti-LOX activities. This highlights the importance of using solvents with different polarities to recover a wider range of bioactive compounds.

4. CONCLUSION

To conclude, the findings of the present study indicated that the antioxidant and anti-peroxidative capabilities of the medicinal plants studied are highly impacted by the extraction solvent and influenced by the plant organ. Methanolic extracts were shown to be more effective in inhibiting radical scavenging and lipid peroxidation, whilst dichloromethane extracts were better at inhibiting hemolysis and lipoxygenase. The phytochemical screening revealed the presence of several bioactive secondary metabolite classes, including phenolic compounds, flavonoids, tannins, sterols, and triterpenes, with their distribution varying according to the plant species, organ, and solvent polarity. These results confirm the relationship between the phytochemical composition of extracts and their biological activities. These findings highlight the importance of the extraction step, where solvents play a crucial role in recovering different classes of bioactive compounds, and support the potential of these Saharan medicinal plants as promising natural sources of antioxidants and anti-inflammatory agents for future pharmaceutical, agricultural, and nutraceutical applications.

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AUTHOR CONTRIBUTIONS

Khaled DJOKHDEM, Amar DJERIDANE and Soumaya HACHANI: Experimental acquisition, investigative processes, formal analysis, drafting of the original manuscript, methodology development, and data organization. Mohamed YOUSFI: Resources and Funding acquisition.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICS STATEMENT

Ethical approval was not required for this study.

DATA AVAILABILITY STATEMENT

All Data supporting the findings of this study are published in this article.

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