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ORIGINAL ARTICLE

Essential Oil Composition and Radical Scavenging Activity of *Piper penangense* C.DC

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Abstract

The genus Piper is among the most important genera in the Piperaceae family. It is known for several medicinally and economically important species that have been used throughout their native range. *Piper* species have great diversity in the world's tropical regions and are represented mainly by aromatic shrubs and trees with significant production of essential oils. The present study reports the chemical composition of the essential oil of *Piper penangense* and its radical scavenging activity. The essential oil was obtained by hydrodistillation and fully characterized by gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS). The antioxidant activity of the essential oil was determined using DPPH free radical scavenging assay. A total of 12 components (84.5%) were successfully identified, which were characterized by humulene epoxide II (31.9%), caryophyllene oxide (9.9%), muurola-4,10(14)-dien-1β-ol (9.1%) and β-ionone (8.3%). The essential oil showed significant activity towards DPPH radical scavenging (concentration 1,000 µg/mL) with a percentage inhibition of 72.5%. This study may provide valuable information and indications for further exploring the potential nutraceutical and pharmaceutical applications of the *Piper* species.

Keywords: essential oil; Piperaceae; *Piper penangense*; humulene epoxide II; radical scavenging activity

Introduction

Antioxidants are important in both the pharmaceutical and food industries since they are widely utilised to prevent the beginning and/or progression of disease and food spoilage caused by reactive oxygen species (ROS), which include free radicals, hydrogen peroxide, and other peroxides (Chaudhary et al., 2023). However, there is rising global concern about the widespread use of synthetic antioxidants such as butylated hydroxyl toluene (BHT) and butylated hydroxyl anisole (BHA), as they have been connected to a variety of negative consequences on human health. As a result, there is a global interest in finding safe antioxidant products made from natural compounds found in plants, such as essential oils, because they

are widely accepted by consumers and can be used for a variety of purposes across multiple industries (Olszowy & Dawidowicz, 2016).

Essential oils as secondary metabolites involve complex mixtures of natural compounds with versatile organic structures representing useful medicinal properties. Essential oils are important natural sources and are used as raw materials for the production of fragrance compounds in cosmetics, as flavouring additives for food and beverages, as scenting agents in a variety of household products, and as intermediates in the synthesis of other perfume chemicals. Essential oils from aromatic and medicinal plants have been known since antiquity to possess biological activities, most notably antibacterial, antifungal, and antioxidant properties (Salleh et al., 2014, 2015, 2016).

The Piperaceae family belongs to the major group Angiosperms, consists of about 5 genera and more than 3000 species. *Manekia*, *Verhuellia*, *Zippelia*, *Piper*, and *Peperomia* are the genera in Piperaceae plant taxonomy. The Piperaceae family is commonly found in warm tropical, and subtropical regions, widespread in South and Central America and central Asia, particularly in India. The genus *Piper* is among the most important genera in the Piperaceae family, consisting of over 2000 species and widely distributed globally, mainly in the Southeast Asian region. It is known for several medicinally and economically important species that have been used throughout their native range. *Piper* species have great diversity in the world's tropical regions and are represented mainly by aromatic shrubs and trees with significant production of essential oils (Salleh et al., 2014).

Piper penangense is commonly found from Thailand to Peninsular Malaysia. It is mostly found in lowland and hill evergreen forest, specifically in shaded areas, along streams, and near waterfalls. This species is similar to *P. sarmentosum* in gross morphology but differs in fruit spine, and free fruit (Burkill, 1966). Following our previous studies, the present work aimed to analyse the chemical composition and radical scavenging activity of *P. penangense* essential oil.

Materials and Methods

Plant material

The leaves of *P. penangense* were collected from Langgun Island, Langkawi (6.35256° N, 99.78974° E) on September 2022, and identified by Shamsul Khamis from Universiti Kebangsaan Malaysia (UKM). The voucher specimen (PT60/22) was deposited at UKMB Herbarium, Faculty of Science and Technology, UKM.

Isolation of essential oil

The fresh leaf (300 g) was subjected to hydrodistillation in Clevenger-type apparatus for 4 h. The essential oil obtained was dried over anhydrous magnesium sulfate and stored at 4-6°C. The essential oil had a spicy odor and yielded 0.12% calculated from the fresh weight of the leaves.

Analysis of essential oil

Gas chromatography (GC-FID) analysis was performed on an Agilent Technologies 7890B equipped with a DB-5 capillary column (30 m long, 0.25 µm thickness, and 0.25 mm inner diameter). Helium was used as a carrier gas at a 0.7 mL/min flow rate. Injector and detector temperatures were set at 250 and 280°C, respectively. The oven temperature was kept at 50°C, then gradually raised to 280°C at 5°C/min, and finally held isothermally for 15 min. Diluted samples (1/100 in diethyl ether, v/v) of 1.0 µL were injected manually (split ratio 50:1). The injection was repeated three times, and the peak area percentages were reported as means ± SD of triplicate. The peak area percentage was calculated using the GC HP Chemstation software (Agilent Technologies).

Gas chromatography-mass spectrometry (GC-MS) chromatograms were recorded using an Agilent Technologies 7890A/5975C MSD equipped with HP-5MS fused silica capillary column (30 m long, 0.25 µm thickness and 0.25 mm inner diameter). Helium was used as the carrier gas at a 1 mL/min flow rate. The injector temperature was 250°C. The oven temperature was programmed from 50°C (5 min hold) to 250°C at 10°C/min and finally held isothermally for 15 min. For GC-MS detection and electron, an ionization system with ionization energy of 70 eV was used. A scan rate of 0.5 s (cycle time: 0.2 s) was applied, covering a mass range from 50-400 amu (Salleh et al., 2016).

Identification of components

For the identification of essential oil components, co-injections with the standards (major components) were used, together with the correspondence of retention indices and mass spectra with respect to those reported in Adams, NIST08 and FFNSC2 libraries (Adams, 2007). Semi-quantification of essential oil components was made by peak area normalization considering the same response factor for all volatile components. Percentage values were the mean of three chromatographic analyses.

DPPH free radical scavenging

The DPPH free radical scavenging assays of phytochemicals were investigated as a previous method with slight modifications (Salleh et al., 2015). The DPPH solution was freshly prepared in MeOH. The samples were in methanol (200 µL) (conc. of 1000 µg/mL) and mixed with the DPPH solution). The mixture was allowed to stand for 30 min at room temperature in the dark, and then the absorbance was recorded at 517 nm. The percentage inhibition of DPPH (%) was calculated using the following formula; $I\% = [A_{blank} – A_{sample} / A_{blank}] \times 100$; where A_{blank} is the absorbance value of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance value of the test compounds. Butylated hydroxytoluene (BHT) was used as a standard and diluted to the same concentration as the samples.

Results and Discussion

The GC-FID and GC-MS analysis of the leaf oil *P. penangense* indicated the identification of 12 chemical components representing 84.5% of the total oil. The identification of chemical components are listed in Table 1. The leaf oil *P. penangense* consisted mainly of oxygenated sesquiterpenes (66.7%) followed by sesquiterpene hydrocarbons (9.5%). The major components identified in the leaf oil were humulene epoxide II (31.9%), caryophyllene oxide (9.9%), muurola-4,10(14)-dien-1β-ol (9.1%), and β-ionone (8.3%). Humulene epoxide II was found as major component in several *Piper* species such as *P. coruscans* (Ecuador: leaf oil, 4.1%) (Gilardoni et al., 2020), *P.donnell-smithii* (Guatamela: leaf oil 11.5%) (Cruz et al., 2011), *P. tuberculatum* (Vanezuella: leaf oil 6.0%) (Krinski & Foerster, 2016) and *P. retalhuleuense* (Guatamela: leaf oil 4.8%) (Cruz et al., 2011). Meanwhile, β-ionone was rarely found in *Piper* essentialoils. It was found as a minor component in the essential oils of *P. pseudolindenii* (Costa Rica: leaf oil 0.1%) (Vila et al., 2003), *P. corrugatum* (Panama: leaf oil 0.4%) and *P. darienense* (Panama: leaf oil 0.3%) (Santana et al., 2016). Furthermore, (*E*)-nerolidol (17.5%) and cedrol (14.8%) were found to be the major components of *P. penangense*, collected from Kepong, Kuala Lumpur (Jantan et al., 1994).

Chemical differences in the essential oil composition of plant species concerning their geographical origins and harvesting season have been reported, showing that the chemical and biological diversity of aromatic and medicinal plants depend on factors such as cultivation area, climatic conditions, vegetation phase, and genetic modifications. In fact, these factors influence the plant's biosynthetic pathways and consequently, the relative proportion of the main characteristic components (Salleh et al., 2015).

Table 1. Chemical components identified from the leaf oil of *P. penangense*

No	Components	MF	KI[a]	KI[b]	Percentage (%)
1	β-Elemene	$C_{15}H_{24}$	1388	1389	1.8
2	Alloaromadendrene	$C_{15}H_{24}$	1459	1458	1.7
3	γ-Gurjunene	$C_{15}H_{24}$	1474	1475	6.0
4	**β-Ionone**	$C_{13}H_{20}O$	1486	1487	**8.3**
5	α-Selinene	$C_{15}H_{24}$	1499	1498	2.8
6	Spathulenol	$C_{15}H_{24}O$	1577	1577	3.8
7	**Caryophyllene oxide**	$C_{15}H_{24}O$	1585	1582	**9.9**
8	**Humulene epoxide II**	$C_{15}H_{24}O$	1607	1608	**31.9**
9	**Muurola-4,10(14)-dien-1-ol**	$C_{15}H_{24}O$	1627	1630	**9.1**
10	β-Eudesmol	$C_{15}H_{26}O$	1647	1649	3.6
11	Cadalene	$C_{15}H_{18}$	1679	1675	4.0
12	Germacra-4(15),5,10(14)-trien-1β-ol	$C_{15}H_{24}O$	1687	1685	1.6
	Sesquiterpene hydrocarbons				**9.5**
	Oxygenated sesquiterpenes				**66.7**
	Others				**8.3**
Total identified					**84.5**

KI: based on the comparison of calculated KI with those reported in Adams
[a] Linear retention index experimentally determined using a homologous series of C6-C30 alkanes
[b] Linear retention index taken from Adams, Wiley or NIST08 and literature

The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability. It has been reported that free radical scavenging activity is greatly influenced by the phenolic components of the samples. In this study, the essential oils demonstrated significant inhibition (72.5% ± 0.22 mg/mL), compared to BHT (93.5% ± 0.25 mg/mL). The activity could be due to the high amounts of oxygenated sesquiterpenes in the essential oils of *P. penangense*. In another studies, several *Piper* essential oils have shown strong radical scavenging activity such as *P. lolot* (IC_{50} value 16.7 µg/mL) (Phong et al., 2022), *P. ecuadorense* (IC_{50} value 1.8 µg/mL) (Valarezo et al., 2021) and *P. magniba*ccum (IC_{50} value 20.5 µg/mL) (Hashim et al., 2017).

The antioxidant activity of essential oil molecules is quite complex and is due to the inherent ability of some of their components, particularly phenols, to stop or delay the aerobic oxidation of organic matter, although the procedure by which the oil is obtained limits the content of phenolics in the final matrix (Amorati et al., 2013). Phenol-free essential oils also display antioxidant behavior. This could be due to the presence of double bonds and to the radical chemistry of some terpenoids (e.g., eucalyptol) and other volatile components (e.g., sulfur-containing components) and has been confirmed in vitro by using different biological and nonbiological methods, such as DPPH and ABTS assays (Aruoma, 1998). The antioxidant effect of sweet orange essential oil, whose main components are D-limonene, octanal and decanal has been demonstrated on isolated brain homogenates via the inhibition of Fe^{2+}-induced lipid peroxidation. Many different in vitro studies have been carried out by using essential oils or their molecules, such as cinnamaldehyde, eugenol, carvacrol or β-caryophyllene, with interesting results (Ademosun et al., 2016).

Conclusion

This work constitutes to report the essential oil composition and radical scavenging activity of *P. penangense* obtained from Malaysia. A study on the essential oil of *P. penangense* revealed the existence of sesquiterpenes as the major class of components, dominated by humulene epoxide II. Further studies are needed to investigate the safety of *P. penangense* essential oil to be used as a therapeutic against neurodegenerative diseases.

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