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Article

Mediterranean Rosemary (*Salvia rosmarinus*) is a producer of cyclobutane-containing terpenes

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Abstract: For the first time, the chemical composition of essential oils obtained from the leaves and flowers of Mediterranean rosemary (*Salvia rosmarinus*) collected in forested areas surrounding Jerusalem has been investigated. Gas chromatographic analysis revealed that the essential oils are dominated by structurally unusual terpenoids containing a cyclobutane ring. A comprehensive survey of the literature further demonstrates that S. rosmarinus is a prolific natural source of cyclobutane-containing terpenes, with approximately 40 such compounds reported to date. These rare terpenoid frameworks are of particular interest due to their uncommon ring strain and distinct biosynthetic origin. In addition, available data on the biological activities of the major cyclobutane-containing terpenes are summarized, highlighting their antimicrobial, anti-inflammatory, antioxidant, and cytotoxic properties. Collectively, these findings underscore rosemary as a unique producer of cyclobutane terpenoids and emphasize the pharmacological potential of this rare class of natural products.

Keywords: Mediterranean rosemary, GC-MS, cyclobutane, terpenes, activity

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1. Plant source

Fresh and dry plant *Salvia rosmarinus* was collected on the slope of Mount Hered on the outskirts of Jerusalem in July-August 2024. A voucher specimen (No. 202308) was deposited in Hebrew University-Pharmacy School.

2. Previous studies

Rosemary has been valued as an aromatic and medicinal plant since antiquity. In ancient Egypt, rosemary-based creams and oils were reportedly used to protect the skin from extreme desert heat, often in combination with other botanical extracts such as chamomile, myrrh, thyme, and marjoram. Archaeological and historical accounts also indicate that rosemary was placed in the tombs of Egyptian pharaohs, where its fragrance was believed to accompany and honor the deceased in the afterlife. According to classical sources, Pedanius Dioscorides (40–90 AD) referred to rosemary as *Libanotis coronaria*, highlighting its invigorating properties and its use as a restorative agent for physical fatigue [1,2].

In contemporary contexts, rosemary (*Salvia rosmarinus*) is widely cultivated for culinary, medicinal, and ornamental purposes. The plant is characterized by fragrant, needle-like leaves and blue to violet flowers and is frequently grown in gardens not only for aesthetic appeal but also for its natural pest-repellent properties. The name rosemary derives from the Latin ros marinus, meaning “dew of the sea,” reflecting its native distribution along Mediterranean coastal regions. Rosemary leaves are commonly used as a culinary spice, particularly in meat-based dishes, where they impart a distinctive aroma and flavor. Medicinally, rosemary exhibits pronounced anti-inflammatory activity, largely attributed to phenolic constituents such as rosmarinic acid. Traditional and modern applications include the treatment of biliary and urinary tract spasms, dyspepsia, spastic colitis, physical and mental fatigue, cardiac neuroses, and menstrual disorders [3,4].

3. Present study

Fresh and air-dried specimens of Salvia rosmarinus were collected on the slopes of Mount Hered, located on the outskirts of Jerusalem. Fresh and dried leaves, as well as fresh blue flowers (25 mg each), were subjected to headspace (HS) analysis. Gas chromatography–mass spectrometry (GC–MS) analyses were performed using an Agilent 7890B GC system coupled with an Agilent 5977B mass selective detector and equipped with a PAL 3 autosampler (RSI 85).

Separation was achieved on an HP-5MS UI capillary column (Agilent Technologies; 30 m × 250 μm i.d., 0.25 μm film thickness). Samples were incubated for 6 min at 80 °C prior to injection. The oven temperature program was as follows: initial temperature of 35 °C held for 5 min, increased to 150 °C at 5 °C min^{-1}, and then ramped to 250 °C at 15 °C min^{-1}. Injector and detector temperatures were set at 250 °C and 280 °C, respectively. Helium was used as the carrier gas at a flow rate of 1.0 mL min^{-1}, with a split ratio of 5:1.

Compound identification was based on comparison with authentic standards, retention times, calculated retention indices, mass spectral fragmentation patterns, and library matching using the NIST/EPA/NIH Mass Spectral Library (2017), Wiley Registry of Mass Spectral Data (11th Edition), FFNSC3 (2015), and the Adams Essential Oils Library.

In parallel, a comprehensive analysis of recent reviews and experimental studies addressing the pharmacological properties of S. rosmarinus was conducted. These data indicate that rosemary extracts exhibit a broad spectrum of biological activities, including antimicrobial, antioxidant, antiproliferative, and antifungal effects [5–8].

Table 1 summarizes reported studies on rosemary essential oils, highlighting the dominant cyclobutane-containing terpenes, the geographic origin of plant material, and their relative abundance. Across multiple regions, α-pinene, β-pinene, and verbenone consistently represent the major cyclobutane-bearing constituents. Their combined contribution to the essential oil composition varies substantially depending on geographic origin and environmental conditions, ranging from approximately 18% to 51% of the total volatile fraction.

Table 2 shows the headspace analysis of fresh and dry leaves and blue flowers of *S. rosmarinus*, respectively. Having analyzed the essential oils of *S. rosmarinus* growing in different regions of the world, we found that this plant species can synthesize a wide variety of cyclobutane-containing terpenes presented in Figure 1.

What is currently known about cyclobutane-containing natural products and their distinctive pharmacological properties? As noted above, rosemary oil has been used since ancient times, long before the chemical composition of the plant was understood, including its high content of cyclobutane-containing terpenoids. Historically, the first naturally occurring cyclobutane frameworks were identified in the 19th century with the isolation of α- and β-pinene, followed shortly thereafter by the discovery of caryophyllene [9–12].

Over the past 25 years, a substantial number of cyclobutane-containing terpenes and their derivatives have been discovered and structurally characterized. Despite these advances, cyclobutanes remain a relatively rare and underexplored class within natural product chemistry. Their inherent ring strain, rigid three-dimensional geometry, and unusual bonding angles confer distinctive physicochemical properties that can profoundly influence molecular recognition and biological activity. Consequently, cyclobutane motifs are frequently encountered in biologically active natural products, where they often play a critical role in modulating pharmacological function.

In Salvia rosmarinus, cyclobutane-containing terpenes are predominantly localized in the stems, flowers, and leaves, although their relative abundance varies markedly with plant tissue type, developmental stage, environmental conditions, harvest season, and exposure to biotic and abiotic stressors. Such variability is characteristic of plant secondary metabolism and reflects the adaptive role of these compounds in defense, signaling, and ecological interactions. In addition, pronounced differences in both qualitative and quantitative metabolite profiles are frequently observed among cultivars and chemotypes of the same species, further contributing to the chemical diversity of rosemary essential oils [13].

Table 1. Major cylobutane-containing components of the essential oils

No.	Major components	%	Gathering place	Ref
1	a-Pinene	35.8	France	[14]
	β-Pinene	4.3		
	Verbenone	4.8		
	β-Caryophyllene	1.4		
	Total	46.3		
2	Verbenone	11.5	Cavusbasi Village/ Istanbul, Turkey	[15]
	α-Pinene	6.8		
	Total	18.3		
3	α-Pinene	26	Sevilla, Spain	[16]
	β-Pinene	6.1		
	Verbenone	2.5		
	Total	34.6		
4	α-Pinene	14	Morocco	[16]
	β-Pinene	9.2		
	Total	23.2		
5	α-Pinene	18.3	Morocco	[16]
	β-Pinene	4.6		
	β-caryophyllene	2		
	Total	24.9		
6	α-Pinene	38.5	Ballouneh, Mount Lebanon, Lebanon	[17]
	β-Pinene	3.8		
	Total	42.3		
7	α-Pinene	45.1	Brazil	[18]
	Verbenone	3.9		
	β-Pinene	2.7		
	Total	51.7		
8	α-Pinene	43.9	Lalehzar, Iran	[19]
	Verbenone	2.6		
	Verbenol	2.2		
	β-Caryophyllene	1.6		
	Total	50.3		
9	α-Pinene	46.1	Kerman, Iran	[19]
	Verbenone	2.3		
	Total	48.4		
10	α-Pinene	25.2	Ponte Don Melillo, Italy	[20]
	Verbenone	4.8		
	β-Pinene	1.1		
	β-Caryophyllene	1		
	Total	32.1		
11	Verbenone	12.9	Western Coastal region, Egypt	[21]
	α-Pinene	9.3		
	Total	22.2		
12	Verbenone	18.9	Ethiopia, Haramaya	[22]
	trans-Caryophyllene	3.4		
	α-Pinene	1.4		
	Caryophyllene oxide	1		
	Total	24.7		
13	Verbenone	17.4	Eastern Cape, South Africa	[23]
	α-Pinene	11.5		
	Total	28.9		
14	α-Pinene	31.3	Irbid City, Jordan	[24]
	α-Pinene	0.31		
	Total	31.6		

Table 2. Head-space analysis of fresh and dry leaves and fresh blue flowers of *S. rosmarinus*

Peak	RT	Fresh leaves %	Dry leaves %	Fresh blue flowers %	Compound	RI
13	11.343	0.58	0.35	0.37	tricyclene	925
14	11.616	1.03	0.39	0.43	α-thujene	929
15	11.824	22.71	**18.29**	**21.23**	**α-pinene**	937
16	12.345	12.01	9.58	9.32	camphene	952
17	12.594	0.00	-	0.30	thuja-2,4(10)-diene	956
18	13.379	8.59	**4.49**	**5.91**	**β-pinene**	979
21	14.013	2.26	1.77	1.10	β-myrcene	991
23	14.406	0.38	0.30	0.36	α-phellandrene	1005
25	14.838	1.34	0.96	0.41	α-terpinene	1017
26	15.119	1.40	1.88	0.27	*p*-cymene	1025
27	15.263	3.32	3.99	4.40	limonene	1030
28	15.335	26.47	28.30	5.38	1,8-cineole	1032
29	15.632	0.06	0.04	0.19	α-ocimene	1039
31	16.281	3.07	2.09	1.69	γ-terpinene	1060
32	17.251	1.00	0.98	1.55	terpinolene	1088
33	17.644	0.42	0.51	1.51	linalool	1099
38	19.015	7.64	15.51	8.53	camphor	1144
39	19.584	**-**	**-**	**0.24**	**pinocarvone**	1164
40	19.672	1.27	3.21	0.53	borneol	1167
41	19.929	**-**	**-**	**1.77**	**_cis_-pinocamphone**	1173
43	20.426	0.54	0.85	0.56	α-terpineol	1189
45	20.987	**-**	**-**	**4.80**	**verbenone**	1205
46	21.252	-	-	0.08	carveol	1219
47	22.791	-	-	0.07	isopiperitenone	1222
48	23.192	1.37	1.01	16.43	bornyl acetate	1284
49	24.931	0.04	0.03	-	α-cubebene	1351
50	25.524	**0.03**	**0.03**	**-**	**ylangene**	1372
51	25.645	**0.17**	**0.18**	**-**	**copaene**	1376
52	26.334	0.02	0.04	-	methyleugenol	1402
53	26.791	**1.79**	**1.74**	**0.49**	**β-caryophyllene**	1419
54	27.023	**0.04**	**0.03**	**-**	**β-copaene**	1433
68	30.158	**0.01**	**-**	**-**	**caryophyllene oxide**	1581

Among the cyclobutane-containing terpenes identified in rosemary, four compounds are particularly notable for their biological relevance: the monoterpene hydrocarbons α- and β-pinene, the monoterpene ketone verbenone, and the sesquiterpene β-caryophyllene. These metabolites often dominate rosemary essential oils and collectively account for many of the plant's pharmacological properties.

The antimicrobial activity of pinene stereoisomers highlights the critical role of chirality in biological function. Studies have demonstrated that only the (+)-enantiomers of α- and β-pinene exhibit significant antimicrobial effects, whereas their (–)-counterparts are largely inactive. Time–kill assays revealed that (+)-α-pinene and (+)-β-pinene are highly fungicidal toward Candida albicans, achieving complete eradication of the inoculum within 60 min [25]. Consistent with its long-standing use in traditional medicine, α-pinene has historically been employed in the treatment of respiratory tract infections and remains an important ingredient in the flavor and fragrance industries. In vitro assays further revealed enantioselective activity profiles, with (+)-α-pinene exhibiting antibacterial effects and (–)-α-pinene demonstrating greater insecticidal activity [26].

Beyond antimicrobial effects, α-pinene has attracted attention for its anticancer potential. It exhibits cytotoxic activity against a range of human cancer cell lines, including ovarian carcinoma, hepatocellular carcinoma, and neuroblastoma cells [27–29]. Mechanistic studies indicate that α-pinene induces apoptosis through mitochondrial dysfunction, increased reactive oxygen species (ROS) generation, activation of caspase-3, chromatin condensation, DNA fragmentation, and externalization of phosphatidylserine on the cell membrane [30]. Moreover, α-pinene isolated from Schinus terebinthifolius has been shown to suppress metastatic progression in melanoma models, underscoring its potential as an anti-metastatic agent [30].

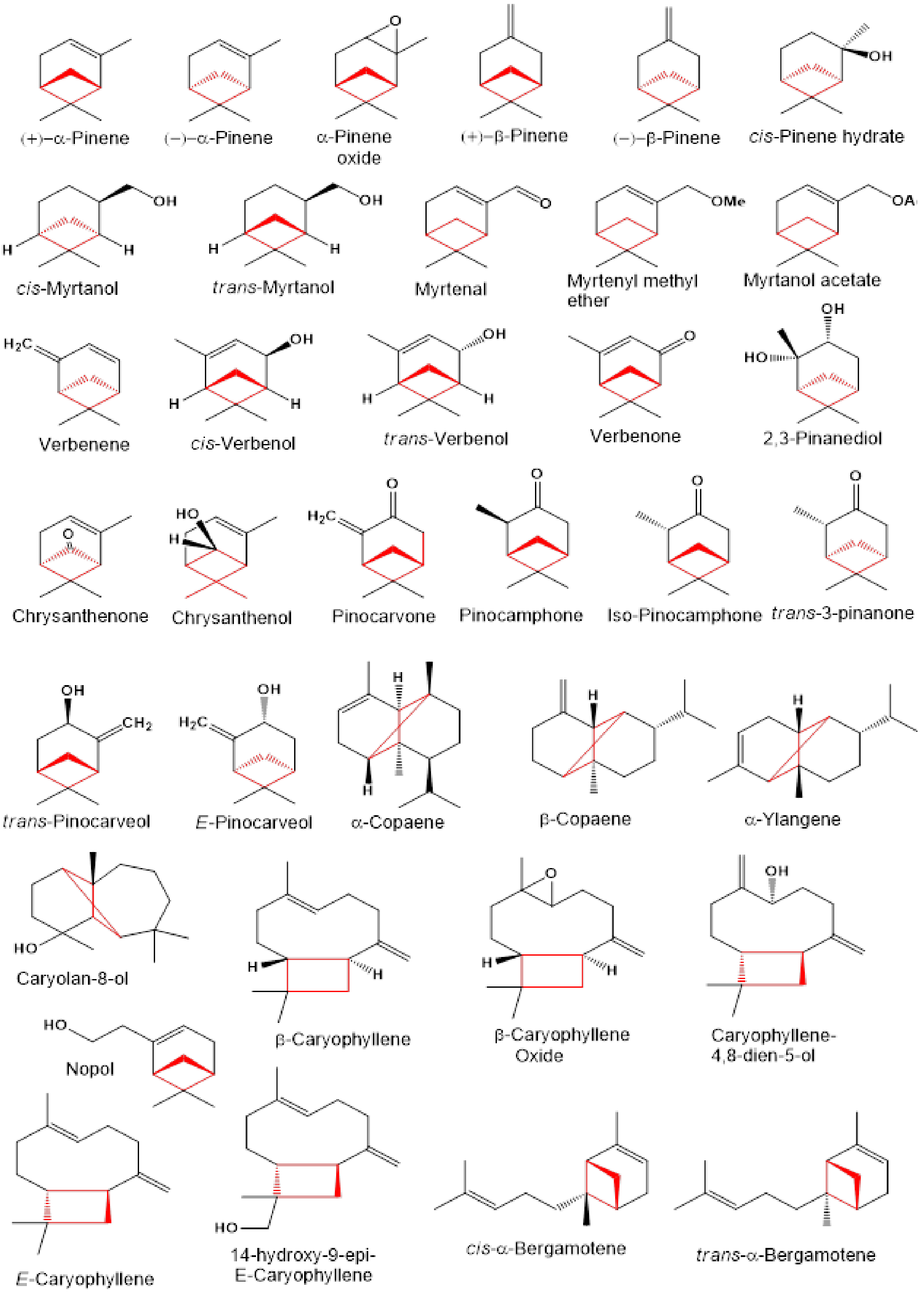

Figure 1. A wide variety of cyclobutane-containing terpenes have been found in the essential oils of *S. rosmarinus*. That is, de facto *S. rosmarinus* is a producer of unique terpenoids. The butane ring is marked in red

β-Caryophyllene (BCP) and its oxidized derivative *β*-caryophyllene oxide (BCPO) represent another biologically significant class of cyclobutane-containing terpenes. Both compounds display pronounced anticancer activity, inhibiting proliferation across multiple cancer cell lines. Notably, BCP enhances the efficacy of conventional chemotherapeutic agents by increasing their intracellular accumulation. BCP is recognized as a dietary phytocannabinoid with high affinity for the cannabinoid receptor type 2 (CB2), but negligible interaction with CB1, suggesting a favorable safety profile. In contrast, BCPO does not bind CB receptors, and its biological effects are mediated through alternative mechanisms. BCPO has been shown to modulate key oncogenic signaling pathways, including MAPK, PI3K/AKT/mTOR/S6K1, and STAT3, which are central to cancer cell survival, proliferation, and inflammation [31].

In addition to anticancer effects, β-caryophyllene exhibits potent neuroprotective and anti-inflammatory activities. These effects are partly attributed to the suppression of pro-inflammatory mediators such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), and the transcription factor NF-κB. Through these mechanisms, β-caryophyllene alleviates chronic inflammatory states and oxidative stress, contributing to its therapeutic potential in metabolic and neurodegenerative disorders [32].

Chirality also plays a decisive role in the biological activity of the monoterpene alcohol verbenol and the monoterpene ketone verbenone. Both enantiomers of verbenone and stereoisomers of verbenol significantly reduced IL-6 levels in HaCaT keratinocytes exposed to Staphylococcus lugdunensis, indicating anti-inflammatory and immunomodulatory effects. These compounds also exhibited broad antimicrobial activity against bacteria, yeasts, and filamentous fungi [33]. Verbenone, in particular, has demonstrated antibacterial efficacy against Staphylococcus aureus, further supporting its pharmacological relevance [34].

Finally, the essential oils obtained from rosemary leaves and flowers collected in forested areas of the southern Jerusalem region were analyzed for the first time using headspace gas chromatography–mass spectrometry (HS-GC–MS). This analysis revealed that cyclobutane-containing terpenes constitute the dominant chemical class in these oils. The major bioactive components were identified, and their reported biological activities were correlated with literature data, reinforcing the conclusion that cyclobutane-containing terpenes play a central role in the medicinal properties of rosemary.

4. Conclusion

This study provides the first comprehensive characterization of cyclobutane-containing terpenes in the essential oils of Salvia rosmarinus collected from forested areas surrounding Jerusalem, highlighting this species as a rich and underappreciated source of structurally unusual terpenoids. Headspace GC–MS analysis revealed that cyclobutane-bearing monoterpenes and sesquiterpenes constitute the dominant components of the leaf and flower essential oils, confirming rosemary as a consistent producer of these strained ring systems.

Cyclobutane-containing terpenes such as α- and β-pinene, verbenone, and β-caryophyllene were shown—based on extensive literature evidence—to exhibit a broad spectrum of biological activities, including antimicrobial, anti-inflammatory, anticancer, neuroprotective, and immunomodulatory effects. Notably, the biological activity of these metabolites is strongly influenced by stereochemistry, with enantiomer-specific effects observed for pinene and verbenone, underscoring the importance of three-dimensional molecular architecture in mediating pharmacological responses. The rigid, compact cyclobutane moiety contributes to unique spatial orientation, enhanced receptor interactions, and metabolic stability, which collectively distinguish these compounds from more common terpene frameworks.

The dominance of cyclobutane-containing terpenes in rosemary essential oils provides a plausible chemical basis for the long-standing medicinal use of this plant in traditional systems of medicine, particularly for the treatment of inflammatory disorders, infections, and neurological conditions. Moreover, modern pharmacological studies reveal that these compounds not only exert direct biological effects but may also act as adjuvants, enhancing the efficacy of conventional drugs through synergistic mechanisms.

Overall, the findings emphasize the importance of strained-ring terpenoids as biologically privileged scaffolds and highlight rosemary as a valuable model for studying the biosynthesis, ecological function, and therapeutic potential of cyclobutane-containing natural products. Future research integrating metabolomics, stereochemical analysis, and mechanistic pharmacology will further elucidate the role of these compounds and support their development as leads for pharmaceutical, nutraceutical, and functional food applications.

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