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Comparative Physicochemical Profiling and Antimicrobial Activity of Pinaceae Leaf Essential Oils against *Staphylococcus aureus* and *Klebsiella pneumoniae*

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ABSTRACT

The research evaluated physical properties, chemical constituents, and antimicrobial activity of Pinaceae oils (*Picea koraiensis*, *Abies nephrolepis*, *Picea abies*, and *Tsuga sieboldii*) to explore their potential as antibiotic alternatives. Leaf oils were extracted via hydrodistillation and assessed against *Staphylococcus aureus* and *Klebsiella pneumoniae* using minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays. Among the oils, *P. koraiensis* oil exhibited the highest density (0.8969 ± 0.0027 g/mL) and refractive index (nD^{20} = 1.4720 ± 0.0001), likely due to its major constituents, including limonene, camphene, and α-pinene. Oil yields ranged from 0.17 ± 0.03% to 3.07 ± 0.05%, with *A. nephrolepis* producing the highest yield. Pinaceae oils were rich in monoterpenes (84.98%–89.49%). Camphor (12.75%) was a major constituent of *P. koraiensis* oil, whereas *A. nephrolepis* oil contained unique terpenoids, including α-terpinyl acetate and the sesquiterpene nerolidol. *A. nephrolepis* and *T. sieboldii* oils showed strong antimicrobial activity against *S. aureus* (MIC: 2%–3%, MBC: 6%–8%), likely due to α-pinene and bornyl acetate. Both oils were also active against *K. pneumoniae* (*A. nephrolepis*: MIC 2%, MBC 3%; *T. sieboldii*: MIC 1%, MBC 2%), associated with abundant limonene (20.24%) and bornyl acetate (25%), respectively. Additionally, the strong antimicrobial activity of these oils may be attributed to their shared minor component, α-bisabolol, which was absent in the other oils. Therefore, *A. nephrolepis* and *T. sieboldii* oils show potential as natural preservatives or respiratory therapeutics, pending further pharmacological and toxicological validation. Physicochemical profiling of Pinaceae oils will aid quality control standard development.

Keywords: essential oils, antimicrobial activity, physical properties, bornyl acetate, limonene, *Abies nephrolepis*, *Tsuga sieboldii*

1. INTRODUCTION

Essential oils hold promise as an alternative strategy for addressing the global threat posed by multidrug-resistant pathogens (Murbach Teles Andrade *et al*., 2014), offering advantages such as lower toxicity, reduced genotoxicity with prolonged use, the ability to target multiple cellular pathways, and cost-effective production (Raut and Karuppayil, 2014). Many studies have explored the antimicrobial effects of various essential oils against microorganisms (Delaquis *et al*., 2002). *Staphylococcus aureus* is a common cause of reported food-

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borne illnesses (Bean *et al*., 1997). This bacterium can grow without specific nutritional or environmental requirements; therefore, *S. aureus* can easily contaminate and grow in food (Oh *et al*., 2007). Cinnamon essential oil exhibits effective antibacterial activity against *S. aureus* by damaging bacterial cells (Zhang *et al*., 2016). *Klebsiella pneumoniae* is a rod-shaped, Gram-negative bacterium commonly present in the normal human intestinal microflora (Yang *et al.,* 2020). It primarily affects individuals with weakened immune systems and is a frequent cause of hospital-acquired infections (Li *et al*., 2014).

Pinaceae has the widest distribution area and the largest population among tree species in South Korea (Ahn and Bae, 2005). Therefore, the commercial development of essential oils may be feasible due to a stable supply of plant material. Numerous investigations on the pharmacological activities of Pinaceae essential oils have been conducted, including antibacterial (Ham *et al*., 2020), antimicrobial (Chouhan *et al*., 2017), and antioxidant (Xie *et al*., 2015) activities. Studies have also examined the bioactivity of wood essential oils extracted from coniferous tree species (*Pinus densiflora*, *Chamaecyparis obtusa*, *Pinus koraiensis*, and *Larix kaempferi*), which are commonly used for timber (Oh *et al*., 2023; Yang *et al.,* 2019). Despite their commercial potential, only a few Pinaceae species have been studied in South Korea. In addition, research on the bioactivity of leaf essential oils from other coniferous tree species remains limited.

Picea koraiensis Nakai (Korean spruce) is native to northeastern Asia, including parts of Korea (Kong, 2006). Gao *et al*. (2005) analyzed the volatile organic compounds of *P. koraiensis* using a thermal-desorption cold trap; the major compounds were limonene (40%) and α-pinene (21.44%). *Abies nephrolepis* Maxim. is an evergreen conifer growing to 20–25 m tall with a trunk diameter of up to 0.75–1 m. It is an economically valuable species utilized for pulp, wood, and timber (Woo *et al*., 2008). *A. nephrolepis* essential oil showed cytotoxic effects in lung cell lines due to inhibition of cell proliferation rather than apoptosis (Ahn *et al*., 2020). The essential oil also showed herbicidal activity (Yun *et al*., 2013). *Picea abies* (L.) H. Karst. is an evergreen conifer introduced for forestry and landscaping in South Korea (Lee, 1977). Fir essential oils have traditionally been used in Europe to treat catarrhal conditions in children by hot-water inhalation (Pauli and Schilcher, 2004). Essential oil from young spruce of *P. abies* possesses antimicrobial activity (Radulescu *et al*., 2011). *Tsuga sieboldii* Carriere is an evergreen conifer native to Japan (Hayashi, 1951). Lagalante and Montgomery (2003) studied the composition of volatile terpenoids from single needles of *T. sieboldii* using headspace solid-phase microextraction; the major terpenoids were α-pinene (20.03%) and isobornyl acetate (21.37%).

Investigating the oil yield, physicochemical properties, chemical composition, and biological activities of essential oils is vital for assessing the feasibility of producing high-quality products. Furthermore, essential oils with potent antimicrobial activity can inform the development of value-added products across industries. Therefore, this study investigated the physical properties, chemical composition, and antimicrobial activity of Pinaceae essential oils (*P. koraiensis*, *A. nephrolepis*, *P. abies*, and *T. sieboldii*) as potential antibiotic alternatives.

2. MATERIALS and METHODS

2.1. Chemicals and reagents

All chemicals and reagents were of analytical grade. Dimethyl sulfoxide (DMSO, extra pure grade, product number: 3047-4460, Duksan, Ansan, Korea), Dulbecco's phosphate-buffered saline (D-PBS, product number: LB01241902, Welgene, Gyeongsan, Korea), and sulfuric acid (95%, extra pure grade, product number: 7664-93-9, Duksan) were used. Resazurin sodium salt (7-hydroxy-3H-phenoxazin-3-one 10-oxide, product number: R7017-1G), barium chloride dihydrate (product number: B0750), and ampicillin (product number: A9393) were purchased from Sigma-Aldrich, St. Louis, MO, USA.

2.2. Plant material and essential oil extraction

The plant materials are listed in Table 1. These species were identified, and voucher specimens were deposited at the herbarium (herbarium code: WFRC), the Warm-Temperate and Subtropical Forest Research Center at the NIFoS (Jeju, Korea). The selected essential oils were obtained from the Essential Oils Bank at the NIFoS. Essential oils were extracted from leaves via hydrodistillation. Essential oil extraction was performed at the NIFoS (Seoul, Korea). Each sample was mixed with distilled water in a ratio of 1:10 (kg:L). Samples in distilled water were heated at 102℃ using a heating mantle (model: MS-DM608, serial number: 201602, Minsung Scientific, Seoul, Korea). Volatiles were condensed using a Dean–Stark trap. Extraction continued until no more essential oil was obtained. The essential oil yield was calculated as:

Essential oil yield (%) = [Essential oil distilled (g) / Plant material (g, dry weight)] × 100. (1)

The essential oils were dehydrated using anhydrous sodium sulfate (98.5%, Samchun, Seoul, Korea) and stored in a deep freezer until required.

2.3. Strains and culture conditions

The microorganisms were *S. aureus* ATCC 25923 and *K. pneumoniae* CCARM0015. These were sourced from the Laboratory Culture Collection at the microbiological laboratories at the NIFoS and stored in 25% glycerol at −40℃. Nutrient agar (product number: 213000, Difco, Franklin Lakes, NJ, USA) was prepared by adding 23 g/L to Mueller-Hinton broth (MHB, product number: 70192, Millipore, Billerica, MA, USA). Frozen stocks were streaked on nutrient agar plates and cultured at 28℃ for 24 h to obtain single colonies. A single colony was inoculated into 4 mL MHB and cultured at 250 rpm overnight.

2.4. Physicochemical analysis

The physical properties of four essential oils were measured according to International Organization for Standardization (ISO) protocols. Relative density and refractive index were measured by ISO 279:1988 and ISO 280:1988, respectively. Relative density was measured with a glass pycnometer, and refractive index with a refractometer (Abbemat 3X00, Anton Paar, Graz, Austria). According to ISO guidelines, data were expressed to four decimal places. Each parameter was evaluated in triplicate.

Table 1. Information on the plants used in the study

Family	Botanical name		Part	Region
Pinaceae	1	*Picea koraiensis* Nakai	Leaves	Pyeongchang
	2	*Abies nephrolepis* Max.	Leaves	Chuncheon
	3	*Picea abies* (L.) H. Karst.	Leaves	Gwangneung
	4	*Tsuga sieboldii* Carrière	Leaves	Ulleungdo Island

2.5. Gas chromatography–mass spectrometry

Qualitative analyses of the selected essential oils were conducted using gas chromatography–mass spectrometry (TRACE 1310/ISQ-LT, Thermo Fisher Scientific, Waltham, MA, USA). A TR-5MS capillary column (30 m × 0.25 mm × 0.25 μm; Thermo Fisher Scientific) was used. The carrier gas was helium at 1 mL/min; inlet pressure, 25 psi. The inlet was 250℃. The oven was held at 40℃ for 3 min, ramped at 3℃/min to 200℃, then at 15℃/min to 340℃, and held for 10 min. Quantitative analyses were performed by GC with a flame ionization detector (FID) using the same column. The FID was 280℃, and the helium flow rate was 40 mL/min. The MS interface and ion source were 280℃ and 250℃, respectively. Individual peaks were identified by Kovats indices (KI) using n-alkanes (C8–C20, product number: 04071, Sigma-Aldrich, Seoul, Korea) and by comparison of MS data with the National Institute of Standards and Technology (NIST) library.

2.6. Determination of minimum inhibitory concentration and minimum bactericidal concentration

Broth microdilution assays were conducted to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for the selected essential oils, following the Clinical and Laboratory Standards Institute (CLSI) protocol M7-A8, with minor modifications. Growth-inhibitory activity was tested against two pathogens. Resazurin solution was used as a growth indicator. Bacterial suspensions were streaked on nutrient agar plates and incubated at 37℃ for 24 h. Overnight cultures were prepared in MHB. For inoculum preparation, cells were suspended in D-PBS. Turbidity was adjusted to the 0.5 McFarland standard with D-PBS as determined using a spectrophotometer (Optizen POP, Mecasys Co., Ltd., Daejeon, Korea) at 600 nm; the corresponding cell density was 1.5×10^8 colony forming units (CFU)/mL (McFarland, 1907). From this, two additional serial dilutions yielded a working suspension of 1.5×10^6 CFU/mL.

MIC and MBC were determined by the resazurin microtiter plate method with slight modifications (Rahman *et al.*, 2004; Sakkas *et al.*, 2016). Essential oils were prepared in DMSO. Stock solutions were 8% and 12% (v/v). Final test concentrations were 12, 8, 6, 4, 3, 2, 1.5, 1, 0.75, 0.5, 0.25, and 0.125% (v/v). Serial twofold dilutions were prepared in 96-well plates containing MHB. Prepared bacterial inoculum (10 μL) was added to each well, giving a final inoculum of 5×10^5 CFU/mL. Resazurin indicator solution (10 μL) was added to every well, including controls. Plates were wrapped loosely with Parafilm to minimize dehydration and incubated at 37℃ for 24 h for MIC determination. MIC was defined as the lowest concentration of essential oil with no visible growth. MBC was determined by subculturing from MIC plates after the MIC trials and further incubation for 48 h. MBC was defined as the lowest concentration that completely killed the inoculum. Color change was assessed visually: blue indicated growth inhibition, while pink to colorless indicated microbial growth or absence of inhibition (Ivanova *et al.*, 2013). Ampicillin was used as a positive control (Barnes *et al.*, 2023). All tests were carried out in triplicate.

2.7. Statistical analyses

Statistical analyses were performed using SAS (version 9.4, SAS Institute, Cary, NC, USA). Physical properties (Table 2) and essential oil yields (Table 3) were analyzed using one-way ANOVA followed by post hoc Tukey's multiple range tests. Assumptions of normality and homogeneity were not formally tested due to the small sample size (n = 3), which may limit statistical power.

Table 2. Relative density and refractive index of four Pinaceae essential oils

Botanical name	Relative density (g/mL)	Refractive index (nD^{20})
Picea koraiensis	0.8969 ± 0.0027[a]	1.4720 ± 0.0001[a]
Abies nephrolepis	0.8645 ± 0.0002[d]	1.4692 ± 0.0002[d]
Picea abies	0.8721 ± 0.0002[c]	1.4704 ± 0.0002[c]
Tsuga sieboldii	0.8743 ± 0.0003[b]	1.4709 ± 0.0002[b]

All measurements were performed in triplicate; results are mean ± SD.
[a–d] Mean values with different letters are significantly different at $p < 0.05$, as analyzed by Tukey's multiple range test.

Table 3. Leaf essential oil yield (% DW) in four Pinaceae species

	Botanical name	Essential oil yield (% DW)
1	*Picea koraiensis*	0.81 ± 0.05[c]
2	*Abies nephrolepis*	3.07 ± 0.05[a]
3	*Picea abies*	0.38 ± 0.04[b]
4	*Tsuga sieboldii*	0.17 ± 0.03[d]

All measurements were performed in triplicate; results are mean ± SD.
[a–d] Mean values with different letters are significantly different at $p < 0.05$, as analyzed by Tukey's multiple range test.

3. RESULTS and DISCUSSION

3.1. Physical characteristics of leaf essential oils

In this study, the aim was to compare the physical characteristics, chemical profiles, and antimicrobial effects of various Pinaceae essential oils. Selecting samples from the same region was not feasible because each species has its own natural habitat. Therefore, leaf samples of Pinaceae species were collected from their natural habitats across South Korea, as recorded in the National Plant Resource Management System (Korea National Arboretum, 2025) operated by the Korea Forest Service, except for the non-native *P. abies*. Specifically, *P. koraiensis* and *A. nephrolepis* samples were obtained from natural habitats in the Pyeongchang and Chuncheon regions, respectively. Leaf samples from *P. abies* were collected from a plantation in Gwangneung. Lastly, *T. sieboldii*, which is native to Ulleungdo Island, was collected on Ulleungdo.

The Pinaceae essential oils were colorless to pale yellow liquids with a characteristic, refreshing, and pleasant odor. Table 2 shows the relative density and refractive index of the Pinaceae essential oils.

The relative density, also known as specific gravity, is an important physical property used to evaluate the quality of essential oils. Most essential oils have a lower density than water, except for a few, such as clove and cinnamon essential oils, which have densities of 1.047 and 1.050 at 20℃, respectively (Oladimeji *et al*., 2004). The relative density of *P. koraiensis* essential oil was approximately 1.04 times that of *A. nephrolepis* essential oil (0.8645 ± 0.0002 g/mL). Consistent with the relative density data, *P. koraiensis* essential oil had the highest refractive index (nD^{20} = 1.4720 ± 0.0001) compared with the other essential oils, which ranged from nD^{20} = 1.4692 ± 0.0002 to 1.4709 ± 0.0002. The refractive index identifies compounds, determines their purity, and analyses the ratio of homogeneous binary mixtures of known components (Ospina *et al*., 2016). According to chemical profiling by GC-MS, the major components of *P. koraiensis* essential oil were limonene (16.76%), camphene (14.13%), and α-pinene (13.27%). The refractive indices of limonene, camphene, and α-pinene at 2

5℃ are approximately 1.4744 (U.S. Coast Guard, 1984), 1.4570 (Weast, 1979), and 1.4632 (Lide, 2005), respectively. Similarly, the refractive index of *P. koraiensis* essential oil at 20℃ is 1.4720, which is relatively high compared with other essential oils due to its major components.

3.2. Yield and chemical compositions of Pinaceae essential oils

The extraction of essential oils usually results in yields of less than 1% of the original plant material (Singh *et al*., 2021; Yang *et al*., 2021). *A. nephrolepis* showed the highest oil yield (3.07 ± 0.05% DW) among the essential oils in this study, which ranged from 0.17 ± 0.03% to 0.81 ± 0.05% DW (Table 3).

Ensuring a consistent supply of plant material is vital when utilizing forest resources for commercial essential oil production. Although *A. nephrolepis* had a high oil yield (3.07 ± 0.05% DW), its development for commercial essential oil production poses significant challenges regarding forest conservation. Specifically, changing climate conditions, such as climate warming, threaten its current habitat on mountaintops, which exists in small, disjunct distributions in South Korea (Lee, 1990). *A. nephrolepis* has been classified as Endangered (EN) by the National Institute of Biological Resources, South Korea. Further research is needed to investigate propagation and cultivation techniques for the development of commercial essential oils.

The essential oil yield of the Pinaceae family varies by species, and comparing yields with references is difficult owing to the many factors that influence oil production. The essential oil yield from *A. nephrolepis* was 0.17 ± 0.05% by steam distillation for 1 h (Yun *et al.*, 2013). However, essential oil from the needles of *A. nephrolepis* in China was obtained through hydrodistillation, yielding 3.6% (v/w; Li *et al.*, 2005). This may reflect not only differences in plant material and distillation time but also the influence of geographic circumstances and climate on essential oil content (Perry *et al*., 1999).

The chemical components in essential oils were identified using GC-MS, and KI were calculated from retention times and compared with KI references for compound confirmation (Table 4). According to Davies (1990), these retention indices are system-independent constants that facilitate peak identification by comparing measured values with tabulated data, thereby enabling reliable analysis across different laboratories and conditions. KI are particularly useful for identifying monoterpenes and sesquiterpenes in essential oils and related natural or synthetic products (Sadgrove *et al.*, 2022). Consequently, the integration of GC-MS with KI has been established as a methodological standard for identifying the chemical composition of essential oils, offering a reliable and reproducible framework for the identification of monoterpenes, sesquiterpenes, and other volatile natural products (Dosoky and Setzer, 2018; Ham *et al.*, 2020; Yang *et al.*, 2022). As shown in Table 4, Pinaceae essential oils were characterized by high monoterpene content (84%–89%) and lower sesquiterpene content (3%–5%). Notably, α-pinene and camphene were dominant constituents in Pinaceae oils, accounting for about 27.40%–38.67% of the total oil content. Unlike the other Pinaceae oils, *P. koraiensis* oil contained a high level of camphor (12.75%). In addition, *A. nephrolepis* oil was characterized by components such as α-terpinyl acetate and the sesquiterpene nerolidol.

Numerous studies have identified monoterpenes as the main components of essential oils extracted from Pinaceae species (Tumen *et al*., 2010). Laakso and Hiltunen (1994) noted that monoterpenes can serve as a taxonomic index for the classification of different species. Santene was present in the Pinaceae essential oils in this study, except in the essential oil of *T. sieboldii*. Kubeczka and Schultze (1987) reported that santene, a terpene homologue, is characteristic of fir

Table 4. Comparative chemical profile (%) of four Pinaceae essential oils

RT (min)	Compound	KI	% in chemical components			
			Picea koraiensis	*Abies nephrolepis*	*Picea abies*	*Tsuga sieboldii*
15.46	Santene	877.14	1.4	3.94	2.39	-
19.05	Tricyclene	915.53	0.84	1.9	1.67	3.46
20.18	α-Pinene	923.69	13.27	19.76	13.04	18.97
22.18	Camphene	938.14	14.13	18.91	20.56	11.02
25.97	β-Pinene	965.51	2.88	1.74	2.17	2.28
27.81	Myrcene	978.8	2.56	-	1.24	8.83
31.28	β-Carene	1,003.86	1.06	0.51	1.92	0.05
35.68	Limonene	1,035.64	16.76	20.24	10.59	9.03
35.95	β-Phellandrene	1,037.59	1.04	-	-	4.21
36.18	Eucalyptol	1,039.26	-	-	0.68	-
43.44	Terpinolene	1,091.69	-	-	0.61	0.33
44.84	Linalool	1,103.94	-	0.66	-	-
47.95	Camphor	1,152.91	12.75	-	3.35	0.09
48.44	Camphene hydrate	1,160.63	1.9	-	-	-
49.59	Borneol	1,178.74	5.05	5.4	8.33	0.5
51.07	α-Terpineol	1,201.69	0.83	0.76	0.75	1.21
52.41	Fenchyl acetate	1,219.12	-	1.27	-	-
57.78	Bornyl acetate	1,288.95	13.88	13.78	18.65	25
62.12	α-Terpinyl acetate	1,359.15	-	0.62	-	-
65.04	α-Gurjunene	1,412.69	-	0.93	-	-
65.71	Methyl undecanoate	1,429.35	1.9	1.64	1.83	1.61
65.83	Caryophyllene	1,432.34	-	0.81	-	1.2
69.55	β-Cadinene	1,531.75	0.58	-	0.73	0.23
70.77	Nerolidol	1,570.48	-	0.92	-	-
73.8	α-Cadinol	1,678.95	1.1	-	0.94	0.25
74.48	α-Bisabolol	1,704.96	-	1.48	-	0.08
Total chemical constituents (%)			91.33	95.27	89.45	88.35

RT: retention time, KI: Kovats index, -: below detection limit.

oils. Hence, santene may serve as an indicator of adulteration with lower-cost conifer essential oils.

The dominant components in *A. nephrolepis* essential oil were monoterpenoids, including limonene (20.24%), α-pinene (19.76%), and camphene (18.91%), which constituted 58% of the total oil content. The chemical profile of *A. nephrolepis* from Korea differed from those of Russian and Chinese oils. Specifically, high concentrations of elemol and sabinyl acetate, the most abundant components in Chinese oil (14.8% and 14.7%, respectively), were absent in both Russian and Korean oils (Kolesnikova and Yu, 1989). Although bornyl acetate was also detected in Chinese oil, it was present in a smaller amount (1.4%) than in Korean oil (13.78%). Therefore, differences in the chemical constituents of *A. nephrolepis* oils from various countries could be attributed to factors such as climatic and geographical conditions (Li *et al.*, 2005). Even among domestically produced *T. sieboldii* essential oils, both qualitative and quantitative variations in chemical composition were observed. In this study, *T. sieboldii* essential oil contained bornyl acetate (25.00%), α-pinene (18.97%), and camphene (11.02%) as the main constituents. However, a previous study reported β-pinene (18.08%) and α-pinene (8.19%) as the primary components in *T. sieboldii* oil (Ham *et al.,* 2020).

3.3. Antimicrobial activity of essential oils from four Pinaceae species

The antimicrobial activity of Pinaceae oils against *S. aureus* varied, with MIC values of 2%–3% and MBC values ranging from 6% to > 12%. Among the selected essential oils, *A. nephrolepis* and *T. sieboldii* showed strong antimicrobial activity against *S. aureus* (MIC: 2%–3%, MBC: 6%–8%; Table 5). Both oils contained major bioactive components, α-pinene and bornyl acetate, which together accounted for 33.54%–43.97% of the total oil, compared with 27.15%–31.69% in the other oils. Notably, the α-pinene content in these oils (18.97%–19.76%) was higher than in the other tested oils (13.04%–13.27%; Table 4). This aligns with a previous study by Leite *et al.* (2007), which reported that α-pinene in pine oil was effective against various strains of *Staphylococcus*. Similarly, hydrodistilled pine-needle essential oil showed strong antimicrobial activity against *S. aureus*, primarily attributed to α-pinene, supporting its biological activity (Zafar *et al.*, 2010). Furthermore,

Table 5. MIC and MBC of four essential oils (% v/v) against two common pathogens

Sample		Microorganisms			
		Staphylococcus aureus		*Klebsiella pneumoniae*	
Essential oils (%, v/v)	*Picea koraiensis*	MIC	3	MIC	1
		MBC	> 12	MBC	12
	Abies nephrolepis	MIC	2	MIC	2
		MBC	8	MBC	3
	Picea abies	MIC	3	MIC	1.5
		MBC	12	MBC	4
	Tsuga sieboldii	MIC	3	MIC	1
		MBC	6	MBC	2
Positive control (mg/mL)	Ampicillin	MIC	0.019	MIC	0.15
		MBC	0.039	MBC	0.50

Ampicillin (10 mg/mL; serial dilution) was used as the positive control.
MIC: minimum inhibitory concentration (% v/v), MBC: minimum bactericidal concentration (% v/v).

bornyl acetate (25%) may be an important contributing component in *T. sieboldii* essential oil. Bornyl acetate, a bicyclic monoterpene, has exhibited various pharmacological activities, including antimicrobial, anticancer, and antiabortifacient effects (Zhao *et al.*, 2023). For example, *Tetraclinis articulata* essential oil, characterized by a high content of bornyl acetate (35.05%), demonstrated strong antimicrobial activity against *S. aureus* (Rabib *et al*., 2020).

The antimicrobial activity of Pinaceae oils against *K. pneumoniae* varied, with MIC values of 1%–2% and MBC values ranging from 2% to 12%. Strong activity against *K. pneumoniae* was observed for *A. nephrolepis* (MIC: 2%, MBC: 3%) and *T. sieboldii* (MIC: 1%, MBC: 2%; Table 5). Considering the chemical profiles, the strong activity of *A. nephrolepis* and *T. sieboldii* may be associated with limonene (20.24%) and bornyl acetate (25%), respectively (Table 4). Limonene disrupts bacterial membranes in both Gram-positive and Gram-negative species, causing leakage of intracellular materials and cell death (Bei *et al.*, 2015; Zahi *et al.*, 2015; Zhang *et al.*, 2014). In particular, limonene has been reported as one of the most effective constituents for antimicrobial activity against *K. pneumoniae* in *Abies cilicica* (Antoine & Kotschy) Carrière subsp. *cilicica* essential oil (Dayisoylu *et al.*, 2009). The strong activity against *K. pneumoniae* may also be attributed to bioactive components such as bornyl acetate and limonene; for example, Jagannath *et al*. (2012) reported notable antimicrobial properties of *Heracleum rigens* seed oil associated with high terpene content, particularly bornyl acetate (51.2%) and limonene (9.62%). In this study, *A. nephrolepis* and *T. sieboldii* oils contained higher combined levels of limonene and bornyl acetate (34.02%–34.03%) than the other oils (29.24%–30.64%; Table 4). Notably, *A. nephrolepis* and *T. sieboldii* showed bactericidal effects against *K. pneumoniae* with lower MBC:MIC ratios of 1.5 and 2, respectively. The MBC:MIC ratio is used to determine whether an antibacterial agent is bactericidal or bacteriostatic *in vitro* (Mogana *et al.,* 2020); a ratio of $\leq$ 4 indicates a bactericidal effect, whereas higher ratios are considered bacteriostatic (Ishak *et al.,* 2025). Thus, *A. nephrolepis* and *T. sieboldii* were particularly effective in inhibiting *K. pneumoniae*, whereas other essential oils may require higher concentrations for bactericidal effects. Additionally, the strong activity of *A. nephrolepis* and *T. sieboldii* may be influenced by their shared minor component, α-bisabolol, which, together with other constituents, could synergistically enhance antimicrobial effects. Notably, α-bisabolol was absent in the other essential oils examined in this study. α-bisabolol, an oxygenated sesquiterpene, is known for its biotechnological potential due to antimicrobial effects on pathogens such as *K. pneumoniae* and *S. aureus* (Farias *et al.*, 2019).

The antimicrobial efficacy of essential oils is influenced by chemical profile, environmental conditions, and bacterial structural features; however, mechanisms remain unclear (Angane *et al*., 2022). Effects may be specific or nonspecific, for example via intracellular molecule–specific interactions or interactions with biomembranes, respectively. Antimicrobial effects may also result from synergistic interactions among multiple constituents rather than a single component (Bang *et al.,* 2020). This study was limited to two bacterial strains; thus, further research should include a broader panel of Gram-positive and Gram-negative bacteria.

4. CONCLUSIONS

Pinaceae species play a significant role in traditional medicine, and pine essential oils are widely valued across industries for their distinctive fragrance and bioactive components. This study examined the physical properties, chemical constituents, and antimicrobial activity of Pinaceae essential oils (*P. koraiensis*, *A. nephrolepis*, *P. abies*, and *T. sieboldii*) as potential alternatives to antibiotics. *P. koraiensis* essential oil showed the highest density (0.8969 ± 0.0027 g/mL) and refractive index (nD^{20} = 1.4720 ± 0.0001), likely due to major components such as limonene (16.76%), camphene (14.13%), and α-pinene (13.27%). Oil yields varied from 0.17 ± 0.03% to 3.07 ± 0.05%, with *A. nephrolepis* producing the highest yield. However, the commercialization of *A. nephrolepis* essential oil is challenging due to the species' endangered status.

Pinaceae essential oils primarily comprised monoterpenes (84.98%–89.49%). Notably, qualitative and quantitative differences in the chemical profiles of Pinaceae oils were observed among specimens, which could influence their aroma and bioactivity. *T. sieboldii* and *A. nephrolepis* essential oils demonstrated strong antimicrobial activity, likely due to bioactive components such as α-pinene, bornyl acetate, and limonene. Additionally, the strong antimicrobial activity of both essential oils may be linked to their shared minor component, α-bisabolol, which was absent in the other samples. Further studies on cytotoxicity, delivery systems, and formulation stability are essential before industrial or clinical applications can be pursued. Therefore, *A. nephrolepis* and *T. sieboldii* essential oils could serve as valuable industrial resources for food preservation and respiratory support treatments, with their utility contingent upon further pharmacological and toxicological validation. Moreover, our findings contribute to the establishment of preliminary quality standards for the selected essential oils.

CONFLICT of INTEREST

No potential conflict of interest relevant to this article was reported.

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