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RESEARCH ARTICLE

Chemical Characterization, Antimicrobial, Antioxidant and Cytotoxic Potential of Essential Oils from *Perilla frutescens* L. found in Nepal

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Abstract

Perilla is widely valued for its culinary, aromatic, and medicinal uses. This study was conducted to characterize the chemical components, enantiomeric distribution, and to explore the antimicrobial, antioxidant, and cytotoxic potential of essential oils from *Perilla frutescens* L. found in Nepal. Essential oils (EOs) obtained through hydrodistillation were analyzed for their chemical constituents using gas chromatography-mass spectrometry (GC-MS), while chiral GC-MS was employed to determine the enantiomeric distribution of chiral terpenoids. The DPPH and ABTS radical-scavenging assays were used to evaluate the antioxidant activities. *In vitro* antimicrobial activity was performed by microbroth dilution. Cytotoxic activities were determined by cell viability using a Cell Counting Kit assay. GC-MS analyses of three *P. frutescens* (PEO) samples had 50 to 54 chemical components, consisting of 91.98 to 99.67 %. PEO was characterized as PK type by the most dominant components, perilla ketone (42.26 to 56.26 %), isoegomaketone (23.68-23.85 %), β-caryophyllene (1.33-7.33 %), and isobicyclogermacrene (3.29-5.27 %), respectively. Twelve chiral terpenoids were identified as enantiomeric distribution with 1-octen-3-ol (100 % as dextrorotatory) in all three PEO samples. PEO collected from Bardiya in summer gave the best antioxidant activity against DPPH with an IC_{50} value of 334.26±0.20 μg/mL and against ABTS with an IC_{50} value of 93.15±1.04 μg/mL. The antimicrobial inhibition against *E. coli*, *C. albicans*, and *A. niger* was observed, with MICs varying from 156.3 to 625 μg/mL. PEO taken from Kavre in summer showed good cytotoxicity with an IC_{50} value of 7.41 μg/mL against the NIH-3T3 cell line and an IC_{50} value of 8.14 μg/mL against the MCF-7 cell line. All three PEOs showed notable chemical complexity and promising biological activities, emphasizing their importance as natural ingredients.

Keywords: *Perilla*, Chemical characterization, Antioxidant, Antimicrobial, Cytotoxicity activity

Introduction

Perilla frutescens L. is a plant in the Lamiaceae family used as a spice, aromatic, and medicine. Its leaves are very popular in Korean cuisine, known as deulkkae or Korean perilla, whereas its seeds are used all over Asian countries as a spice and to produce perilla oil. It is an annual plant native to Southeast Asia. It has characteristic leaves, which are easily identifiable due to their distinct deep parallel-veined tear-shaped leaves in a bushy plant that grows up to two meters. It has light pink to light purple flowers and aromatic purple/red/green leaves, according to the major active constituents present. The young, huge, raw leaves are frequently used to

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wrap and eat pork meat to cut the fattiness off the meat. They are used as garnish, in sushi, and as a component in soups [1]. The leaves are used medicinally to treat food poisoning and for their anti-allergic and anti-inflammatory properties. Anti-tumor-promoting compounds found in perilla plants have been extensively studied [2, 3]. *Perilla* extract and rosmarinic acid, a potent antioxidant, have been shown to prevent liver damage induced by D-galactosamine and lipopolysaccharide [4].

In Nepal, only a single plant species of this genus, *P. frutescens* (L.) Britton, Silaam (Nep.), is described by the Annotated Checklist of the Flowering Plants of Nepal [5]. It is important to identify the chemical constituents, explore the structural activity association, and establish the biological assessment of EOs as possible sources for further utilization. Knowing this fact, the present study has been conducted on the chemistry of *Perilla* essential oil samples from two geographical locations.

Both cultivated varieties (the green and red colors) of *P. frutescens* in Egypt consisted of perillaldehyde, caryophyllene oxide, limonene, and caryophyllene [6]. *P. frutescens* essential oil was found to have the main components tylfuran, perillaldehyde, caryophyllene, laurolene, 2-hexanoylfuran, 2-butylamine, asarone, farnesene, caryophyllene, and (*Z*, *E)* farnesene [7]. On analysis of purple perilla, the major components were found to be carvone (32.55 %), perilaldehyde (20.52 %), caryophyllene (9.85 %), 2-furyl methyl ketone (7.53 %), 2,6-ethyl-6-(4-methyl-3-pentenyl)-2-norpinene (5.17 %), and terpinyl acetate (3.41 %) [8]. The main components of *P. frutescens* were perillaldehyde, β-dehydro-elsholtzia ketone, *perilla* ketone, limonene, β-caryophyllene, (*Z*, *E*)-α-farnesene, shisofuran, and *trans*-shisool [9]. Perillaldehyde is the main compound of *P. frutescens* essential oil [10]. Perillaldehyde (54.35 %), limonene (23.81 %), *trans*-caryophyllene (7.2 %), *cis, trans*-α-farnesene (7.02 %), and linalool (2.40 %) are the major components of the essential oil of red *P. frutescens,* and perillaldehyde (65.26 %), limonene (12.49 %), *cis, trans*-α-farnesene (7.31 %), *trans*-caryophyllene (5.91 %), and linalool (2.75 %) are the major components of the essential oil of green *P. frutescens* [11].

PEO had high antioxidant activity with an IC_{50} value of 2.60 μg/mL [12]. Two varieties of *P. frutescens* essential oils in Egypt showed good antimicrobial activities against *A. niger, C. albicans, B. subtilis, and E. coli* [6]. The major compound, perillaldehyde, was responsible for the antimicrobial activity of the essential oil of *P. frutescens* with moderate and broad-spectrum activity against several Gram-positive and Gram-negative bacterial strains as well as fungi [13]. PEO had antibacterial activity against *S. aureus* and *E. coli,* and the MICs for them was 500 μg/mL and 1250 μg/mL, respectively [14]. PEO showed strong anticancer, antidepressant, and anti-inflammatory effects in an *in vivo* study [10]. PEO showed strong antioxidant and antifungal activity [7]. PEO inhibits *Candida albicans*, oral inflammation, and the apoptosis of normal cells to improve the survival rate of mice in oropharyngeal candidiasis [12]. *P. frutescens* essential oil inhibited bacterial reproduction and prevented food deterioration through damaging the cell membrane of *Enterococcus faecalis* [15, 16]. Chemical components such as perillaldehyde, rosmarinic acid, and isoestrogen have shown anticancer effects [17].

P. frutescens cultivars from China and Japan, essential oil was extracted and chemically characterized similarly to our study, with major constituents perilla ketone, elemicin, and β-caryophyllene in the Chinese *Perilla* cultivars and myristicin, perilla ketone, and β-caryophyllene in the Japanese

cultivars [16].

Seven chemotypes—the perilla ketone (PK) type, perilla ketone; the myristicin (PM) type, perilla ketone; the unknown (PU) type, perilla ketone; the β-caryophyllene myristicin (PB) type, perilla ketone; the myristicin unknown (PMU) type, perilla ketone; the elemicine myristicin β-caryophyllene (PEMB) type, and the perilla ketone, limonene, β-caryophyllene, myristicin (L) type—were characterized [18]. PEO content was highest before the flowering stage. Polymorphism in essential oil composition and morphological traits in the Perilla accessions was observed with harvest time and geographical origin [19].

In China wild-collected *P. frutescens* had PK (majority) and PA chemotypes, whereas cultivated types had five chemotypes: PK-type, PA-type (majority), PL-type, PP-type, and EK-type. The phenological differences can be seen as PK-type leaves of *P. frutescens* var. acuta are green, while the PA-type leaves are reddish purple. Among the main 31 constituents, perillaketone (0.93-96.55 %), perillaldehyde (0.10-61.24 %), perillene (52.15 %), caryophyllene (3.22-26.67 %), and α-farnesene (2.10-21.54 %) were the major constituents [20]. The chemical structures of the major compounds of EOs from *Perilla* species are shown in **Figure 1**.

The Perilla leaves collected at three phenological periods during nutrition, flowering, and frutescence and three times a day at 7 am, 12 pm, and 6 pm showed volatile oil yields between 0.08 % and 0.96 %; it descended in accordance to nutrition>flowering> fructescence, nutrition period: PA type>PK type>PL type [21]. Chemotypes seem genetically determined and not affected by the growth and development. The best yield of essential oil and major components for PA type is when it's harvested at 7am, for PK type at noon of all the periods, and for PL type at noon of the nutrition period [21].

The literature review shows that there is no

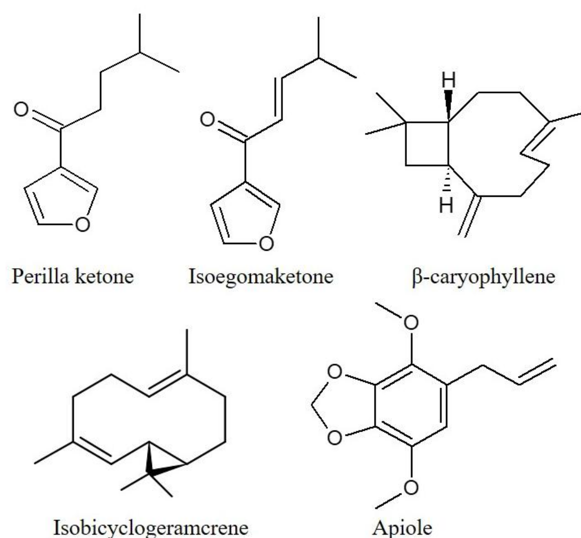

Figure 1: Chemical structures of some major chemical compounds in the essential oils of *Perilla* species.

extensive study on this species in Nepal. Therefore, the present study investigates the variation in the volatile constituents of essential oils of *P. frutescens* from two locations in Nepal. Using GC-MS analysis, we aimed to identify and quantify the major compounds across seasons, locations, and their antimicrobial, antifungal, antioxidant, and cytotoxic activities. As one of the most comprehensive studies to date on *Perilla* EOs from Nepal, the findings may support their potential applications and promote the sustainable use of the country's biodiversity.

Materials and Methods

Collection of plant materials

The plant samples of *P. frutescens* (L.) Britton, locally known as Silaam (Nepali) and commonly referred to as acute common perilla (English), were collected from two geographical regions: Kavre and Bardiya during the summer and winter seasons. The collected sites included Dhulikhel, Kavre (27° 36' 59.4'' N, 85° 32' 13.5'' E) at an altitude of 1351 m, where samples were gathered in December 2021 (S_1) and August 2022 (S_2); and Bansgadhi, Bardiya (28°14' 32.9'' N, 81°31' 15.9'' E) at 159 m altitude, with a sample collected in August

2022 (S_3). The plant samples were collected from wild habitat and shade-dried at room temperature (**Figure 2**). The plant species were identified by taxonomist Ms. Rita Chhetri (senior research Officer), National Herbarium and Plant Laboratories (KATH), Lalitpur, Government of Nepal. The herbarium voucher specimen numbers are KVR-06, KVR-021, and BRD-028, respectively.

Figure 2: Photograph of *Perilla frutescens* taken during sample collection

Extraction and Isolation of Essential Oils

The EOs were obtained by hydro-distillation using a Clevenger-type apparatus for 3 h from 100 g (n=3) following the standard protocol [22]. The aerial parts of plant material and water in 1:5 ratio (w/v) were taken during the essential oil extraction process. The essential oil thus obtained was dried with anhydrous sodium sulfate and was stored in vials at 4 °C for further analysis and screening. The yield was calculated in percentage based on the ratio of volume extracted to weight of dried plant material [23].

Gas Chromatography-Mass Spectrometry analysis

The GC-MS analysis of both essential oil samples was used for identification of the EOs components and was carried out by comparison of the mass spectral fragmentation patterns (over 80 % similarity match) as well as the comparison of the retention indices, calculated using a homologous series of n-alkanes (C8-C40), with those reported in the literature and the Aromatic Plant Research Center's library using the Lab Solutions GC-MS solution software version 4.45 (Shimadzu Scientific Instruments, Columbia, MD, USA). Chiral GC-MS was carried out as previously described. The enantiomers were identified by comparing retention times and mass spectral fragmentation patterns with authentic samples acquired from Sigma-Aldrich (Milwaukee, WI, USA), and the enantiomer ratios were calculated from the peak areas [24, 25, 26].

Antimicrobial Activity

The *in vitro* antimicrobial activities of two EO samples were evaluated in terms of minimum inhibitory concentration (MIC) using the micro-broth dilution technique on the bacteria *Bacillus cereus* (ATCC 14579), *Staphylococcus aureus* (ATCC 29213), and *Staphylococcus epidermidis* (ATCC 14990); and the fungal strains *Aspergillus niger* (ATCC 16888), *Candida albicans* (ATCC 18804), and *Aspergillus fumigatus* (ATCC 96918) [27, 24].

Cytotoxicity Assay of Essential Oils against Cancer Cell Lines

The cytotoxicity assay against NIH-3T3 (mouse embryonic fibroblast) and MCF-7 (human breast ductal carcinoma) cell lines was carried out. The cell viability in the presence or absence of essential oils was determined using a Cell Counting Kit-8 kit (Dojindo Molecular Technologies, Inc., Rockville, MD, USA). The NIH-3T3 and MCF-7 cell lines were a kind gift from Prof. Shiro Watanabe and were maintained in standard Dulbecco's modified Eagle's medium (DMEM) with 10 % fetal bovine serum (FBS) supplemented with 0.1 % $NaHCO_3$ and 1% antibiotic–antimycotic solution. For the cytotoxicity experiments, exponentially growing cells were harvested and plated in 96-well plates (1×10^4/well) in DMEM at 37 °C under humidified 5 % CO_2 and 95 % air for 24 h. After the cells were washed with phosphate-buffered saline (PBS), the medium was changed to serially diluted test samples in Dulbecco's

Modified Eagle Medium (DMEM), with the control and blank in each plate. After three days/72 h of cell incubation, cells were washed with PBS two more times, and 100 µL of DMEM containing 10 % WST-8 cell counting kit solution was added to each well containing cells. After a 3 h incubation, the absorbance at 450 nm was measured using a Multiscan Sky High plate reader (Thermo Fisher Scientific). Cell viability was calculated from the mean values from three wells using the following equation.

Cell viability = [(Abs (test sample) – Abs (blank))/ (Abs (control) – Abs (blank))] × 100 %.

Gemcitabine (GEM) was utilized as a positive control. Various concentrations of PEO samples were tested: 100, 50, 25, 12.5, and 6.25 to 3.125 µg/mL. The experiment was carried out in three replications, and IC_{50} values were determined from the non-linear regression of the mean values ± SD of the entire data set. Only active samples were taken, and morphological assessment was done just before adding CCK-8. Advanced judgment was carried through using different concentrations for samples with percentage inhibition of 50 % and above to calculate the IC_{50} (median inhibitory concentration). The cell growth suppression rates are depicted as IC_{50}. Cell viability can be calculated using the ratio of total live/total cells (live and dead). Staining also facilitates the visualization of overall cell morphology. Here, EVOS FL (10×magnification) was used for this study. The term cell index (%) shows the ability of EO to kill the cells at very high concentration (100 µg/mL) but activate cancer cell growth at low concentration. Therefore, its application as an anticancer agent would be minimal [28].

Antioxidant Activity

DPPH Free Radical-scavenging Assay

The free radical-scavenging activity of the two EO samples was determined by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) as previously reported. The IC_{50} (µg/mL) value (concentration of sample required to scavenge 50 % of free radicals) was calculated by using a non-linear regression model. Ascorbic acid and BHT were used as positive controls. The experiments were conducted in triplicate [29].

ABTS Radical-Scavenging Assay

The ABTS (2,2′-azinobis-3-ethylbenzo-thiazoline-6-sulfonic acid) radical-scavenging potential of essential oils was determined by using the standard protocol as reported previously [30]. A sufficient volume of 7 mM ABTS (38.402 mg of ABTS in 10 mL distilled water) (Glentham Life Sciences, United Kingdom) was mixed in a 1:1 ratio with potassium persulfate (6.6 mg) (10 mL, 2.45 mM) and allowed to stand at room temperature for 14-16 h to form a stable oxidized ABTS radical. This working ABTS stock solution was diluted with methanol until the absorbance at 734 nm was 0.70±0.02. Now 2 mL of ABTS stock solution was added to each 1 mL of sample preparation, incubated for 10 minutes at a dark room temperature, and the triplicate absorbance was measured at 734 nm using a 96-microplate reader (Bio Tek, EPOCH). Using the given equation [31], the linear % inhibition concentration (IC%) was calculated, and an IC_{50} % value was compared with the standard.

IC% = [(mean Abs. of control - mean Abs. of sample)/mean Abs. of control] × 100.

As a control sample, 1 mL of ethanol was mixed with 2 mL of ABTS stock solution. Ascorbic acid (Fischer Scientific, Bengaluru, India) was used as a standard.

Results and Discussion

This study of the *Perilla* plant in Nepal is one of the first studies done on this particular plant species. Not much literature could be found on this plant of Nepalese origin. This study will be a pioneer in the field, characterizing the chemical composition, enantiomeric distribution, and several biological activities, such as antioxidant, antimicrobial, and cytotoxic activities.

Yields of Essential Oils

The yields of essential oils from the three *P. frutescens* samples were as follows: 0.78±0.13 % for Kavre (winter, S_1), 0.87±0.07 % for Kavre

(summer, S_2), and 0.80±0.02 % for Bardiya (summer, S_3). The EO yields were found to be relatively comparable to previous research done on the same plant. The differences in EO yields may be due to various reasons, like geographical origin and seasonal variation. The PEOs were pleasant-smelling, transparent, slightly viscous liquids and pale yellow in color.

Chemical Composition of Essential Oils

The GC-MS analyses of hydro-distilled essential oils of three *P. frutescens* samples revealed the identification of 50 to 54 chemical components, representing 91.98 to 99.67 % of the oils, respectively (**Table 1**). *P. frutescens* essential oil was characterized by the most dominant component, perilla ketone (42.26 to 56.26 %). Other important components in *P. frutescens* were found to be isoegomaketone (23.68-23.85 %), β-caryophyllene (1.33-7.33 %), isobicyclo germacrene (3.29-5.27 %), and apiole (9.16 %) (**Figure 3**).

All three samples of *P. frutescens* essential oil were dominated by their oxygenated monoterpene fraction, and the contents were about 69.46 to 83.05%. Perilla ketone and isoegomaketone were the major oxygenated monoterpene compounds in this EO. The *P. frutescens* also contained a considerable proportion of sesquiterpene hydrocarbons (7.41 to 15.42 %), and the main sesquiterpene hydrocarbons were β-caryophyllene and isobicyclogermacrene, respectively, whereas this essential oil had very low amounts of monoterpene hydrocarbons and oxygenated sesquiterpenes. Although there is variation in the percentage of major constituents, they remain a high-percentage of composition.

These results show similarity with the previous findings with perilla ketone (0.17-97.9%), perilla aldehyde (0.45-82.15 %), and β-dehydro-elsholtzia ketone (58.03-67.75 %) as the major components in PEO from Iraq [32]. The present findings of the research work are in agreement with those investigated by Gwari *et al.*, who reported perilla ketone (44.7-69.2 %), isoegomaketone (7.3-27.6 %), *trans*-caryophyllene (0.1-17.8 %), and linalool (0.3-5 %) as the most abundant volatile compounds from India [33, 34]. The essential oil of *Perilla frutescens* (L.) Britt contains perillaldehyde (7.01-82.12 %), perilla ketone (75.09-97.69 %), β-dehydro-elsholtzia ketone (67-79 %), limonene (3.18-14.85 %), shisofuran (7.71 %), farnesene (*Z*, *E*, α) (1.03-6.86 %), β-caryophyllene (1.28-6.88 %), and *trans*-shisool (0.04-3.52 %). The difference was explained by different ecological factors (cultivated conditions, soil, environment, and weather) that determine the content and chemical composition of secondary metabolites, which, as a result, may lead to various biological activities [35].

From **Table 2** below, we can see that oxygenated monoterpenes were the major constituents in all three samples regardless of geographical location or the harvest time, followed by sesquiterpene hydrocarbons. There are variations caused by the harvest time and geographical location.

There is slight variation in the yield percentage of essential oil among the three samples of *P. frutescens* (L.) Britton, Silaam (Nep.), collected from Kavre (winter, S_1) (0.78±0.13 %), Kavre (summer, S_2) (0.87±0.07 %), and Bardiya (summer, S_3) (0.80±0.02 %) (**Table 3**).

Enantiomeric Distribution Analysis

The chiral GC-MS analysis for the identification and composition of various chiral components in *P. frutescens* essential oils is presented in **Table 4**. In total, 12 chiral terpenoid components were evaluated for their enantiomeric distributions in the essential oil of *P. frutescens*, namely, α-pinene, sabinene, β-pinene, limonene, 1-octen-3-ol, linalool, α-terpineol, β-caryophyllene, germacrene D, δ-cardinene, (*E*)-β-ionone, and (*E*)-nirolidol. This analysis displays that 1-octen-3-ol was detected in enantiomerically pure form (100%) as dextrorotatory in all samples of *P. frutescens*,

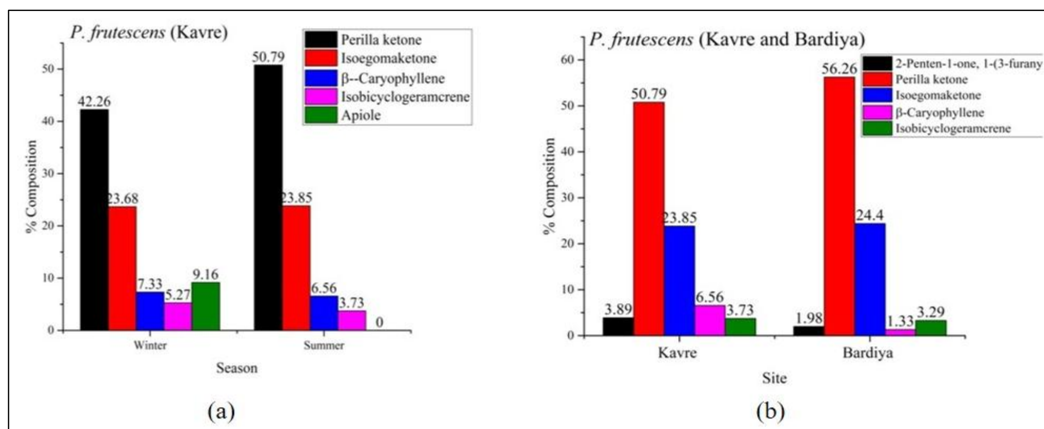

Figure 3: Variation in major components of essential oils of *Perilla frutescens* (a) from winter to summer in Kavre and (b) from Kavre to Bardiya sites.

Table 1: Chemical composition of *Perilla. frutescens* essential oil.

Lit. RI	Exp. RI	Components	PEO (S_2) (%)	PEO (S_1) (%)	PEO (S_3) (%)
Monoterpene hydrocarbons			0.09 %	0.51 %	0.32 %
932	931	α-Pinene	-	0.05	0.05
969	972	Sabinene	0.02	0.04	0.04
974	978	β-Pinene	0.05	0.05	0.05
988	989	Myrcene	-	0.04	-
1024	1028	Limonene	0.02	0.31	0.18
1054	1058	γ-Terpinene	-	0.02	-
Oxygenated monoterpenoids			76.59 %	69.46 %	83.05 %
1026	1031	1,8-Cineole	-	0.01	-
1046	1046	Dihydro tagetone	0.04	0.02	0.06
1095	1099	Linalool	1.23	0.86	1.86
1148	1157	Menthone	-	0.04	0.04
1160	1163	Pinocarvone	-	0.02	0.02
1186	1195	α-Terpineol	0.05	0.02	0.02
1195	1198	Methyl chavicol	-	0.02	0.1
1245	1241	Perilla ketone	50.79	42.26	56.26
1252	1252	*trans*-Piperitone epoxide	-	0.02	0.02
-	1310	Egomaketone	-	1.96	24.4
1327	1319	Methyl geranate	0.11	0.12	0.21
-	1325	*iso*-egomaketone	23.85	23.68	-
1356	1357	Eugenol	0.6	0.5	0.3
1366	1358	Piperitenone oxide	-	0.05	-
Sesquiternepe hydrocarbons			13.39 %	15.42 %	7.41 %
1335	1334	δ-Elemene	0.07	0.04	0.09
1374	1376	α-Copaene	0.1	0.04	0.14
1383	1380	*trans*-β-Damascenone	0.03	0.01	0.05
1387	1382	β-Bourbonene	0.11	0.03	0.13
-	1391	*trans*-β-Elemene	0.19	0.12	0.12

1408	1405	(*Z*)-Caryophyllene	0.02	-	-
1417	1418	β-Caryophyllene	6.56	7.33	1.33
1434	1430	g-Elemene	0.05	0.02	0.07
1442	1443	6,9-Guaiadiene	0.03	0.01	0.01
1454	1452	(*E*)-β-Farnesene	-	0.73	-
1452	1454	α-Humulene	0.88	0.83	0.9
1484	1484	Germacrene D	1.19	0.75	0.78
-	1489	Isobicyclogeramcrene	3.73	5.27	3.29
1505	1504	(*E,E*)-α-Farnesene	0.24	0.18	0.28
1522	1519	δ-Cadinene	0.14	0.06	0.22
1559	1560	Germacrene B	0.05	-	-
Oxygenated sesquiterpenoids			1.09 %	10.02 %	1.20 %
1561	1561	*trans*-Nerolidol	0.24	0.23	0.23
1582	1578	Caryophyllene oxide	0.46	0.31	0.51
1595	1584	6-Methoxy elemicin	-	0.03	-
1601	1594	*trans*-β-Elemenone	0.13	0.19	0.11
1608	1612	Humulene epoxide II	0.05	0.03	0.07
-	1627	*iso*-Spathulenol	0.04	-	0.04
1638	1640	epi-α-Cadinol	0.03	-	0.06
1644	1642	epi-α-Muurolol	0.03	-	0.05
1652	1655	α-Cadinol	0.11	-	0.13
1677	1672	Apiole	-	9.16	-
Others			8.51 %	2.46 %	8.37 %
846	850	(*2E*)-Hexenal	0.04	0.03	0.06
844	860	(*3Z*)-Hexanol	0.06	0.08	0.09
863	864	n-Hexanol	0.02	0.02	-
859	863	(*2E*) -Hexenol	-	0.04	0.04
952	960	Benzaldehyde	0.28	1.3	1.1
974	982	1-Octen-3-ol	0.83	0.52	0.62
988	996	3-Octanol	0.09	0.09	0.06
981	986	6-Methyl-5-hepten-2-one	-	0.01	-
979	984	3-Octanone	-	0.02	-
1036	1036	Benzene acetaldehyde	0.02	-	0.02
1100	1105	n-Nonanal	0.05	-	0.05
-	1113	5-hydroxy-4,6-dimethyl- 6-Hepten-3-one	0.02	-	-
1150	1150	2-trans-6-cis-Nonadienal	0.02	-	0.02
1190	1191	Methyl salicylate	0.05	-	-
-	1200	1-(3-furanyl)-4-methyl-2-Pentanone	0.59	-	0.69
-	1220	1-(3-furanyl)-4-methyl-2-Penten-1-one	3.89	-	3.32
-	1261	1H-Pyrazole-4-carboxylic acid	2.32	-	
1487	1481	(*E*)-β-Ionone	-	0.02	-
-	1529	Dodec-5-en-11-olide	0.04	-	-

-	1571	(3Z) -Hexenyl benzoate	0.03	-	-
1517	1518	Myristicin	-	0.12	-
1555	1548	Elemicin	-	0.06	-
-	1562	Isoelemicin	-	0.03	-
1620	1616	Dill apiole	-	0.07	-
2500	2498	Pentacosane	0.05	-	0.06
2700	2698	Heptacosane	0.03	-	0.05
		Total	99.67 %	97.87 %	91.98 %

Note: RI= Literature, Exp. RI = retention index values calculated with respect to a series of n-alkanes (C8-C40) on a ZB-5ms column; components are listed in order increasing RI values.

Table 2: Chemical composition of terpene classes of *Perilla frutescens* essential oil.

Name of PEO	Classes of Terpene				
samples	MH	OM	SH	OS	OT
P. frutescens (S_1)	0.51	69.46	15.42	10.02	2.46
P. frutescens (S_2)	0.09	76.59	13.39	1.09	8.51
P. frutescens(S_3)	0.32	83.05	7.41	1.2	8.37

Table 3: Percentage yields and organoleptic properties of EOs of *Perilla frutescens.*

EO Samples	Yields (%)	Appearance	Color	Aroma
PEO (S_1)	0.78±0.13	Transparent liquid	Pale yellow	Pleasant odor
PEO (S_2)	0.87±0.07	Transparent liquid	Pale yellow	Pleasant odor
PEO (S_3)	0.80±0.02	Transparent liquid	Pale yellow	Sweet odor

Note: Yield values are mean±standard deviation of three samples of each *Perilla* species, analyzed individually in triplicate.

whereas, α-terpineol and β-caryophyllene were in pure form in the samples from Kavre (summer) and Bardiya (summer). Similarly, δ-cardinene, *(E)*-β-ionone, and *trans*-nirolidol were in the optically pure form in the samples from Kavre (winter) and Bardiya (summer). This is about the detailed reports on the enantiomeric distribution of chiral chemicals as representatives found in *P. frutescens* essential oils because there have been no previous reports on it.

In-vitro Antimicrobial Activity

P. Frutescens essential oil displayed good results against fungi, Gram-positive, and Gram-negative bacteria (**Table 5**). Varying degrees of inhibition were found against *E. coli*, *C. albicans*, and *A. niger*, with MICs varying from 156.3 to 625 µg/mL. The result was also good against *P. aeruginosa*, with an MIC value of 625 µg/mL. In contrast, all samples showed the lowest inhibition against *B. cereus* and *S. aureus.* MICs on *S. aureus* and *E. coli* were 500 µg/mL and 1250 µg/mL, respectively [28].

Table 4: Enantiomeric distributions of chiral compounds in the essential oil of *P. frutescens.*

Compounds	RT	RT	*P. frutescens* (S_2)		*P. frutescens* (S_1)		*P. frutescens* (S_3)	
	(+)	(–)	(+)	(-)	(+)	(-)	(+)	(-)
α-Pinene	16.40	15.92	-	-	47.08	52.92	48.20	51. 80
Sabinene	19.74	20.60	-	-	58.8	41.2	-	-
β-Pinene	20.27	20.62	22.48	77.52	57.35	42.65	54.08	45.92
Limonene	25.99	25.06	20.2	79.8	4.08	95.92	10.69	89.31
1-Octen-3-ol	33.95	NA	100	0	100	00	100	0
Linalool	44.69	45.30	15.85	84.15	8.74	91.26	15.80	84.20
α-Terpineol	60.58	59.73	0	100	-	-	100	0
β-Caryophyllene	NA	69.33	0	100	-	-	0	100
Germacrene D	73.48	73.73	54.01	45.99	54.81	45.19	54.11	45.89
δ- Cardinene	77.33	76.50	-	-	100	0	-	-
(*E*)-β- Ionone	80.10	80.25	-	-	100	0	100	0
(*E*)-Nirolidol	83.40	83.59	-	-	0	100	-	-

Note: RT = Retention time (min), dextrorotatory (+), levorotatory (-), and'-'= not detected.

Perilla aldehyde moderately inhibits a broad range of bacteria in the range of 0.125–1.00 µg/mL [13]. In a previous antimicrobial study of this plant, there was no inhibition against *Salmonella* spp., *S. aureus*, and *E. coli.* The primary components perilla ketone (42.26–56.26 %), β-caryophyllene (1.33–7.33 %), exert potent antifungal properties [29, 30]. Antimicrobial properties of a few compounds from perilla were also recently confirmed in *S. aureus* and *C. albicans* [26]. This activity of essential oils is probably due to the presence of active constituents that could suppress or inhibit the growth of bacterial strains either individually, synergistically, or antagonistically.

Table 5: Minimum inhibitory concentrations of *P. frutescens* essential oil against tested bacteria and fungi.

Name of microorganism	MICs (µg/mL)		
	(S_2)	(S_1)	(S_3)
Bacillus cereus (ATCC 11778)	1250	1250	1250
Staphylococcus aureus (ATCC 6538)	1250	1250	1250
Pseudomonas aeruginosa (ATCC 9027)	625	625	1250
Escherichia coli (ATCC 8739)	1250	625	625
Candida albicans (ATCC 10231)	312.5	156.3	625
Aspergillus niger (ATCC 16888)	312.5	156.3	312.5

Note: Standards used for assays: Gentamicin for bacteria and amphotericin B for fungi (MIC< 19.5 µg/mL).

In-vitro Cytotoxicity Evaluation

P. frutescens essential oils (PEO) were tested against cell lines MCF-7 and NIH-3T3 to explore their cytotoxicity and antiproliferative capacity. The cytotoxicity was expressed as the IC_{50} values, which represent the concentration of EOs preventing 50 % of cell viability and are shown in **Table 6**. The PEO showed an inhibitory effect on the cell lines, depending on the variations in their concentrations. The cytotoxicity was expressed as IC_{50} values, which indicate the concentrations at which 50 % of cells were killed in DMEM.

PEO showed higher cytotoxic potential against the cell lines tested, with IC_{50} values varying from 7.41 to 23.76 µg/mL. *P. frutescens* (Kavre-summer, S_2) showed good values with IC_{50} values ranging from 7.41 to 8.14 µg/mL, highly cytotoxic against NIH-3T3 and MCF-7 cell lines, respectively. The viability of cells significantly decreased at high concentrations of the essential oils. The cytotoxic potential of positive control gemcitabine had IC_{50} values of 0.4977 µg/mL and 0.5175 µg/mL for MCF-7 and NIH-3T3 cell lines, respectively.

Table 6: Cytotoxicity of essential oils in terms of IC_{50} values, µg/mL.

EOs and standard	Cell lines	
	NIH-3T3 (IC_{50}, µg/mL)	MCF-7 (IC_{50}, µg/mL)
P. frutescence (Summer, S_2)	7.41	8.14
P. frutescens (Winter, S_1)	23.76	12.12
P. frutescens (Summer, S_3)	21.70	12.53
Gemcitabine	0.517	0.4966

Note: IC_{50} is median inhibitory concentration, '-'= Not determined.

Perilla essential oils' cytotoxic effects are comparatively stronger among Lamiaceae plants [31]. PEO could be a good agent to inhibit breast cancer cells [39, 40]. This cytotoxic activity of PEO is attributed to dominant components like perilla ketone, isoegomaketone, and the synergistic effect of minor constituents present in the essential oil sample. PEO 100 ppm (100 g/mL) was effective against cell lines HCT116 and PACA2 with 100 % inhibition and A431 with 81.2 % inhibition. PEO showed cytotoxicity on cancer cell lines with IC_{50} values of 48.8, 66.5, 30.8, and 100 µg/ml against PACA2, A431, HCT116, and BJ1 cell lines, respectively [41]. According to published guidelines [42], IC_{50} values of 10-100 µg/mL represent promising inhibitory capacity against cancer cells. Some major compounds, mainly sesquiterpenes and the synergism of minor constituents, are responsible for the cytotoxicity of EOs [43, 40, 44].

Antioxidant Activity

DPPH Activity

In the DPPH radical scavenging assay, good

antioxidant activity was observed with an IC50 value of 359.17±0.11 µg/mL for essential oils extracted from *P. frutescens* (winter, S1) collected from Kavre. Similarly, *P. frutescens* (summer, S2) collected from Kavre showed antioxidant activity with an IC_{50} value of 343.263±0.09 µg/mL, and *P. frutescens* (summer, S_3) collected from Bardiya gave an IC_{50} value of 334.26±0.20 µg/mL. The positive standards used were ascorbic acid, BHT, and quercetin. In the DPPH assay, ascorbic acid and BHT each had an IC_{50} value of 6.4±0.34 µg/mL and 12.5±0.05 µg/mL, respectively (**Table 7**).

Table 7: Antioxidant activities of *P. frutescens* essential oil in terms of IC_{50} value from DPPH and ABTS assays

EO Samples	DPPH (Mean±SD) IC_{50} (µg/mL)	ABTS (Mean±SD) IC_{50} (µg/mL)
P. frutescens (Winter, S_1)	359.17±0.11	140.1±1.23
P. frutescens (Summer, S_2)	343.263±0.09	129.5±1.21
P. frutescens (Summer, S_3)	334.26±0.20	93.15±1.04
Ascorbic Acid	6.4±0.34	2.0±1.20
BHT	12.5±0.05	-
Quercetin	-	7.8±0.65

Note: Values are mean ± standard deviations from three experiments (n=3)

PEO was collected at three locations: Can Tho City, An Giang, and Vinh Long Province. The EOAG (IC_{50} = 0.68) and EOVL exhibited strong radical scavenging activity with percentage inhibition of 61.82±0.15 % and 60.36±0.23 % at 1% concentration [45]. The IC_{50}, expressed as the concentration capable of scavenging 50 % of the DPPH radical, was 10.20 for PEO. PEO showed a strong radical-scavenging effect (83.40 ± 1.08 %) at 32 µg/ml (IC_{50} = 10.20), which is lower than those recorded for ascorbic acid (IC_{50} = 16.6) and scavenging effect (89.846 ± 0.296 %) [41].

ABTS Radical-scavenging Activity

In the ABTS assay, ascorbic acid and quercetin had IC_{50} values of 2.0±1.20 µg/mL and 7.8±0.65 µg/mL, respectively. Comparatively, the three essential oil samples extracted from three perilla plants showed good radical-scavenging activity against ABTS free radicals, with the sample from Bardiya showing the best inhibitory activity.

In the ABTS radical-scavenging assay, good antioxidant activity was observed with an IC_{50} value of 140.1±1.23 µg/mL measured for essential oils extracted from *P. frutescens* (winter, S_1) collected from Kavre. Similarly, *P. frutescens* (summer, S_2) collected from Kavre showed antioxidant activity with an IC_{50} value of 129.5±1.21 µg/mL, and *P. frutescens* (summer, S_3) collected from Bardiya gave an IC_{50} value of 93.15±1.04 µg/mL **(Table 7)**.

PEO collected at three locations, Can Tho City, An Giang, and Vinh Long Province, exhibited similar ABTS scavenging ability with IC50 values ranging from 0.80±0.01 to 0.99±0.01%, with the best antioxidant capacity shown by EOVL, followed by EOCT, and the lowest by EOAG [45]. *P. frutescence* EO showed the lower percentage of inhibition values (59.14% at 32 µg/mL) but was lower than ascorbic acid (95.38% at 32 µg/mL). The IC_{50} values ranged from 0.16 µg/mL for ascorbic acid to 8 µg/mL for *P. frutescens* [41].

Conclusions

This study explored the volatile constituents, enantiomeric distribution, and biological efficacies of *Perilla frutescens* essential oil samples sourced from Nepal. The essential oils were classified as PK type, characterized by a high content of perilla ketone, along with notable amounts of

isoegomaketone, β-caryophyllene, isobicyclogermacrene, and apiole. Enantiomeric analysis identified 12 chiral terpenoids, with 1-octen-3-ol as the enantiomerically pure dextrorotatory form. These chemical and chiral profiles may serve as indicators of the authentication and identification of *Perilla* essential oils. The essential oils demonstrated stronger antimicrobial activity against *E. coli*, *C. albicans*, *A. niger*, and *P. aeruginosa* compared to *B. cereus* and *S. aureus*. PEO from Bardiya collected during the summer exhibited the highest antioxidant activity (as assessed by DPPH and ABTS assays), while the summer sample from Kavre showed significant cytotoxicity against MCF-7 breast cancer cell lines. Overall, *P. frutescens* EO displays strong potential as an antimicrobial, antioxidant, and cytotoxic agent for future pharmaceutical applications with proper validation.

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Author's Contributions Statement:

P. N. Paudel: Conceptualization, Methodology, Investigation, Experimentation, Data analysis, Writing-original draft, writing-review and editing **A. Shrestha:** Writing-original draft preparation, writing-review and editing**, P. Satyal, W. N. Setzer**, **S. Awale, R. Satyal, R. Gyawali, S. Watanabe, J. Maneenet:** Data analysis, Validation, Formal analysis

Conflict of Interest:

They declare that there is no conflict of interest.

Data Availability Statement:

All data are available in the manuscript. The data supporting this study's findings are available from the corresponding author upon reasonable request.

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