

ORIGINAL ARTICLES

ESSENTIAL OILS FROM *PINUS SYLVESTRIS*, *PINUS NIGRA* AND *PINUS PINASTER*: COMPOSITION, EXTRACTION, AND THERAPEUTIC POTENTIAL

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ABSTRACT

INTRODUCTION: Essential oils obtained from the most widespread representatives of the genus *Pinus* in the Republic of Bulgaria—*P. sylvestris* L., *P. nigra* Arn., and *P. pinaster* Sol.—are characterized by a similar aromatic profile; however, their chemical composition and physiological effects may differ significantly.

Their composition comprises more than 50 identified volatile compounds, varying both qualitatively and quantitatively. The observed therapeutic effects are determined not only by the diversity of their terpene composition but also by the extraction method employed. Understanding these relationships enables a more precise and scientifically grounded application of pine essential oils.

AIM: The aim of the present study is to present a laboratory method for the isolation of essential oils from needles and young shoots of *P. sylvestris* L., *P. nigra* Arn., and *P. pinaster* Sol., and to determine their composition using GC–MS analysis.

MATERIALS AND METHODS: For the purposes of this study, a steam distillation setup was employed, designed to simulate a real steam distillation system, along with needles and thin branches of *P. sylvestris*, *P. nigra*, and *P. pinaster*, and GC–MS instrumentation.

RESULTS: The proposed extraction method yielded essential oils with reproducible chromatographic profiles. GC–MS analysis revealed both common and species-specific constituents across the three *Pinus* samples.

CONCLUSION: The successful integration of extraction methodologies with comprehensive GC–MS analysis provides a robust foundation for future research on the sustainable utilization of pine-derived essential oils.

Keywords: *pinus*, essential oils, composition, therapeutic potential

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Received: May 3, 2026

Accepted: June 18, 2026

INTRODUCTION

Essential oils obtained from the most widespread representatives of the genus *Pinus* in the Republic of Bulgaria—*P. sylvestris* L., *P. nigra* Arn., and *P. pinaster* Sol.—are characterized by a similar aromatic profile; however, their chemical composition and physiological effects may differ significantly (1).

Their composition comprises more than 50 identified volatile compounds, varying both qualitatively and quantitatively. The observed therapeutic effects are determined not only by the diversity of their terpene composition but also by the extraction method employed. Understanding these relationships enables a more precise and scientifically grounded application of pine essential oils (2).

Essential oils extracted from the needles of coniferous tree species represent an important group of natural products with wide applications in medicine, pharmacy, cosmetics, and aromatherapy. Of particular interest are the oils derived from various *Pinus* species, due to their rich chemical composition and well-documented biological activities.

Accumulated scientific evidence indicates that pine needle essential oils exhibit a wide range of biological activities, making them a valuable subject of research both from a fundamental scientific perspective and for the development of new therapeutic and cosmetic products.

Phytochemical Profile and Pharmacological Classification of Terpenoid Compounds

The therapeutic efficacy of botanical extracts is dictated by the synergistic interaction of diverse terpenoid classes (3). These compounds are categorized by their molecular complexity—ranging from high-volatility monoterpenes to more structurally complex diterpenes—each contributing a specific set of biological activities.

1. Monoterpene Hydrocarbons: Respiratory and Stimulant Effects

The monoterpene hydrocarbon fraction (4) featuring α -pinene, β -pinene, limonene, myrcene, Δ -3-carene (Fig. 1), constitutes the primary volatile component of many essential oils. These molecules are rapidly absorbed and exert systemic effects (5).

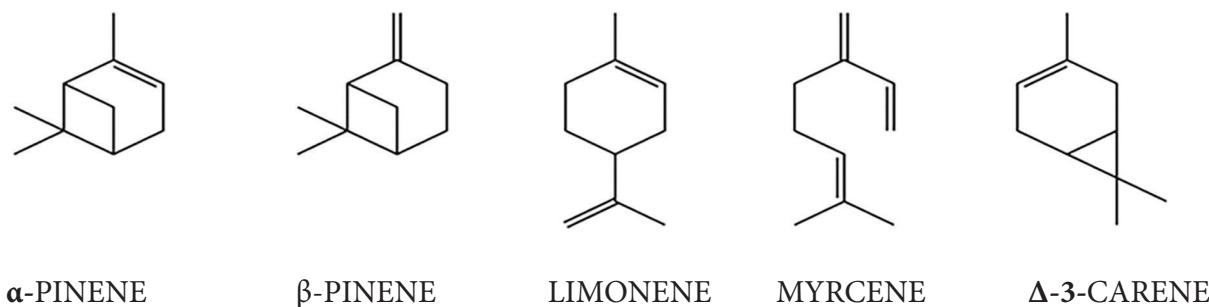


Fig. 1. 2D chemical structures of monoterpene hydrocarbons.

Essential oils from *Pinus sylvestris*, *Pinus nigra*, and *Pinus pinaster* are characterized by a high content of monoterpenes, among which α -pinene, β -pinene, and δ -3-carene are predominant, along with various oxygenated compounds such as borneol and terpineol. The relative proportions of these constituents vary considerably depending on species, geographical origin, environmental conditions, and extraction methods, leading to the formation of different chemotypes with distinct properties.

Respiratory modulation: These compounds act as potent bronchodilators and expectorants, facilitating the clearance of mucus and improving pulmonary airflow (6).

Antisepsis & inflammation: They exhibit intrinsic antiseptic properties and exert mild anti-inflammatory activity by inhibiting specific pro-inflammatory pathways (7).

Neurological impact: This class is associated with central nervous system (CNS) stimulation, enhancing cognitive alertness and metabolic focus (8).

2. Oxygenated Monoterpenes: Anti-Inflammatory and Regenerative Synergetics (9)

Oxygenated monoterpenes, including borneol, bornyl acetate, and terpinen-4-ol (Fig. 2), represent a more chemically stable group with enhanced bioavailability.

pounds characterized by a pronounced affinity for specific molecular targets, such as the CB2 receptor in the case of β -caryophyllene.

Analgesic & anti-inflammatory potency: These compounds offer strong anti-inflammatory activity, specifically targeting musculoskeletal inflammation.

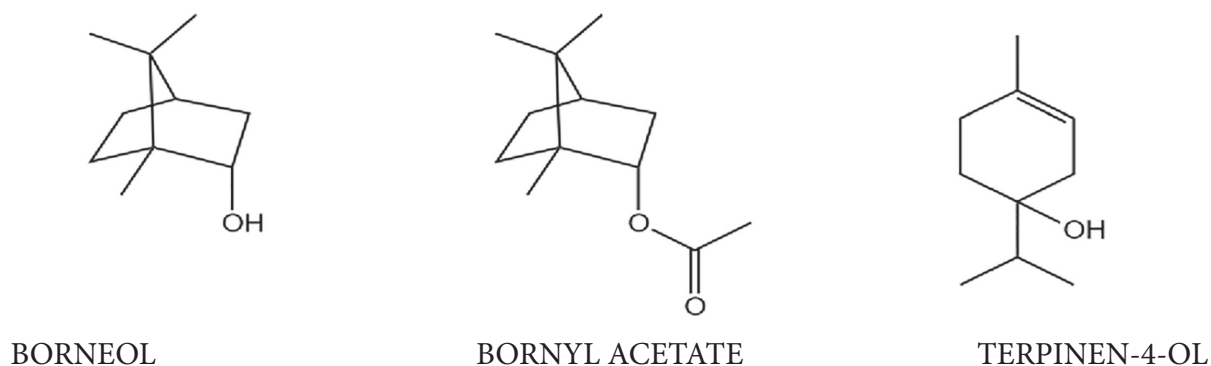


Fig. 2. 2D chemical structures of oxygenated monoterpenes.

Synergistic modulation: These molecules serve to modulate the stimulatory effects of pinenes, preventing over-excitation of the nervous system while providing a grounding, anxiolytic effect.

Antimicrobial & dermal recovery: This fraction displays robust antimicrobial action and supports tissue regeneration, making it vital for cellular repair and immune defense.

3. Sesquiterpenes: Deep Tissue Analgesia and Chronic Pain Modulation

The sesquiterpene fraction, including β -caryophyllene, germacrene D and cadinenes (10) (Fig. 3), comprises higher-molecular-weight com-

Homeostasis: They are particularly effective in managing chronic conditions, providing long-term nervous system stabilization and pain relief for joints and muscular tissues.

4. Diterpenes: Immunomodulation and Structural Support

The diterpene fraction, exemplified by manol oxide (11) (Fig. 4), comprises higher-molecular-weight and less volatile terpenoids, often requiring specialized extraction techniques, such as supercritical CO₂ extraction (SFE), for efficient isolation.

Immunomodulatory activity: Diterpenes are recognized for their significant immunomodulatory

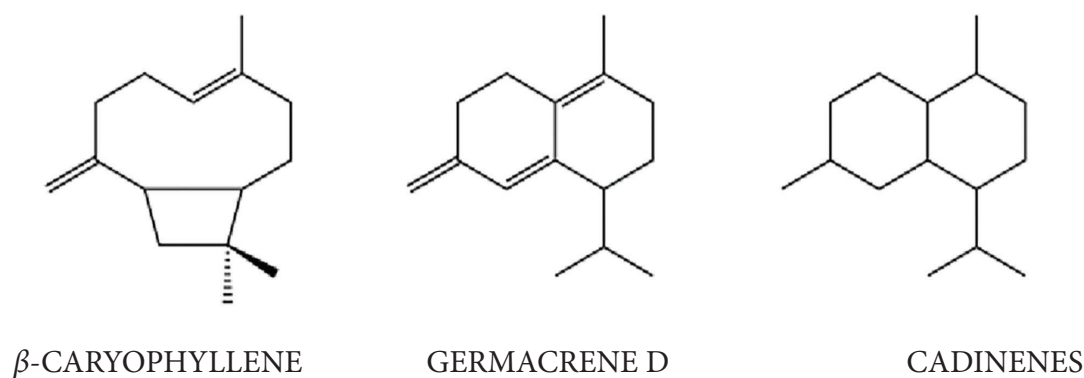
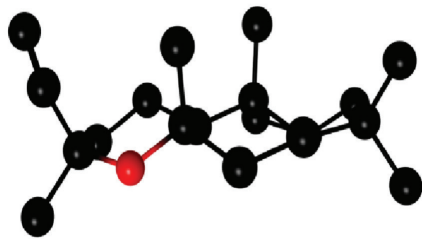


Fig. 3. 2D chemical structures of sesquiterpenes.



MANOOL OXIDE

Fig. 4. 3D chemical structure of manool oxide.

properties, contributing to the regulation of the immune response.

Regenerative capacity: Through high-level regenerative pathways, they support structural cellular integrity and provide a foundational anti-inflammatory barrier against systemic stress.

A summarized relationship between the presence of specific chemical constituents, their primary therapeutic effects, and the symptoms they influence is presented in Table 1.

Relationship between Extraction Method, Chemical Composition, and Biological Activity

Essential oils from *Pinus sylvestris*, *Pinus pinaster*, and *Pinus nigra* exhibit different physiological effects determined not only by their specific terpene composition but also by the extraction method employed. (12).

Characteristics of Extraction Methods (13)

1. Steam Distillation

A widely used method for isolating volatile compounds, particularly essential oils, but associated with several limitations. High temperatures may cause thermal degradation (14) of thermolabile components, while the presence of water and steam can induce hydrolysis and chemical transformations. The simultaneous extraction of multiple compounds without precise control results in relatively low selectivity. Due to these limitations and its high energy consumption—especially during prolonged processes—this method is mainly suitable for volatile monoterpenes and water-insoluble compounds (15).

2. Vacuum Distillation (16)

A modification of conventional distillation in which reduced pressure allows operation at lower temperatures. Nevertheless, partial thermal stress remains unavoidable, and the selectivity is comparable to that of atmospheric distillation. Combined with

Table 1. Pharmacological profiles and therapeutic applications of select terpenoids.

Compound	Primary Biological Activity	Therapeutic Applications
α -Pinene	Bronchodilation	These compounds are employed in the treatment of respiratory pathologies (e.g., sinusitis and bronchitis), contributing to the reduction of airway resistance.
β -Pinene	Antiseptic & Antimicrobial	Exhibits broad-spectrum inhibitory effects against pathogens; utilized as an adjuvant in treating infectious etiologies.
Δ -3-Carene	Desiccative Effect	Induces mucosal drying; clinical utility in alleviating hypersecretion associated with rhinitis and rhinorrhea.
Limonene	Immunomodulatory	Enhances leukocyte response and cytokine regulation; indicated for fatigue syndromes and immune system optimization.
Bornyl acetate	Sedative & Anxiolytic	Modulates the central nervous system to induce relaxation; utilized in clinical protocols for stress reduction and sleep disorders.
β -Caryophyllene	Anti-inflammatory & Analgesic	Attenuates musculoskeletal inflammation via cannabinoid receptor interaction; effective in chronic pain management.
Germacrene D	Homeostatic Stabilization	Promotes physiological equilibrium; contributes to the long-term management of chronic metabolic and systemic imbalances.

higher technological complexity, these factors limit its broader application in essential oil extraction.

3. Supercritical CO₂ Extraction (17)

A modern and highly efficient method, although less effective for strongly polar compounds unless co-solvents (e.g., ethanol) are used. It is particularly suitable for extracting sesquiterpenes and diterpenes but requires sophisticated equipment and highly qualified personnel.

The influence of extraction methods on chemical composition and the resulting physiological effects is summarized in the following table provides a high-level scientific overview of how various extraction techniques influence the chemical composition, molecular integrity, and subsequent pharmacological efficacy of the resulting essential oils and extracts (18).

The dependence of essential oil composition on pine species is clearly expressed, as different species vary significantly in their dominant constituents and corresponding practical applications.

In the case of *Pinus sylvestris* (Scots pine), the essential oil is primarily obtained by steam distillation. Its chemical composition is characterized by a high content of α -pinene (32–60%), β -pinene (5–

22%), and limonene (7–12%). This combination of monoterpenes provides pronounced expectorant, antiseptic, and refreshing effects on the respiratory system. Consequently, Scots pine essential oil is widely used in the management of acute respiratory conditions, such as colds, bronchitis, and inflammation of the upper respiratory tract.

For *Pinus pinaster* (Maritime pine), steam distillation is also the primary extraction method. The resulting oil contains an even higher proportion of α -pinene (71–85%) and β -pinene (11–20%), while limonene is present in lower amounts (1–7%). This composition accounts for its strongly stimulating and pronounced antiseptic properties. The oil is commonly used for air disinfection purposes and in formulations designed to provide invigorating and tonic effects.

In contrast, *Pinus nigra* (Austrian pine) essential oil is often obtained through vacuum distillation or carbon dioxide (CO₂) extraction. Its composition is dominated by sesquiterpenes such as β -caryophyllene and germacrene D, compounds known for their stronger anti-inflammatory and analgesic activities. As a result, Austrian pine oil is primarily applied in products intended for the relief of

Table 2. Comparative analysis of terpenoid extraction methodologies and phytochemical yields.

Extraction Methodology	Dominant Phytochemical Fractions	Degradation & Molecular Alterations	Pharmacological Profile & Bioavailability
Steam Distillation	Primarily low-molecular-weight Monoterpenes (α -pinene, β -pinene, limonene, Δ -3-carene)	Significant loss of Sesquiterpenes ; susceptibility to partial ester hydrolysis due to steam exposure.	Rapid-onset bronchodilatory and antiseptic effects; optimized for acute respiratory relief.
Hydrodistillation	Monoterpenes with trace inclusions of heavier volatile fractions.	High risk of thermal degradation and aqueous hydrolysis of sensitive thermolabile compounds.	Attenuated therapeutic potency; milder physiological response compared to steam-based methods.
Vacuum Distillation (Reduced Pressure)	High retention of Monoterpenes and Oxygenated Terpenoids (esters/alcohols).	Minimal oxidative stress and thermal degradation due to significantly lower boiling points.	Synergistic anti-inflammatory and respiratory benefits; preserves the native „full-spectrum“ aromatic profile.
Supercritical CO ₂ Extraction	Comprehensive profile: Monoterpenes , Sesquiterpenes , Diterpenes , and lipophilic Resins .	Negligible ; non-thermal and non-aqueous process ensures maximum molecular structural integrity.	Potent immunomodulatory , analgesic , and systemic anti-inflammatory activity; high therapeutic density.

muscle and joint pain, as well as in conditions associated with inflammatory processes.

Thus, variations in chemical composition among pine species directly determine their specific therapeutic applications, highlighting the importance of botanical origin when selecting an essential oil for practical use (19).

The choice of extraction method depends on the physicochemical properties of the target compounds, their thermal stability, required selectivity, and economic constraints. The method acts as a selective factor determining which terpene compounds dominate the final product and, consequently, its physiological and therapeutic effects.

AIM

The aim of the present study is to present a laboratory method for the isolation of essential oils from needles and young shoots of *P. sylvestris* L., *P. nigra* Arn., and *P. pinaster* Sol., and to determine their composition using GC–MS analysis.

MATERIALS AND METHODS

For the purposes of this study, a steam distillation setup was employed, designed to simulate a real steam distillation system, along with needles and thin branches of *P. sylvestris*, *P. nigra*, and *P. pinaster*, and GC–MS instrumentation.

Steam distillation was carried out using a 10L steam generator connected to a 10L distillation flask, three condensers connected in series with silicone tubing for water circulation, and a distillate receiver (Fig. 5). The system produced 1 L of distillate in 41 minutes without plant material and in 37 minutes when steam was passed through the plant material.

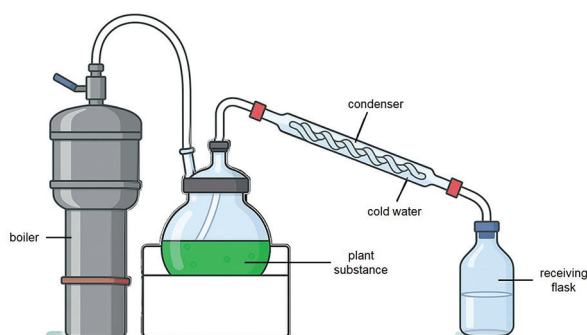


Fig. 5. Schematic representation of the steam distillation setup.

Needles and thin branches (350 g) of *Pinus pinaster*, *P. nigra*, and *P. sylvestris* were subjected to steam hydrodistillation. Each cycle lasted approximately 1 hour, yielding 2 L of floral water for each species. The floral waters were extracted twice with 20 mL of pentane. The organic phases were concentrated under a stream of argon (20 mL/min) and analyzed by GC–MS.

RESULTS AND DISCUSSION

The proposed extraction method yielded essential oils with reproducible chromatographic profiles. GC–MS analysis revealed both common and species-specific constituents across the three *Pinus* samples (Table 3).

The essential oil of *Pinus sylvestris* was characterized by the predominance of α -terpineol (18.72% of TIC), sclareol (15.26% of TIC), β -caryophyllene (7.15% of TIC), and verbenone (6.18% of TIC), indicating a composition rich in oxygenated monoterpenes. In contrast, *Pinus pinaster* exhibited elevated levels of (3E)-cembrene A (12.98% of TIC), neophytadiene (10.71% of TIC), and n-pentadecanal (8.27% of TIC), reflecting a profile dominated by diterpenoids and fatty acid derivatives. The essential oil of *Pinus nigra* contained primarily α -pinene (25.85% of TIC), δ -cadinene (12.83% of TIC), β -caryophyllene (10.85% of TIC), and γ -muurolene (7.30% of TIC), consistent with a resinous composition rich in sesquiterpenes.

Steam distillation enabled the isolation of the primary volatile fraction of the essential oils.

Subsequent liquid–liquid extraction with pentane facilitated the extraction of residual oil constituents remaining in the aqueous phase after distillation, likely due to partial solubility or incomplete phase separation. The use of pentane offers several advantages, including efficient recovery of nonpolar compounds, increased overall extraction yield, and facile removal prior to GC–MS analysis owing to its low boiling point.

However, this approach may also lead to the co-extraction of non-volatile or low-volatility lipophilic constituents, such as diterpenoids, waxes, and fatty acid derivatives, which are not typically considered part of the essential oil fraction. This may account for certain discrepancies observed between the experimental results and previously reported literature data.

Table 3. Comparative analysis of the chemical composition of essential oils obtained by steam distillation from *P. sylvestris*, *P. nigra*, and *P. pinaster*.

RT	RI	Name	% of TIC	RT	RI	Name	% of TIC	RT	RI	Name	% of TIC
17.48	1184	α -Terpineol	18.72	39.07	2210	(3E)-Cembrene A	12.98	9.24	933	α -Pinene	25.85
38.31	2244	Sclareol	15.26	42.26	2299	Neophytadiene	10.71	26.06	1522	β -Cadinene	12.83
23.58	1416	P-Caryophyllene	7.15	23.58	1416	n-Pentadecanal	8.27	23.58	1416	P-Caryophyllene	10.85
16.00	1145	cis-Verbenol	6.18	30.55	1708	n-Hexadecanol	5.94	25.15	1477	γ -Muurolene	7.30
15.84	1134	trans-Sabinol	5.83	36.48	2085	2-keto-Mannool oxide	5.45	25.93	1513	γ -Cadinene	4.48
39.41	2213	Sandaracopimaral	4.93	38.39	2133	13-epi-Mannool	5.11	24.98	1466	9-epi-(E)-Caryophyllene	4.07
17.82	1201	Verbenone	4.40	35.76	2020	Cembrene	4.77	15.84	1133	trans-Sabinol	3.96
36.64	2075	Dehydroabietanal	4.34	34.12	1938	Mannool	4.46	17.48	1190	α -Terpineol	3.67
34.56	1950	Cembrene	4.23	35.57	1986	Limonene	4.16	29.26	1661	α -Cadinol	2.86
35.33	1971	Sandaracopimaradiene	3.86	33.95	1875	Mannool oxide	2.69	16.00	1144	cis-Verbenol	2.81
25.13	1482	Germacrene D	3.29	39.60	2236	(2E,6E)-Farnesyl acetate	2.54	16.79	1171	α -Phellandren-8-ol	2.45
40.40	2238	Methyl pimarate	2.80	33.09	1815	Methyl pimarate	2.33	17.81	1202	Verbenone	2.17
12.39	1023	Eucalyptol	2.70	36.24	2063	Abieta-8,12-diene	2.21	25.56	1485	Germacrene D	1.95
16.81	1180	α -Phellandren-8-ol	2.42	33.58	1848	Methyl isopimarate	2.03	15.41	1104	Camphen-6-ol	1.67

Therefore, the chemical profiles obtained in the present study should be interpreted in the context of the combined extraction strategy involving both steam distillation and pentane extraction.

CONCLUSION

The results obtained from the applied distillation and extraction methods for essential oils from three *Pinus* species demonstrate their suitability for chemotaxonomic applications.

GC–MS analysis enabled the identification of distinct, species-specific terpene profiles, allowing for reliable differentiation among the investigated pine species.

Such findings are of considerable importance for biodiversity conservation and sustainable development, as they support the development of environmentally friendly alternatives to synthetic processes across various industrial applications.

The successful integration of extraction methodologies with comprehensive GC–MS analysis provides a robust foundation for future research on the sustainable utilization of pine-derived essential oils.

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