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Phytochemical screening and antioxidant evaluation of *Deverra tortuosa* aerial parts from Eastern Libya

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Keywords: Antioxidant activity, Apiaceae, coumarins, DPPH assay, flavonoids, phenolics

Abstract: *Deverra tortuosa* (Desf.) DC. is a notable xerophytic medicinal plant belonging to the Apiaceae family. Despite its recognized pharmacological value, phytochemical and antioxidant characterization of populations native to eastern Libya remains limited. This study evaluated the qualitative and quantitative phytochemical profiles and *in vitro* antioxidant capacity of *Deverra tortuosa* aerial parts collected in Benghazi, eastern Libya. Powdered plant material was extracted with 96.0% methanol and sequentially fractionated using n-hexane, ethyl acetate, and methanol. Qualitative screening detected phenols, tannins, flavonoids, coumarins, terpenoids, steroids, saponins, carbohydrates, proteins, amino acids, and cardiac glycosides, whereas alkaloids and free anthraquinones were absent. Spectrophotometric quantification demonstrated substantial reserves of specialized secondary metabolites, dominated by total coumarins (39.57±0.97), followed by terpenoids (29.31±0.96), flavonoids (27.82±0.93), and phenolics (8.58±0.14 mg GAE/g extract). The crude methanolic extract exhibited potent, concentration-dependent DPPH radical-scavenging activity, with percentage inhibition increasing from 57.30±0.56% at 250 µg/mL to 74.30±0.70% at 500 µg/mL, yielding a linear regression-derived IC_{50} of 129.90 µg/mL. Qualitative TLC-DPPH testing corroborated strong free-radical scavenging capacity. These findings establish *Deverra tortuosa* from eastern Libya as a rich natural source of bioactive coumarins and natural antioxidants.

Introduction

The family Apiaceae comprises numerous aromatic and medicinal plants widely recognized for their rich chemical diversity and therapeutic applications [1]. Xerophytic taxa within this family, such as those belonging to the genus Deverra (formerly *Pituranthos*), possess specialized metabolic adaptations that enable survival in arid environments, often generating unique secondary metabolite profiles [2]. *Deverra tortuosa* (*D. tortuosa*, Desf.) DC. is a prominent North African medicinal shrub traditionally used to treat gastrointestinal disorders, respiratory ailments, inflammation, and infections [3, 4]. Pharmacological studies have documented antioxidant, anti-inflammatory, and antimicrobial activities [3-6]. Coumarins, furanocoumarins, flavonoids, and essential oil terpenes constitute the primary bioactive markers of *D. tortuosa* [4, 5]. Climatic conditions, soil characteristics, and environmental stressors strongly modulate secondary metabolite synthesis in wild plants. Consequently, specimens collected from different geographic locations frequently exhibit quantitative and qualitative chemical

variations [7]. Although *D. tortuosa* has been investigated in western Libya and neighboring North African regions, detailed phytochemical and antioxidant data on wild populations from eastern Libya remain scarce. To address this knowledge gap, the present study provides targeted phytochemical screening and *in vitro* antioxidant evaluation of *D. tortuosa* aerial parts harvested in Benghazi, eastern Libya. Qualitative screening, quantitative spectrophotometric assays of major metabolite classes, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging tests were performed to establish its bioactive profile.

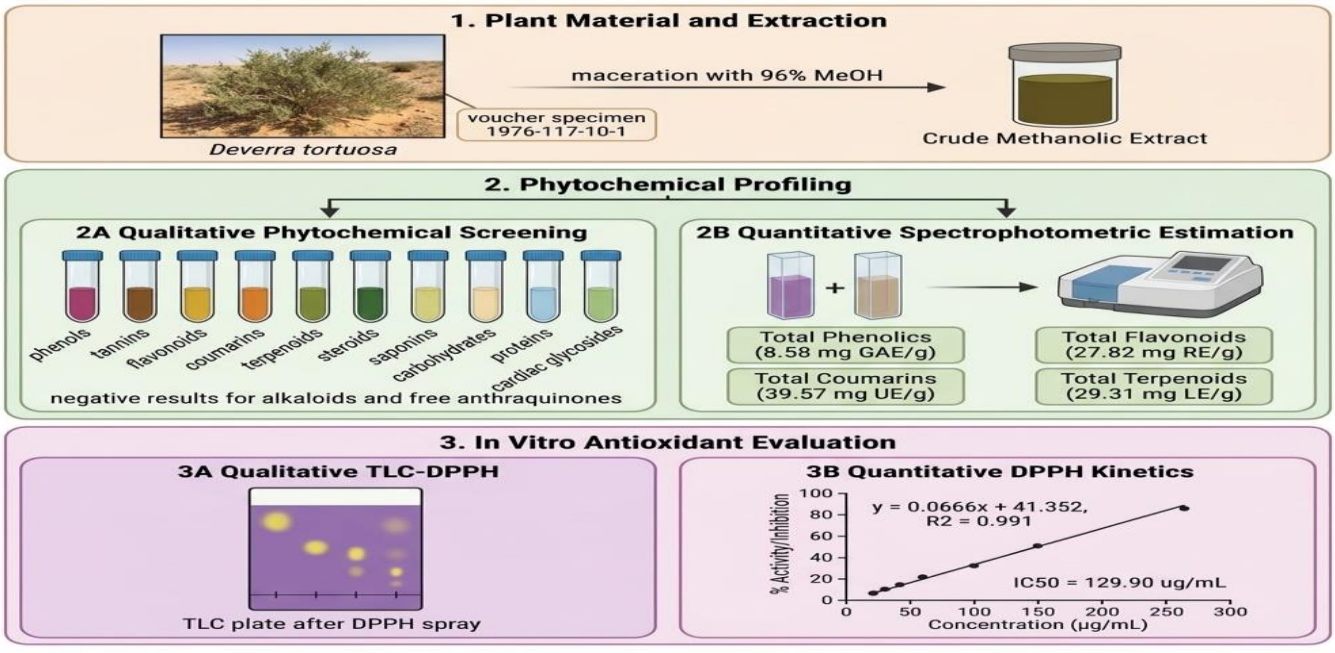
Materials and methods

Plant material collection and authentication: Aerial parts of *D. tortuosa* were collected in Benghazi, eastern Libya, in April 2025. Botanical identification and authentication were performed by M. Maklouf and M. Abuhadra (Department of Botany, Faculty of Science, University of Tripoli, Tripoli, Libya). A voucher specimen (catalogue number 1976-117-10-1) was deposited in the institutional herbarium.

Extraction and fractionation procedure: Plant material was shade-dried at room temperature for two weeks and finely pulverized. For crude extraction, 50 g of powdered material was macerated in 96.0% methanol (1: 10 w/v) at room temperature (25.0±2.0°C) for three days with regular agitation. The mixture was filtered through Whatman No. 1 paper and evaporated under reduced pressure at temperatures below 40.0°C. The extraction yield was calculated as: yield (% w/w)=(weight of dried extract/weight of dry plant material)×100.

For sequential fractionation, 100 g of powdered plant material was extracted successively with 600 mL n-hexane, 600 mL ethyl acetate, and 500 mL 96.0% methanol (three days per solvent step). The residues were dried prior to each subsequent solvent treatment. Concentrated extracts were stored at 4.0°C. The conceptual experimental workflow is summarized in **Figure 1**.

FIGURE 1: SCHEMATIC OVERVIEW OF EXPERIMENTAL WORKFLOW FOR PHYTOCHEMICAL CHARACTERIZATION AND ANTIOXIDANT EVALUATION OF *DEVERRA TORTUOSA* AERIAL PARTS FROM EASTERN LIBYA



Qualitative phytochemical screening: Qualitative phytochemical tests were conducted on the crude methanolic extract using established protocols [8-13]. Assays were performed to evaluate the presence of proteins, amino acids, carbohydrates, phenols, tannins, flavonoids, saponins, cardiac glycosides, terpenoids, steroids, alkaloids, coumarins, and free anthraquinones.

Quantitative spectrophotometric estimation: Total phenolic content (TPC) was quantified via the Folin-Ciocalteu colorimetric method. Briefly, 1.0 mL of methanolic extract solution was mixed with 2.5 mL of 10.0% Folin-Ciocalteu reagent and 2.0 mL of 2.0% sodium carbonate. After 15 min incubation at 25°C, absorbance was measured at 765 nm. Gallic acid was used as a standard, and results were expressed as mg gallic acid equivalents per gram of extract (mg GAE/g).

Total flavonoid content was estimated using the aluminum chloride assay. Extract solution (1.0 mL) was combined with 3.0 mL methanol, 0.2 mL of 10.0% aluminum chloride, 0.2 mL of 1.0 M potassium acetate, and 5.6 mL distilled water. Following 30 min incubation, absorbance was recorded at 420 nm. Rutin was used as the standard (mg rutin equivalents per gram extract, mg RE/g).

Total coumarin content was measured using umbelliferone as the standard. Extract (1.0 mL) was treated with 1.0 mL of 0.1 M NaOH and diluted to 10 mL with 95.0% ethanol. Absorbance was read at 320 nm after 30 min in darkness, reported as mg umbelliferone equivalents per gram extract (mg UE/g).

Total terpenoid content was determined using sulfuric acid colorimetry with linalool as standard. Extract (1.0 mL) in 2.0 mL chloroform was layered over 1.5 mL concentrated sulfuric acid. Absorbance was measured at 538 nm after 2.0 hrs. (mg linalool equivalents per gram extract, mg LE/g). All assays were executed in triplicate [12].

In vitro DPPH radical-scavenging assay: Antioxidant activity was evaluated using the DPPH radical-scavenging method [14]. For qualitative visualization, 10.0 µL extract was spotted onto a silica gel TLC plate and sprayed with 0.1 mM DPPH in methanol. The plate was incubated in the dark for 30 min; yellow spots against a purple background indicated radical-scavenging activity. For quantitative analysis, extract solutions (250-500 µg/mL) were mixed with 2.0 mL of 0.1 mM DPPH methanolic solution and incubated in the dark at 25°C for 30 min. Absorbance was recorded at 517 nm against a blank. Scavenging percentage was calculated as: DPPH Inhibition (%)=[(A0-As)/A0]×100, where A0 is the control absorbance and As is the sample absorbance. The IC_{50} value was calculated using linear regression analysis.

Statistical analysis: Assays were performed in triplicate, and data are presented as mean±standard deviation (SD). Linear regression analysis was used to determine the IC_{50} value.

Results

Extraction yield and preliminary phytochemical screening: The extraction yield of crude methanolic extract was 2.33%w/w. Sequential solvent fractionation yielded 1.025% w/w for n-hexane, 1.002% w/w for ethyl acetate, and 1.812% w/w for the final methanolic fraction. Qualitative phytochemical screening confirmed the presence of phenols, tannins, flavonoids, coumarins, terpenoids, steroids, saponins, carbohydrates, proteins, amino acids, and cardiac glycosides. Alkaloids and free anthraquinones were absent (**Table 1**).

Table 1: Qualitative phytochemical screening of *Deverra tortuosa* methanolic extract

Phytochemical group	Findings	Phytochemical group	Findings
Proteins and amino acids	+	Terpenoids	+
Carbohydrates	+	Steroids	+
Phenols and tannins	+	Alkaloids	-
Flavonoids	+	Coumarins	+
Saponins	+	Free anthraquinones	-
Cardiac glycosides	+		

+: Detected/present and −: Not detected/absent

Quantitative phytochemical composition: Spectrophotometric quantification revealed substantial secondary metabolite concentrations. Coumarins constituted the predominant chemical class (39.57±0.97 mg UE/g extract), followed by terpenoids (29.31±0.96 mg LE/g extract) and flavonoids (27.82±0.93 mg RE/g extract). Total phenolic content was recorded at 8.58±0.14 mg GAE/g extract (**Table 2**).

Table 2: Quantitative phytochemical composition of *Deverra tortuosa* crude methanolic extract

Phytochemical class	Standard reference	Wavelength (nm)	Content (mg equivalent/g extract)*
Total phenolics	Gallic acid	765	8.58±0.14 mg GAE/g
Total flavonoids	Rutin	420	27.82±0.93 mg RE/g
Total coumarins	Umbelliferone	320	39.57±0.97 mg UE/g
Total terpenoids	Linalool	538	29.31±0.96 mg LE/g

* Values are presented as Mean±Standard Deviation of triplicate determinations.
GAE: Gallic acid equivalents; RE: Rutin equivalents; UE: Umbelliferone equivalents; LE: Linalool equivalents

In vitro DPPH antioxidant scavenging activity: The crude methanolic extract showed marked, concentration-dependent DPPH free-radical scavenging activity. Scavenging percentages increased progressively across the evaluated range: 57.30±0.56% at 250.0 µg/mL, 61.40±0.46% at 300.0 µg/mL, 65.70±0.61% at 350.0 µg/mL, 68.10 ±0.60% at 400 µg/mL, 71.10±0.56% at 450.0 µg/mL, and 74.30±0.70% at 500.0 µg/mL (**Table 3**).

Table 3: In vitro DPPH free-radical scavenging activity and linear regression parameters of *Deverra tortuosa* methanolic extract

Concentration (µg/mL)	DPPH Inhibition (%) *	Linear Regression Parameters (y=mx+c, R^2=0.991)		
		Slope (m)	Intercept (c)	Extrapolated IC_{50} (µg/mL)
250	57.30±0.56	0.0666	41.35	129.90
300	61.40±0.46	—	—	—
350	65.70±0.61	—	—	—
400	68.10±0.60	—	—	—
450	71.10±0.56	—	—	—
500	74.30±0.70	—	—	—

* Values represent the mean ± Standard Deviation of triplicate measurements.
Linear regression equation: y=0.0666x+41.352 (R^2=0.991)

In **Figure 2**, linear regression analysis (y=0.0666x+41.352, R^2=0.991) yielded an extrapolated IC_{50} value of 129.90 µg/mL. TLC-DPPH qualitative screening confirmed rapid decolorization of the radical. Thus, **Figure 2** shows a linear regression plot of DPPH radical-scavenging activity (R^2=0.991) for the methanolic extract of *D. tortuosa* aerial parts, yielding an IC_{50} of 129.90 µg/mL.

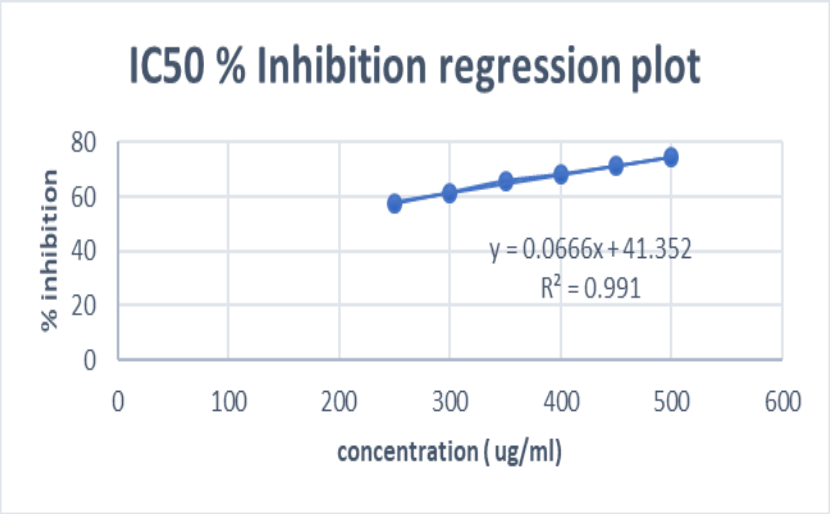


Figure 2: DPPH radical-scavenging activity linear regression plot (R^2=0.991) for the crude methanolic extract of *Deverra tortuosa* aerial parts, demonstrating concentration-dependent inhibition (250-500 µg/mL) and yielding an extrapolated IC_{50} value of 129.90 µg/mL

Discussion

Xerophytic medicinal plants indigenous to the hyper-arid and semi-arid ecosystems of North Africa have evolved specialized metabolic adaptations as defensive survival mechanisms against environmental stressors, including intense solar radiation, thermal fluctuations, and severe drought [1, 2]. In the present study, qualitative phytochemical profiling of *D. tortuosa* aerial parts collected from Benghazi, eastern Libya, established a robust chemical baseline. The extract displayed a rich accumulation of phenolic compounds, tannins, flavonoids, coumarins, terpenoids, and saponins, whereas alkaloids and free anthraquinones were noticeably absent. This qualitative pattern aligns closely with previous pharmacognostic evaluations of Deverra species across North Africa and the Middle East, confirming a conserved biosynthetic profile within the Apiaceae family [3, 5]. Quantitative spectrophotometric estimation provided further insight into metabolite distribution, revealing that coumarins constitute the predominant chemical class within the crude extract (39.57±0.97 mg UE/g). Coumarins and furanocoumarins serve as key chemotaxonomic markers for the genus Deverra (formerly classified under *Pituranthos*), playing vital ecological roles in plant defense against oxidative stress and herbivory [2, 3]. Furthermore, the substantial co-occurrence of terpenoids (29.31±0.96 mg LE/g) and flavonoids (27.82±0.93 mg RE/g) points to a multi-component phytochemical matrix. When compared to prior reports on North African populations-such as investigations by Guetat et al. [3] and Elshibani et al. [6] on western Libyan populations -the eastern Libyan specimens analyzed here demonstrate notable quantitative variations. These subtle chemical shifts are largely driven by geo-climatic heterogeneity, edaphic composition, microclimates, and seasonal harvesting stress, which collectively influence secondary metabolite accumulation [4, 6].

In evaluating radical-scavenging capacity, the crude methanolic extract demonstrated marked, concentration-dependent *in vitro* antioxidant activity in the DPPH assay. The scavenging percentage rose steadily from 57.30±0.56% at 250 µg/mL to 74.30±0.70% at 500 µg/mL, displaying excellent linearity (R^2=0.991) across the regression model (y=0.0666x+41.352). This yielded an extrapolated IC_{50} value of 129.90 µg/mL. In natural product screening, an IC_{50} value falling between 50 and 200 µg/mL represents strong antioxidant potential for an unpurified crude extract [15]. Mechanistically, this observed radical-scavenging efficacy is attributed to the synergistic interplay of hydrogen-atom-transfer and single-electron-transfer pathways facilitated by phenolic hydroxyl groups and oxygenated coumarin and flavonoid skeletons present in the matrix [14, 15]. Excess free

radicals and reactive oxygen species (ROS) are well-known triggers of cellular oxidative damage, membrane lipid peroxidation, and chronic inflammatory pathologies [15-17]. By effectively neutralizing DPPH free radicals-further validated by distinct yellow spot decolorization on silica TLC plates-eastern Libyan *D. tortuosa* demonstrates meaningful antioxidant capacity. These findings strongly support its traditional ethnomedicinal use and highlight its potential value as a plant-derived source of natural antioxidants for pharmaceutical and health applications. These findings support the medicinal potential of eastern Libyan *D. tortuosa* and warrant further bio-guided isolation of its active coumarin constituents.

***Conclusion**:* This study provides a targeted phytochemical and antioxidant evaluation of the aerial parts of *Deverra tortuosa* from eastern Libya. The results demonstrate that the plant contains high reserves of bioactive secondary metabolites, dominated by coumarins, terpenoids, and flavonoids, alongside significant DPPH radical-scavenging capacity.

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Authors' contributions: SAL conceived and designed the study, collected data, and performed and interpreted the data. SAL & SOHA drafted the manuscript and revised the important intellectual context. Both authors reviewed and approved the final version of the manuscript.
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