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Bioprospecting Terpenoid-Rich Himalayan Plants for Use in Food Preservation and Natural Flavouring Agents

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Abstract: The Indian Himalayan Region (IHR) is known for its distinct flora and fauna. Many aromatic plants from IHR are used in food preservation, as ingredients in food items, and in folk herbal medicines, and their fragrance is used to improve mood and relieve depression. In continuation with this, essential oils (EOs) of five selected aromatic plants from Dronagiri hills of Uttaranchal (India) were analysed by gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS). Volatile oil of *Tanacetum nubigenum* Wall. contained *β*-eudesmol and isobornyl propionate as major compounds, *Pleurospermum angelicoides* (DC.) Klotzsch was found to contain *p*-cymene, camphene and *α*-asarone as major compounds; *Rhododendron anthopogon* D. Don was found rich in *α*-pinene, whereas germacrene-D and limonene were the major components of *Selinum wallichianum* (DC.) Raizada & Saxena, and *Juniperus wallichiana* Brandis were rich in *β*-pinene, sabinene, and *α*-pinene. Four out of five plant species were found to be rich in monoterpenoids, while one was noted for its abundance of oxygenated sesquiterpenoids. *Tanacetum nubigenum, Pleurospermum angelicoides,* and *Selinum wallichianum* are identified as chemotypes, while *Juniperus wallichiana* exhibits a composition similar to that previously reported. Several essential oils (EOs) derived from various traditional plants have demonstrated enhanced antimicrobial and antioxidant properties. They have also successfully extended the shelf lives of cereal products and improved the standard of food safety. Without compromising the quality, the main classes of essential oils (EOs), such as terpenes and aromatic volatile chemicals are crucial for food safety. Essential oils investigated may be used as alternative preservatives to extend the shelf life of grains and cereals, owing to their various properties, including antibacterial and antioxidant effects.

Keywords: Essential oils; chemotype; monoterpenoids; food preservative; Dronagiri plants.

1. Introduction

Essential oils (EOs) are naturally occurring volatile secondary metabolites synthesised by a wide range of aromatic and medicinal plants. They are primarily derived from members of several angiosperm families, including Apiaceae, Lamiaceae, Rutaceae, Myrtaceae, Lauraceae, Verbenaceae, Zingiberaceae, and Asteraceae [1]. These oils are complex mixtures of low-molecular-weight compounds, predominantly composed of terpenoids. Essential oils are stored in specialised secretory structures such as glandular trichomes, oil ducts and resin canals within the plant tissues. They are distributed in different parts of the plant, including leaves, stems, flowers, seeds, fruits, bark, and roots [2]. The characteristic fragrance or flavour of each plant species is largely attributed to the composition of its essential oil. The term

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chemotype refers to a chemically distinct entity within a plant species, characterised by differences in the composition of its secondary metabolites, particularly essential oils, despite sharing similar morphological features. Terpenoid-rich Himalayan plants are believed to possess strong antioxidant and antibacterial properties. Foodborne infections can be efficiently inhibited, and these bioactive chemicals can delay deterioration. These plants have distinct phytochemical profiles, making them viable options for natural food preservation. Furthermore, as organic flavourings, their fragrant terpenoids may improve the food's sensory appeal. By using functional evaluation and phytochemical analysis, this study seeks to validate these potentials. These compounds play crucial ecological roles, such as attracting pollinators and deterring herbivores or pathogens [3]. Essential oils are known for their antimicrobial, antioxidant, anti-inflammatory, and insecticidal properties. They have been used traditionally in herbal medicine, perfumery, and culinary practices across various cultures. In recent years, their application has expanded into pharmaceuticals, food preservation, and cosmetic formulations. Their natural origin and broad-spectrum bioactivity make them attractive alternatives to synthetic chemicals. As such, essential oils continue to gain attention in research focused on sustainable and bio-based solutions for health and agriculture [4,5]. Research on aromatic plants in the Himalayan region is of great relevance to the fields of agriculture and food chemistry due to several compelling factors, including the region's status as a rich biodiversity and agroecological hotspot, its deep reservoir of traditional ethnobotanical knowledge, and the proven medicinal and therapeutic potential of its flora. These plants serve not only cultural and healthcare roles but also offer economic value through their applications in natural products, nutraceuticals, and agro-based industries. Essential oils have garnered increased interest in the food industry due to their potential as natural preservatives, in addition to their pharmacological and antibacterial properties. They are potential options for improving food safety and shelf life because of their broad-spectrum antibacterial activity against foodborne pathogens and spoilage organisms [4]. Additionally, essential oils satisfy consumer demand for clean-label and chemical-free food products by providing a natural alternative to artificial preservatives [5]. Whether through direct addition, encapsulation, or active packaging, the use of essential oils in food systems is effective in preventing oxidative deterioration and microbial growth, which helps maintain food safety and quality [4-5].

To explore novel bioactive compounds for use in food preservation, flavouring, and functional foods, we selected five aromatic species native to the Indian Himalayan Region (IHR): *Tanacetum nubigenum, Pleurospermum angelicoides, Selinum wallichianum, Juniperus wallichiana,* and *Rhododendron anthopogon* for detailed investigation of their essential oil composition. Although local communities already utilise these species for medicinal and aromatic purposes, their chemical characterisation and standardisation using advanced analytical techniques (e.g., GC-MS) are essential to unlock their potential for applications in food safety, natural additives, and green agriculture. This approach supports sustainable use, value addition, and integration of Himalayan botanicals into the broader agricultural and food sciences landscape.

The genus Tanacetum, commonly known as tansy, belongs to the family Asteraceae. Six *Tanacetum* species, *T. nubigenum, T. arteminiodes, T. gracile, T. tibeticum, T. senecionis* and *T. longifolium* have been identified from high altitudes in the Himalayan region of Uttarakhand [6]. *T. nubigenum* Wallich ex DC, an aromatic herb that grows on stony slopes (3600 to 4300 m), has tall stems, small flower heads, and leaves that are three times cut into linear acute lobes. It is a silvery tufted plant, usually with many stems arising from the rootstock [7].

Pleurospermum angelicoides Benth. (Apiaceae) is an herb native to India, Yunnan province, China and Nepal. The roots are used in folk medicine as an antipyretic and diaphoretic agent [8]. It is locally known as “Chippi” in the Himalayan region and used for the treatment of typhoid and dysentery [9].

The genus *Rhododendron* belongs to the Ericaceae family of plants and comprises approximately 850 species [10]. This genus is known for its flavonoids, tannins, essential oils, chromones, terpenoids, and steroid content [11-12].

Selinum has 35 species distributed worldwide. The plant species are perennial, branched herbs belonging to the family Apiaceae. Plants of this genus grow on humus-rich slopes in the temperate to alpine zones of the Himalayas, South Africa, and the Andean mountains. Commonly, *Selinum* is known as Bhutkesh. In India, five species of *Selinum* have been recorded from Uttarakhand *viz*. *Selinum wallichianum, S. elatum, S. candollii, S. striatum, S. vaginatum* [13].

Juniperus L. (Cupressaceae), a genus of evergreen, aromatic shrubs or trees, is distributed in the temperate and cold regions of the Northern Hemisphere. Four species of *Juniperus viz. J. communis, J. wallichiana, J. recurva* and *J. squamata* have been reported from the north-western Himalaya of Uttarakhand [14].

2. Materials and Methods

2.1. Plant Material

The fresh aerial part of Aromatic plants (Table 1) was collected from Dronagiri (Coordinates 30°35'6.45"N and 79°51'27.66"E, Elevation 13045 Feet), Uttarakhand (India), Plant herbaria were identified from the Botanical Survey of India, Dehradun and voucher specimen were deposited in the Phytochemistry laboratory, Chemistry Department, Kumaun University, Nainital.

Table 1. Plants Collected from Dronagiri (Chamoli), Uttarakhand, India

S.No.	Plant Species	Common Name	Abbreviation	Acc. No. (BSI)
1.	*Tanacetum nubigenum* Wall.	Tancy	TN	113959
2.	*Rhododendron anthopogon* D. Don	Tesu	RA	113961
3.	*Juniperus wallichiana* Brandis	Bhitaru	JW	113965
4.	*Selinum wallichianum* (DC.) Raizada & Saxena	Bhutkesh	SW	113967
5.	*Pleurospermum angelicoides* (DC.) Klotzschwere	Choru	PA	113976

BSI=Botanical Survey of India

2.2. Extraction of Oil and Preparation of Extract

The plant materials (400 g) were subjected to steam distillation. The distillates were saturated with NaCl and extracted with *n*-hexane and dichloromethane. The organic phase was dried over anhydrous sodium sulphate, and the solvents were distilled off. The essential oil was extracted by hydro distillation following the method reported previously [15]. Oil yields are as follows:

Table 2. Oil Yield of Plants Collected from Dronagiri (Chamoli), Uttarakhand, India

S. No.	Plant Species	Oil Yield (v/w%)
1.	*Tanacetum nubigenum* Wall.	0.7
2.	*Rhododendron anthopogon* D. Don	0.4
3.	*Juniperus wallichiana* Brandis	0.9
4.	*Selinum wallichianum* (DC.) Raizada & Saxena	0.7
5.	*Pleurospermum angelicoides* (DC.) Klotzschwere	0.8

2.3. GC and GC-MS Analysis

The oils were analysed by using a Nucon 5765 gas chromatograph (Rtx-5 column, 30 m × 0.32 mm ID, 0.25 µm), split ratio 1: 48, N_2 flow of 4 kg/cm^2 and on Thermo Quest Trace GC 2000 interfaced with MAT Polaris Q Ion Trap Mass spectrometer fitted with a Rtx-5 (Restek Corp.) fused silica capillary column (30 m x 0.25 mm; 0.25 µm film coating). Analyses of essential oil and extracts were performed by following the method discussed by Mathela *et al.* [15].

2.4. Identification of Constituents

The essential oil was fractionated by column chromatography (CC) on silica gel CC (230-400 mesh, Merk 600 x 25 cm column) packed with hexane, and eluted with hexane followed by gradient elution by Et_2O/hexane (1-20%). The identification was done on the basis of Linear Retention Index (LRI), determined with reference to homologous series of *n*-alkanes (C_9-C_{24}, Polyscience Corp., Niles IL, under identical experimental conditions), co-injection with standard (Sigma and Aldrich) of major compounds, MS Library search (NIST and WILLEY), by comparing with the MS literature data[11]. The relative amounts of individual components were calculated based on the GC peak area (FID response) without the use of a correction factor.

3. Results and Discussion

A total of fifty-five compounds were identified in the studied oil samples. This accounted for 92.35% to 99.47% of the total composition of the oils (Table 3). This study is based on a limited sample size.

3.1. Tanacetum nubigenum (TN)

Oxygenated monoterpenes, 18.6%, and oxygenated sesquiterpenes, 60.4%, were the major fractions present in the aerial part of the plant (Table 2). The main constituent was identified as *β*-eudesmol (54.3%). In previous investigations on *T. nubigenum* collected from two regions of the Himalaya, two chemotypes were revealed. The first chemotype, collected from Milam village (Pithoragarh), was shown to be dominated by bornyl acetate (39.7%). In contrast, the second chemotype, collected from Pindari (Bageshwar), was dominated by (3*R*,6*R*)-linalool oxide acetate (69.4%). On the other hand, *T. nubigenum* collected from Chamoli (Garhwal Himalayas) was shown to contain (-)-*cis*-chrysanthenol (37.0%) as the main component [17-19]. Our sample contains *β*- eudesmol as a principal constituent, which makes it a new chemotype from previous samples investigated from that region.

3.2. Pleurospermum angelicoides (PA)

Pleurospermum angelicoides (PA) was rich in monoterpene hydrocarbons (82.4%). The major constituents were *p*-cymene (50.4%) and camphene (21.9%). In comparison, previously *Pleurospermum angelicoides* collected from Milam glacier at an altitude of 3600 m contained limonene (48.4%) and *α*-asarone (23.2%) along with a minor presence of *β*-asarone (4.8%), followed by α-pinene and perilla aldehyde in its leaf oil [20]. Our sample contains *p*-cymene-rich essential oil, which is another principal chemotype marker of the species.

3.3. Rhododendron anthopogon (RA)

Volatile constituents of the aerial parts of *Rhododendron anthopogon* **(**RA**)** were found to rich in monoterpene hydrocarbons (49.25%) and oxygenated monoterpenes (20.1%). The major constituents identified as α-pinene (31.2%) and β-pinene (11.7%). In early investigations on the essential oil of *Rhododendron anthopogon* was found to contain *α*-Pinene (21.5–37.4), *δ*-cadinene (9.1–13.8), *β*-pinene (9.5–16.0), limonene (5.9–13.3), cis-ocimene (5.3), *δ*-amorphene (4.6), *α*-muurolene (4.5), and *(E)*-caryophyllene [21-22].

3.4. Selinum wallichianum (SW)

Monoterpene hydrocarbons (41.85%) and Sesquiterpene hydrocarbons (39.2%) were the major fractions found in the essential oil of *Selinum wallichianum* (SW). The major constituents were germacrene D (18.7%) and limonene (15.5%). In early reports on its essential oil composition, it was found to contain limonene, elemol, terpineol, geraniol, and eudesmol as the major constituents. 3,5-nonadiyne was also found in its root oil [23-25].

3.5. Juniperus wallichiana (JW)

Juniperus wallichiana (JW) was found to be rich in monoterpene hydrocarbons (83.7%). The major constituents were identified as sabinene (50.1%) and α-pinene (23.6%). This was similar to *Juniperus wallichiana*, collected from areas near Milam village, which was dominated by sabinene (46.7%), along with α-pinene (6.6%) and terpinen-4-ol (6.5%) in its leaf oil [26].

Table 3. Essential oil Composition of *Tanacetum nubigenum* (TN), *Pleurospermum angelicoides* (PA), *Rhododendron anthopogon* (RA), *Selinum wallichianum* (SW), and *Juniperus wallichiana* (JW)

No	Compounds	LRI	%Composition (FID)					Mode of Identification
			TN	PA	RA	SW	JW	
1	*α*-Thujene	932	-	3.1	0.1	0.2	0.1	a,b
2	*α*-Pinene	941	0.1	0.1	**31.2**	4.3	**23.6**	a,b,c
3	Camphene	946	-	**21.9**	0.6		0.7	a,b
4	Benzaldehyde	952	-	0.3	1.59	1.59		a,b
5	Sabinene	978	0.1	0.26	0.26	0.26	**50.1**	a,b,c
6	*β*-pinene	982	**5.3**	0.14	**11.7**	**13.1**	0.5	a,b
7	Myrcene	994	-	*t*	*t*	*t*	0.3	a,b
8	*α*-Terpinene	1019	0.8	0.6	-	**5.4**	-	a,b
9	*p*-Cymene	1029	-	**50.4**	1.1	1.5	1.6	a,b
10	Limonene	1034	1.7	**5.6**	2.7	**15.5**	**6.8**	a,b,c
11	1,8-Cineole	1038	2.7	1.6	1.9	0.5	1.9	a,b
12	(*Z*)-*β*-Ocimene	1041	*t*	0.1	2.5	3.1	2.5	a,b
13	(*E*)-*β*-Ocimene	1054	0.2	0.2	0.3	0.1	0.3	a,b
14	*cis*-Sabinene hydrate	1069	0.7	0.2	0.13		0.6	a,b
15	Linalool	1101	1.4	1.2	1.2	0.3	1.3	a,b
16	*trans*-*p*-Mentha-2-enol	1145	0.3	*t*	*t*	-	-	a,b
17	Benzyl acetate	1160	-	-	**7.3**	-	-	a,b
18	Borneol	1167	0.3	0.2	0.23	0.3	0.9	a,b
19	Terpinene-4-ol	1180	1.2	0.2	0.56	0.5	3.5	a,b
20	Methyl chavicol	1198	0.2	*t*	0.14	-	*t*	a,b
21	Geraniol	1155	1.5	-	0.1	-	*t*	a,b
22	(*Z*)-Anethole	1249	-	*t*	0.3	1.5	-	a,b
23	Bornyl acetate	1285	0.2	-	0.2	-	-	a,b
24	(*E*)-Anethole	1287	-	1.3	1.24	-	-	a,b
25	Thymol	1290	0.3	-	0.6	-	-	a,b
26	*δ*-Elemene	1335	-	*t*	2.4	**6.6**	2.4	a,b
27	*α* -Cubebene	1345	*t*	*t*	0.2	1.1	*t*	a,b
28	α-Terpinyl acetate	1352	0.2	-	0.1	-	*t*	a,b
29	α-Copaene	1374	-	0.2	0.3	*t*	*t*	a,b
30	Isobornyl proponiate	1381	**9.4**		0.4	*t*	0.3	a,b
31	*β*-Elemene	1389	T	0.1	0.1	*t*	0.1	a,b
32	*α*-Gurjunene	1410	0.4	-	0.1	-	0.2	a,b
33	*β*-Caryophyllene	1418	3.3	*t*	**8.5**	**9.6**	0.5	a,b
34	*β*-Gurjunene	1431	-	*t*	0.2	-	0.1	a,b
35	(*Z*)-*β*-farnesene	1440	-	*t*	0.3	-	-	a,b
36	*α*-Humulene	1457	0.4	*t*	0.1	*t*	-	a,b
37	(E)-*β*-Farnesene	1459	2.3	*t*	0.2	-	-	a,b
38	*γ*-Muurolene	1479	0.3	*t*	0.1	-	-	a,b
39	Germacrene D	1481	2.2	0.4	4.5	**18.7**	0.3	a,b
40	Bicyclogermacrene	1497	0.2	0.3	0.1	-	-	a,b
41	*δ*-Cadinene	1529	0.3	*t*	0.1	**10.9**	-	a,b
42	γ-Asarone	1572	-	*t*	0.1	*t*	-	a,b
43	Germacrene D-4-ol	1578	0.6	0.4	t	*t*	-	a,b
44	Caryophyllene oxide	1581	-	0.3	0.1	*t*	0.1	a,b
45	Guaiol	1604	0.9	*t*	0.2	-	-	a,b
46	*β*-Asarone	1616	-	*t*	0.2	-	-	a,b
47	10-*epi*-*γ*-Eudesmol	1622	-	1.1	0.2	-	-	a,b
48	*β*-Eudesmol	1652	**54.3**	-	**7.9**	1.2	-	a,b,c
49	Selin-11-en-4-*α*-ol	1658	2.2	-	-	*t*	-	a,b
50	*α*-Asarone	1675	-	**7.67**	-	*t*	-	a,b
51	5-Allyl-4,6,7-trimethoxy-benzo [1,3] dioxide	-	-	1.6	-	*t*	-	a,b
52	*α*-Bisabolol	1685	1.9	-	-	*t*	-	a,b
53	Aristolone	1758	0.5	-	-	-	-	a,b
54	(*E*)-Spiroketalenol-ether polyyne	1877	*t*	-	-	-	-	a,b
55	(*Z*)-Spiroketalenol-ether polyyne	1886	*t*	-	-	-	-	a,b
	Monoterpenoids		8.0	82.4	49.25	41.85	83.7	
	Oxygenated Monoterpenoids		18.6	5.2	20.1	14	13.7	
	Sesquiterpenoids		9.4	0.8	14.3	39.2	1.2	
	Oxygenated Sesquiterpenoids		60.4	11.07	8.7	1.2	0.1	
	Total		96.4	99.47	92.35	96.25	98.7	

a= Identification from LRI, b= Identification from MS data, c= co-injection with standard (Sigma and Aldrich); *t: trace <0.1*

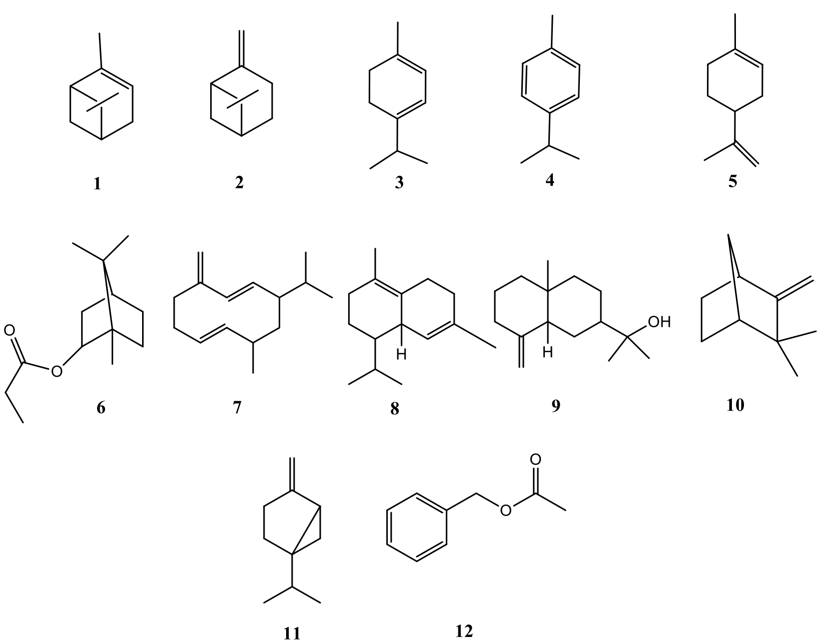

Figure 1. Structures of the Biochemical Markers (major constituents) (**1.** *α*-Pinene, **2.** *β*-Pinene, **3.** *α*-Terpinene, **4.** *p*-Cymene, **5.** Limonene, **6.** Isobornyl proponiate, **7.** Germacrene D, **8.** *δ*-Cadinene, **9.** *β*-Eudesmol, **10.** Camphene, **11.** Sabinene, **12.** Benzyl acetate)

Monoterpenes, comprising hydrocarbons (Figure 1), are the largest class of plant secondary metabolites and are found in essential oils of aromatic plants. Monoterpenes, hydrocarbons 41.85% to 83.7%, are the major classes of compounds identified in the four samples of the present study, *viz. Pleurospermum angelicoides* (PA), *Rhododendron anthopogon* (RA), *Selinum wallichianum* (SW), and *Juniperus wallichiana* (JW), while *Tanacetum nubigenum* (TN) contains oxygenated sesquiterpenes 60.4% as a major class of compounds. Furthermore, Monoterpenes and their derivatives are key ingredients in the design and production of new biologically active compounds [27]. *o*-Cymene, *p*-cymene *α*-phellandrene, *α*-pinene, *α*-thujene, camphene, sabinene, *β*-pinene, myrcene, limonene, linalool, neral, nerol, carvone, *γ*-terpinene, 1,8-cineole and linalyl acetate are a few examples of those monoterpenes which are reported to be much more active against different pathogens [28]. Numerous significant components, including *α*-pinene, *β*-eudesmol, and *p*-cymene, have been identified and recognised for their bioactive properties. *α*-Pinene mainly affects ion transport systems and microbial membrane integrity to provide its antibacterial actions [29]. By disrupting the production of bacterial cell walls and inducing oxidative stress reactions, *β*-eudesmol has shown antibacterial potential [30]. Although it is less effective on its own, *p*-cymene is known to disrupt cell membranes, allowing other antimicrobial drugs to enter and resulting in synergistic effects [31]. These mechanisms support the observed antimicrobial activity and highlight the potential application of these compounds in food preservation and pharmaceutical formulations. Consequently, the compounds found in our samples may play a cardinal role in agricultural, biological and medicinal applications. Monoterpenes, a major class of volatile organic compounds found in essential oils extracted from various plant parts, have long been valued for their fragrance and therapeutic effects. In the context of agriculture and food packaging, these compounds have attracted significant attention due to their broad-spectrum bioactivity and natural origin. Monoterpenes exhibit strong antimicrobial, antioxidant, and antiparasitic properties, making them ideal candidates for use in natural food preservation and plant protection. Their relatively simple molecular structure, low production cost, and ease of extraction or synthesis enhance their practical applicability in sustainable agricultural practices. In food systems, monoterpenes can inhibit the growth of spoilage microorganisms and extend shelf life without the need for synthetic preservatives. As bioactive agents, they are increasingly incorporated into edible coatings, biodegradable films, and active packaging materials to provide continuous protection against microbial contamination and oxidation. Furthermore, their GRAS (Generally Recognised as Safe) status by regulatory bodies, such as the FDA, supports their use in consumable goods. The growing demand for clean-label, eco-friendly food packaging solutions has positioned monoterpenes as promising natural alternatives. Their integration into agricultural and food packaging technologies not only enhances food safety and quality but also aligns with global sustainability goals by reducing chemical inputs and environmental impact. In summary, monoterpenes hold immense promise in modern agri-food systems as multifunctional agents for natural preservation, crop protection, and eco-friendly packaging innovations. The study's essential oils can be used to enhance safety and preservation in various food systems. For instance, essential oils high in α-pinene have been effectively used to prolong the shelf life of meat products by preventing the growth of pathogenic bacteria, such as Listeria monocytogenes [32]. Similarly, p-cymene-containing essential oils have demonstrated antibacterial activity in fresh fruit packaging to prevent fungal deterioration and preserve sensory quality [33]. Additionally, by lowering oxidation and microbial contamination, the use of terpenoid-rich oils in edible coatings has demonstrated promise in maintaining the freshness of fruits and vegetables [34]. The viability of using essential oils produced from plants, as these examples demonstrate, is a natural food preservative. However, future research should focus on evaluating the preservative efficacy of the essential oil through in situ experiments within various food systems, which will assess both microbial inhibition and sensory impact.

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