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# Chemical Composition and Biological Activity of Peucedanum Dhana A. Ham Essential Oil

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# Abstract

The essential oil of *Peucedanum dhana* A. Ham growing in Thailand was investigated for their volatile compounds and screened for its antimicrobial, antioxidant, and cytotoxic activities. Forty-two compounds were identified, with the most important compounds being trans-piperitol, o-cymene, β-pinene, γ-terpinene, and limonene. Essential oil of P. dhana has remarkable antimicrobial activity against tested pathogens with minimum inhibitory concentration and minimum microbicidal concentration values, ranging from 62.50–250 µg/mL and 250-1,000 µg/mL, respectively. The antioxidant activity of *P. dhana* oil was measured using DPPH and ABTS scavenging activity assays. The $IC_{50}$ values using the DPPH and ABTS method were 9.13 and 9.36 mg/mL, respectively. The total phenolic compounds in *P. dhana* oil was 183.05 mg GAE/100 g dry weight. The cytotoxic effect of *P. dhana* oil was tested against Hela, A549, SW480, and 3T3L1 cells. The essential oil had cytotoxicity against all cancer cells, with significant cytotoxicity towards the SW480 cell. The results indicate that the *P. dhana* essential oil could be used as a source of functional ingredients in food and pharmaceutical applications.


# Introduction

Natural products have been traditionally used for therapeutic purposes against many infectious diseases caused by various pathogenic microorganisms. Essential oils produced by aromatic plant organs such as flowers, seeds, leaves, and roots are evaluated as one of the natural products that contain various medicinal benefits. Essential oils are composed of secondary metabolites, which can be associated with various functions for plant survival and microorganismal defense[1]. They are rich in bioactive compounds such as terpenes and phenols which are responsible for their antimicrobial and antioxidant activities[2]. Antiviral, antifungal, antitoxigenic, antiparasitic, and insecticidal properties of essential oils have also been reported[3]. Therefore, essential oil is an alternative herbal source for the development of new drugs and new applications in human health owing to their biological action mode.

The genus *Peucedanum* belongs to the Apiaceae family comprising of more than 120 species[4]. Most species are widely cultivated in Europe, Asia, and Africa[4]. According to the ethnopharmacological history of this genera, several species are widely used in various traditional medicines for the treatment of cough, sore throat, cold, headache[5,6], angina[7], asthma, cramps, epilepsy, gastrointestinal disorders, rheumatism, gout, and cardiovascular disease[8]. Some species are also used as a chemopreventive[8] and antifebrile agents[9]. Moreover, some species were reported to possess antimicrobial, antifungal, antioxidant, anti-inflammatory, and anticancer properties[10–13]. Previous phytochemical studies on *Peucedanum* indicated the presence of several bioactive compounds such as coumarins, polyphenols, amines, glycosides, flavonoids, phenolic acids, and diterpenes[10]. Essential oils were also produced by some plant species including *P. officinale*, *P. alsaticum*, *P. austriacum*, *P. oreoselinum*, *P. longifolium*, *P. ruthenicum*, *P. paniculatum*, and *P. cervaria*[10,14,15].

The *P. dhana* A. Ham is a plant species in the *Peucedanum* genus, which is evaluated as a rare herb[4]. It is a glabrous perennial tree with small yellow flowers and is calyx-teeth obsolete. It has a whitish, hairy patterned fruit with short or long hairs, and a waxy coating. This plant was reported to have high medicinal value 4. Its roots were used as a tonic that promotes sexual desire[16]. Fruits of *P. dhana* produce essential oil with aromatic smell[17]. However, the chemical composition, and the antimicrobial and antioxidant activities of *P. dhana* essential oil are rarely explored in the literature. Thus, this study aims to investigate the chemical composition of the essential oil extracted from *P. dhana* fruits and to evaluate its antimicrobial, antioxidant, and cytotoxicity activities.

## Results And Discussion

# Chemical composition of P. dhana essential oil

The essential oil of *P. dhana* fruits was obtained by hydrodistillation with a yield of 0.76%v/w on dry weight basis. The chemical composition of the *P. dhana* essential oil is presented in Table 1. In total, 42 volatile compounds were identified, representing 99.27% of the oil. Trans-piperitol (50.9%), o-cymene (11.4%), β-pinene (11.0%), γ-terpinene (9.2%), and limonene (4.9%) were the major volatile compounds of the P. dhana essential oil. Trans-arbusculone (2.4%), cis-4-caranone (2.0%), β-cubebene (1.5%), yomogi alcohol (1.4%), and isobornyl acetate (1.3%) were detected as minor components. These results showed a high content of monoterpene hydrocarbons over 97.7% in *P. dhana* essential oils.

Table 1
Volatile compounds of *P. dhana* essential oil by GC-MS

| Compound | RI* | RI** | % area | Compound | RI* | RI** | % area |
|---|---|---|---|---|---|---|---|
| heptanal | 911 | 901 | t*** | pinocarvone | 1161 | 1160 | t |
| tricyclene | 928 | 921 | t | borneol | 1167 | 1165 | t |
| artemisia triene | 931 | 923 | t | neoiso-isopulegol | 1168 | 1167 | t |
| α-thujene | 934 | 924 | 0.05 | terpinen-4-ol | 1179 | 1174 | 0.06 |
| α-pinene | 940 | 932 | 0.90 | α-terpineol | 1193 | 1186 | 0.07 |
| camphene | 954 | 946 | 0.10 | cis-4-caranone | 1200 | 1200 | 2.03 |
| benzadehyde | 966 | 952 | t | decanal | 1207 | 1201 | 0.22 |
| sabinene | 979 | 969 | 0.10 | trans-piperitol | 1217 | 1207 | 50.91 |
| β-pinene | 983 | 979 | 11.02 | isobornyl acetate | 1285 | 1283 | 1.26 |
| cis-meta-mentha-2,8-diene | 991 | 987 | t | β-cubebene | 1390 | 1388 | 1.49 |
| myrcene | 996 | 990 | 0.20 | decyl acetate | 1410 | 1407 | 0.55 |
| dehydroxy-trans-linalool oxide | 1002 | 992 | t | E-caryophyllene | 1417 | 1417 | 0.08 |
| yomogi alcohol | 1007 | 999 | 1.35 | δ-amorphene | 1519 | 1511 | 0.14 |
| δ-2-carene | 1014 | 1001 | t | α-calacorene | 1539 | 1539 | t |
| α-terpinene | 1020 | 1014 | t | maaliol | 1578 | 1566 | t |
| o-cymene | 1028 | 1022 | 11.37 | 2-ethylbutyric acid, octyl ester | 1581 | 1581 | 0.77 |
| limonene | 1031 | 1024 | 4.92 | cis-dihydro-mayurone | 1604 | 1595 | t |
| γ-terpinene | 1061 | 1054 | 9.20 | guaiol | 1615 | 1600 | t |
| trans-arbusculone | 1073 | 1066 | 2.39 | muurola-4,10(14)-dien-1-β-ol | 1624 | 1630 | t |
| terpinolene | 1091 | 1086 | t | α-muurolol | 1643 | 1646 | t |
| linalool | 1103 | 1095 | 0.07 | pogostol | 1650 | 1651 | 0.36 |

*Calculated retention indices obtained on a DB-5 column.

**Retention indices on a DB-5 column obtained from Adams.[54]

*** trace amount < 0.05

The results agreed with previous study on the chemical composition of essential oils extracted from *Peucedanum* genus reporting monoterpenes and sesquiterpenes as major constituents. Aggarwal et al.[18] reported that β-caryophyllene oxide was identified as main components in the *P. dhana* essential oil. The major chemical compounds of the essential oil obtained from *P. longifolium* were monoterpene hydrocarbons, with myrcene as the dominant compound[13]. Alavi et al.[10] identified 31 volatile compounds in the essential oil obtained from *P. ruthenicum* M. Bieb., and the major compounds were 1,8-cineole and camphor. In addition, volatile components of the essential oils extracted from *P. japonicum*[11], *P. austriacum*[19], *P. cervaria*, and *P. alsaticum*[20] have also been reported, with similar main components including α-pinene and β-phellandrene. Meanwhile sabinene was the main constituent of *P. ostruthium* essential oil[21]. Moreover, the major components in the essential oil obtained from *P. paniculatum* L. were β-cyclolavandulyl and β-isocyclolavandulyl esters[14]. Essential oil of *P. officinale* presented limonene and α-pinene as main components[22]. It is noted that it is possible to detect a diversity of terpenoids, especially monoterpenes in plant species of the *Peucedanum* genus. However, different factors including plant age, cultivation, climatic and micro-environmental conditions, seasons, harvest time, chemotypic variation, and geography play important roles in the variations of essential composition of plants[23].

# Antimicrobial activity

The antimicrobial activity of the *P. dhana* essential oil was evaluated against seven pathogenic microorganisms, using the disc diffusion and the broth microdilution methods. The zone of inhibition diameter, MIC, and MMC of the *P. dhana* essential oil and chloramphenicol for the tested microorganisms are shown in Table 2. Both Gram-positive and Gram-negative bacteria were susceptible to the essential oil of *P. dhana* fruits. The MIC of the bacterial pathogens ranged from 62.50 µg/mL to 250 µg/mL, whereas the MIC for fungus was 125 µg/mL. The *P. dhana* essential oil was effective in inhibiting the growth of all tested microbial strains. The data obtained from the disc diffusion method indicated that *P. aeruginosa* ATCC 27853 was the most significantly sensitive (40.80 mm) to 1 mg/mL of *P. dhana* essential oil compared to those obtained from other tested pathogens ranging between 10.70–19.60 mm. In agreement with the results from the disc diffusion method, the results of the MIC determination indicated that the *P. dhana* essential oil had the lowest MIC value (62.50 µg/mL) for all Gram-negative bacterial pathogens, including *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922, and *E. aerogenes* ATCC 13048. Meanwhile Gram-positive bacteria and fungus showed higher MIC values, ranging from 125 µg/mL to 500 µg/mL. As can be seen in Table 2, the lowest MMC value was 250 µg/mL for *P. aeruginosa* ATCC 27853, while the highest MMC value was 1,000 µg/mL for *S. aureus* ATCC 25923. In addition, the zone of inhibition diameter of the *P. dhana* essential oil for all microbial strains was similar to those obtained from chloramphenicol that was used as a positive control.

Table 2
Antimicrobial activity of *P. dhana* essential oil and chloramphenicol

| Microorganism | Chloramphenicol | | | *P. dhana* essential oil | | |
|---|---|---|---|---|---|---|
| | MIC (µg/mL) | MMC (µg/mL) | Zone of inhibition diameter (mm) | MIC (µg/mL) | MMC (µg/mL) | Zone of inhibition diameter (mm) |
| **Gram-positive bacteria** | | | | | | |
| *S. aureus* ATCC 25923 | 125.00$^{c}$ | 500.00$^{b}$ | 19.10 ± 0.01$^{c}$ | 250.00$^{c}$ | 1000.00$^{c}$ | 18.20 ± 0.20$^{b}$ |
| *S. epidermidis* ATCC 12228 | 125.00$^{c}$ | 500.00$^{b}$ | 19.50 ± 0.01$^{c}$ | 125.00$^{b}$ | 500.00$^{b}$ | 19.25 ± 0.25$^{b}$ |
| *B. subtilis* ATCC 6051 | 62.50$^{b}$ | 500.00$^{b}$ | 20.40 ± 0.01$^{b}$ | 125.00$^{b}$ | 1000.00$^{c}$ | 19.60 ± 0.31$^{b}$ |
| **Gram-negative bacteria** | | | | | | |
| *E. coli* ATCC 25922 | 62.50$^{b}$ | 500.00$^{b}$ | 10.10 ± 0.00$^{e}$ | 62.50$^{a}$ | 500.00$^{b}$ | 12.90 ± 0.20$^{c}$ |
| *P. aeruginosa* ATCC 27853 | 31.25$^{a}$ | 250.00$^{a}$ | 40.30 ± 0.01$^{a}$ | 62.50$^{a}$ | 250.00$^{a}$ | 40.80 ± 0.20$^{a}$ |
| *E. aerogenes* ATCC 13048 | 62.50$^{b}$ | 500.00$^{b}$ | 19.20 ± 0.03$^{c}$ | 62.50$^{a}$ | 500.00$^{b}$ | 18.70 ± 0.30$^{b}$ |
| **Fungus** | | | | | | |
| *C. albicans* ATCC 10231 | 62.50$^{b}$ | 500.00$^{b}$ | 17.50 ± 0.20$^{d}$ | 125.00$^{b}$ | 500.00$^{b}$ | 10.70 ± 0.20$^{d}$ |

*The data are mean ± standard deviation. Different letters indicate significant differences ($p < 0.05$)*

Regarding the antimicrobial activity, *P. dhana* essential oil was able to inhibit the growth of microbial pathogens, especially the Gram-negative bacteria with MIC value of 62.50 µg/mL. The antibacterial potential against pathogenic bacteria of several species from the genus *Peucedanum* has been reported. Ilić et al.[13] demonstrated that essential oils extracted from *P. longifolium* fruits exhibited slight antibacterial activity against the tested bacterial strains in vitro. According to Pirbalouti et al.[24], essential oil extract from *P. membranacea* Boiss exhibited a significant antibacterial activity against the Proteus vulgaris strain. The essential oil of *P. ruthenicum* M. Bieb. fruits showed antibacterial activities against *S. aureus*, *S. epidermidis*, and *B. cereus*[10]. Essential oils from several species, including *P. ferulaefolium*[25], *P. ruthenicum*[26], *P. japonicum*[11], and *P. alsaticum*, and *P. cervaria*[27], had antibacterial properties. However, there is no report on the antifungal activity from essential oil extracted from *Peucedanum* species. The

data obtained in this work show a growth reduction potential for bacterial pathogens. Moreover, the essential oil of *P. dhana* fruits possesses antifungal activity against *C. albicans*. The antimicrobial activity of essential oil is mainly associated with the presence of active components, including monoterpenes, sesquiterpenes, and their derivatives, as reported by Burt[3]. Essential oils of Spanish *Mentha rotundifolia*[28] and *Ocimum canum*[29] were rich in piperitol and presented weak antibacterial activity[29]. Thus, the strong antimicrobial activity of *P. dhana* oil could be explained by the synergetic effect of major and minor compounds[30–32]. These active compounds could enhance the antibacterial property of trans-piperitol. It was found that the mode of action of *P. dhana* oil could depend on the hydrophobicity of their functional groups, which enables them to partition the lipids of the bacterial cell membrane disturbing the cell wall and the cytoplasmic membrane, leading to lysis and leakage of intracellular compounds[33]. In addition, Moleyar and Narasimtram[34] described that the antimicrobial activity of volatile compounds in essential oil resulted from the combined effect of direct and indirect vapor absorption on pathogenic strains through the aqueous medium. The vapor absorption on microbial pathogens was detected by their membrane permeability. The absorption into aqueous media is examined by solubility, volatility, and stability of their volatile components. However, there is a limited detailed information about the antimicrobial activity mechanism of major volatile compounds. Thus, the antimicrobial potential of pure compounds presented in *P. dhana* oil is also required.

# Antioxidant activity and total phenolic content

The antioxidant activity of *P. dhana* essential oil was investigated using the DPPH and ABTS-scavenging assays. Results of the antioxidant activity of *P. dhana* essential oil and trolox are shown as $IC_{50}$ (Table 3). The $IC_{50}$ of *P. dhana* essential oil was 9.13 ± 0.11 mg/mL and 9.36 ± 0.21 mg/mL using the DPPH and ABTS assays, respectively, which was significantly higher than those from trolox with DPPH (0.66 ± 0.04 mg/mL) and ABTS (0.78 ± 0.04 mg/mL) assays. The total phenolic content of the *P. dhana* essential oil was 183.05 ± 0.21 mg GAE/100 g dw (Table 3).

Table 3
Total phenolic content and antioxidant activities of *P. dhana* essential oil and trolox

| Sample | yield | Total phenolic content | $IC_{50}$ (mg/mL) | |
|---|---|---|---|---|
| | (%w/w) | (mg GAE/100 g dry weight) | DPPH | ABTS |
| *P. dhana* essential oil | 0.76 ± 0.21 | 183.05 ± 0.21 | $9.13 \pm 0.11^{a}$ | $9.36 \pm 0.21^{a}$ |
| Trolox | - | - | $0.66 \pm 0.04^{b}$ | $0.78 \pm 0.04^{b}$ |
| *The data are mean ± standard deviation. Different letters indicate significant differences ($p < 0.05$)* | | | | |

The antioxidant activity and total phenolic content of the *P. dhana* essential oil have not yet been reported. However, there are few reports about the antioxidant activity and total phenolic content of essential oils obtained from *Peucedanum* species. Normally, essential oils were evaluated as a weak antioxidant[35]. Our study agreed with previous studies. Tepe et al.[36] studied the antioxidant properties of

*P. longifolium* and *P. palimbioides* essential oils. Their results revealed that the inhibition percentage of free radical DPPH of *P. longifolium* and *P. palimbioides* essential oils was 8.59–41.87% and 10.67–47.26%, respectively, while the standard compounds – butylated hydroxytoluene and butylated hydroxyanisole had strong inhibition percentage of free radical DPPH of 93.85% and 94.98%, respectively. Matejić et al.[37] reported that the total phenolic contents of the crude extracts obtained from the aerial parts of *P. aegopodioides*, *P. alsaticum*, *P. officinale*, and *P. longifolium* varied from 52.18 mg GAE/g to 118.32 mg GAE/g of extract. In another study, the total phenolic contents of polyphenolic extract and hydroalcoholic extract from the aerial parts of *P. pastinacifolium* Boiss. & Hausskn was 117.1 ± 6.2 mg GAE/g and 44.3 ± 1.7 mg GAE/g of extract, respectively[38]. The antioxidant activity of the essential oil of *P. dhana* might be due to the occurrence of major monoterpenic constituents like β-pinene, γ-terpinene, and limonene. Beta-pinene, which is the major compound in many essential oils such as turpentine oils, exhibited outstanding antioxidant activity[39]. Previous studies have also confirmed that γ-terpinene[40] and limonene[41] possessed good antioxidant activity. Moreover, the antioxidant activity of essential oil may be correlated to the volatile compounds listed in Table 1. Although the phenol compounds are evaluated mainly as the antioxidant compounds due to their oxidation and redox properties, terpenes have more potential in inhibiting free radicals. Their strong antioxidant activity is due to the activated methylene group[42]. Moreover, the phenolic content of *P. dhana* essential oil differed from those obtained from other species, which may be attributed to environmental and genetic factors[43].

# Cytotoxicity

Due to the cytotoxicity of essential oils, they have been applied as potential antitumor agents[44,45]. Therefore, the cytotoxicity of essential oil of *P. dhana* fruits was evaluated in both cancer and normal cell lines using MTT assay by comparing the $IC_{50}$ values to those of doxorubicin (Table 4). The *P. dhana* essential oil had cytotoxicity against all tested cancer cell lines, with $IC_{50}$ values ≤ 60 µg/mL, and demonstrated significantly low dose-dependent cytotoxicity in all cell lines compared to those obtained from the positive control, doxorubicin. The $IC_{50}$ values obtained from *P. dhana* essential oil were 56.63 ± 0.11 µg/mL, 51.67 ± 0.23 µg/mL, 18.24 ± 0.11 µg/mL, and 961.36 ± 0.11 µg/mL for Hela, A549, SW480, and 3T3L1 cell lines, respectively. Meanwhile, the $IC_{50}$ values obtained from doxorubicin were 0.55 ± 0.21 µg/mL, 0.46 ± 0.16 µg/mL, 0.34 ± 0.13 µg/mL, and 876.34 ± 0.16 µg/mL for Hela, A549, SW480, and 3T3L1 cell lines, respectively. Cell lines of SW480 were more sensitive to *P. dhana* essential oil than the other cancer cell lines. These $IC_{50}$ values, which are less than those obtained from antimicrobial activity tests of *P. dhana* oil, suggest that the *P. dhana* oil has moderate to strong cytotoxic effect, depending on the cell lines. This result agreed with the previous studies on essential oils of various plant species[46].

Table 4
Cytotoxicity of *P. dhana* essential oil and doxorubicin

| Cell line | $IC_{50}$ (µg/mL) | |
|---|---|---|
| | P. dhana essential oil | doxorubicin |
| cervical cancer (Hela) | 56.63 ± 0.11[c] | 0.55 ± 0.21[c] |
| human lung adenocarcinoma (A549) | 51.67 ± 0.23[b] | 0.46 ± 0.16[b] |
| human colonic adenocarcinoma (SW480) | 10.24 ± 0.11[a] | 0.34 ± 0.13[a] |
| murine fibroblast (3T3L1) | 961.36 ± 0.11[d] | 876.34 ± 0.16[d] |
| *The data are mean ± standard deviation. Different letters indicate significant differences ($p < 0.05$)* | | |

The study of Sylvestre et al.[2] reported the cytotoxic effect of essential oils. Based on their study, $IC_{50}$ values between 10–50 µg/mL indicate strong cytotoxic activity, while $IC_{50}$ values between 50–100 µg/mL, 100–200 µg/mL, and 200–300 µg/mL were indicate moderate, weak, and very weak cytotoxic properties, respectively. In addition, $IC_{50}$ values higher than 300 µg/mL indicates no cytotoxicity. Considering the cytotoxic activity on the four tested cells based on the MTT assay, *P. dhana* oil showed a moderate cytotoxic activity against the Hela, and A549 cells. However, a strong cytotoxic activity of *P. dhana* oil was demonstrated in SW480 cells. The doxorubicin, used as a positive control in this study, had a strong cytotoxic effect on cancer cells, which is considered as one broad spectrum of antitumor antibiotics of the most extensively used agents in chemotherapy regimens of cancer patients. The molecular mechanism of the cytotoxicity of *P. dhana* oil may appear according to its lipophilic property which accumulated in the cell membrane. The lipophilic property may increase its permeability reducing membrane-bound enzymes, metabolism activity, and induction of apoptosis[47]. In addition, β-pinene, α-pinene, and γ-terpinene have been considered as the major components responsible for some of the cytotoxic activities[48,49]. However, the cytotoxic effect of other components that are present at lower concentrations in the *P. dhana* oil essential oil must be considered. Examples of such components are limonene[50], β-cubebene[51], α-terpineol[52], and camphene[53], which are previously known to have cytotoxic effects against different cell lines. Considering an investigation about the cytotoxic effect of *Peucedanum* spp. on tumor cells, Yeong et al.[54] reported that the essential oil of *P. japonicum* Thunb revealed a cytotoxic activity against A549 cells with $IC_{50}$ of 0.04192% v/v. The obtained results in this study also present the importance of *P. dhana* as an alternative herbal source of essential oil that can be used as a cytotoxic agent against cancer cells, especially in human colonic adenocarcinoma.

# Conclusion

The major volatile components of the essential oil obtained from the *P. dhana* fruits were trans-piperitol, o-cymene, β-pinene, γ-terpinene, and limonene. The *P. dhana* essential oil had strong antimicrobial activity

against Gram-negative bacteria, Gram-positive bacteria, and fungus *C. albicans*. The essential oil showed antioxidant activity mainly through the formation inhibition of DPPH and ABTS, and also had cytotoxic effects against the tested cancer cells, especially SW480 cells. The strong biological activity attributed to the *P. dhana* essential oil may be due to the synergistic interactions of terpenes in its composition. These results highlight the potential of *P. dhana* as a source of essential oil for pharmaceutical applications such as antimicrobials and health promoters.

## Material And Methods

# Plant material

*P. dhana* A. Ham fruits were collected at Thung Hua, Wang Nuea district, located in Lampang province, Northern Thailand (latitude: 19°23'75.3"S, longitude: 99°57'50.6"W, altitude: 720 m) in December 2019. The plant was identified and a voucher specimen (No. 10124) was deposited in the Mae Fah Luang Botanical Garden, Mae Fah Luang University, Chiang Rai, Thailand.

# Essential oil extraction

The dried *P. dhana* fruits (500 g) were subjected to hydrodistillation for 4 h using a Clevenger-type apparatus. The obtained fruit essential oil was collected and combined with anhydrous sodium sulfate to eliminate the water. The essential oil was collected in a sealed vial and stored at 4 °C.

# Identification of chemical composition by gas chromatography-mass spectrometry (GC-MS)

First, the essential oil was diluted with dichloromethane (1:100 v/v). The chemical composition of the *P. dhana* essential oil was identified using Agilent 6890 N gas chromatograph connected to a mass spectrometer (Agilent 5973 network mass selective detector, Agilent Technologies, Santa Clara, CA, USA). The fused-silica capillary DB5-MS (30 m × 0.25 mm i.d., 0.25 μm) (J&W Scientific, USA) was used in the system. A total of 1.0 μL of the sample was injected using a split ratio of 1:50. The oven temperature started at 60 °C, then programmed to 240 °C at a rate of 3 °C/min. Helium (99.99% purity) was used as a carrier gas with a flow rate of 1 mL/min. Electron impact ionization was used, with the electron energy set to 70 eV. The ion source temperature was set to 250 °C. The acquisition was performed in a scan mode (m/z 30–300). Compounds were identified using their retention indices and mass spectra. Retention indices were calculated using linear interpolation of the retention times of $C_9$-$C_{17}$ n-alkanes and were compared with corresponding reference standard data reported by Adams[55] and the mass spectra from W8N08 and Wiley 7N libraries. Quantitative analysis of volatile components was performed using a relative peak area percentage on the spectra taken using an Agilent 6890 N gas chromatograph connected to a flame ionization detector (Agilent Technologies, Santa Clara, CA, USA). The same parameter as those in identifying the essential oil was used. The injector and detector temperatures were 250 °C and 280 °C, respectively.

# Antimicrobial activity

## Microbial strains

Seven human pathogens were used in this study including three Gram-positive bacteria: *Staphylococcus aureus* ATCC 25923, *S. epidermidis* ATCC 12228, *Bacillus cereus* ATCC 11778, three Gram-negative bacteria: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Enterobacter aerogenes* ATCC 13048, and one fungus: *Candida albicans* ATCC 10231. All bacterial strains were obtained from the Department of Medical Science, Ministry of Health, Bangkok, Thailand while the fungus *C. albicans* ATCC10231 was obtained from the culture collection of the Faculty of Dentistry, Khon Kaen University.

# Disc diffusion test

The antimicrobial screening by disc diffusion method was conducted using a modified method[56]. All bacterial strains were sub-cultured in Müller-Hinton broth (YM, Difco, USA) while the fungus was sub-cultured in sabouraud dextrose broth (Becton, Dickinson and Company, Sparks, USA). All strains were incubated at 37 °C for 24 h. The turbidity of the cell suspension was measured at 600 nm and adjusted with broth media to reach a 0.5 McFarland standard. The *P. dhana* essential oil and chloramphenicol were prepared at a concentration of 1.00 mg/mL using 10% dimethylsulfoxide (DMSO) and sterilized distilled water, respectively. Each bacterial strain was spread on a sterile Petri dish containing Müller-Hinton or sabouraud dextrose agar using a sterile cotton swab. Then, 30 µL of each sample was dropped on a sterilized paper disc with 6 mm diameter (WhatmanTM, USA) and placed on a Petri dish. All bacterial plates were incubated at 37 °C for 24 h, while fungal plates were incubated at 28 °C for 48 h. The diameter of the inhibition clear zone after incubation was measured by a vernier caliper. A 10% DMSO and chloramphenicol were used as negative and positive controls, respectively. Each experiment was carried out in triplicate.

## Determination of minimum inhibitory concentration (MIC) and minimum microbicidal concentration (MMC)

MIC and MMC values were measured by a broth microdilution method according to a modification method[57]. The essential oil and chloramphenicol were dissolved in 10% DMSO and sterilized distilled water, respectively, with the following concentrations: 1,000, 500, 250, 125, 62.50, 31.25, 15.62, and 7.81 µg/mL. A two-fold dilution procedure was used for all mixtures. The experiment was performed in 96-well microtiter plates. A combination of 10 µL of microbial suspension ($10^6$ CFU/mL) of each pathogen and 0.675% resazurin (Sigma-Aldrich, USA) were added to each well containing 50 µL of essential oil samples or chloramphenicol. All plates were covered with the sterile cap. The bacterial plates were incubated at 37 °C for 4 h, while fungal plates were incubated at 28 °C for 4 h. A 10% DMSO and chloramphenicol served as negative and positive controls, respectively. The color of each well was compared to those obtained from the negative control. The lowest concentration with the same color as the negative control was interpreted as the MIC. To determine the MMC, 50 µL of sample in each well,

with the same color as the negative control, were streaked onto an agar medium. Muller-Hinton agar was used for bacteria, while sabouraud dextrose agar was used for the fungus. All bacterial plates were incubated at 37 °C for 24 h while fungal plates were incubated at 28 °C for 48 h. The lowest concentration of the essential oil sample that did not show colony formation on the agar medium was determined as the MMC.

# Antioxidant activity

## 2,2-diphenyl-2-picrylhydrazyl radical (DPPH) assay

The scavenging capacity against DPPH of the *P. dhana* essential oil was evaluated according to the modified method[57]. The essential oil samples and a standard reference, trolox, were prepared in methanol with the following concentrations: 1,000, 500, 250, 125, 62.50, 31.25, 15.62, and 7.81 µg/mL. Two-fold dilution was employed for each mixture. A 0.05 mL of various essential oil and trolox concentrations was mixed with 1.95 mL of 0.2 mol/L DPPH solution. The mixture was shaken vigorously and kept in the dark at 27 °C for 30 min. The absorbance of the mixture was determined at 517 nm using a PerkinElmer spectrophotometer. Methanol was used as blank solution. The scavenging capacity were calculated as $[(A_C\text{-}A_s/A_c) \times 100$ where $A_C$ and $A_S$ correspond to the absorbance of the control and sample, respectively. The antioxidant activity of P. dhana essential oil and trolox was reported as IC50. Each sample was tested for antioxidant activity in triplicate.

## 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium radical cation (ABTS) assay

The scavenging activity against ABTS of the *P. dhana* essential oil was determined according to the modified method[57]. The ABTS radical cation was prepared by mixing 7 mM ABTS solution with 2.45 mM potassium persulfate and kept in the dark at 27 °C. For each concentration, 50 mL of the essential oil solution prepared in methanol was mixed with 150 mL of ABTS solution before shaking vigorously and kept in the dark at 27 °C for 5 min. The absorbance of the solution was determined at 734 nm using a PerkinElmer spectrophotometer. Methanol was used as a blank solution. Trolox was also used as a standard reference. The scavenging capacity was calculated using equation described above. Trolox was used as a standard reference. The antioxidant activity of *P. dhana* essential oil and trolox was reported as $IC_{50}$. Each sample was tested for antioxidant activity in triplicate.

# Antioxidant activity

The total phenolic content in the *P. dhana* essential oil was determined spectrophotometrically using the Folin-Ciocalteu reagent[58]. The essential oil solutions were diluted with methanol. One milliliter of solution was mixed vigorously with 5 mL of the Folin-Ciocalteu reagent. After 5 min, 4 mL of 15% $Na_2CO_3$ and 5 mL distilled water were added. The mixture was kept in the dark at 27 °C for 1 h. The absorbance of the solution was determined at 765 nm using a PerkinElmer spectrophotometer. The standard reference curve

for gallic acid was performed for the following concentrations: 1,000, 500, 250, 125, 62.50, 31.25, 15.62, and 7.81 µg/mL. Methanol was used as a blank solution. Trolox was also used as a standard reference. The regression equation and correlation coefficient were calculated and expressed in mg GAE/g dry weight. All measurements were performed in triplicates. The total phenolic content was determined and expressed as mg gallic acid equivalents (GAE/g dry weight) using a standard curve as a reference.

# Cytotoxicity activity

## Cell line and culture

The human colonic adenocarcinoma (SW480), human lung adenocarcinoma (A549), cervical cancer (Hela) cell lines, and murine fibroblast (3T3L1) were purchased from the China Center for Type Culture Collection. These cell lines were cultured and maintained in a humidified incubator at 37 °C and in 5% $CO_2$ atmosphere. Dulbecco's Modified Eagle medium supplemented with 10% fetal bovine serum, 100 units/mL penicillin, and 100 µg/mL streptomycin was used as the culture medium for all cell lines.

## 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

The effect of the *P. dhana* essential oil on the cell viability of three human cancer cell lines was determined by the MTT method[59]. Cells were treated with different concentrations of essential oil (1,000, 500, 250, 125, 62.50, 31.25, 15.62, and 7.81 µg/mL) in 96-well plates containing 100 µL medium with a density of $1 \times 10^4$ cells per well. The plates were incubated for 24 h. The MTT working solution (10 µL of 5 mg/mL in stock solution) was added to each well and was further incubated for 4 h. For each well. 200 µL of DMSO was added after removing the MTT solution, prior to shaking gently for 10 min to dissolve the obtained purple crystals. The presence of solubilized cells was determined using a microplate reader (BioTek Instruments, Inc.) at 570 nm. DMSO was used as a blank solution. Doxorubicin was used as a positive control. The cytotoxic activity of *P. dhana* oil against all cell lines was reported as $IC_{50}$ values.

# Statistical analysis

Results are expressed as the mean ± standard deviation (SD). All experiments were performed in triplicates. Analysis of variance (ANOVA) was performed to measure the antimicrobial and antioxidant activities, total phenolic content, and cytotoxicity characteristics of the *P. dhana* essential oil. The mean comparison was based on Student's t-test at $p < 0.05$. All statistical tests were performed using the SPSS statistics software (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY, IBM Corp).

## Declarations

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# Author contributions

S.K., T.S. and N.R. performed experiments. S.K. performed GC-MS analysis, analyzed data, and wrote the first draft of the manuscript. R.C. and P.P. reviewed the manuscript. P.P. supervised the work.

# Competing interests

The authors declare no competing interests

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