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GC–MS Profile and Antioxidant Activity of *Euphorbia viguieri* leaf: a Gateway to Valuable Bioactive Compounds

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ABSTRACT

Exploring *Euphorbia viguieri*'s overlooked chemistry via GC–MS unveils novel bioactive metabolites, bridging phytochemical gaps in a scarcely studied species. To profile *E. viguieri* leaf volatiles, phenolics, flavonoids, and antioxidant activity, enabling comparative insights within the diverse *Euphorbia* genus. Fresh *E. viguieri* leaves from Gorgan, Iran were dried, ground, and macerated in 80% methanol for 72 hours. The extract was concentrated, freeze-dried, and analyzed via GC–MS (HP-5MS column). Total phenolics (Folin–Ciocalteu), flavonoids (aluminum chloride), and DPPH radical scavenging activity were assessed. All assays were performed in triplicate, with data expressed as mean ± SD (Tukey's test, $p < 0.05$). GC–MS analysis of *E. viguieri* leaf extract identified ten major compounds, including monoterpenes ((S)-limonene, 1,8-cineole, 3-carene, isoborneol), phenylpropanoids ((E)-cinnamaldehyde, eugenol). These compounds exhibit defensive, antimicrobial, and protective roles. Quantitative assays revealed substantial total phenolic content (0.886 ± 0.01 mg GAE/g DW) and exceptionally high flavonoid levels (0.947 ± 0.01 mg QE/g DW). The extract demonstrated potent antioxidant activity with a DPPH value of 0.784 ± 0.01 µmol TE/g DW. The presence of eugenol, known for anti-inflammatory properties, alongside cuticular wax constituents, suggests both chemical defense and stress adaptation strategies. These findings establish *E. viguieri* as a rich source of bioactive phytochemicals with notable free-radical-scavenging capacity. *E. viguieri* exhibits potent antioxidant and diverse phytochemical profiles, warranting further pharmacological exploration and medicinal application studies.

Keywords: *Euphorbia viguieri*, GC–MS profiling, Bioactive metabolites, Antioxidant capacity, Euphorbiaceae

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INTRODUCTION

Euphorbia viguieri is a relatively lesser-known species of the Euphorbiaceae family that naturally occurs in tropical climates. As a member of the large *Euphorbia* genus, it possesses distinct morphological characteristics that differentiate it from other species. Its stems are typically succulent and contain white latex canals that exude sap when damaged; in many *Euphorbia* species, this latex plays a protective role against herbivores and also contains bioactive secondary metabolites in its chemical composition. The leaves are simple, often arranged in opposite or alternate pairs, and relatively thick in texture, which helps the plant withstand high temperatures and intense sunlight. The inflorescences are usually in the form of cyathia, a characteristic structure of the *Euphorbia* genus, composed of tiny flowers

accompanied by colored or green bracts. The seeds are trigonous and contain starchy endosperm, and their dispersal mechanism is typically explosive or facilitated by wind and insects. Scattered findings from botanical sources and reliable websites indicate that *E. viguieri*, due to its specific ecological niche and limited distribution, has been less studied in terms of chemical composition and potential medicinal applications. This makes it a valuable candidate for phytochemical and pharmacological investigations [1].

The *Euphorbia* genus includes more than 2,000 known plant species that inhabit a wide range of environments and produce a rich array of secondary metabolites. These compounds include diterpenes (particularly euphorbanes), monoterpenes, sesquiterpenes, flavonoids, tannins, fatty acid esters, and phenolic compounds. In certain species, such as *E. milii* and *E. hirta*, GC–MS studies have revealed a considerable range of volatile and non-volatile compounds exhibiting diverse biological activities, including antibacterial, antifungal, anti-inflammatory, and antioxidant effects[2, 3]. It has also been reported that compounds such as eugenol, limonene, borneol, farnesene, and unsaturated fatty acids are present in various parts of these plants, including leaves, stems, and latex. Many of these metabolites, owing to their strong antioxidant properties, play vital roles in plant defense against biotic stresses (diseases, insects) and abiotic stresses (drought, temperature) [4]. Recent studies on different *Euphorbia* species have shown that these plants are not only chemically diverse but also possess high potential for the development of drugs and plant-based products. For example, research conducted on *E. milii* has reported the presence of numerous terpenoid and phenolic compounds with significant free radical scavenging and anti-inflammatory effects[5]. In *E. trigona*, the abundance of oxygenated monoterpenes such as 1,8-cineole and terpenoid alcohols, along with high flavonoid content, has been associated with antibacterial and cytoprotective properties[6]. In contrast, available information on *E. viguieri* remains very limited, with only one comprehensive report by [1] providing a brief overview of its botanical characteristics. This scientific gap presents an important opportunity for researchers to employ modern analytical techniques such as GC–MS and LC–MS/MS to identify the complete chemical profile of this species and determine its potential medicinal value.

Given the limited background information, the present study was conducted to comprehensively analyze the chemical constituents of *E. viguieri* leaf extract using GC–MS and to evaluate its phenolic, flavonoid, and antioxidant capacities. This approach not only provides new insights into a poorly studied species but also allows for comparison with other *Euphorbia* species, leading to a better understanding of the chemical ecology of the genus.

MATERIALS AND METHODS

This study was conducted at the Faculty of Agriculture, University of Hormozgan (Bandar Abbas, Iran). Fresh, fully developed leaves of *Euphorbia viguieri* were collected from the Farhikhtegan Greenhouse Complex, Gorgan, Golestan Province, Iran. Fully mature leaves of Euphorbia viguieri were collected in 2025 from the Farhikhtegan Greenhouse Complex (Shahrak-e Golkhaneh-ye Farhikhtegan), Gorgan, Golestan Province, Iran. The plant was identified by Mehrdad Babarabie, and the voucher specimen (No. 9821) is deposited at the Herbarium of the Islamic Azad University, Gorgan Branch. The leaves were air-dried at room temperature (22–25 °C) in the shade until a constant weight was achieved, then ground using a stainless-steel mill and sieved through a 40-mesh screen to prepare for extraction.

Samples of Euphorbia viguieri were collected from the research and commercial greenhouse "Shahrak-e-Golkhaneh-i Farhikhtegan" located in Gorgan City, Golestan Province, Iran, to enable reliable evaluation of the plant's chemical constituents. The geographical location of the sampling site is shown in figure 1, and Figure 2 presents a view of the greenhouse where the plants were maintained and cultivated, along with a view of the Euphorbia viguieri plants themselves.

For crude extract preparation, maceration in 80% methanol was employed, a method widely recognized as efficient for extracting polar and semi-polar secondary metabolites [7, 8]. In this procedure, 50 g of leaf powder was soaked in 500 mL of methanol: water (80:20, v/v) for 72 hours at room temperature with continuous shaking (120 rpm). The mixture was filtered through Whatman No. 1 filter paper, and the residue was re-extracting three additional times under identical conditions using fresh solvent to ensure complete extraction. The combined extracts were concentrated under reduced pressure at a maximum temperature of 40 °C using a rotary evaporator. The concentrated extract was then freeze-dried to remove any remaining solvent completely. For GC–MS analysis, a stock solution of the extract (1 mg/mL) was prepared in HPLC-grade methanol and filtered through a 0.22 µm syringe filter. Compounds were tentatively identified by comparing their mass spectra with the NIST/Wiley library.

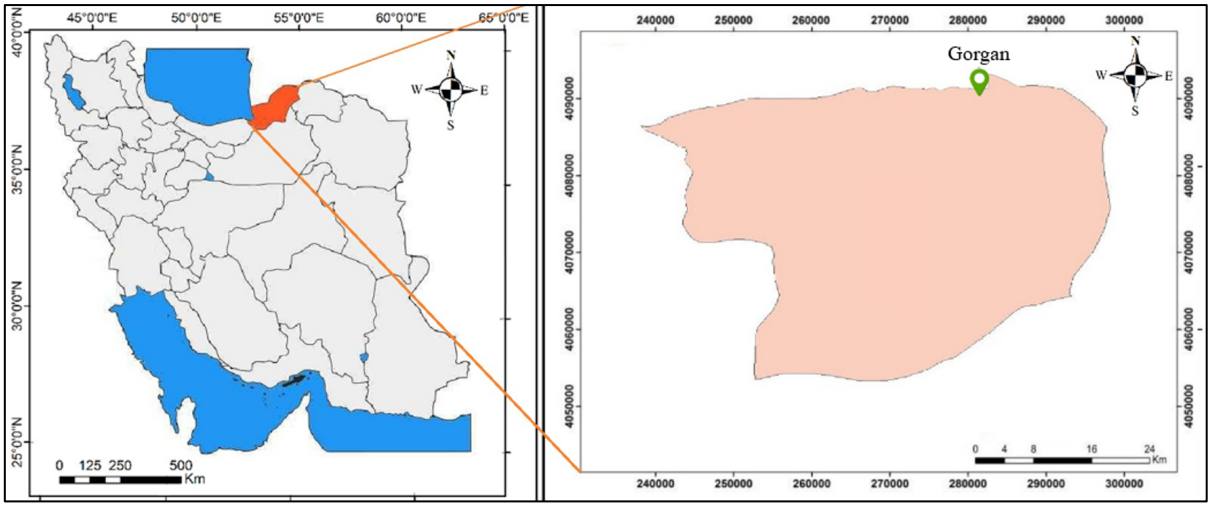

Fig. 1 *Euphorbia viguieri* plant sample collection area



Fig. 2 *Euphorbia viguieri* (Photographed by the authors from the Farhikhtegan greenhouse in Gorgan, Iran, 2025).

Identification was further supported by comparing calculated retention indices (RI) with literature values for non-polar columns. For compounds where authentic standards were not available, identifications are reported based on mass spectral matching and literature-based retention behavior. Gas chromatographic separation coupled with mass spectrometric detection was conducted on an Agilent 7890A chromatograph interfaced with a 5977B quadrupole mass analyzer, utilizing a fused-silica capillary column coated with a 5% phenyl–methylpolysiloxane stationary phase (HP-5MS, 30 m length × 0.25 mm internal diameter, 0.25 µm film thickness). High-purity helium served as the mobile phase, maintained at a constant volumetric flow rate of 1.0 mL min^{-1}. A 1.0 µL aliquot of the specimen was introduced via splitless injection, with the injector port thermostatically regulated at 250 °C. The thermal gradient applied to the column oven commenced at 60 °C (isothermal for 2 min), subsequently elevated to 180 °C at a rate of 3 °C min^{-1}, then further ramped to 280 °C at 10 °C min^{-1}, concluding with a 10 min isothermal hold. Mass fragmentation patterns were acquired under electron-impact ionization (70 eV) across a mass-to-charge ratio interval of 40–550 Da. Analyte confirmation was accomplished by spectral matching against the NIST 11 reference database, complemented by congruence of calculated retention indices with literature values [9]. It should be noted that the GC–MS analysis was limited to the detection of volatile and semi-volatile compounds under the operating conditions and does not represent the entire chemical profile of the polar extract, including non-volatile high-molecular-weight constituents. Retention indices (RI) were calculated for all identified compounds by analyzing a homologous series of n-alkanes (C8–C40) under identical operating conditions. The experimental RI values were compared with established values from the literature to validate the identity of each compound, providing a higher level of confidence alongside mass spectral matching.

The total phenolic burden was quantified employing the Folin–Ciocalteu chromogenic procedure. Briefly, standard solutions of gallic acid and test extracts were reacted with diluted Folin reagent and aqueous sodium carbonate; following a 30 min incubation period, optical density was recorded at 765 nm. Quantification was expressed as milligram equivalents of gallic acid per gram of dry extract mass (mg GAE/g DW). Total flavonoid concentration was ascertained via aluminum chloride complexation assay [10]. Specimens were sequentially combined with sodium nitrite, aluminum chloride, and sodium hydroxide; after a 15 min reaction interval, absorbance measurements were taken at 415 nm. Calibration was performed against a rutin reference curve, with final values denoted as milligram rutin equivalents per gram of dry extract weight (mg RE/g DW). The corresponding standard calibration curve and its regression equation are provided in Supplementary Information (Fig. 3). Radical-scavenging capacity was evaluated by means of the DPPH (2,2-diphenyl-1-picrylhydrazyl) decolorization assay, following the protocol delineated by Stankovic *et al.* [10]. The attenuation in absorbance of a 0.1 mM DPPH methanolic solution was monitored at 517 nm subsequent to a 30 min reaction period. Trolox served as the reference standard, and antioxidant efficacy was represented either as the half-maximal inhibitory concentration (IC_{50}) or as Trolox equivalent antioxidant capacity (TEAC).

All experimental determinations were carried out in triplicate, with data reported as arithmetic means accompanied by standard deviation (SD). Intergroup comparisons were executed via one-way analysis of variance (ANOVA), and pairwise mean differences were assessed employing Tukey's post hoc test, with statistical significance established at $p < 0.05$.

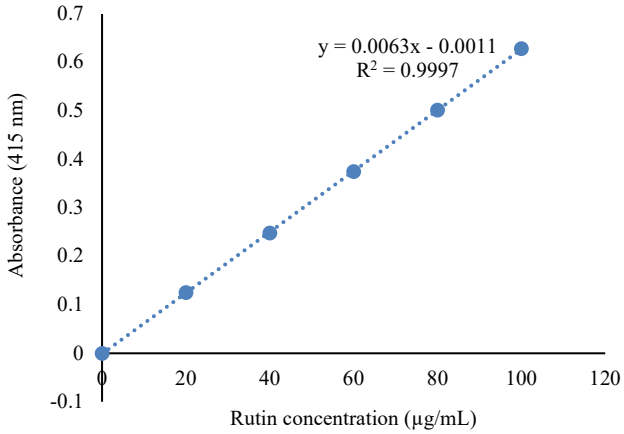

Fig. 3 Rutin standard curve for total flavonoid content determination

RESULTS

GC–MS Profile of *Euphorbia viguieri* Leaf Extract

Gas chromatography–mass spectrometry (GC–MS) analysis of the hydroalcoholic leaf extract of *E. viguieri* revealed the presence of a wide range of phytochemical compounds, including monoterpenes, phenylpropanoids, and fatty acid/ester derivatives. The identified compounds showed retention times (RT) ranging from 5.43 to 16.51 min. A summary of the corrected compound names, retention times, Kovats retention indices, chemical classes, and characteristic mass spectral ions is presented in Table 1.

Gas chromatography–mass spectrometry (GC–MS) analysis of the hydroalcoholic leaf extract of E. viguieri revealed the presence of a wide range of phytochemical compounds, including monoterpenes, phenylpropanoids, and fatty acid/ester derivatives.

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Their characteristic fragment ions were also considered for confirmation of the proposed structures. Since experimentally calculated Kovats retention indices using an n-alkane series were not available for these compounds under the present analytical conditions, these compounds were tentatively identified based on mass spectral library matching.

Table 1 Phytochemical compounds identified in the leaf extract of *Euphorbia viguieri*

No.	Compound	RT (min)	Reference RI	Molecular formula	Chemical class	Identification basis
1	3-Carene	5.43	1008	$C_{10}H_{16}$	Monoterpene hydrocarbon	NIST 11 MS library and RI comparison
2	(S)-Limonene	5.46	1024	$C_{10}H_{16}$	Monoterpene hydrocarbon	NIST 11 MS library and RI comparison
3	Eucalyptol (1,8-Cineole)	5.67	1026	$C_{10}H_{18}O$	Oxygenated monoterpene	NIST 11 MS library and RI comparison
4	Isoborneol	6.51	1155	$C_{10}H_{18}O$	Oxygenated monoterpene	NIST 11 MS library and RI comparison
5	Borneol	6.62	1165	$C_{10}H_{18}O$	Oxygenated monoterpene	NIST 11 MS library and RI comparison
6	(E)-Cinnamaldehyde	7.42	1267	C_9H_8O	Phenylpropanoid	NIST 11 MS library and RI comparison
7	Eugenol	8.19	1356	$C_{10}H_{12}O_2$	Phenylpropanoid	NIST 11 MS library and RI comparison

The compounds are arranged according to the corrected Kovats retention index order where available. RT: experimental retention time; KI: Kovats retention index. Dash (—) indicates that the value was not experimentally available under the present analytical conditions.

At RT = 5.43 min, the compound cyclohexene, 1-methyl-4-(1-methylethenyl) -, (S)- (p-mentha-1,8-diene), commonly known as (S)-limonene, with the molecular formula $C_{10}H_{16}$ and CAS number 5989-54-8, was identified as a hydrocarbon monoterpene.

The peak at RT = 5.46 min corresponded to (1,8-cineole, $C_{10}H_{18}O$; CAS 470-82-6)**,** characterized by major ions at m/z 43, 81, 84, 93, 108, 111, 139, 154, and 155.

At RT = 5.67 min**,** 1,4-cyclohexadiene, 1-methyl-4-(1-methylethyl)- (3-carene, $C_{10}H_{16}$; CAS 99-85-4) was detected, with predominant mass fragments at m/z 93, 91, 136, 77, 69, and 105.

The chromatographic peak at RT = 6.51 min corresponded to isoborneol ($C_{10}H_{18}O$; CAS 124-76-5)**,** whose base ion appeared at m/z 93/9.

The phenylpropanoid compound (E)-cinnamaldehyde (C_9H_8O; CAS 14371-10-9) was observed at RT = 7.42 min, with a base ion at m/z 131.

Table 2 Total phenolic content, total flavonoid content, and antioxidant capacity of *E. viguieri* leaf extract

Assay / Parameter	Absorbance (Mean ± SD)
Total phenolic content (TPC)	0.886 ± 0.01
Total flavonoid content (TFC)	0.947 ± 0.01
Antioxidant capacity (DPPH)	0.784 ± 0.01

DISCUSSION

The identified phytochemicals in the hydroalcoholic extract of *E. viguieri* are consistent with compounds known to play critical biological roles in plant survival and adaptation. The identified monoterpenes, phenylpropanoids, and fatty acid/ester derivatives contribute significantly to plant defense mechanisms against herbivores and pathogens, drought resistance, and the maintenance of cuticular integrity under environmental stress [11]. *Cyclohexene, 1-methyl-4-(1-methylethenyl), (S)* (Limonene) is a cyclic monoterpene with a strong citrus aroma and one of the most characteristic components of plant essential oils. It plays a crucial role in attracting pollinators and deterring pests. Its identification in the GC–MS profile of this study confirms its position as a major constituent of *E. viguieri* essential oil. The antibacterial mechanism of limonene involves disruption of cell membrane permeability, alteration of proton diffusion capacity, and disturbance of the electrochemical balance of the membrane [12].

In addition, its antioxidant properties have been attributed to its ability to scavenge hydroxyl and superoxide radicals. Comparison with other species shows that the concentration of limonene in *E. heterophylla* is about 12–20%, which is lower than that measured in *E. viguieri* [13]. This difference likely reflects metabolic adaptation of the studied species to specific ecological conditions [14]. The variability in concentration suggests metabolic adaptation, potentially influencing the plant's defense mechanisms against environmental stressors and oxidative damage [15].

1,8-cineole is an oxygenated monoterpene with known anti-inflammatory activity, clearly identified in the GC–MS profile. Its anti-inflammatory function occurs through inhibition of the NF-κB pathway, downregulation of iNOS and COX-2 expression, and thus suppression of nitric oxide production in macrophages [16]. Besides its anti-inflammatory role, 1,8-cineole is recognized as a signature compound in drought-adapted plants. Its presence in significant concentration has been reported in *E. tirucalli* and is associated with mucolytic and antitussive effects in clinical models [17]. 3-Carene is a bicyclic monoterpene known for its antifungal and antibacterial activities, acting against pathogens such as *Candida albicans* and *Botrytis cinerea* by disrupting cell wall and membrane integrity[18]. Its detection in this study, with a higher relative percentage compared to *E. retusa*, may indicate habitat-specific inhibition effects and metabolic adaptation in *E. viguieri*.

Isoborneol is an oxygenated monoterpene with a norbornane skeleton and a hydroxyl group, clearly identified in this study. It exhibits antiviral and antibacterial properties through the disruption of the lipid layer of viruses and the inhibition of key bacterial enzymes [19]. The higher concentration of this compound compared with the essential oils of *E. esula* and *E. helioscopia* emphasizes its potential role in the plant's chemical defense system. The accumulation of isoborneol as a bioactive monoterpene highlights a chemical adaptation for plant protection, as these compounds play a critical role in mitigating biotic stress by managing insect pests and modulating insect-associated microbial communities [20]. (E)-Cinnamaldehyde is an aromatic aldehyde with an unsaturated three-carbon chain identified in the GC–MS chromatogram of this study. This compound exhibits strong antibacterial activity through the disruption of DNA and

protein synthesis and the inactivation of bacterial metabolic enzymes [21]. Its anti-inflammatory effects are supported by inhibition of the p38 MAPK and NF-κB pathways. In *E. pekinensis*, this compound has been reported at a lower concentration [22], whereas its higher content in this study may indicate greater antioxidant potential. Cinnamaldehyde has been reported to inhibit pathogen growth and induce defense responses in plant tissues, supporting its potential role in the chemical defense of *E. viguieri* [23]. Isoborneol (Bicyclo[2.2.1] heptan-2-ol, 1,7,7-trimethyl-, exo is a saturated bicyclic alcohol, recognized as a derivative of borneol. It may act as a feeding deterrent for insects and simultaneously function as part of the plant's defensive profile. Although also reported in *E. helioscopia*, its higher concentration in this study indicates enhanced ecological defensive potential. Recent studies on borneol-containing derivatives have demonstrated notable fungicidal activity, supporting the view that this compound may contribute to the defensive chemical profile of *E. viguieri.* In addition to its anti-inflammatory effects, its insecticidal properties have also been reported [24]. The higher content of this compound in *E. viguieri* compared with *E. lathyris* may contribute to its stronger bioprotective efficiency.

CONCLUSION

Comparative analysis showed that the dominant compounds in each category in *E. viguieri* exhibited different percentage patterns compared to related species such as *E. retusa*, *E. hirta*, and *E. esula*. These differences may be associated with climatic conditions. The simultaneous presence of 1,8-cineole, isoborneol, methyl α-linolenate, and cinnamaldehyde increases the likelihood of synergistic antimicrobial and antioxidant activity, making this species a valuable candidate for developing pharmaceutical and sustainable agricultural products, although comprehensive safety and toxicity assessments are still required.

Conflict of Interests

All authors declare no conflict of interest.

Ethics Approval and Consent to Participate

No human or animals were used in the present research.

Ethical Issues

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors. The authors have adhered to ethical standards, including avoiding plagiarism, data fabrication, and double publication. Some sentences in this manuscript were revised with the assistance of artificial intelligence, using language models to improve clarity and readability.

Consent for Publication

All authors read and approved the final manuscript for publication.

Availability of Data and Material

All data generated or analyzed during this study are included in this published article. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

Conceptualization: M.B.
Data curation: M.M.
Formal analysis: A.Gh.
Investigation: A.M.D.
Methodology: M.B., B.F-N.
Project administration: M.B.
Resources: All authors.
Software: B. F-N.
Supervision: M.B.
Validation: M.B., B.F-N.
Visualization: F.Z.
Writing–original draft: M.B.
Writing–reviewing & editing: All authors.

Informed Consent

The authors declare not used any patients in this research.

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