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Screening the Physical Properties, Chemical Profile and Antimicrobial Activity of Five Essential Oils

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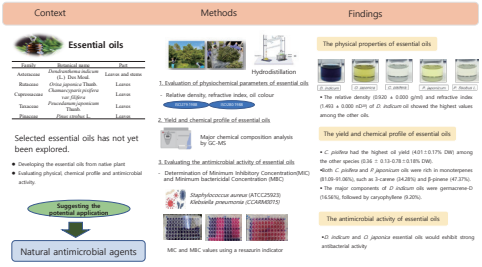
Abstract: Given the global challenge of drug-resistant pathogens, essential oils would be new pharmacological supplements for antibacterial action with low toxicity and low or no resistance. This study investigated the physical properties, chemical profile and antimicrobial activity of native essential oils to develop antibiotic alternatives. Specifically, essential oils were extracted from *Dendranthema indicum*, *Orixa japonica*, *Chamaecyparis pisifera* var. *filifera*, *Peucedanum japonicum*, and *Pinus strobus* using hydrodistillation. The antimicrobial activity of essential oils against *Staphylococcus aureus* and *Klebsiella pneumonia* was assessed by Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). The essential oils from *D. indicum* with blue colour showed lower *b values (3.21±0.78) than other species (14.94±0.51-15.84±0.78). *D. indicum* oil showed the highest values in relative density (0.920±0.000g/mL) and refractive index (1.493±0.000nD^{20}) among the other oils. These results might be attributed to the major component of camphor in oils, known for its high density and refractive index. *C. pisifera* had the highest oil yield (4.01±0.17% DW) among the other species (0.36 ± 0.13-0.78±0.18% DW). Both *C. pisifera* and *P. japonicum* oils were rich in monoterpenes (81.09-91.06%), such as 3-carene (34.28%) and β-pinene (47.37%). Yet, the other oils were abundant in sesquiterpenes (13.82-36.45%). For example, the major components of *D. indicum* oils were germacrene-D (16.56%), followed by caryophyllene (9.20%). *D. indicum* and *O. japonica* oils were particularly effective in inhibiting both bacterial growths (MIC:0.5-1.5% and MBC:4-8%), whereas the other essential oils might require higher concentrations for bactericidal effects (MIC:1-3% and MBC:3-≥12%). The strong antibacterial effect of *D. indicum* and *O. japonica* oils might be attributed to the major components of camphor (23.05%) and linalool (10.92%), respectively. Therefore, *D. indicum* and *O. japonica* essential oils would exhibit strong antibacterial activity, highlighting a potential antimicrobial agent in food and health industrial resources.**



Key words: essential oils, *Dendranthema indicum*, *Orixa japonica*, antimicrobial activity, physical properties

1 Introduction

Historically, many plant oils and extracts such as tea tree (*Melaleuca alternifolia*), myrrh (*Commiphora myrrha*), and clove (*Eugenia caryophyllata*) have been used as topical antiseptics, owing to their antimicrobial properties[1,2]. Terpenes, key bioactive compounds in these oils, exhibit antibacterial effects and are used in food, cosmetics, and healthcare[3,4].

Given the global challenge of drug-resistant pathogens, essential oils offer a promising alternative to control multi-drug-resistant bacteria[5]. *Staphylococcus aureus*, a gram-positive bacterium, is considered one of the most significant foodborne pathogens worldwide[6]. Causative toxins produced by *S. aureus* can cause food poisoning, toxic shock syndrome, and human dermatitis[7]. Essential oils extracted from lemon (*Citrus limon*), sweet orange (*Citrus sinensis*) and bergamot (*Citrus bergamia*) show antimicrobial activity against food-related bacteria[8]. The bioactive components of carvacrol, citronellol, geraniol, and nerol have been reported to possess strong antibacterial

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activity[9]. *Klebsiella pneumoniae*, a gram-negative bacterium, is a clinically significant organism causing broad spectra of diseases[10]. In particular, the respiratory tract is one of the sites where *K. pneumoniae* is frequently encountered[11]. Meng et al.[12] found that essential oils extracted from *Juniperus rigida* show strong antibacterial activity against *K. pneumoniae*. Specifically, essential oils would cause irreversible damage to the cell wall and membrane, leading to the leakage of protein.

Essential oils extracted from medicinal crops have received little attention and insufficient scientific studies in Korea. Especially, Medicinal and aromatic crops have been more extensively investigated concerning their biological properties of extract as a functional food ingredient[13, 14]. In this study, we selected the five essential oils from＜Essential oils bank＞established by the National Institute of Forest Science (NiFoS). Selected species were chosen due to their traditional medicinal use, known phytochemical content, and ecological availability, which make them promising candidates for evaluating the antimicrobial properties. Especially, evaluating the antimicrobial activity of essential oils would provide insight into value-added products by applying them to various industries. *Dendranthema indicum* spreads across East Asia, and it has been commonly used as a traditional medicine for a long time[15]. Most studies focused on the *D. indicum* essential oils extracted from flowers by identifying the chemical profile of oils[16, 17]. *Orixa japonica* is a dioecious shrub belonging to the family *Rutaceae*[18]. Several studies of *O. japonia* extract have evaluated its biological activities[19−21]. Among the selected essential oils in this study, there are insufficient scientific studies of *C. pisifera* var. *filifera* is a subspecies of *C. pisifera*. And the chemical components and biological effects of its leaf essential oils have not been extensively studied. *Peucedanum japonicum* is a perennial herbaceous plant, and it is commonly consumed as a vegetable and herbal medicine in South Korea[22]. There have been few studies on the essential oils extracted from its roots[23]. *Pinus strobus* is one of the most important Pinus species for timber production. Most research focuses on the breeding and plantation for afforestation, but there is limited research on essential oils[24−26]. Therefore, the study investigated the chemical profile of selected essential oils with antibacterial activity against *S. aureus* and *K. pneumoniae*. These results would contribute to a better understanding of the physical, chemical profile, and antimicrobial activity of selected essential oils, suggesting potential applications.

2 Experimental Procedures

2.1 Chemicals and reagents

All incidental chemicals and reagents used were of analytical grade. Dimethyl sulfoxide (DMSO, extra pure grade, Product number: 3047-4460, Duksan) and Dulbecco's Phosphate buffered saline (D-PBS, Product number: LB01241902, Welgene), Sulfuric acid (sulfuric acid 95%, extra pure grade, Product number: 7664-93-9, Duksan) were used. Resazurin sodium salt (7-hydroxy-3H-phenoxazin-3-one10-oxide, product number: R7017-1G), barium chloride dihydrate (Product number: B0750), and ampicillin (Product number: A9393) were purchased from Sigma-Aldrich, St Louis, USA.

2.2 Plant material and essential oils extraction

The plant materials are listed in Table 1. These species were identified and voucher specimens were deposited at the herbarium (herbarium code: WFRC), Warm-Temperate and Subtropical Forest Research Center at the National Institute of Forest Science (Jeju, South Korea). The essential oils used in this study were obtained from the Essential oils Bank at the National Institute of Forest Science (Seoul, South Korea).

The essential oils were extracted from the different plant parts via hydro-distillation. Essential oils extraction was performed at the National Institute of Forest Science (Seoul, South Korea). Specifically, each sample was mixed with distilled water (DW) in a ratio of 1:10 (kg: L). Samples soaked in the DW were heated at 102℃ using a heating mantle (Model: MS-DM608, Serial number: 201602, Minsung Scientific. Co. Ltd., Korea). Then, the volatiles were condensed using a Dean-Stark trap. Extraction continued until no more essential oils were obtained. The yield of essential oils was calculated using the following equation.

$$\text{Essential oil yield}(\%) = [\text{essential oils distilled}(g)/\text{plant material}(\text{DW } g)] \times 100\%$$

Table 1 Information on the plants used in the study.

Family	Botanical name	Part	Region
Asteraceae	*Dendranthema indicum* (L.) Des Moul.	Leaves and stems	Yeongju
Rutaceae	*Orixa japonica* Thunb.	Leaves	Jeju
Cupressaceae	*Chamaecyparis pisifera var. filifera*	Leaves	Pocheon
Taxaceae	*Peucedanum japonicum* Thunb.	Leaves	Yeongju
Pinaceae	*Pinus strobus* L.	Leaves	Pocheon

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

2.3 Strains and culture conditions

The microorganisms used in this study were *S. aureus* ATCC 25923 and *K. pneumoniae* CCARM0015. These microorganisms were sourced from the Laboratory Culture Collection at the microbiological laboratories at the NIFoS, and stored in 25% glycerol stock at −40℃. Nutrient agar (Nutrient agar, Product number: 213000, Difco, USA) was prepared by adding 23 g/L agar to Muller Hinton Broth (MHB, Product number: 70192, Millipore, USA). The frozen storage bacteria were inoculated on a nutrient agar plate and cultured at 28℃ for 24 hours to ensure a single colony. A single colony was inoculated into 4 mL MHB broth and then cultured with 250 rpm overnight.

2.4 Evaluation of the physiochemical parameters of essential oils

The physical properties of five essential oils were determined according to the protocol described by the International Organisation for Standardisation (ISO). Specifically, the essential oils' relative density and refractive index were measured by ISO 279:1988 and ISO 280:1988, respectively. A glass pyknometer measured the relative density and the refractive index was measured by a refractometer (Abbemat 3X00, refractometer, Anton Paar, Graz, Austria). According to the ISO guideline, the data were expressed as four decimal places. The colour of essential oils was measured using E-EYE (IRIS Visual Analyser 400, Alpha MOS, Toulouse, France). The colour of essential oils was evaluated as L^* value (lightness/darkness), a^* value (redness/greenness), and b^* value (blueness/yellowness). Specifically, the L^* measures the whiteness value of colour and ranges from black at 0 to white at 100. The chromaticity coordinate a^* measures red when positive and green when negative, and the chromaticity coordinate b^* measures yellow when positive and blue when negative[27]. Each parameter was evaluated three times.

2.5 Analysis of gas chromatography-mass (GC-MS)

Qualitative analyses of the selected essential oil were conducted using GC-MS (Trace 1310/ISQ-LT, ThermoScientific, USA). The Tr-5MS capillary column (30 m × 0.25 mm × 0.25μm; ThermoScientific, USA) was used. The carrier gas was helium at a flow rate of 1 mL/min, 25 psi. The sample inlet was maintained at 250℃. Further, an oven was maintained at 40℃ for 3 min, after which the temperature was increased by 3℃ until it reached 200℃ and then was raised by 15℃ until it reached 340℃ for 10 mins. Quantitative analyses were performed by GC analysis of the selected essential oils using an FID and using the same column. FID was maintained at 280℃, and the flow rate for helium was 40 mL/min. The temperatures of the interface of a mass spectrometer and ion source were maintained at 280℃ and 250℃, respectively. The identification of individual peaks was done using their Kovats Indices (KI) using n-alkanes (C_8-C_{20}, Product number: 04071, Sigma-Aldrich, Korea) and MS data were compared to those for standard compounds in the MS database of the National Institute of Standards and Technology (NIST).

2.6 Determination of Minimum Inhibitory Concentration (MIC) and Minimum bactericidal Concentration (MBC)

Broth micro-dilution assays were performed to determine the MIC and MBC for the selected essential oil, according to the Clinical and Laboratories Standards Institute (CLSI) protocol, M7-A8 with some modifications. The growth inhibitory activity of essential oil was tested against two pathogens. Resazurin solution was used as an indicator of growth for the microbial assays. The bacterial suspensions were inoculated on nutrient agar plates and incubated at 37℃ for 24 hours. Overnight cultures were prepared in MHB. For the preparation of the bacterial suspensions, the inoculum was suspended in D-PBS solution. The turbidity of the bacterial suspension was compared to 0.5 McFarland standard adjusted with D-PBS solution until the bacterial suspension matched the McFarland standard as determined using a spectrometer (Optizen POP, South Korea) at 600 nm wavelength, and the number of bacteria recorded as Colony Forming Units (CFU) was 1.5×10^8 CFU/mL[28]. From this initial solution, two additional serial dilutions were made to acquire a working solution of 1.5×10^6 CFU/mL.

MIC and MBC of each essential oil were determined by using the resazurin microtiter assay plate method with a slight modification[29, 30]. A preliminary experiment confirmed that the stock concentration of only 8% (serial dilution) does not differ in MIC and MBC values among essential oil samples. Therefore, the concentration of samples in this study was further refined and set more specific levels. The essential oils were prepared in DMSO. A stock solution of the essential oils to be tested was prepared (8% and 12% essential oil v/v). The samples were subdivided into the concentration of 12% and 8% essential oils. The final concentrations of each essential oil were 12, 8, 6, 4, 3, 2, 1.5, 1, 0.75, 0.5, 0.25, and 0.125 (v/v). Subsequently, serial double-fold dilutions were prepared in 96-well plates containing MHB. Prepared bacterial inoculum (10 μL) was transferred to each well containing the serial double-fold dilutions of the essential oil, giving a final bacterial concentration of 5×10^5 CFU/mL. Resazurin indicator solution (10 μL) was added to every well including the control well. Each plate was wrapped loosely with the parafilm to ensure that bacteria did not become dehydrated. The 96-well plates were incubated at 37℃ for 24 hours to

obtain MIC. MIC is defined as the lowest concentration of the essential oil at which the microbial strain does not show visible growth. MBC was determined by serial subcultivation of MIC plates after the MIC trials and further incubation for 48 hours. MBC is defined as the lowest concentration of essential oil which inoculated bacteria was completely killed. The colour change was assessed visually. A blue-coloured solution indicated growth inhibition in the test wells, while a pink-to-colourless solution indicated microbial growth or absence of inhibition[31]. Ampicillin is used as a positive control[32]. All tests were carried out in triplicate.

2.7 Statistical analysis

Statistical analyses were performed using SAS (Version 9.4, SAS Institute INC., Cary, NC, USA) software. The physical properties of essential oils (Table 2) and essential oils yield (Table 3) data were analysed using one-way ANOVA followed by post-hoc Tukey's multiple range tests.

3 Results

3.1 The physical properties of selected essential oils

The relative density, refractive index, and colour (L^*, a^*, b^*) of five essential oils are given in Table 2. The relative density of *D. indicum* essential oils was 0.920 ± 0.000g/mL, showing the highest values among the other samples (0.860 ± 0.000-0.880 ± 0.010g/mL). The relative density of *O. japonica* essential oils (0.880 ± 0.010g/mL) was significantly higher compared to *C. pisifera* and *P. strobus* essential oils. However, no significant difference in relative density was observed between *C. pisifera* and *P. strobus* essential oils (0.860 ± 0.000g/mL and 0.861 ± 0.012g/mL, respectively). Consistent with the relative density data, *D. indicum* essential oil had the highest refractive index (1.493 ± 0.000nD^{20}) compared to the other essential oils, which ranged from 1.470 ± 0.000 to 1.478 ± 0.000nD^{20}.

D. indicum essential oil was observed to be a clear blue liquid, while the other essential oils appeared colourless to golden yellow with the unaided eye (Fig. 1). As shown in Table 2, the L^*, a^*, and b^* values of *D. indicum* essential oil were significantly lower than those of *O. japonica*, *P. japonica*, *P. strobus*, and *C. pisifera* essential oils. In particular, *D. indicum* essential oil showed the lowest b^* value (3.21 ± 0.78) among the samples, five times lower than the b^* values of the other essential oils (from 14.94 ± 0.51 to 15.84 ± 0.01). Excluding the *D. indicum* essential oil, there was no significant variation in L^* and a^* values among the other four essential oils.

Table 3 Essential oil yield (% DW) for five different species.

	Botanical name	Essential oil yield (% DW)
1	*D. indicum*	0.78 ± 0.18^{b}
2	*O. japonica*	0.42 ± 0.11^{cd}
3	*C. pisifera*	4.01 ± 0.17^{a}
4	*P. japonicum*	0.72 ± 0.22^{bc}
5	*P. strobus*	0.36 ± 0.13^{d}

* All tests were done in triplicate and results were given in average ± standard deviation (SD).
* $^{a\text{-}c}$Mean values with different letters were significantly different at $p < 0.05$ as analysed by Tukey's multiple tests.

3.2 The oil yield and chemical profile of essential oils

The essential oils were obtained with a yield of 0.36 ± 0.13% to 4.01 ± 0.17% by hydrodistillation (Table 3). In particular, *C. pisifera* had the highest oil yield (4.01 ± 0.17% DW), among the other species (0.36 ± 0.13-0.78 ± 0.18% DW). Following that, the oil yield of *D. indicum* and *P. japonicum* was 0.78 ± 0.18% and 0.72 ± 0.22%, respectively. *O. japonica* and *P. strobus* showed the lowest oil yields at 0.42 ± 0.11 and 0.36 ± 0.13% DW, respectively, with no significant differences between these two species.

In this study, we analysed the major chemical composition of essential oils using GC-MS analysis (Table 4). *C. pisifera* and *P. japonicum* essential oils were rich in monoterpenes, comprising 91.06% and 81.09%, respectively. Both essential oils predominantly contained mono-

Table 2 The physical characteristics of five essential oils.

Species	Relative density (g/ml)	Refractive Index (nD^{20})	Colour		
			L^*	a^*	b^*
D. indicum	0.920 ± 0.000^{a}	1.493 ± 0.000^{a}	66.88 ± 0.53^{b}	−8.85 ± 1.11^{b}	3.21 ± 0.78^{e}
O. japonica	0.880 ± 0.010^{b}	1.476 ± 0.000^{c}	85.70 ± 0.01^{a}	−3.95 ± 0.05^{a}	15.84 ± 0.01^{a}
C. pisifera	0.860 ± 0.000^{c}	1.470 ± 0.000^{d}	85.60 ± 0.13^{a}	−4.06 ± 0.04^{a}	15.66 ± 0.15^{ab}
P. japonicum	0.870 ± 0.010^{bc}	1.478 ± 0.000^{b}	85.71 ± 0.00^{a}	−3.96 ± 0.01^{a}	15.80 ± 0.05^{ab}
P. strobus	0.861 ± 0.012^{c}	1.478 ± 0.000^{b}	85.76 ± 0.02^{a}	−3.76 ± 0.12^{a}	14.94 ± 0.51^{b}

* All tests were done in triplicate and results were given in average ± standard deviation (SD).
* $^{a\text{-}c}$Mean values with different letters were significantly different at $p < 0.05$ as analysed by Tukey's multiple tests.

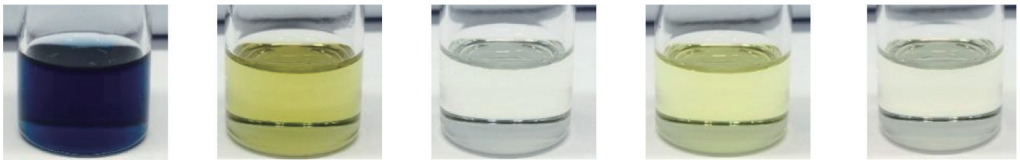

Fig. 1 The colour of five essential oils by observing the human eyes.

Table 4 The major chemical profile of five essential oils.

	RT	KI	Name	Chemical profile of essential oils (%)				
				D. indicum	*O. japonica*	*C. pisifera*	*P. japonicum*	*P. strobus*
1	26.85	927.69	α-Pinene	2.57	2.43	21.25	19.12	25.69
2	29.49	943.11	Camphene	2.18	–	1.11	0.22	2.43
3	33.73	967.87	Sabinene	0.24	1.78	0.03	0.60	–
4	34.71	973.60	β-Pinene	2.59	–	0.38	47.37	15.16
5	34.96	975.34	1-Octen-3-ol	–	–	0.05	–	–
6	37.33	988.90	β-Myrcene	0.59	1.47	10.68	1.67	2.72
7	39.94	1007.77	α-Phellandrene	–	–	0.05	0.15	1.24
8	40.34	1012.68	3-Carene	–	–	34.28	–	–
9	42.17	1032.17	*m*-Cymene	0.54	–	0.04	0.99	0.14
10	42.68	1037.75	Limonene	1.22	0.43	3.14	1.40	1.95
11	42.82	1039.28	β-Phellandrene	–	–	0.34	3.83	3.14
12	42.96	1040.81	Eucalyptol	4.51	–	–	–	–
13	43.42	1045.84	(*E*)-Ocimene	1.45	2.22	0.01	–	–
14	44.37	1056.24	(*Z*)-Ocimene	0.29	–	–	–	–
15	45.33	1066.74	γ-Terpinene	0.37	0.55	0.33	2.57	0.11
16	47.37	1089.06	Terpinolene	–	–	3.02	0.27	0.88
17	48.28	1099.45	Linalool	–	10.92	0.07	–	–
18	48.36	1099.89	Filifolone	1.13	–	–	–	–
19	48.77	1105.36	Thujone	0.56	–	–	–	–
20	49.83	1119.57	Chrysanthenone	0.65	–	–	–	–
21	51.27	1138.87	Pinocarveol	0.58	–	–	–	–
22	51.79	1145.84	Camphor	23.05	–	0.11	–	–
23	53.49	1168.63	α-Phellandren-8-ol	0.27	–	0.02	–	–
24	53.63	1170.51	Borneol	1.22	–	0.32	–	–
25	53.91	1174.90	Isocamphopinone	–	–	–	–	0.16
26	54.30	1179.49	Terpinen-4-ol	0.87	1.65	0.27	0.32	0.13
27	54.65	1184.83	*p*-Cymen-8-ol	–	–	0.07	–	–
28	55.45	1194.91	α-Terpineol	0.36	3.31	0.31	1.64	1.29
29	55.59	1196.78	Myrtenal	0.28	–	–	–	–
30	55.70	1198.26	Estragole	–	–	–	0.94	–
31	57.90	1228.53	Nerol	–	0.60	–	–	–
32	59.94	1255.99	Linalyl acetate	–	5.85	–	–	–
33	62.46	1289.23	Bornyl acetate	0.29	–	11.22	–	–

Table 4 Continued.

	RT	KI	Name	Chemical profile of essential oils (%)				
				D. indicum	*O. japonica*	*C. pisifera*	*P. japonicum*	*P. strobus*
34	65.74	1352.49	α-Terpinenyl acetate	–	–	1.32	–	–
35	66.15	1361.00	Nerol acetate	–	0.87	1.32	–	–
36	67.05	1379.67	Geranyl acetate	–	19.00	1.32	–	–
37	67.31	1384.38	α-Copaene	0.31	–	–	0.10	0.13
38	67.85	1395.63	β-Elemene	1.02	0.44	–	1.02	0.51
39	68.94	1424.04	Methyl undecanoate	2.12	–	–	2.57	2.20
40	69.24	1432.24	Caryophyllene	9.20	5.07	0.06	0.35	1.44
41	70.13	1456.56	(*E*)-β-Famesene	0.34	–	0.04	–	–
42	70.57	1468.58	Humulene	0.81	0.43	–	–	0.41
43	71.17	1484.97	(+)-α-Muurolene	0.42	–	–	0.13	0.68
44	71.48	1493.44	Germacrene-D	16.56	–	–	0.66	4.78
45	71.54	1495.90	(*E*)-Methyl isoeugenol	–	2.77	–	–	–
46	71.78	1502.00	Valencene	0.25	–	–	–	0.49
47	71.95	1507.67	γ-Elemene	2.10	3.83	–	–	–
48	72.47	1525.00	γ-Cadinene	0.24	–	–	–	1.55
49	72.58	1528.67	δ-Cadinene	1.27	0.77	–	0.10	4.76
50	73.64	1565.00	(*E*)-Nerolidol	–	1.36	–	–	–
51	74.72	1600.00	Caryophyllene oxide	1.07	0.35	–	–	0.18
52	75.25	1619.41	Carotol	–	–	–	0.17	–
53	75.88	1642.49	Cubenol	0.54	–	–	–	0.31
54	76.29	1657.51	τ-Muurolol	1.33	0.46	–	–	1.58
55	76.81	1676.56	Juniper camphor	0.99	–	–	0.21	–
56	77.37	1697.07	α-Bisabolol	–	–	–	0.13	–
57	77.98	1718.42	Farnesol	–	1.11	–	–	–
58	86.55	1967.62	Isophyllocladen	–	0.55	–	–	–

*RT: Retention time (min), *KI: Kovats index *-: below detection limit

terpene hydrocarbons, α-pinene, which constitutes nearly 20% of total oils. The major component of *C. pisifera* essential oil was 3-carene(34.28%), whereas *P. japonicum* essential oil contained β-pinene as its predominant compound(47.37%). However, both essential oils had low levels of sesquiterpenes, accounting for only 0.1% and 2.87%, respectively. On the contrary to the low level of sesquiterpenes in *C. pisifera* and *P. japonicum* essential oils, sesquiterpenes were notably present in *D. indicum*, *O. japonica*, and *P. strobus* essential oils. The *D. indicum* essential oils exhibited a high sesquiterpene content of sesquiterpenes(36.45%), whereas *C. pisifera* essential oils contained only 0.1%. The predominant components of *P. strobus* were α-pinene(25.69%)and β-pinene(15.16%), yet these chemical components were lower in *D indicum* and *O. japonicum* essential oils(below 3%). The major component of *D. indicum* essential oils was camphor (23.05%), followed by eucalyptol(4.51%). In the case of *O. japonica* essential oils, the major chemical profiles were linalool(10.92%)and geranyl acetate(19.00%), respectively.

3.3 Evaluating the antimicrobial activity of essential oils against two pathogens

The antimicrobial activity of essential oils was evaluated against *S. aureus* and *K. pneumoniae* by determining the MIC and MBC(Table 5). The essential oils showed varying degrees of antimicrobial activity(MIC: 0.5–3 % and MBC: 3- >12%). *D. indicum*, *O. japonica*, and *C. pisifera* essential oils showed active antimicrobial activity towards *S. aureus* with comparatively low MIC and MBC values of 1-1.5% and 3-4%, respectively. However, the other essential oils showed comparatively high MIC(3%)and MBC(> 12%)against *S. aureus*. In the case of *K. pneumoniae*, *D.*

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

Sample		Microorganisms			
		S. aureus		*K. pneumoniae*	
Essential oils (%, v/v)	*D. indicum*	MIC	1	MIC	0.5
		MBC	4	MBC	8
	O. japonica	MIC	1.5	MIC	1
		MBC	4	MBC	4
	C. pisifera	MIC	1	MIC	2
		MBC	4	MBC	>12
	P. japonicum	MIC	3	MIC	2
		MBC	>12	MBC	12
	P. strobus	MIC	3	MIC	1
		MBC	>12	MBC	12
Positive control (mg/mL)	Ampicillin	MIC	0.019	MIC	0.15
		MBC	0.039	MBC	0.50

* MIC: Minimum Inhibitory Concentration in % (v/v); MBC: Minimum Bactericidal Concentration in % (v/v)
* Ampicillin (10 mg/mL: serial dilution) was used as a positive control.

indicum essential oil had a low MIC of 0.5%, but required 8% for the complete bactericidal action. In particular, MBC values were generally higher with *C. pisfera*, *P. japonica*, and *P. strobus* requiring ≥ 12% to exhibit the bactericidal activity. These results suggested that *D. indicum* and *O. japonica* essential oils were particularly effective in inhibiting bacterial growth, whereas other essential oils might require higher concentrations for bactericidal effects.

4 Discussion

4.1 The physical properties of selected essential oils

Physical parameters are widely used to evaluate the purity and quality of essential oils[33]. For example, any impurities or adulteration in essential oils could be easily detected by comparing the physical characteristics of the essential oils with established standards. For instance, commercial tea tree oil (*Melaleuca alternifolia*) is represented as follows: 0.885 to 0.906 for a relative density at 20℃, and 1.475 to 1.482 for a refractive index at 20℃ (ISO 4730:2017). However, the samples in this study are not commercial essential oils, so there are no established standards for physical parameters, including but not limited to relative density, refractive index, and optical density.

The density of essential oils is one of the most important categories, in determining the quality of essential oils used in cosmetics, pharmaceuticals, and agri-food industries[4]. In this study, the relative density of five essential oils varied depending on species (ranging from 0.861 ± 0.012 to 0.920 ± 0.000 g/mL). These values are lower than one, which is the density of water. Most essential oils are less dense than water, except for some essential oils such as clove and cinnamon essential oils (1.047 and 1.050 at 20℃, respectively)[34]. The relative density of *D. indicum* essential oils was 0.920 ± 0.000 g/mL, showing the highest values among other essential oils. This result is in agreement with the relative density of essential oil from the dried leaves, stems, and flowers of *D. indicum* obtained by hydrodistillation was 0.91 g/mL[15]. There was no specific data for the relative density of other essential oils. The refractive index of liquids or fatty acids depends on the number of carbon atoms, double bonds, and the presence of ketone or hydroxyl groups[35]. For this reason, the refractive index is one of the simple parameters used to assess the purity of essential oils[36]. Line with the data for the relative density, the refractive index of *D. indicum* essential oils also showed the highest refractive index values among the samples. Higher amounts of camphor in *D. indicum* essential oils might affect the physical properties such as relative density and refractive index. Specifically, pure camphor's density and refractive index at 20℃ are 0.992g/mL and 1.5462nD[20], respectively.

Typical essential oils appeared colourless to yellow and green, including the essential oils in this study. Yet, *D. indicum* essential oil was observed to be a clear blue liquid showing the lower values in b^* values (3.21 ± 0.78). This result aligned with a study conducted in China, where *D. indicum* essential oils extracted from the air-dried flower heads have a blue colour[37]. Liu, Chen[38] explained

that the blue colour of essential oils is because of the presence of azulene, producing blue, green, blue-purple, and red-purple. Also, it contains a matricin which is the result of a series of chemical reactions (dehydration, hydrolysis, and decarboxylation) during the extraction process. The blue colour of *D. indicum* essential oils might be explainable that thermolabile components converted into azulene or azulene-like derivatives during the steam distillation process[39, 40]. The differences in the physicochemical properties of essential oils, even within the same species, can be attributed to various factors, including but not limited to physiological variations, environmental conditions, geographic differences, and extraction methods. These variations also affect the oil yield, chemical profile, and biological activity[41]. Taken together, these findings could be conducive to establishing the quality standards of essential oils. This would be a benefit to both producers and consumers by ensuring the purity and quality of essential oils.

4.2 The yield and chemical compositions of essential oils

In this study, the essential oils vary among species. In particular, *C. pisifera* has the highest oil yield (4.01 ± 0.17% DW) among the other species (0.36 ± 0.13-0.78 ± 0.18% DW). According to the study performed by Kim and Lee[42], the oil yield of *C. pisifera* is a comparatively lower value of 1.16% relative to our study. The sample was collected from Chonbuk province in Korea and essential oils were extracted from the dried leaves by hydrodistillation for six hours. However, *C. pisifera* essential oils in this study were extracted from the frozen leaves by hydrodistillation for 33 hours until no more essential oils were obtained. Hence, the difference in oil yield within the same species could have arisen from the chemotype, plant materials, and extraction condition. Especially, the drying of the plant material before distillation might result in reduced essential oil yield, but the decreasing rate can differ in various plants[43]. Furthermore, the yield of essential oils varies owing to the different parts of plants within the same species. In this study, essential oils were extracted from leaves to explore the potential for a sustainable essential oils industry using unused forestry biomass residues. The oil yield of *D. indicum* essential oils was 0.78% (DW), yet the essential oils from the flower head was 0.16% (v/w)[37]. Likewise, the oil yield of *P. japonicum* leaf essential oils was 0.72% in this study, while the essential oils from the root were 0.10%[23]. The *P. strobus* essential oils showed the lowest oil yield of 0.36% among the samples in this study. The essential oils yield of the same species in Argentina was 0.50% DW[44]. The various factors affecting oil yield make it difficult to compare the results with the references. Also, there is limited research on the selected essential oils.

The GC-MS analysis identified the major 58 constituents in five essential oils. The differences in qualitative and quantitative analysis of essential oils in the same species (a single plant species) would be attributed to different harvest times, climatic and seasonal factors, storage duration of medicinal herbs as well as extraction conditions, and these differences would result in the different biological activities based on their chemical profile of essential oils[45].

C. pisifera and *P. japonicum* oils were dominated by monoterpenes (81.09-91.06%). *C. pisifera* primarily contains 3-carene (34.28%), while *P. japonicum* is dominated by β-pinene (47.37%). The *C. pisifera* leaf essential oils extracted by hydrodistillation in this study had a similar chemical profile, but differed in relative components, to that previously reported owing to the sample condition and extraction time[42]. Despite these quantitative differences, the chemical profile of *C. pisifera* leaf essential oils has the same major components such as 3-carene, α-pinene, and bonyl acetate. The difference in quantitative difference might be related to distillation time (six hours) and sample preparation (air-dried leaves)[42]. The obtained result resembled some previously reported data regarding the chemical profile of *P. japonicum* essential oils. Specifically, the major component of hydrodistilled *P. japonicum* essential oils extracted from the whole plants were β-pinene (66.07%)[46].

The essential oils of *D. indicum*, *O. japonica*, and *P. strobus* are rich in sesquiterpenes (13.82 –36.45%). *D. indicum* essential oils had 37 major compounds representing 84.38% of the oils and the main components were camphor (23.05%), followed by germacrene-D (16.56%). This result aligned with the study performed by Choi and Kim[47] showing that camphor (43.8%) was the major component of *D. indicum* essential oils, even though the oils were extracted from the flower. However, *D. indicum* essential oils extracted from aerial parts including leaves, stems and flowers contained chamazulene (15.93%), β-caryophyllene (13.78%) and germacrene-D (9.11%)[15]. In this study, the predominant components of *O. japonica* oil were linalool (10.92%) and geranyl acetate (19.00%). Park and Shin[48] studied the fumigant activity of *O. japonica* oils, though no study identified the chemical constituents in oils.

P. strobus essential oils contained monoterpenes (55.04%) and sesquiterpenes (16.82%). Although *P. strobus* essential oils were mainly composed of monoterpenes (55.04%), it contained significant levels of sesquiterpenes (16.82%) including germacrene-D (4.78%) and γ-cadinene (1.55%). These findings agreed with the results obtained by Jeon and Lee[26].

4.3 Antimicrobial activity of selected essential oils

Essential oils pose antimicrobial activity by acting on the defence systems of cells, such as efflux pumps, quorum sensing, biofilm formation, and ATPase pump inhibition[49].

In this study, the antimicrobial activity of selected essential oils was evaluated by MIC and MBC values against *S. aureus* and *K. pneumonia*.

D. indicum, *O. japonica*, and *C. pisifera* essential oils showed active antimicrobial activity towards *S. aureus* with comparatively low MIC and MBC values of 1-1.5% and 3-4%, respectively. The antibacterial activity of *D. indicum* flower oils has been confirmed against *S. aureus*, *Escherichia coli* and *Streptococcus pneumoniae*[50]. The strong antimicrobial activity of *D. indicum* essential oils could be associated with the abundant camphor components (23.05%) in oils. Viljoen, Van Vuuren[51] mentioned that camphor as well as 1,8-cineole were well-known chemical constituents with pronounced antimicrobial properties against *S. aureus*. Furthermore, the minor chemical components of α-terpineol and terpinen-4-ol in oils could also contribute to the antimicrobial effects, as a synergic effect. There is limited research on *O. japonica* leaf oils, although several studies have explored the biological activities of *O. japonica* extracts from both the leaves and roots[19, 52]. In particular, the leaf extract of *O. japonica* showed significant antimicrobial activity against various foodborne pathogens including *S. aureus*[19]. In this study, the strong antimicrobial activity of *O. japonica* leaf essential oils could be associated with a high linalool content (10.92%). The chemical component of linalool (0.1%, v/v) showed antimicrobial activity against *S. aureus*, owing to its functional destabilisation of bacterial membranes[53]. In this study, *C. pisifera* essential oils might pose a strong antimicrobial activity because of 3-carene[54]. Specifically, 3-carene strongly inhibited biofilm formation in *S. aureus* and the MIC was 10.39+ mg/mL[55]. The strong antimicrobial activity of *O. japonica* leaf essential oils against *K. pneumonia* could be associated with a high linalool content (10.92%). Likewise, *T. algeriensis* essential oils with high linalool content (18.44%) exhibited antibacterial effects against *K. pneumonia*[56]. Specifically, the chemical component of linalool disrupts the bacterial membrane by inducing oxidative stress[57]. Yet, *P. strobus* showed weak antimicrobial activity against both pathogens with high values of MIC and MBC. This was in line with the result of Lis-Balchin, Deans[58] also found that the needle pine oil with a high content of α- and β-pinenes showed a lack of antimicrobial activity.

MBC:MIC ratio ≤ 4 was considered to have a bactericidal effect, 4-32 as a bacteriostatic effect, and >32 with a bacteria-resistant effect[59]. The antimicrobial activity of *D. indicum* and *C. pisifera* essential oils against *S. aureus* demonstrated a bactericidal effect, with an MBC:MIC ratio of 4. In comparison, ampicillin exhibited a bactericidal effect with a significantly lower MBC:MIC ratio of 2. Similarly, *O. japonica* essential oils showed a bactericidal effect against *K. pneumoniae* with an MBC:MIC ratio of 4, closely matching the MBC:MIC value of ampicillin (3.3). The mechanism of antimicrobial activity in essential oils is complicated. It can be specific or non-specific (e.g. intracellular molecule-specific interaction or interaction with biomembranes, respectively) and may also be a result of synergistic effects between multiple constituents, not a single chemical component[60]. The combination of *D. indicum* and *O. japonica* oils might enhance their antimicrobial effectiveness, taking advantage of their synergistic and additive effects. Herman, Tambor[61] found that essential oils in combination with linalool showed the enhanced antimicrobial efficacy. Owing to the abundant components of camphor and linalool in the combination of *D. indicum* and *O. japonica* oils, their antimicrobial activity might be strengthened compared to using each essential oil individually. Hence, further research is needed to compare the antimicrobial activity of essential oils alone and in combination with both essential oils.

To sum up, *D. indicum* and *O. japonica* oils were particularly effective in inhibiting both bacterial growths (MIC:0.5-1.5% and MBC:4-8%), whereas other essential oils might achieve higher concentrations for bactericidal effects (MIC:1-3% and MBC:3- ≥ 12%). These results supported the notion that these essential oils might have a role as pharmaceuticals and food preservatives.

5 Conclusion

The increasing application and markets for essential oils could bring new opportunities for forestry resources. Especially, utilising the unused forestry biomass for essential oils could offer a sustainable, cost-effective, and profitable strategy for both forestry management and the essential oils industry. Essential oils (*D. indicum*, *O. japonica*, *C. pisifera*, *P. japonicum*, and *P. strobus*) were obtained by hydrodistillation, and their chemical composition was analysed by GC-MS. Additionally, the physical properties and antimicrobial activity of selected essential oils were studied to gain insight into the industrial applications by establishing the quality standards and confirming their biological activities.

Compared to other yellow essential oils, *D. indicum* essential oil was observed to be a clear blue with lower values in *b**. This result might be attributed to the conversion of thermolabile components into azulene-like derivatives during the steam distillation process. *D. indicum* oil showed the highest values in relative density (0.920 ± 0.000 g/mL) and refractive index (1.493 ± 0.000nD) among the other oils. The pure camphor's high density and refractive index in oils might be surmised.

The yields of the different oil samples ranged from 0.36 ± 0.13% to 0.78 ± 0.18%, with the highest yield for the *C. pisifera* oil (4.01 ± 0.17%). *C. pisifera* and *P. japonicum* oils were rich in monoterpenes (81.09-91.06%), with sig-

nificant levels of 3-carene (34.28%) and β-pinene (47.37%). Conversely, the other oils contained a high percentage of sesquiterpenes (13.82-36.45%). Especially, the major components of *D. indicum* oils were germacrene-D (16.56%) and caryophyllene (9.20%). Compared to the other essential oils, *D. indicum* (MIC:0.5-1% MBC:4-8%) and *O. japonica* (MIC:1-1.5% MBC: 4%) essential oils showed a strong antimicrobial activity against *S. aureus* and *K. pneumonia*. The components of camphor (23.05%) and linalool (10.92%) might play a key role in demonstrating the strong antimicrobial activity of these oils, respectively. When comparing the MBC:MIC ratio of ampicillin and five essential oils, *O. japonica* essential oil exhibited a bactericidal effect against *K. pneumoniae* (MBC:MIC = 4), closely resembling that of ampicillin (MBC:MIC = 3.33).

The result suggested that *D. indicum* and *O. japonica* essential oils might be valuable industrial resources for the food preservation and respiratory supportive treatment. Furthermore, the physical and chemical properties of selected essential oils would provide the fundamental data, such as the quality and standardisation parameters for commercial essential oils.

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Conflicts of Interest

The authors declare no conflict of interest.

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