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Characterization of lipophilic oil fraction from *Primula vulgaris*: Chemical profile, antioxidant capacity, and DNA damage assessment

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ABSTRACT

Primula vulgaris (Primulaceae) is a perennial plant widely recognized for its traditional therapeutic and cosmetic applications; however, information regarding the organ-dependent lipophilic composition and associated antioxidant properties remains limited. In this study, a comparative characterization of lipophilic oil fractions obtained from the root, leaf, and flower of *P. vulgaris* by hexane-based Soxhlet extraction was performed to elucidate differences in their chemical profiles and bioactivities. GC–MS-based profiling, supported by FTIR analysis, provided a lipophilic profiling supported by authentic reference standards and library matching, revealing clear compositional variations among plant organs. The flower oil was enriched in γ-linolenic acid methyl ester, the leaf oil was dominated by stearic and oleic acid methyl esters, while oleic acid methyl ester was the predominant component in the root oil. Antioxidant properties evaluated by DPPH radical scavenging, CUPRAC, total phenolic content, and total flavonoid assays demonstrated that the root oil exhibited the highest reducing and radical scavenging capacity, whereas the leaf oil contained the highest flavonoid content, indicating functional divergence among organs. DNA interaction studies showed that none of the oil samples exerted genotoxic effects on plasmid DNA, although no protective activity against Fenton-induced DNA damage was observed. Overall, this organ-based comparative study highlights *P. vulgaris* as a promising source of lipophilic antioxidant constituents and provides useful characterization data with potential relevance for food-related and potential source of bioactive lipophilic constituents.

1. Introduction

Humans have historically relied on plants to meet essential needs related to nutrition, protection, and health [1–3]. Medicinal and aromatic plants continue to play an important role in food, pharmaceutical, and cosmetic industries due to their bioactive constituents and functional properties, particularly their antioxidant potential [4,5].

Primula vulgaris (Primulaceae) is a perennial hemicryptophyte widely distributed across Eurasia, commonly inhabiting woodlands and lowland areas where its growth is influenced by soil moisture and climatic conditions [6]. The species is cultivated worldwide for ornamental purposes and has also been traditionally used for its therapeutic and cosmetic applications [7,8]. Previous studies have reported that species within the *Primula* genus contain various secondary metabolites, including flavonoids and phenolic compounds, which contribute to their biological activities [9,10]. However, existing research has predominantly focused on polar extracts or specific biological effects, while the *organ-dependent lipophilic composition* of *P. vulgaris* has received comparatively limited attention.

Extraction methodology plays a decisive role in determining the chemical characteristics of plant-derived oils. Solvent-assisted extraction techniques, such as Soxhlet extraction, are widely applied due to their high efficiency, reproducibility, and suitability for isolating lipophilic constituents from plant matrices [11,12]. Despite the development of alternative extraction technologies, Soxhlet extraction remains a benchmark method for comparative studies owing to its consistent performance [13,14]. Lipophilic oil fractions obtained through solvent-based methods represent complex mixtures of hydrocarbons and oxygenated compounds that are relevant for food-related applications as well as for their antioxidant functionality [15,16].

Oxidative stress, resulting from an imbalance between reactive oxygen species and antioxidant defense mechanisms, is associated with lipid peroxidation, protein oxidation, and DNA damage, thereby contributing to the development of various chronic diseases [17,18].

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Plant-derived antioxidants, particularly lipophilic compounds, have attracted increasing interest due to their potential interactions with lipid-rich systems and biological membranes [19,20]. Consequently, the characterization of lipophilic antioxidant fractions from different plant organs is of growing importance in food measurement and potential source of bioactive lipophilic constituent's research.

In this context, the present study aims to perform a comparative characterization of lipophilic oil fractions obtained from the root, leaf, and flower of *Primula vulgaris* using hexane-based Soxhlet extraction. GC–MS-based lipophilic profiling, supported by authentic reference standards and mass spectral library matching, together with FTIR analysis, was employed to elucidate organ-dependent compositional differences. Antioxidant properties were evaluated using DPPH radical scavenging, CUPRAC, total phenolic content, and total flavonoid assays. In addition, the interaction of these oil fractions with plasmid DNA was examined to assess their DNA damage assessment under oxidative conditions. This organ-based approach provides new characterization data that may support the evaluation of *P. vulgaris* as a potential source of lipophilic antioxidant constituents for food and potential source of bioactive lipophilic constituents.

2. Material and methods

2.1. Materials and chemicals

All chemicals used in this study were of analytical grade (≥99.9%) and were purchased from Sigma–Aldrich (St. Louis, MO, USA). Organic solvents were of HPLC grade. Plant materials were milled using a laboratory grinder (Retsch ZM200, Haan, Germany). Soxhlet extraction was performed using a standard glass Soxhlet apparatus (250 mL) equipped with a heating mantle. Solvent removal was carried out under reduced pressure using a rotary evaporator (Heidolph Laborota 4001, Schwabach, Germany). Nitrogen content was determined using a LECO FP-528 nitrogen analyzer, and ash content was measured using a muffle furnace. FTIR spectra were recorded using a PerkinElmer Spectrum 65 spectrophotometer. GC–MS analyses were performed on a Thermo Scientific Trace 1310 GC coupled with an ISQ single quadrupole mass spectrometer. Antioxidant assays were conducted using a Thermo Scientific Multiskan Go microplate reader. DNA damage and protection experiments were evaluated by agarose gel electrophoresis using a Major Science electrophoresis system and gel documentation unit.

2.2. Plant material collection and lipophilic oil fraction extraction

Primula vulgaris samples were collected from the Yalak Plateau near Yalak Village, İskilip district, Çorum province (Türkiye) on March 11, 2023 (Fig. 1). Plant identification was confirmed by Dr. Taner Özcan (Balıkesir University, Department of Biology) according to standard floristic references [21, 22].

Fig. 1. *Primula vulgaris*.

Flower, leaf, and root samples were separated and air-dried in the shade at ambient temperature for 10 days. The dried materials were milled to a uniform particle size prior to extraction [23,24]. The dried and milled sample was passed through a 40-mesh sieve (425 μm) before extraction. For each plant part, 5.0 g of powdered material was extracted with n-hexane using a Soxhlet apparatus. n-Hexane was selected as the extraction solvent to recover the lipophilic constituents of *P. vulgaris*. Approximately 500 mL of solvent was used during the extraction procedure, ensuring continuous reflux, efficient solvent circulation, and exhaustive extraction of the non-polar fraction. The extraction process was performed for 2 h at approximately 75 °C. After extraction, solvents were removed under reduced pressure at 45 °C. Oil yields were calculated gravimetrically and expressed as percentage (*w*/w %) according to Eq. (1). Following extraction, the lipophilic fractions were kept in their n-hexane solutions and stored under nitrogen in amber glass vials at 4 °C until analysis in order to minimize light-induced and oxidative degradation. The experiment were performed in triplicate and results are expressed as mean ± standard deviation.

$$\text{Oil\%} = (\text{Fixed Oil Amount (g)}/\text{Sample Amount (g)}) \times 100 \quad (1)$$

2.3. Determination of nitrogen, ash, and total fat content

Nitrogen content (%) of flower, leaf, and root samples was determined using the DUMAS combustion method with a LECO FP-528 analyzer, which provides rapid and reproducible measurements [25]. Approximately 0.25 g of each sample was analyzed.

Ash content (%) was determined by incinerating 3.0 g of each sample in a muffle furnace at 550 °C for 5 h. After cooling in a desiccator, ash percentages were calculated gravimetrically [26].

Total fat content (%) was determined using an ANKOM XT15 extraction system following petroleum ether extraction at 90 °C for 40 min [27]. Oil content was calculated according to Eq. (2).

$$\begin{aligned}\text{Total fat\%} = (&(\text{The first weight of filter paper} \\ &- \text{The last weight of filter paper}) \\ &/\text{Weighed sample quantity}) \times 100\end{aligned} \quad (2)$$

All experiments were performed in triplicate and results are expressed as mean ± standard deviation.

2.4. GC–MS analysis

Prior to GC–MS analysis, fatty acids present in the lipophilic fractions were converted into their methyl ester derivatives to improve their volatility and chromatographic performance. Briefly, each lipophilic oil fraction (0.1–0.3 mL) was transferred into a screw-capped glass tube (15 mL), followed by the addition of n-hexane (10 mL). The mixture was vigorously vortexed, after which approximately 0.5 mL of 2.0 M potassium hydroxide solution in methanol was added. The tube was vortexed again vigorously and allowed to stand until two distinct phases were formed. Subsequently, using a syringe, 0.5 mL of the upper organic phase containing fatty acid methyl esters was transferred into a 2 mL GC vial containing n-hexane (1.5 mL) over a PTFE syringe tip filter (45 μm). The vial was vortexed thoroughly before GC–MS analysis using a THERMO TriPlus RSH Trace 1310–ISQ LT system. Method optimization was initially performed using two to three trial injections per sample; following optimization, compositional analyses were performed using a single injection for each sample [28].

GC–MS analysis was performed using a Thermo Trace 1310 gas chromatograph coupled to an ISQ single-quadrupole mass spectrometer (Thermo Fisher Scientific, Austin, TX, USA) equipped with a TriPlus RSH autosampler. Separation was achieved using a TG-WAXMS capillary column (60 m × 0.25 mm i.d., 0.25 μm film thickness). Helium was used as the carrier gas at a constant flow rate of 1.2 mL min^{-1}. The oven

temperature was maintained at 80 °C for 2 min, increased to 240 °C at 4 °C min^{-1}, and held at 240 °C for 45 min. A 3.0 μL aliquot of each prepared sample was injected. The ion source and detector temperatures were maintained at 250 °C, and mass spectra were acquired over the *m/z* range of 55–550. Compound identification was performed by comparison of mass spectra with the NIST and Wiley 9 spectral libraries. For compounds lacking authentic standards, identification was based on mass spectral library matching (NIST, Wiley) and should therefore be considered tentative. GC–MS analysis was used for semi-quantitative compositional profiling rather than absolute quantification. Major fatty acid methyl esters were additionally confirmed by comparison of retention times and mass spectra with the Supelco 37 Component FAME Mix (CRM47885, lot XA16568V; varied concentrations in dichloromethane; Supelco, Bellefonte, PA, USA). A certified C7–C40 saturated alkanes standard mixture (49452-U, lot LRAC7894; 1000 μg mL^{-1} of each component in hexane; Sigma-Aldrich, St. Louis, MO, USA) was analyzed under the same chromatographic conditions for retention-index determination.

2.5. FTIR analysis

FTIR spectra of lipophilic oil fraction samples were collected over the range of 4000–400 cm^{-1} to identify major functional groups. FTIR spectra of the lipophilic fractions were recorded on a PerkinElmer Spectrum 65 spectrophotometer equipped with a diamond ATR crystal. Approximately 1–2 drops of each sample was deposited directly on the cleaned ATR crystal and spectra were obtained from 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1} with 16 scans per sample. Before each measurement the clean ATR crystal was recorded a background spectrum. The lipophilic fractions were characterized comparatively by FTIR analysis on the basis of functional groups, without the need of an external reference standard.

2.6. Antioxidant activity assays

2.6.1. DPPH radical scavenging assay

DPPH radical scavenging activity was evaluated according to a modified method of Villano et al. [19]. Oil samples were prepared at a concentration of 1 mg mL^{-1} in dimethyl sulfoxide and further diluted as required. Aliquots of 50 μL sample solution were mixed with 250 μL of 0.01 mM DPPH solution and incubated in the dark for 30 min. The mixture was transferred into a 96-well plate using a micropipette. In addition, a blank sample without any sample was prepared. Trolox and ascorbic acid were also added to the plate after similar procedures for reference purposes. The absorbance of the mixtures was measured at 517 nm with a Multiscan Go ELISA reader spectrophotometer. For reference standard solutions, solutions containing 0.2, 0.5, 1.0, 2.0, and 4.0 μg of ascorbic acid and Trolox were prepared. The experiment were performed in triplicate and results are expressed as mean ± standard deviation ($n = 3$).

2.6.2. CUPRAC assay

Cupric ion reducing antioxidant capacity was determined using the CUPRAC method described by Apak et al. [29]. Copper (II) chloride solution (2.0×10^{-2} M), neocuproine (Nc) solution (1.5×10^{-2} M), and ammonium acetate buffer solution (pH = 7) were transferred to the test tube. An oil sample (10 μL) was added, followed by pure water until the final volume reached 300 μL. The tubes were closed and allowed to sit for 1 h. Absorbance at 450 nm was measured spectroscopically using with a Multiscan Go ELISA reader spectrophotometer. The standard calibration curve for each antioxidant compound was established as absorbance-concentration with 0.3, 0.6, 1.0, 1.4, 1.8, 2.4, 3.0, and 6.0 μg μL^{-1} ascorbic acid and Trolox standard substances. Results were expressed as Trolox and ascorbic acid equivalents. The experiment were performed in triplicate and results are expressed as mean ± standard deviation ($n = 3$).

2.6.3. Total flavonoid content

Total flavonoid content was determined using a modified aluminum chloride colorimetric method [30]. Lipophilic oil solutions (25 μL of a 1 mg mL^{-1} solution) were prepared in the reaction medium containing 10% aluminum chloride, 5% aqueous sodium nitrite, and a 1 N sodium hydroxide solution mixture. After a 40-min incubation at room temperature, absorbance was determined spectrophotometrically at 415 nm. Total flavonoid concentration was calculated using quercetin as a standard. Total flavonoid contents in oil samples were calculated from the standard curve prepared with quercetin and expressed as micrograms of quercetin equivalents. Prepared quercetin standard solutions contained 1.0, 2.5, 5.0, 10.0, 20.0, 40.0, 60.0, and 80.0 μg of quercetin. The results were expressed as quercetin equivalents. The experiment were performed in triplicate and results are expressed as mean ± standard deviation ($n = 3$).

2.6.4. Total phenolic content

Total phenolic content was determined using the Folin–Ciocalteu method with minor modifications [31]. For total phenolic content experiments, 10 μL of sample (0.5 mg mL^{-1}) was added to the reaction medium. Then, 133.3 μL of Folin–Ciocalteu reagent (FCR, 0.5 N) and Na_2CO_3 solution (10%) were added, respectively, and the final volume was completed to 300 μL with distilled water. Then, it was incubated for 30 min at room temperature in the dark. The absorbance at 760 nm was recorded spectroscopically. For the blank solution, 133.3 μL of FCR + 133.3 μL of Na_2CO_3 (10%) solution was added, and the final volume was completed to 300 μL with distilled water. For standard solutions, 0.1, 0.25, 0.5, 1.0, 2.0, 4.0, and 6.0 solutions containing 8.0 μg gallic acid were prepared. The results were expressed as gallic acid equivalents. The experiment were performed in triplicate and results are expressed as mean ± standard deviation ($n = 3$).

All samples were analyzed in duplicate.

2.7. DNA interaction assay

DNA interaction experiments were performed using pBR322 plasmid DNA following a previously reported method [32]. The harmful effects of Fenton solution and the protective effects of lipophilic oil fractions (root, leaf, and flower) on DNA conformation were assessed using the pBR322 plasmid DNA (Thermo Scientific pBR322, catalog number: SD0041, concentration: 0.5 μg μL^{-1}, quantity: 100 μg). 1 mg mL^{-1} dimethyl sulfoxide solutions of each fraction were prepared. For the experimental design, two distinct concentrations (1.0 mg mL^{-1} and 0.5 mg mL^{-1}) of each fraction were used to generate negative (just plasmid DNA) and positive control (Fenton + plasmid DNA) groups. A commercially available quercetin compound with known biological activities was used at a concentration of 1 mg mL^{-1} in addition to comparing the results. The reaction mixtures that were to be incubated were as follows: 3 μL of pBR322 plasmid DNA, 5 μL of Fenton solution (30 mM H_2O_2, 50 mM ascorbic acid, and 80 mM $FeCl_3$), and 5 μL of the synthesized/prepared compound solution from various concentrations (1000 mg L^{-1} and 10 mg L^{-1}). The final volume was completed to 20 μL with distilled water. After that, this mixture was incubated for 30 min at 37 °C. Five microlitres of 6× loading dye were added to the reaction mixture following incubation. An EtBr (ethidium bromide)-stained 0.8% agarose gel was loaded with 10 μL of the mixture. The agarose gel was run for 60 min at 100 V. A gel-imaging device was then used to capture gel images.

3. Results and discussions

The present research is among the studies carried out within the scope of phytochemistry. The *Primula vulgaris* plant was collected from Çorum province. The plant's lipophilic oil fractions, ash content, total nitrogen content, and total fat content were investigated. Studies were performed on the chemical composition of the obtained lipophilic oil

fractions using GC–MS, functional groups using FTIR, antioxidant activity, and DNA protection activity.

3.1. Lipophilic oil fraction yield, nitrogen, ash, and total fat content

The quantitative characteristics of *Primula vulgaris* flower, leaf, and root samples are summarized in Table 1. Lipophilic oil fraction yields obtained by hexane-based Soxhlet extraction showed pronounced organ-dependent differences. The highest oil yield was observed in the leaf sample (4.81 ± 0.61%), followed by the flower (3.39 ± 0.82%) and the root (2.02 ± 0.61%). These results clearly demonstrate that lipophilic constituents are unevenly distributed among different anatomical parts of the plant.

When interpreting the antioxidant, phytochemical analyses, and DNA damage assessment findings obtained from these lipophilic oil samples, several methodological considerations should be taken into account. Although prolonged Soxhlet extraction can promote partial oxidation of unsaturated lipid components, to minimize oxidative degradation, the extracts were subsequently stored under nitrogen in n-hexane solution in amber-colored glass vials at 4 °C. Furthermore, all samples were subjected to the same extraction, storage, and processing procedures. Therefore, the observed organ-specific differences in chemical composition and antioxidant behavior are thought to primarily reflect biological variation among plant organs.

Oil yields determined using the ANKOM XT15 system with petroleum ether were consistently lower than those obtained by Soxhlet extraction. The oil contents were measured as 2.87 ± 0.76% for leaves, 1.84 ± 0.45% for flowers, and 0.66 ± 0.13% for roots. This discrepancy can be attributed to differences in solvent polarity and extraction efficiency. Soxhlet extraction using *n*-hexane provides prolonged solvent–matrix contact, enabling more exhaustive recovery of lipophilic compounds compared to the relatively rapid extraction process of the ANKOM system [11,14].

The plant material analyzed in this study was collected from the Yalak Plateau at approximately 1100 m altitude. Previous studies have reported considerably lower oil yields for *Primula vulgaris* collected at similar altitudes when Soxhlet extraction was performed using more volatile solvents. For example, Yaylı et al. reported lipophilic oil fraction yields of 0.48–0.58% for *P. vulgaris* samples collected between 1100 and 1200 m using diethyl ether [33]. In comparison, the higher oil yields obtained in the present study are most likely related to differences in solvent polarity and extraction conditions rather than altitude alone, reflecting the broader recovery of lipophilic fractions by hexane-based Soxhlet extraction.

Nitrogen content analysis revealed that the leaf sample contained the highest nitrogen percentage (3.64 ± 0.16%), whereas the flower and root samples exhibited lower values of 1.97 ± 0.13% and 1.40 ± 0.11%, respectively. These findings are consistent with the metabolic role of leaves as the primary site of protein synthesis and photosynthetic activity [6]. Ash content values ranged from 7.98 ± 0.77% to 8.91 ± 0.72% and did not differ substantially among organs, indicating a relatively uniform mineral distribution across plant tissues.

Table 1
Determination of lipophilic oil fraction % with Soxhlet (hexane) and ANKOM XT15 (Petroleum ether, P.E.), total nitrogen %, ash content %.

Sample	Lipophilic oil fraction % (Soxhlet, hexane)	Lipophilic oil fraction % (ANKOM XT15, P. E.)	Total Nitrogen % (DUMAS)	Ash Content %
Flower	3.39 ± 0.82	1.84 ± 0.45	1.97 ± 0.13	8.91 ± 0.72
Root	2.02 ± 0.61	0.66 ± 0.13	1.40 ± 0.11	8.44 ± 0.89
Leaf	4.81 ± 0.61	2.87 ± 0.76	3.64 ± 0.16	7.98 ± 0.77

3.2. GC–MS-based lipophilic profiling of oil fractions

GC–MS analysis was applied to obtain a tentative lipophilic profile of the lipophilic oil fractions following methyl ester derivatization. Representative chromatograms for flower, leaf, and root oils are presented in Figs. S1–S27, and the relative abundance of tentatively assigned components is listed in Table 2.

The flower oil exhibited the most chemically diverse profile. The dominant tentatively assigned components were γ-linolenic acid methyl ester (26.57%), oleic acid methyl ester (25.18%), and palmitic acid methyl ester (19.63%). In addition, several long-chain hydrocarbons and minor fatty acid methyl esters, including tricosane (7.69%), 10-octadecenoic acid methyl ester (7.68%), 3-hexanol (4.30%), tetracosane (3.19%), and dodecanoic acid methyl ester (1.98%), were detected. The relatively high proportion of γ-linolenic acid methyl ester is noteworthy, as this compound is widely recognized for its physiological and nutritional relevance [34].

In contrast, the leaf oil displayed a more simplified profile dominated by stearic acid methyl ester (48.71%) and oleic acid methyl ester (23.91%), together accounting for over 70% of the total tentatively assigned components. Palmitic acid methyl ester (15.12%) constituted another major fraction, while minor components such as linoleic acid methyl ester (2.68%), cis-10-heptadecenoic acid methyl ester (2.49%), and palmitoleic acid methyl ester (1.12%) contributed to the overall profile. Comparable fatty acid dominance patterns have been reported in leaf-derived oils of other *Primula* species [35].

The root oil showed the least complex profile among the analyzed samples, with oleic acid methyl ester accounting for 58.85% of the total composition, followed by palmitic acid methyl ester (27.74%). Additional minor components included cis-10-heptadecenoic acid methyl ester (6.03%), arachidic acid methyl ester (2.73%), and palmitoleic acid methyl ester (1.54%). The dominance of monounsaturated fatty acid derivatives in the root oil suggests a lipid storage function consistent with underground plant organs [36–38].

Across all samples, 3-hexanol, palmitic acid methyl ester, and oleic acid methyl ester were common constituents, although their relative abundances differed markedly. Oleic acid methyl ester was the most abundant component in all oils, with the highest proportion observed in the root sample. Such organ-dependent variations in fatty acid composition have also been documented for *P. vulgaris* and related species growing at different altitudes and under varying environmental conditions [37].

Compound identification was achieved by comparison of retention times and mass spectra with those of authentic reference standards for major fatty acid methyl esters. For minor constituents lacking reference standards, identification was performed by mass spectral library matching and should therefore be considered tentative. This combined approach provides a reliable and conservative characterization of the lipophilic profiles of the lipophilic oil fractions [39].

Major components like oleic acid methyl ester, palmitic acid methyl ester, and stearic acid methyl ester were confirmed using authentic reference standards, while minor constituents were identified through library matching.

3.3. FTIR characterization of lipophilic oil fractions

FTIR spectra of the flower, leaf, and root oil samples (Fig. 2) exhibited characteristic absorption bands associated with fatty acid methyl ester-rich matrices. In all samples, strong bands observed at approximately 1739–1727 cm^{-1} were attributed to carbonyl (C=O) stretching vibrations of ester groups, confirming the predominance of esterified fatty acids.

Broad absorption bands in the region of 3314–3376 cm^{-1} were assigned to O—H stretching vibrations, while bands near 3010–3072 cm^{-1} corresponded to alkene ($C(sp^2)$–H) stretching, indicating the presence of unsaturated components. Aliphatic C—H stretching

Table 2
Primula vulgaris flower, leaf, and root samples hexane extract (lipophilic oil fraction) of the flower, leaf, and root samples of *Primula vulgaris* was analyzed using GC–MS to identify their components*.

No	RT	Component Name	Identification**	Flower	Leaf	Root
				Area %	Area%	Area%
1	7.42	3-Hexanol (CAS)	Lib	4.30	1.42	1.65
2	25.14	Dodecanoic acid methyl ester (CAS)	Std	1.98	–	1.46
3	31.00	Tetradecanoic acid methyl ester (CAS)	Std	1.22	–	–
4	34.45	Docosane (CAS)	Std	2.56	–	–
5	34.65	Palmitic acid methyl ester (C16:0)	Std	19.63	15.12	27.74
6	36.33	Palmitoleic acid methyl ester (C16:1)	Std	–	1.12	1.54
7	37.23	Trichosan (CAS)	Std	7.69	–	–
8	37.50	2-Hexadecenoic acid methyl ester	Std	–	1.57	–
9	38.82	Tetracosane (CAS)	Std	3.19	–	–
10	39.56	Cis-10-Heptadecenoic acid methyl ester (C17:1)	Std	–	2.49	6.03
11	40.01	10-Octadecenoic acid methyl ester (CAS)	Lib	7.68	–	–
12	40.60	Oleic acid methyl ester (18:1n9c)	Std	25.18	23.91	58.85
13	41.24	Linoleic acid methyl ester (18:2n6c)	Std	–	2.68	–
14	42.46	γ-Linolenic acid methyl ester (18:3n6)	Std	26.57	–	–
15	42.68	Stearic acid methyl ester (C18:0)	Std	–	48.71	–
16	42.90	Arachidic acid methyl ester (C20:0)	Std	–	–	2.73
		Total Percentage (%)		100%	97.02%	100%

*Only compounds meeting the identification criteria based on mass spectral matching and/or authentic reference standards are reported. Peaks not meeting identification criteria were excluded from the reported composition table. **Identification; Std: confirmed with authentic standard; Lib: assigned by MS library matching.

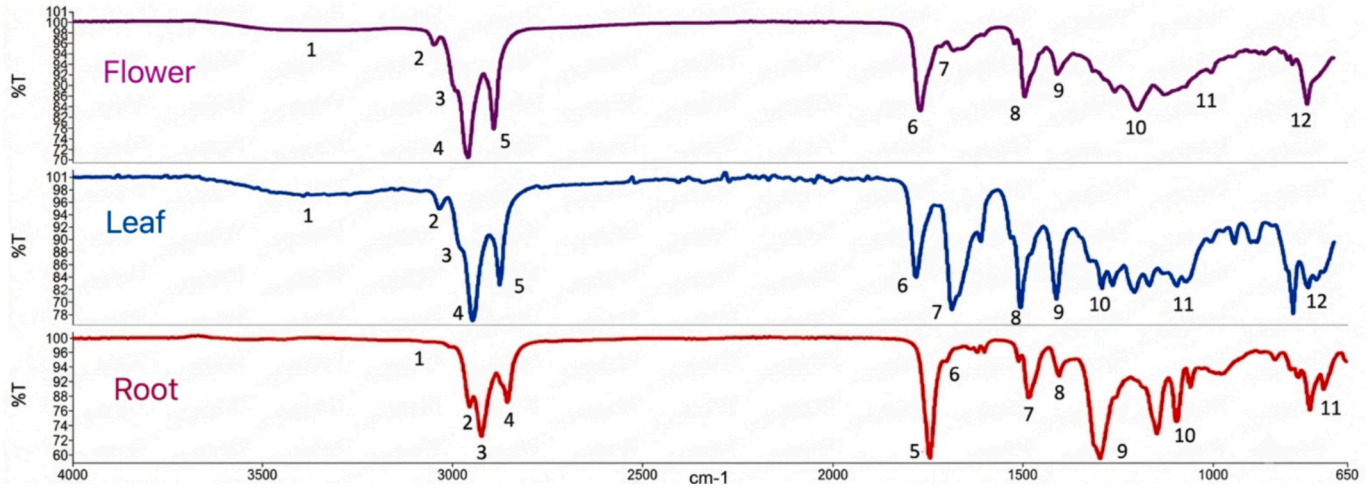

Fig. 2. ATR-FTIR spectra of flower, leaf, and root lipophilic fractions of *P. vulgaris*.

vibrations of –CH_2 and –CH_3 groups were observed between 2850 and 2950 cm^{-1}. Additional bands at approximately 1460–1370 cm^{-1} were attributed to CH_3 bending vibrations, and bands near 1058–1096 cm^{-1} corresponded to C—O stretching. These spectral features are consistent with previously reported FTIR profiles of lipid-rich plant oils and corroborate the GC–MS-based profiling results [40,41].

3.4. Antioxidant activity

The antioxidant properties of the lipophilic oil fractions obtained from the root, leaf, and flower of *Primula vulgaris* were evaluated using DPPH radical scavenging, CUPRAC, total phenolic content (TPC), and total flavonoid content (TFC) assays (Table 3). The combined use of these complementary methods enabled a comprehensive assessment of both radical scavenging and reducing capacities of the lipophilic extracts. However, these assays provide preliminary chemical antioxidant information and do not necessarily reflect *in vivo* efficacy.

By taking 50 µL of 1 mg mL^{-1} solutions of root, leaf, and flower essential oils obtained from *Primula vulgaris* in dimethyl sulfoxide, the DPPH• radical scavenging effect was investigated. For this purpose, standard calibration curves, as shown in Fig. S31 (given in *Supplementary material),* were created using Trolox and ascorbic acid standard

Table 3
Antioxidant activity of the samples (DPPH, CUPRAC), total flavonoid amount and total phenolic compound amount.

Lipophilic oil fraction	DPPH		CUPRAC		Total Phenolic Compound	Total Flavonoid
	Ascorbic acid µg $µL^{-1}$	Trolox equivalent µg $µL^{-1}$	Ascorbic acid equivalent µg $µL^{-1}$	Trolox equivalent µg $µL^{-1}$	Gallic acid equivalent µg $µL^{-1}$	Quercetin equivalent µg $µL^{-1}$
Flower oil	0.0149 ± 0.0007	0.0326 ± 0.0022	0.2054 ± 0.0189	0.0005 ± 0.0000	0.0085 ± 0.0003	0.1674 ± 0.0025
Leaf oil	0.0156 ± 0.0008	0.0272 ± 0.0018	0.1917 ± 0.0138	0.0005 ± 0.0000	0.0094 ± 0.0004	0.2327 ± 0.0042
Root oil	0.0166 ± 0.0010	0.0407 ± 0.0030	0.2309 ± 0.0226	0.0006 ± 0.0000	0.0099 ± 0.0006	0.1831 ± 0.0029

materials. The DPPH• radical scavenging effect of the root, leaf, and flower essential oils obtained from *Primula vulgaris* was determined spectrophotometrically using the standard calibration curves. The antioxidant amounts of the flower, leaf, and root samples presented in Table 3 were calculated as ascorbic acid and Trolox equivalents.

In the DPPH assay, the root oil exhibited the highest radical scavenging activity, expressed as 0.0166 μg μL^{-1} ascorbic acid equivalent and 0.0407 μg μL^{-1} Trolox equivalent. The flower and leaf oils showed lower but comparable activities. This trend suggests that the higher abundance of unsaturated fatty acid derivatives in the root oil, particularly oleic acid methyl ester, may contribute to hydrogen-donating ability in nonpolar systems, consistent with previous observations for lipophilic antioxidant fractions [42]. The root oil fraction exhibited the highest DPPH radical scavenging activity among the investigated organs, followed by the leaf and flower oils. Similar organ-dependent differences in antioxidant activity have been reported for several Primula species, although the organ showing the highest activity varies depending on the species and extraction procedure employed [38,43]. For example, flower extracts of *Primula veris* subsp. *columnae* showed stronger DPPH scavenging activity than leaf extracts, whereas strong antioxidant activity has also been reported for root/rhizome extracts of other Primula species [38,44]. Direct quantitative comparison with the present results should be discussed with difficulty because most previous studies investigated polar solvent extracts and expressed antioxidant activity using different assay formats and units [45].

The distinctive advantage of the CUPRAC method over other electron transfer (ET) methods in analyzing total antioxidant capacity (TAC) is that the pH can be easily adjusted, the reagents are user-friendly and stable, and the method is low-cost and straightforward. It can be applied to both lipophilic and hydrophilic antioxidants [29]. For determining the Cu(II) ion-reducing antioxidant capacity (CUPRAC) method, ascorbic acid and Trolox were used as standard reference substances to obtain the calibration curves, as shown in Fig. S32 (given in *Supplementary material)*. A similar pattern was observed in the CUPRAC assay, where the root oil demonstrated the highest Cu(II)-reducing capacity (0.2309 μg μL^{-1} ascorbic acid equivalent), followed by the flower (0.2054 μg μL^{-1}) and leaf (0.1917 μg μL^{-1}) oils. The agreement between DPPH and CUPRAC results suggests that electron-transfer mechanisms may contribute to the antioxidant behavior of the root oil fraction. The effectiveness of the CUPRAC method in evaluating lipophilic antioxidants further highlights its suitability for lipophilic oil fraction characterization [29].

Total phenolic compound analysis followed the Folin-Ciocalteau (FCR) method, utilizing 10 μL of 0.5 mg mL^{-1} solutions of root, leaf, and flower essential oils derived from the *Primula vulgaris* plant in dimethyl sulfoxide. For this purpose, a gallic acid standard calibration curve, shown in Fig. S33 (given in *Supplementary material)*, was created to determine the total phenolic compound amounts in the root, leaf, and flower essential oils. In contrast to the radical scavenging and reducing assays, TPC values were uniformly low across all samples, ranging from 0.0086 to 0.0099 μg μL^{-1} gallic acid equivalents. This outcome is consistent with the use of hexane as a nonpolar solvent, which preferentially extracts lipophilic constituents rather than phenolic compounds. Despite these low TPC values, measurable antioxidant activity was still observed, suggesting that non-phenolic lipophilic components may contribute to the overall antioxidant potential of the oils.

Subsequently, the total flavonoid content was assessed. A standard calibration curve (given in *Supplementary material, Fig. S34*) was established using quercetin as a standard reference substance, and total flavonoid contents were calculated as quercetin equivalents. TFC analysis revealed greater variability among the samples. The leaf oil exhibited the highest flavonoid content (0.2327 μg μL^{-1} quercetin equivalent), followed by the root (0.1831 μg μL^{-1}) and flower (0.1674 μg μL^{-1}) oils. This finding suggests that certain flavonoid-derived or flavonoid-associated compounds may still be co-extracted under nonpolar conditions, particularly from leaf tissue. Similar organ-dependent differences in flavonoid-associated antioxidant activity have been reported for *Primula* species extracted using more polar solvents, although at substantially higher absolute concentrations [37,46].

In the literature, there is a research on the differences in the phenolic content and antioxidant activity of the flower, leaf, and root parts of *Primula veris* grown in Romania. The extracts of *Primula veris* were obtained from ethanol by maceration for seven days. The results indicated that ethanolic extracts of *Primula veris* contained high phenolic substances, varying between 136 and 159 mg gallic acid equivalent (GAE) L^1 for leaves, 131–168 mg GAE L^{-1} for roots, and 133–219 mg GAE L^{-1} for flowers. A significant DPPH inhibition percentage was recorded in all plant parts (roots 16.4–23.2%, leaves 16.0–17.0%, and flowers 14.9–24.7%) [38]. The antioxidant properties of essential oils from basil (*Ocimum basilicum L.*) and rosemary (*Rosmarinus officinalis L.*), naturally growing in the vicinity of Balıkesir, were evaluated using DPPH scavenging antioxidant activity and phenolic substance methods, showing higher values than other oils. The essential oils in the flowers and leaves of *Primula veris* subsp. *columnae*, the antioxidant and antimicrobial (antibacterial and antifungal) properties of methanol extracts were determined. The antioxidant properties of the *Primula veris* subsp. *columnae* plant were found to be relatively high [37].

When the results of all antioxidant assays are evaluated collectively, the root oil can be regarded as the most effective fraction in terms of radical scavenging and reducing capacity, whereas the leaf oil is distinguished by its relatively higher flavonoid-associated antioxidant contribution. These observations underscore the importance of organ-specific chemical composition in determining antioxidant behavior and demonstrate that lipophilic extracts of *P. vulgaris* possess measurable and differentiated antioxidant properties despite their low phenolic content.

3.5. DNA interaction and protection assessment

DNA interaction studies were performed using pBR322 plasmid DNA and agarose gel electrophoresis to evaluate both the genotoxic potential and DNA protective capacity of the lipophilic oil fractions (Fig. 3). In the absence of Fenton reagents (wells 1–6), all lipophilic oil samples obtained from the root, leaf, and flower of *Primula vulgaris* preserved the native plasmid DNA conformation (Form I) at both tested concentrations (1.0 and 0.5 mg mL^{-1}) in lines 1–6. The maintenance of supercoiled (Form I) and open circular (Form II) DNA forms indicates that none of the oils induced detectable DNA strand breaks under the experimental conditions, demonstrating their non-genotoxic character.

The goal of DNA damage assessment analysis is for the oil samples to stop or prevent DNA damage. Fenton, plasmid, and the 1.0 mg mL^{-1} and 0.5 mg mL^{-1} solutions were applied to the wells as flower, leaf, and root, respectively. The presence of two bands (Forms I and II) in the gel image obtained from lanes 7–12 indicates that DNA damage occurred, despite the inclusion of flower, leaf, and root samples in the medium. The control lanes were assessed to elucidate the response of plasmid DNA to the oxidative conditions applied. The typical pattern of an intact plasmid with a supercoiled Form I band was seen in lane 13 with plasmid DNA and water. Lane 14 with plasmid DNA and the Fenton reagent, in contrast, displayed conversion of Form I to Form II with marked reduction of the supercoiled band, confirming oxidative DNA strand damage [47]. No obvious band for Form III (linear DNA) was detected, which suggested that the Fenton conditions used primarily led to single-strand DNA breaks rather than substantial double-strand cleavage. In lane 15, quercetin alone (1 mg mL^{-1}) did not cause detectable DNA damage. However, the supercoiled Form I band was not preserved or restored in the presence of both quercetin and the Fenton reagent in lane 16. Hence, with the experimental conditions employed, no detectable protection of plasmid DNA against Fenton-induced damage was observed by quercetin [48]. This result suggests that the oxidative challenge imposed by the Fenton system may have exceeded the protective capacity of quercetin under the experimental conditions

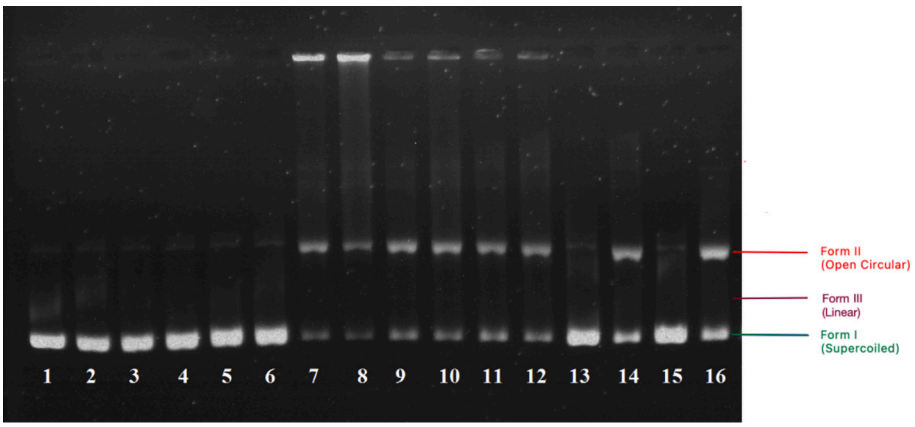

Fig. 3. Effect of *Primula vulgaris* root, leaf, and flower lipophilic oil fractions on H_2O_2-induced plasmid DNA damage assessment analysis using agarose gel electrophoresis. Form I: Supercoiled plasmid DNA; Form II: Open circular plasmid DNA, and Form III: Linear plasmid DNA (not observed). (1; 1 mg mL^{-1} flower + plasmid, 2; 0.5 mg mL^{-1} flower + plasmid, 3; 1 mg mL^{-1} leaf + plasmid, 4; 0.5 mg mL^{-1} leaf + plasmid, 5; 1 mg mL^{-1} root + plasmid, 6; 0.5 mg mL^{-1} root + plasmid, 7; 1 mg mL^{-1} flower + plasmid + Fenton, 8; 0.5 mg mL^{-1} flower + plasmid + Fenton, 9; 1 mg mL^{-1} leaf + plasmid + Fenton, 10; 0.5 mg mL^{-1} leaf + plasmid + Fenton, 11; 1 mg mL^{-1} root + plasmid + Fenton, 12; 0.5 mg mL^{-1} root + plasmid + Fenton, 13; negative for damage (plasmid + water), 14; positive for damage (plasmid + water + Fenton), 15; negative for damage (plasmid +1 mg mL^{-1} quercetin), 16; positive for damage (plasmid +1 mg mL^{-1} quercetin + Fenton).

employed. In light of these results, it was concluded that the presence of flower, leaf, and root lipophilic oil materials did not cause DNA damage in biological systems and also did not exhibit a protective effect against the DNA damage that occurred, indicating insensitivity.

Upon exposure to Fenton reagents, pronounced DNA strand cleavage was observed in all treated samples, as evidenced by the conversion of supercoiled DNA to relaxed and linear forms. None of the lipophilic oil fractions were able to prevent or significantly reduce hydroxyl radical–induced DNA damage at the tested concentrations. These findings indicate that, despite exhibiting measurable antioxidant activity in chemical assays, the lipophilic oil fractions lack sufficient reactivity to counteract severe oxidative stress at the DNA level.

Comparable discrepancies between antioxidant capacity measured by chemical assays and DNA protection efficacy have been reported for other lipophilic plant-derived extracts [33,46.] Such observations suggest that antioxidant mechanisms effective in radical scavenging or electron-transfer assays do not necessarily translate into direct protection against highly reactive hydroxyl radicals generated during Fenton reactions [49–51].

Overall, the absence of genotoxic effects combined with the lack of DNA-protective activity indicates that *P. vulgaris* lipophilic oil fractions cause no plasmid DNA damage under tested conditions but exhibit limited capacity to mitigate oxidative DNA damage. This distinction highlights the importance of integrating DNA-based assays alongside conventional antioxidant tests when evaluating the biological relevance of lipophilic plant extracts.

The results indicate that the tested lipophilic fractions did not exhibit a detectable protective effect against hydroxyl radical-induced plasmid DNA damage under the experimental conditions employed. Further studies involving cellular and mechanistic assays are required to better elucidate the biological relevance of these findings.

3.6. Structure–activity considerations

The structure–activity relationship analysis indicates that antioxidant performance is influenced by both the degree of unsaturation and the relative abundance of dominant fatty acid methyl esters in the lipophilic oil fractions. Oils characterized by higher proportions of unsaturated fatty acid derivatives, particularly oleic and γ-linolenic acid methyl esters, tended to exhibit enhanced radical scavenging and reducing capacities in the applied assays.

Notably, the leaf oil, despite its moderate level of unsaturated fatty acids, displayed the highest flavonoid content, suggesting that non-lipid phytochemicals may also contribute to antioxidant behavior. These observations indicate that the functional properties of *P. vulgaris* lipophilic oil fractions are governed by a combined effect of organ-specific lipid composition and auxiliary phytochemical constituents rather than by lipid composition alone.

Collectively, this organ-based evaluation supports the interpretation of the experimental results within a structure–activity framework, highlighting the multifactorial nature of antioxidant behavior in lipophilic plant extracts.

4. Conclusions

This study provides a comprehensive, organ-based characterization of the lipophilic composition and functional properties of *Primula vulgaris* lipophilic oil fractions obtained by hexane-based Soxhlet extraction. Clear organ-dependent differences were observed in both oil yield and chemical profiles, as revealed by GC–MS-based lipophilic profiling supported by authentic reference standards and FTIR analysis. Flower, leaf, and root oils exhibited distinct fatty acid methyl ester distributions, with γ-linolenic acid derivatives predominating in flower oil, stearic and oleic acid derivatives dominating the leaf oil, and oleic acid methyl ester representing the major component of the root oil. Although oleic, palmitic, stearic and γ-linolenic acid derivatives are common constituents of plant lipids, their comparative distribution among different organs of *P. vulgaris* has been reported only sparsely. The significance of the present study is therefore based on the organ-specific characterization of the lipophilic profiles and not on the occurrence of the individual fatty acids. Antioxidant evaluation using DPPH, CUPRAC, total phenolic, and total flavonoid assays demonstrated that the root oil exhibited the highest overall reducing and radical scavenging capacity, whereas the leaf oil was distinguished by its higher flavonoid content. These findings suggest that antioxidant performance in *P. vulgaris* oils is influenced by both the degree of lipid unsaturation and the contribution of auxiliary phytochemicals. DNA interaction studies showed that none of the lipophilic fractions caused detectable plasmid DNA damage under the tested conditions. However, more cellular and mechanistic studies are required to fully assess their biological effects and safety profiles. However, the oils did not exhibit protective effects against hydroxyl radical–induced DNA strand breakage, suggesting that their antioxidant activity is more pronounced in chemical assay systems than at the level of severe oxidative DNA stress. Overall, this work represents one of the first integrated organ-specific evaluations of the lipophilic composition, antioxidant behavior, and DNA interaction profile of *P. vulgaris* lipophilic

fractions. The results broaden current phytochemical knowledge of this species, and indicate its potential as a source of bioactive lipophilic constituents. At the same time the limitations of its bioactivity at the level of DNA protection are outlined.

CRediT authorship contribution statement

Baki Çiçek: Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Mehmet Oğuz İpekçi:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Ümit Çalışır:** Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Ümit Çakır:** Writing – original draft, Validation, Supervision, Methodology, Investigation, Conceptualization.

Clinical trial number

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jchromb.2026.125203.

Data availability

All experimental data (GC–MS outputs, FTIR spectra, antioxidant measurements, and gel documentation) supporting the conclusions of this article are available from the corresponding author upon reasonable request. The authors affirm that no proprietary or restricted-access data were used in this study.

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