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The UHPLC-HRMS profiling, *in vitro*-antioxidant and pancreatic lipase inhibitory activities of *Peronema canescens* leaves extract and fractions from Indonesia

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ABSTRACT

This study aims to identify metabolite compounds and determine the *in vitro* antioxidant and pancreatic lipase inhibitory activity of *Peronema canescens* Jack extracts and fractions. The results of phytochemical content showed that the ethyl acetate (FEA), *n*-butanol (FB), and aqueous (FA) fractions had significantly higher amounts of phenolic and flavonoid compounds than other fractions. It is linear with the antioxidant activity of FEA, FB, and FA, producing very strong to strong antioxidant activity. *N*-hexane fraction (FH) showed significant terpenoid content and a strong inhibitory effect on the lipase enzyme. Phytoconstituents in the extracts and fractions were profiled using ultra-high-performance liquid chromatography-high resolution mass spectrometry equipped with principal component analysis to classify chemical compounds. Diosmetin and pinoquercetin compounds were identified as being present abundantly in FEA. Meanwhile, terpenoid compounds and fatty acids are abundant in FH, including 7-oxodehydroabietic acid, oleanonic acid, oleanolic acid, docosanamide, eleostearic acid, ethyl linolenic acid, 1-stearoylglycerol, 9-oxo-10 (E), 12 (E)-octadecadienoic acid, and 12-oxo-phytodienoic acid. These findings indicate that FEA contains bioactive compounds that increase antioxidant activity, while FH contains many bioactive compounds with pancreatic lipase inhibitory effects. Further research is required to determine the correlation between the amount of active compound content and the bioactivity of *P. canescens* leaf extracts and fractions. In addition, further research using experimental animals and optimization of safer solvents will provide more comprehensive bioactivity information and increase our best knowledge of ethnomedicine based on *P. canescens* herbal preparations.

INTRODUCTION

Based on data from the World Health Organisation, more than 80% of the world's population depends on traditional herbal medicine as their main means of getting fundamental healthcare [1]. Due to the emergence of negative impact and the development of intolerance to chemically based medications, individuals have shifted their attention to ethno-pharmacognosy. Some individuals often assert the benefits of particular natural or herbal products. The bioactive compounds found in medicinal plants, such as alkaloids, phenolics, and terpenoids, are important in their biological effects. These effects include their ability to reduce inflammation, prevent the incidence of diabetes, and act as antioxidants [2]. Due to the diverse groups of chemical compounds in plants, the extraction process to obtain bioactive compounds with desired health benefits remains an important issue [3]. Prior research has indicated that the effectiveness of extracting compounds is mainly influenced by factors such as pH, temperature, the ratio of sample to solvent, and the polarity of the solvent [3].

Some extraction procedures, such as conventional and advanced methods, can be used to extract the plants. Conventional methods, i.e., maceration, are generally applied to extract medicinal plants. The advantage of this method is that it is cheap, but the maceration method needs a high extraction time, extra solvent requirements, and the loss of

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volatile compounds [4]. Meanwhile, microwave-assisted extraction (MAE) and ultrasonic-assisted extraction (UAE) methods, which are modern, have the advantages of less time, lesser solvent requirements, and lower capital investment in the extraction process. The advantage of UAE compared to MAE is that the UAE method requires less power or energy and higher heat-sensitive compound retention [4,5]. Therefore, the extraction method used in this study was UAE.

Plant-derived medications have been globally employed in traditional medical methods to treat various illnesses. Plants utilized in traditional medicine possess a diverse array of chemical compounds that have the potential to cure both chronic and infectious disorders [6] effectively. Duraipandiyan *et al.* [6] stated that numerous phytochemicals obtained from plants can function as potent and safe alternative options for medical purposes with minimal adverse effects. Numerous beneficial biological activities have been documented from natural materials, which include the ability to inhibit the growth of cancer cells, inhibit the growth of microbes, scavenge free radicals, inhibit the occurrence of diarrhea, provide analgesics, and accelerate wound healing. Populations of people worldwide have long employed plants possessing medicinal uses as raw materials for traditional remedies and nutritional supplements [7]. Herbal medicines in Asia have a rich history that reflects the deep connection between humans and the environment. It is now widely recognized that the medicinal properties of plants are derived from the bioactive compounds that induce specific physiological responses in the human body.

Peronema canescens Jack, often known as Sungkai or Jati Sabrang, is an indigenous plant species in Indonesia that originates in the *Lamiaceae* family, specifically in ethnobotany. This plant is commonly found in the Sumatra and Kalimantan islands, and its stems, leaves, flowers, and seeds have been used as folk medicines in the local community [8]. Fransisca *et al.* [9] stated that the tribe of Dayak on Kalimantan Island traditionally employs *P. canescens* as a medication to treat flu, fever, stomach ache, and worms (ringworms), as well as as a mouthwash to prevent toothache. Empirically, the Lembak people in the Bengkulu, Sumatra island, have used *P. canescens* plants as traditional remedies for fever, worms, antiseptics, diarrhea, malaria, and toothaches [10]. By brief bibliometric analysis, we have noted around 33 *P. canescens* or Sungkai articles found in the Scopus database. The development of this research was started in 2014 (1 article) by exploring the wood quality of the sungkai tree. The works on this plant have been continued in 2020 until mid-2024 which focus on the uses of *P. canescens* leaves for health. This lack number of articles on the database showed that this plant has not been optimally explored. To date, Indonesia has published the most works about *P. canescens* leaves, followed by Malaysia in second place. Since 2022, people started to pay attention to the antioxidant activity of *P. canescens* plant as well as their activities as anti inflammatory and immunomodulator. However, the ability of *P. canescens* to treat and maintain health through various bioactivity has not been much investigated. Moreover, the research related to the isolation and identification of active compounds that play a role in *P. canescens* leaves bioactivity is still very limited.

Various secondary metabolites are known present in the leaves of *P. canescens*, including phenolic compounds, flavonoids, alkaloids, terpenoids, steroids, tannins, and saponins. The compounds in question have been identified as exerting specific pharmacological actions [9,11]. The extract of *P. canescens* leaf in the ethanol solvent shows the strongest antioxidant activity, along with high levels of flavonoids and phenolic compounds [12–14]. In their research finding, Sutomo *et al.* [15] reported that *P. canescens* extracted with methanol exhibits significant antioxidant activity, demonstrated by an IC_{50} value of 63.977 ppm. The extract has been qualitatively confirmed to contain phenolic, flavonoid, steroid, alkaloid, tannin, and saponin compounds. The characterization of secondary metabolites derived from the analysis results utilizing GC-MS indicated that *P. canescens* leaf extract comprises 17 chemical compounds categorized within the fatty acid, steroid, terpenoid, phenolic, and carbohydrate groups. Phenolic compounds, including phenol, 2-methoxy-4-vinylphenol, caryophyllene, and phytol, have been identified for their significant antioxidant activity [16].

Previous research by Muharni *et al.* [17] showed that *P. canescens* extract has compounds called betulinic acid and stigmasterol that are very good at lowering cholesterol levels *in vitro* assays. In their research results, Latief *et al.* [18] also stated that *P. canescens* extract contains β-sitosterol and 5,7-dihydroxy isoflavone compounds. Betulinic acid is a terpenoid compound, while stigmasterol and β-sitosterol are steroid compounds (phytosterols) that play an important role as anticholesterol, antihyperlipidemic, and pancreatic lipase inhibitors [19–21]. *In vivo*, Pratiwi *et al.* [22] have proven that *P. canescens* extract has significant antihyperlipidemic activity in experimental animals. Therefore, the pancreatic lipase inhibition assays are important to be carried out in this study to obtain information on the pancreatic lipase inhibitory capacity of the extract and all fractions of *P. canescens* and to determine the bioactive compounds that play a role in this activity.

As far as we studied, the pancreatic lipase inhibitor activity of *P. canescens* leaves has not been investigated. Furthermore, the determination of *P. canescens* antioxidant activity yielded inconsistent results. This study focused on comprehensively characterizing the phytochemicals in *P. canescens* extract and fraction samples, followed by determining antioxidant activity and the ability to inhibit pancreatic lipase activity. The metabolite profile of *P. canescens* was also found using ultra-high-performance liquid chromatography-high resolution mass spectrometry (UHPLC-HRMS) and principal component analysis (PCA). This approach allowed for the classification of chemicals in *P. canescens* extracts and fractions. Identifying active compounds by metabolite profile and determining their biological activity will improve our knowledge of ethnomedicine based on herbal preparations of *P. canescens*.

MATERIALS AND METHODS

Materials

Peronema canescens leaves have been acquired from Bengkulu, located on the island of Sumatera in Indonesia. It has been identified (voucher specimen BO-1618223) by the Botanical

Characterization Laboratories, a division of the National Research and Innovation Agency of Indonesia. The analytical chemicals and standards utilized in this investigation, including chloroform, ethyl acetate, methanol, *Folin-Ciocalteu*, gallic acid, ascorbic acid, perchloric acid, and glacial acetic acid, were procured from Merck, Germany. Quercetin, trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), dimethyl sulfoxide, potassium persulfate, p-nitrophenylbutyrate, and standard ursolic acid have been obtained from Sigma Aldrich (St. Louis, MO, USA). Orlistat (Xenical) was obtained from a local distributor.

Extraction process

Unexposed to direct sunlight, the leaves of *P. canescens* were dry in the air for 7 days. The moisture content of *P. canescens* leaf simplicia has been measured, and the results obtained are 5.49% ± 1.45%. Dried leaves of *P. canescens* were then powdered and subjected to ultrasound-assisted extraction (UAE, 220-240 V, 50/60 Hz, and 550 W), employing 96% ethanol as the solvent for 1.5 hours, formulated with a solid-to-solvent ratio of 1:6 (w/v). The process of extraction was carried out three times at ambient temperatures. The extracted sample underwent filtration, collection, and concentration utilizing a BUCHI Rotavapor® R-300 rotary evaporator at a temperature of 50°C to get a crude extract. Finally, the resultant extract was dehydrated by freeze-drying (BUCHI Lyovapor™ L-200), and the percentage of extract obtained was determined using the following formula [23]:

$$\textit{Extract yield}\ (\%) = \frac{\textit{weight of the extract after evaporating solvent and freeze drying}}{\textit{dry weight of the sample}} \times 100.$$

Fractionation

An 80-g ethanol extract (EE) sample was mixed in 300 ml of water. The resulting mixture was then separated using the liquid–liquid partitioning method, with the solvents *n*-hexane, chloroform, ethyl acetate, and *n*-butanol in equal quantities. This process was repeated thrice, each partitioning step lasting 30 minutes. This partitioning aimed to obtain fractions based on increasing solvent polarity. Finally, the resulting fractions from the solvents *n*-hexane, chloroform, ethyl acetate, *n*-butanol, and aqueous (FH, FC, FEA, FB, and FA, respectively) were evaporated and lyophilized for further assays. Each series of extraction methods with ethanol and fractionation with various solvents was conducted thrice, so three sets of yield data (%) were obtained.

Total phenolic content

The *Folin-Ciocalteu* reaction method was used to measure the amount of phenolic group in the extract and fractions in this study, with minor adjustments [24]. A 250 µl aliquot of a 1 mg/ml sample was blended with 3.75 ml of water that was distilled and 250 ml of *Folin-Ciocalteu* solution, then vortexed until homogeneous. The resulting solution was then incubated for 8 minutes at 25°C–28°C. After the incubation, 750 ml of a Na_2CO_3 solution (20% w/v in water) was put in, vortexed, and then subjected to a further 2-hour incubation while protected from light. Afterward, 200 µl of every mixture was placed into a 96-well microplate. The absorbance was measured by a microplate reader at 765 nm wavelength using the instrument Multiskan Go from Thermo Scientific™. Values of absorbances were compared with a standard gallic acid within the concentration range of 60–300 µg/ml. All experiments were conducted three times, and the resulting data have been presented as milligrams of gallic acid equivalent (GAE) per gram of extract or fractions.

Total flavonoid content

Total flavonoids were quantified by the modified aluminum chloride colorimetric method [25] with slight modification. A 10 µl sample (0.5 mg/ml) and standard quercetin solution with various concentrations (25–600 µg/ml) were put into a 96-well microplate. Furthermore, 60 µl methanol, 10 ml of potassium acetate, 10 ml of aluminum chloride, and 120 ml of water that had been distilled were administered. After incubating the resulting mixture for 30 minutes, the absorbance was measured at a wavelength of 415 nm. A reference standard was established using quercetin, and the total amount of flavonoids was quantified as milligrams of quercetin equivalent (QE) per gram of extract or fractions.

Total terpenoid content

Terpenoid quantity was conducted using a colorimetric technique [26] with slight modifications. Extract and fractions samples were prepared with 800 µg/ml concentration in methanol p.a. Subsequently, a solution of ursolic acid was made at various concentrations ranging from 10 to 500 µg/ml. A volume of 0.1 ml of sample solution and standard ursolic acid with various concentrations were put into a test tube with a dark screw. Then, 0.2 ml of 5% vanillin and 1 ml of concentrated perchloric acid (60%) were added. Then, the mixture that had been prepared was incubated in a water immersion maintained at a temperature of 60°C for 45 minutes. After 45 minutes of incubation, the resultant mixture remained at ambient temperature for 15 minutes. Next, 2.5 ml of pure acetic acid (glacial) was added and vortexed until homogeneous. After homogenization, 200 µl of the resulting mixture was added to the 96-well microplate, and the absorbance value was measured at 548 nm using a microplate reader (Multiskan Go, Thermo Scientific™). This study expressed the quantitative measure of terpenoid content as milligrams of UAE per gram of extract or fraction, using ursolic acid as the reference standard.

DPPH radical scavenging assay

The free radical scavenging capacity of the extract samples and all fractions of *P. canescens* was determined *in vitro* by the 2,2-DPPH assay, following the methodology published by Mahmood *et al.* [27] with a few modifications. The details of this method can be seen in Supplementary Material 1.

ABTS radical scavenging assay

Assessment of free radical scavenging activity using the ABTS method was carried out using the procedure previously described by Re *et al.* [28] with slight modification.

Table 1. Yield (%), TPC, TFC, and TTEC of *P. canescens* leaf extract and fraction.

Samples	% Yield	TPC (mg GAE/g)	TFC (mg QE/g)	TTeC (mg UAE/g)
EE	8.31 ± 0.20^{c}	84.17 ± 2.88^{d}	182.50 ± 1.13^{c}	351.25 ± 3.13^{b}
FH	22.10 ± 1.94^{b}	26.80 ± 1.30^{f}	98.25 ± 1.66^{d}	465.14 ± 2.32^{a}
FC	28.11 ± 0.43^{a}	72.83 ± 1.56^{e}	63.04 ± 1.88^{e}	462.08 ± 3.18^{a}
FEA	7.48 ± 0.64^{c}	250.81 ± 5.68^{a}	486.58 ± 1.51^{a}	181.94 ± 0.87^{c}
FB	18.44 ± 0.49^{b}	157.33 ± 1.08^{c}	185.00 ± 1.42^{c}	110.00 ± 1.88^{e}
FA	4.56 ± 0.44^{d}	164.47 ± 3.33^{b}	246.50 ± 0.38^{b}	125.00 ± 0.64^{d}

The mean of triplicate measurements is represented by each value, different superscripted letter at the same column indicates significant differences.

Table 2. The result of IC_{50} antioxidant activity of *P. canescens* extract and fraction.

Sample	IC_{50} (µg/ml) (DPPH method)	IC_{50} (µg/ml) (ABTS method)	FRAP (Trolox Equivalen) (mg TE/g sampel)
EE	134.57 ± 6.96^{c}	62.16 ± 1.54^{e}	135.50 ± 0.51^{d}
FH	894.374 ± 24.22^{a}	186.14 ± 11.47^{b}	71.57 ± 0.33^{f}
FC	623.883 ± 20.50^{b}	107.94 ± 2.19^{d}	93.69 ± 0.35^{e}
FEA	39.09 ± 0.^{4}6e	33.85 ± 0.41^{f}	171.22 ± 0.57^{a}
FB	93.51 ± 3.41^{d}	165.43 ± 2.75^{c}	152.59 ± 0.85^{c}
FA	103.714 ± 4.46^{d}	224.38 ± 1.19^{a}	156.43 ± 1.68^{b}
AA*	16.08 ± 0.46^{f}	-	
Trolox	-	13.40 ± 0.26^{g}	

The mean of triplicate measurements is represented by each value, different superscripted letter at the same column indicates significant differences
AA* = Ascorbic acid.

Details of this method are available in the Supplementary Material 1.

Ferric-reducing antioxidant power (FRAP) assay

The ferric-reducing capacity of the plant extracts and fractions was investigated using the Benzie and Strain [29] method with slight modification. A description of this method can be viewed in Supplementary Material 1.

Assay of inhibiting pancreatic lipase

The effectiveness of the extract and fractions in suppressing pancreatic lipase activity was evaluated. The experiment was accomplished by utilizing spectrophotometry to measure the rate at which nitrophenol was formed due to pancreatin hydrolyzing p-nitrophenylbutyrate [30]. First, the extract and fractions were diluted in dimethyl sulfoxide to create solutions with 10 and 100 mg/ml concentrations, respectively. As a consequence, the final concentration of the mixture was 0.38 and 3.8 mg/ml, respectively. Ten ml of completely dissolved extract and fraction were mixed with an equivalent volume of pancreatin solution (made from 1 mg of pancreatic enzyme dissolved in 1 ml of saline phosphate buffer solution, pH 6.8). Next, the mixture was incubated for 5 minutes at 37°C. After incubation, 240 ml of substrate (A solution of 0.165 mM p-nitrophenylbutyrate in PBS) was added to the solution mixture. Then, the absorbance value of the mixture was measured at a specific wavelength of 415 nm with two observations, namely at intervals of 0 and 35 minutes. The lipase inhibitory action was quantified as the amount of µM nitrophenol produced per minute. As a positive control, 120 µg/ml orlistat was employed. To establish a nitrophenol standard curve, nitrophenol was diluted into solutions with concentrations of 0, 1, 2, 5, 10, and 20 µg/ml [31].

Table 3. Pancreatic lipase inhibitory activity of *P. canescens* extract and fraction.

Sample	Inhibitory activity (%)
EE	24.73 ± 1.52
FH	42.94 ± 13.11
FC	NA
FEA	1.25 ± 0.16
FB	NA
FA	NA
Orlistat (46 µg/ml)	72.63 ± 3.56

The mean of triplicate measurements is represented by each value; NA= no activity.

Identification of phytochemical constituents using UHPLC-HRMS

Liquid chromatography (Thermo Scientific™ Vanquish™ UHPLC Binary Pump) and Orbitrap high-resolution mass spectrometry (Thermo Scientific™ Q Exactive™ Hybrid

Table 4. The chemical compound of *P. canescens* extract and fractions.

No	Compound	Chemical formula	MW (g/mol)	RT	MS/MS fragmentation	Class	Biological activities	References
1	Glycitein	C16 H12 O5	284.07	12.40	95.04,930; 119.04,910; 124.01,542; 145.02,824; 167.03,371; 242.05,693; 270.05,188; 285.07,523	Flavonoids	Antioxidant	[35]
2	Diosmetin	C16 H12 O6	300.06	11.37	121.02,841; 167.03,392; 168.00,507; 257.04,437; 258.05,203; 286.04,694; 301.03,552; 301.06,979	Flavonoids	Antioxidant	[36]
							Anti-inflammatory	[37]
3	Rutin	C27 H30 O16	610.15	7.73	167.03,381; 243.06,465; 299.05,496; 302.04,163; 317.06,473; 479.11,734	Flavonoids	Antioxidant, Antityrosinase	[38]
4	Isoprunetin 7-O-glucoside	C22 H22 O10	446.12	7.55	85.02,869; 167.03,365; 242.05,698; 270.05,188; 285.07,523; 286.08,063	Flavonoids	Antioxidant	[39]
5	Quercetin 3,7-dimethyl ether	C17 H14 O7	330.07	10.78	108.02,065; 136.01,524; 145.10,110; 154.02,582; 168.05,684; 242.05,687; 270.05,179; 298.04,642; 331.08,014	Flavonoids	Antioxidant, Anti-inflammatory	[40]
6	Caffeic acid	C9 H8 O4	180.04	5.44	93.07,026; 107.04,932; 117.03,360; 135.04,402; 145.02,834; 163.03,886; 163.11,108; 181.04,929	Phenylpropanoids	Antioxidant	[41]
7	Acteoside	C29 H36 O15	624.20	6.15	135.04,396; 145.02,832; 163.03,883; 301.07,028; 325.09,088	Phenylpropanoid glycoside	Antioxidant, Antiinflamatory, Anticancer, Antidiabetes	[42]
8	4',5,7-trihydroxy-3-methoxyflavone-7-O-β-D-glucopyranoside	C22 H22 O11	462.12	6.58	85.02,868; 167.03,381; 185.04,388; 258.05,200; 283.06,079; 286.04,678; 301.06,992	Flavonoids	TNF-α inhibitory, NO-inhibitory, Antioxidant	[43]
9	2-Amino-3-methyl-1-butanol	C5 H13 N O	103.10	1.11	60.08,125; 87.04,444; 103.96,835; 103.29,041; 104.10,706; 104.24,332	Amino acids	Antibacterial	[44]
10	9-Oxo-10(E),12(E)-octadecadienoic acid	C18 H30 O3	294.22	13.36	93.07,011; 107.08,566; 135.11,673; 167.10,661; 179.14,288; 259.20,508; 277.21,591	Linolenic Acids	Antifungal	[45]
11	Quinic acid	C7 H12 O6	192.06	1.21	76.07,607; 83.04,940; 97.02,857; 111.04,417; 118.08,625; 129.05,453; 157.04,932; 193.15,547	Carboxylic Acids	Antioxidant, tyrosinase inhibitory	[46]
12	Pinoquercetin	C16 H12 O7	316.06	7.69	121.02,842; 123.04,401; 137.02,321; 167.03,374; 243.06,473; 271.05,975; 274.04,678; 317.06,473	Flavonoids	Anti-inflammatory	[47]
13	Chlorogenic acid	C16 H18 O9	354.09	4.69	117.03,359; 135.04,401; 145.02,832; 163.03,882; 181.04,953; 337.09,097; 355.16,983	Carboxylic Acids	Antioxidant, anti-inflammatory, antibacterial, antiviral, hypoglycemic, lipid-lowering, antimutagenic, anticancer, immunomodulatory	[48]
14	Asiatic acid	C30 H48 O5	488.35	13.76	147.11,664; 187.14,783; 201.16,336; 205.15,839; 219.17,413; 389.32,007; 407.32,971; 425.34,073; 453.33,530	Triterpenoid	Anti-inflammatory, antidiabetes, antibacterial,	[49]
15	Verbasoside	C20 H30 O12	462.17	3.79	145.02,821; 163.03,879; 181.04,922; 265.07,068; 325.09,094; 457.13,577; 457.24,188	Phenylpropanoid glycosides	Antioxidant	[50]
16	Isochlorogenic acid A	C25 H24 O12	516.13	7.04	135.04,396; 163.03,883; 145.02,832; 177.05,397; 319.08,084; 337.09,137; 355.11,569; 499.12,192	Carboxylic Acids	Anti-inflammatory, antioxidant	[51]
17	Triethanolamine	C6 H15 N O3	149.11	1.10	68.04,990; 70.06,558; 86.06,024; 88.07,600; 90.90,343; 132.10,179; 150.02,637; 150.11,240	Amino Alcohols	antibacterial and antifungal	[52]
18	Calceolarioside	C23 H26 O11	478.15	6.09	145.02,829; 163.03,882; 165.05,449; 263.09,140; 325.09,094; 307.08,054	Phenylpropanoid glycoside	Anti-inflammatory, analgesic, immunomodulatory, and antioxidant	[53]

Continued

No	Compound	Chemical formula	MW (g/mol)	RT	MS/MS fragmentation	Class	Biological activities	References
19	Phloroglucinol	C6 H6 O3	126.03	1.72	69.03,396; 81.03,385; 85.06,510; 109.02,853; 121.67,186; 127.03,898; 127.04,931	Benzenetriol	Antioxidant	[54]
20	Oleanonic acid	C30 H46 O3	454.34	14.65	409.34,607; 437.34,091; 455.35,559	Triterpenoid	Anti-inflammatory, antioxidant, anticancer, and hepatoprotective	[55]
21	Tetradecylamine	C14 H31 N	213.25	10.68	71.08,598; 85.10,160; 95.04,948; 141.00,037; 158.02,687; 165.44,937; 173.17,258; 214.25,255	Alkylamine	Antibacterial	[56]
22	Quinoline-4-carboxylic acid	C10 H7 N O2	173.05	1.45	83.04,953; 87.04,442; 111.04,423; 128.04,953; 130.06,508; 146.05,997; 174.05,490	Carboxylic acid	Antioxidant, antiviral, Antibacterial	[57]
23	Docosanamide	C22 H42 O4	370.31	17.64	129.05,458; 143.07,036; 147.06,506; 241.17,873; 259.19,016; 268.26,511	Fatty amide	Antibacterial	[58]
24	Oleanolic acid	C30 H48 O3	456.36	15.93	109.10,133; 187.14,787; 189.16,353; 203.17,911; 217.19,481; 393.35,095; 411.36,151; 439.35,754	Terpenoids	Antiviral, antibacterial, antifungal, anticarcinogenic, antidiabetes, anti-inflammatory, antioxidant, hepatoprotective, gastroprotective, hypolipidemic and anti-atherosclerotic	[59]
25	12-Oxo-phytodienoic acid	C18 H28 O3	292.20	12.20	107.08,568; 147.11,667; 149.09,602; 189.11,192; 257.18,942; 275.20,041; 293.21,008	Fatty acid	Antioxidant	[60]
26	Tricin 7-O-glucoside	C23 H24 O12	492.13	6.54	163.03,883; 177.05,449; 288.06,238; 316.05,698; 331.08,038; 339.10,699; 493.20,724	Flavonoids	Anti-inflammatory	[61]
							Antioxidant	[62]
27	Naringenin 7-O-beta-D-glucoside	C21 H22 O10	434.12	5.43	135.04,396; 145.02,826; 151.03,896; 163.03,883; 229.08,530; 273.07,571; 435.16,223; 435.23,578	Flavonoids	Antioxidant, anti-inflammatory	[63]
								[64]
28	Cholestenone	C27 H44 O	384.34	18.72	109.06,495; 123.08,035; 159.11,658; 241.19,556; 255.21,129; 367.33,588; 385.34,558	Steroid	Anti-obesity and lipid-lowering activity, anti-inflammatory	[65]
29	7-Oxodehydroabietic acid	C20 H26 O3	314.19	15.32	95.04,937; 105.07,005; 189.12,711; 255.17,413; 265.15,860; 283.16,913; 311.16,330	Terpenoid	Antibacterial, antiplasmodial	[66]
							anti-inflammatory	[67]
							antioxidant	[68]
							pancreatic lipase inhibitory activity	[69]
30	Ethyl linolenate	C20 H34 O2	306.26	15.17	95.08,578; 109.10,131; 123.11,678; 173.13,179; 243.21,051; 261.22,073; 307.26,273	Fatty Acids	Anti-inflammatory	[70]
							antioxidant	[71]
							cholesterol-lowering activity	[72]
31	1-Stearoylglycerol	C21 H42 O4	358.31	15.82	95.08,573; 97.10,134; 137.13,213; 249.25,748; 267.26,804; 285.27,930; 341.304,011	Fatty alcohol	Antibacterial, antioxidant	[73]
32	Eleostearic acid	C18 H30 O2	278.22	15.65	93.07,016; 123.11,674; 137.13,260; 149.02,319; 173.13,214; 220.17,693; 279.23,145	Fatty Acids	Anti-inflammatory, antidiabetes, triglyceride-lowering activity	[74]

Quadrupole-Orbitrap™ High-Resolution Mass Spectrometer) were employed to assess the phytochemical composition of the extract and fractions. The extract was dissolved in 1 ml MS grade methanol, vortexed for 30 seconds, sonicated for 30 minutes, and filtered through a 0.22 µm filter. MS-grade water (A) and MS-grade methanol (B) were the mobile phases employed in the experiment. Each mobile phase contained 0.1% formic acid. The gradient method was implemented, and the discharge rate was set at 0.3 ml/minute. Initially, the concentration of mobile phase B was modified to 5%, progressively increasing to 90% within 16 minutes. After that, maintain a 90% concentration for 4 minutes before reverting to the initial state of 5% of mobile phase B for 25 minutes. The injection volume was 3 µl, and the column temperature was maintained at 40°C. A comprehensive MS/dd-MS2 collection technique was used for the untargeted screening, incorporating either positive or negative ionization polarities/states. The voltage of spraying was adjusted to 3.30 kilovolts (kV), the capillary temperature was set at 320°C, and the auxiliary gas heater was kept at a temperature of 30°C. In both positive and negative ionization modes, the scan range was conducted from 66.7 to 1,000 m/z, with a resolution of 70,000 for full MS and 17,500 for dd-MS2. Furthermore, the Compound Discoverer 3.2 software from Thermo Scientific™ was employed to evaluate the raw chromatography results. The analysis included the utilization of the two locally stored and online databases, such as ChemSpider (www.chemspider.com) and mzCloud (www.mzcloud.org) [32]. The validation method run in this work related to linearity, limit of detection (LOD), and limit of quantification (LOQ) was explained by Wenzi *et al.* [33] method, which involved ten repetitions to obtain precise data. On the other hand, Aydoğan [34] stated that the quantification value of the acteoside compound was higher than the LOQ and LOD, which were 58 ng/ml and 64 ng/ml, respectively. Meanwhile, other polyphenol compounds had lower quantification values than the LOD and LOQ.

Chemometrics study based on metabolomic analysis

In phytochemical investigations, PCA was employed to distinguish and classify the metabolite compound variable. The chemical compounds in *P. canescens* extracts and fractions can be separated into some principal components with PCA. The chemometrics analysis was conducted using the publicly accessible MetaboAnalyst 6.0 software, which may be accessed at https://www.metaboanalyst.ca. The compound areas derived from untargeted metabolomic analysis were utilized as variables in PCA, and then 32 variables were selected for chemometric analysis. Chemometrics analysis was conducted using six samples, which included EE, FH, FC, FEA, FB, and FA. Thirty-two variables were chosen from the metabolomics analysis to be included in the chemometrics investigation. The Compound Discoverer software applied a filtering process that considered the compound names, preferred the most precise match finds using mzCloud, and considered the DDA fragments for the specified ion. The purpose of the filter was to decrease the number of variables from the initial magnitude. Before chemometric analysis, the data underwent Pareto scaling to ensure optimal variation. PCA was analyzed using a PCA score plot, PCA Biplot, and variable importance of projections value [32].

RESULTS

Yield, total phenolic content (TPC), total flavonoid content (TFC), and total terpenoid content (TTeC) of *P. canescens* leaf extract and fraction

The percentage yield information from *P. canescens* that was extracted with 96% ethanol and subsequently fractionated with *n*-hexane, chloroform, ethyl acetate, *n*-butanol, and aqueous as solvents is presented in Table 1. According to

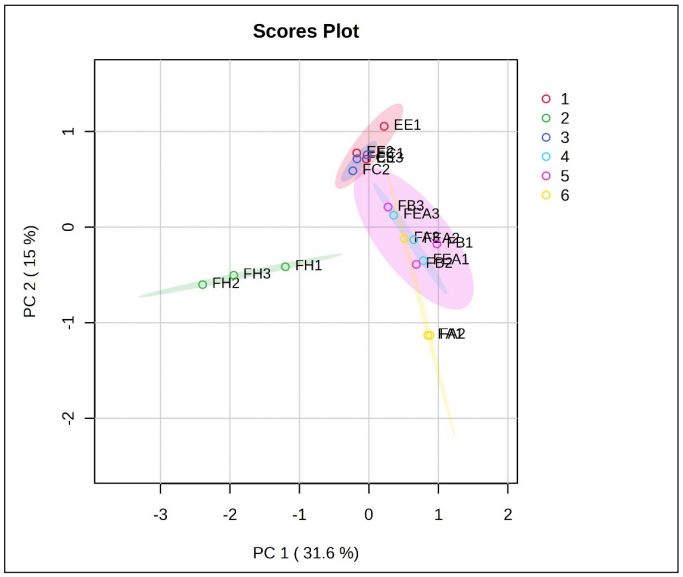

Figure 1. Score plot of PCA based on different extract and fractions of *P. canescens*.

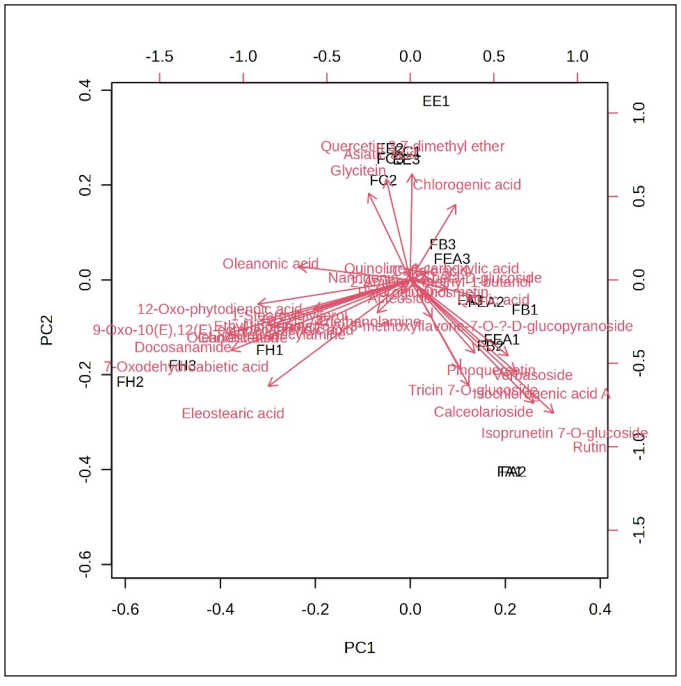

Figure 2. Biplot of PCA of the phytochemical compound of different extract and fractions of *P. canescens*.

Table 1, the fraction using chloroform as the solvent had the highest extraction yield (28.11% ± 0.43%). Instead, the aqueous fractions demonstrated the lowest extraction yield (4.56% ± 0.44%).

In addition, the results of the determination of the total phenolic, flavonoid, and terpenoid compounds are also shown in Table 1. The result of TPC and TFC from extract and fraction of *P. canescens* in this study showed linear results. The highest TPC was shown in the FEA with a value of 250.81 ± 5.68 mg GAE/g, and the highest TFC was also demonstrated in the FEA with a value of 486.58 ± 1.51 mg QE/g. Phytochemicals from the terpenoid group were also determined in this study (Table 1). The TTeC obtained in this study ranged from 110.00 ± 1.88 to 465.14 ± 2.32 mg UAE/g, with the highest total terpenoid content values found in FH and FC (465.14 ± 2.32 and 462.08 ± 3.18, respectively).

Antioxidant activity of *P. canescens* leaf extract and fraction

The results of the antioxidant activity assays of the extract and fractions samples of *P. canescens* in this study are presented in Table 2. The results obtained are shown in IC_{50} values. According to the DPPH free radical scavenging activity analysis, the FEA showed the highest antioxidant activity, with an IC_{50} value of 39.09 ± 0.46 µg/ml. Furthermore, FEA also has

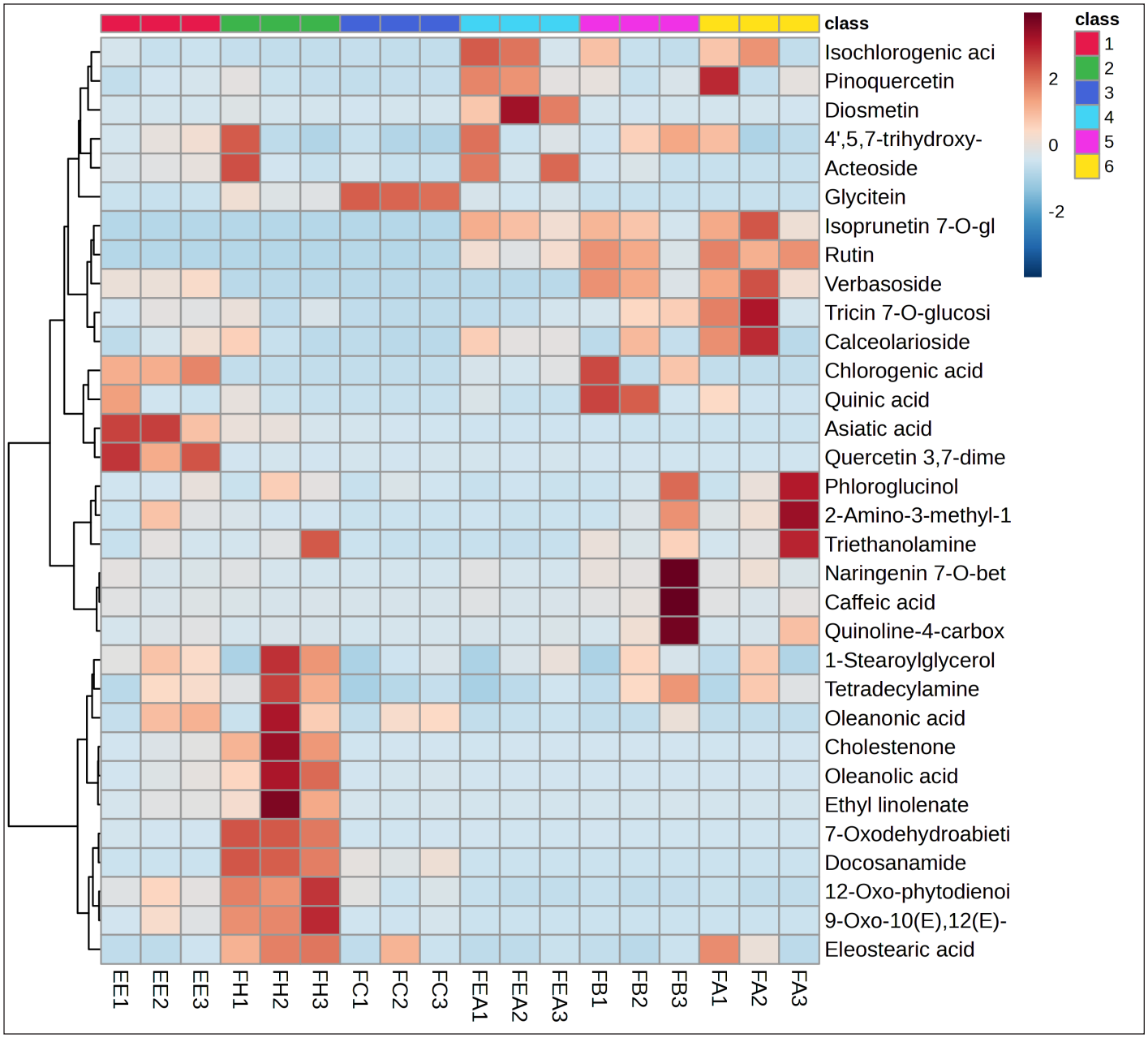

Figure 3. Heat map of top 32 phytochemicals compound correlation with different extract and fraction of *P. canescens* (class 1=Ethanol extract; 2= n-hexane fraction; 3=chloroform fraction; 4= ethyl acetate fraction; 5=n-butanol fraction; 6= aqueous fraction).

the highest ABTS free radical scavenging ability, with an IC_{50} value of 33.85 ± 0.41 µg/ml. The samples of extract and fraction were also evaluated for their ability to reduce power in the redox reaction using the FRAP assays. The results revealed that the FEA had the highest level of reducing activity, measuring 171.22 ± 0.57 mg TE/g.

Pancreatic lipase inhibition assay

The results of this investigation demonstrated that the *n*-hexane fraction exhibited the highest level of inhibition of pancreatic lipase, with a percentage of 42.94%. Furthermore, the EE exhibited moderate inhibition, with a percentage of 24.73%. In contrast, the pancreatic lipase enzyme was not inhibited by the fractions derived from aqueous solvents, *n*-butanol, ethyl acetate, or chloroform, as demonstrated in Table 3.

Principle component analysis of phytochemical constituent

The PCA performed in this study effectively classified the variables that indicate the region of each chemical compound in the extract and fractions (Table 4). The bioactivity of plant extracts and fractions can be affected by chemical compounds. The PCA was used to illustrate the correlation between the amounts of phytochemicals and the bioactivity of extracts and fractions. Figure 1 illustrates the score plot of PCA for evaluating 32 chemical compounds in extract and fraction samples with various solvents. Based on the data set obtained, the first principal component identified 31.6% of the total variation in the data set, while the second principal component represented 15.0%. Together, these two components represented a considerable variable region, 46.60% of the data set.

The investigation results, as illustrated by the Biplot and Score Plot PCA (Figs. 1 and 2), indicate that FH has a significant amount of chemical components classified as terpenoid and fatty acid compounds. In the interim, flavonoid compounds dominate in fractions that contain semi-polar to polar solvents, specifically the FEA and FB groups. Furthermore, the Hierarchical Clustering Heatmaps software application by MetaboAnalyst 6.0 was employed to visualize the clustering of multivariate data. The highest or lowest value in the data set is represented by each colored cell on the map, as illustrated in Figure 3.

DISCUSSION

Yield, TPC, TFC, and TTeC of *P. canescens* Jack. leaf extract and fraction

The phytochemicals are extracted from plants through one series of processes, such as milling, grinding, homogenizing, and extraction. The primary technique employed to extract and separate phytochemicals from plant sources is extraction [75]. The effectiveness of an extraction is influenced by the chemical composition of the plant, which specifies the selection of extraction techniques, sample particle size, type of solvent, and the presence of interfering compounds. The extraction yield can be affected by various factors such as solvent polarity, pH, temperature, extraction time, and sample composition [76]. In this study, the extraction method used was UAE. UAE is an extraction method that uses ultrasonic waves. With the help of the kinetic energy of the ultrasonic wave motion, the cell wall membrane will break, and chemical compounds will be mass-diffused into the solvent [77]. Ultrasonic frequencies range from 18 to 40 kHz, effectively extracting bioactive compounds from plant materials, including compounds with antioxidant potential [78]. Therefore, this study used an ultrasonic frequency of 20 kHz to extract bioactive compounds from *P. canescens* leaves. The optimal time required for extraction is an important factor in determining each plant extract antioxidant capacity, TPC, and TFC. Several research results state that with the UAE method, the optimal extraction time to obtain the best antioxidant activity, TPC, and TFC is 10 minutes [79], 20–120 minutes [80], 0–30 minutes [81], and 60 minutes [82]. Yim *et al.*[83] stated that an extraction time longer than the maximum limit required will cause a decrease in the antioxidant potential and polyphenol content of plant extracts. Due to the interaction of phenol with other plant components, the extraction process of phenol compounds is hampered. The extraction method of *P. canescens* leaves can be optimized for further research by shortening the extraction time to 30–60 minutes and using a combination of ultrasound and microwave-assisted extraction methods. This method combines microwaves and ultrasounds so that when high momentum and energy are applied, the ruptured plant cells will release more metabolite compounds in a shorter time.

This study used various solvents to fractionate the ethanolic crude extract of *P. canescens*. The different polarities of the solvent were applied during extraction to get the desired metabolites. Solvents with different polarities are expected to obtain different metabolites in each fraction. Polar solvents such as methanol, ethanol, and water form hydrogen bonds with other molecules. Higher phenolic compounds were extracted using a more polar solvent [84]. Some plant metabolites have hydrophobic properties, and these compounds can only be extracted by non-polar solvents [85]. For example, in a previous study, sesquiterpene artemisinin, which has highly active antimalarial activity, was extracted using hexane [86].

The results obtained in this study showed that the percentage yield of the 96% EE was 8.31%. Compared to the results obtained by Fadlilaturrahmah *et al.* [87] (yield of 7.28%), which also used 96% ethanol as a solvent, the percentage yield in this study was higher. It may be due to differences in extraction methods and the location of origin of *P. canescens* leaves. Fadlilaturrahmah *et al.* [87] obtained extracts from the maceration method, and the location of origin of *P. canescens* leaves were from the province of South Kalimantan. Differences in the geographical location of the plant origin determine the concentration and type of metabolites contained in the plant. Plants growing in areas with geographical conditions with high ecological pressure will produce more metabolite compounds than those growing in nutrient-rich areas [31,88]. Benhssain *et al.* [89] in their research stated that the results of extract yield, polyphenol, flavonoid, and tannin content of a plant are highly dependent on the geographical, bioclimatic, and edaphic conditions at each location where the plant was sampled.

Next, the raw EE (80 g) performed liquid-liquid partitioning using a series of solvents with progressively higher polarities. Thus, chemical compounds are separated based on

their affinity for the solvent [90]. In this study, the fraction using a semi-polar solvent, ethyl acetate, showed linear results for total phenolic and flavonoid compounds. Meanwhile, the fraction with non-polar solvents, specifically *n*-hexane and chloroform, produced the highest quantity of terpenoid compound. The linear correlation between the total phenolic and total flavonoid contents of *P. canescens* suggests that the predominant phenolic compounds present are semi-polar to polar flavonoids.

Furthermore, the ethyl acetate solvent used in the fractionation technique significantly attracts the flavonoid compounds in the crude EE [91]. Similar to the findings of Muharni *et al.* [92], the present investigation confirms that the ethyl acetate fraction of *P. canescens* exhibited the most significant levels of total phenolic and flavonoid content compared to the hexane and methanol fractions. However, the total content of phenolic and flavonoids obtained by the current study exceeded the results of Muharni *et al.* [92]. It could be due to differences in extraction and fractionation methods and the source of *P. canescens* used.

Among all of the extracts and fractions of *P. canescens* investigated in the current study, FH and FC showed the highest content of chemical compounds in the terpenoid group. The results obtained from the present study are consistent with those of Juswardi *et al.* [16]. Some main chemicals are found in young, mature, and old *P. canescens* are carbohydrates, fatty acids, steroids, and terpenoids. The chemical substances mentioned are methyl stearate, resibufogenin, hexadecanoic acid, pregnan-20-one, 3-(acetyloxy)-5,6:16, 17-diepoxy-, and butyl 4,7,10,13,16,19-docosahexaenoate.

Antioxidant activity of *P. canescens* leaf extract and fraction

The IC_{50} value of a substance is inversely proportional to its antioxidant activity. This value demonstrated the quantity of antioxidants necessary to reduce the concentration of DPPH by 50%. The resultant value is calculated by interpolating using linear regression analysis. Chemical compounds with lower IC_{50} values demonstrate stronger antioxidant activity [93]. The antioxidant activity of the crude extract and fractions of *P. canescens* is examined in this research using a variety of assays, including DPPH, ABTS, and FRAP. Several *in vitro* assays were developed to quantify the effectiveness of antioxidants in plant and food samples. It has been established that various testing methods are required to comprehensively evaluate the antioxidant capacity of a sample against various sources of free radicals. Various assays differ in their fundamental principles and experimental conditions [94].

Among all the assessment methods, the FEA had the highest antioxidant activity. The antioxidant activity of the fraction is attributed to its high concentration of phenolic and flavonoid compounds. The results align with prior research showing a linear association between the quantity of polyphenols and their ability to act as antioxidants [95]. Following Ait Chaouche *et al.* [96], the presence of phenolic compounds is helpful as a key indicator of the antioxidant capability of plants. The plant predominantly comprises phenolic and flavonoid compounds, renowned for their potent antioxidant properties [97]. The chemical structure of phenolic and flavonoid compounds, particularly the benzene ring, and the quantity and placement of -OH functional groups are accountable for their antioxidant activity. The mechanism of phenolics and flavonoids as antioxidants can be through radical scavenging, metal chelation, or enzyme inhibition [98,99].

Antioxidant free radicals and a non-radical substrate were obtained by donating the H-atom from the phenolic compound to the free radical substrate. For example, gallic acid. Its benzene ring stabilizes the radicals due to the resonance effects, while the -OH functional group on the phenolic compound contributes to forming antioxidant free radicals [99]. Another mechanism of phenolic and flavonoid compounds as antioxidants is metal chelation. The phenolics and flavonoids chelate with the metal ions, such as copper or iron. The flavonoid or phenolic compounds can inhibit the reduction of metal ions, consequently preventing reactive oxygen species (ROS) formation [98,99]. Xanthine Oxidase is an example of an enzyme in the body that needs to be inhibited because it can directly or indirectly produce ROS in the body. This enzyme has been reported to be inhibited by flavonoids [100].

The research results of this study accord with the findings stated by Muharni *et al.* [92], which suggests that the fraction with ethyl acetate as a solvent exhibits the strongest antioxidant activity among the other fractions examined, with an IC_{50} value of 320 ug/ml. Many more studies have performed antioxidant examinations on extracts derived from *P. canescens*. Pindan *et al.* [8] investigated the capacity of antioxidants of *P. canescens* by employing different solvents for the fractionation process. The values of the IC_{50} for the crude extract of ethanol were determined to be 25,549 ppm; for the *n*-hexane fraction, it was 607,475 ppm; for the ethyl acetate fraction, 12,986 ppm; and for the remains of the ethanol fraction, it was 15,766 ppm [8]. The differences in the findings of this investigation compared to other studies may be due to many factors, such as variations in secondary metabolite content, solvents used for extraction, and extract composition [101]. The extraction procedures and conditions also affect the antioxidant activity, such as temperature and time [102].

In this study, as positive controls, the antioxidants used were Ascorbic acid and Trolox. The IC_{50} values produced by Ascorbic acid and Trolox were 16.08 ± 0.46 and 13.40 ± 0.26, respectively. This IC_{50} value is higher than the IC_{50} value of ascorbic acid produced in the study by Irnameria and Okfrianti [103], which was 5,440 ppm. However, the results of these two positive control assays are still within the range of very strong antioxidant activity (IC_{50} <50 ppm) [104]. The difference in IC_{50} values from the ascorbic acid standard in this study can be caused by several environmental factors during testing, including temperature, humidity, and light exposure that affect the stability of the ascorbic acid molecular structure [105].

Pancreatic lipase inhibition assay

Pancreatic lipase is an enzyme that has an essential function in absorbing triacylglycerols from the diet. This enzyme causes the breakdown of triacylglycerol into 2-monoacylglycerol and fatty acids. It has been established that the intestinal tract cannot directly absorb fat from the diet before interacting with pancreatic lipase [106]. Several natural compounds, such as terpenoids, tannins, and saponins,

can inhibit intestinal lipase, preventing dietary fat breakdown. Consequently, inhibiting pancreatic lipase activity by natural compounds would limit the decomposition of dietary lipids, reducing the incidence of hypercholesterolaemia [107].

The measurement results accord with the elevated total terpenoid level obtained in the *n*-hexane fraction. The *n*-hexane fraction, known for its abundant terpenoid content, is likely to contain specific chemicals that exhibit an inhibitory effect against pancreatic lipase. Multiple research findings have indicated that the substances oleanolic acids (triterpenes) and 7-Oxodehydroabietic acid (diterpenes) had a potent ability to hinder the activity of the enzyme pancreatic lipase [108].

Principle component analysis of phytochemical constituent

Identifying active compounds in herbal plant extracts and fractions is essential to determining a suitable application technique [109]. This research employed the UHPLC-HRMS combined with chemometric analysis to perform untargeted metabolomics profiling and classify the chemical compounds of *P. canescens* extract and fractions. Chemometrics is an effective statistical technique widely applied to analyze secondary metabolites from plants. Identifying bioactive compounds derived from untargeted metabolomic analysis helps obtain specific compounds in each treatment group [110].

Based on the score plot PCA, the *n*-hexane fraction of *P. canescens* is separated from other fractions by PC1 (Fig. 1). Meanwhile, the chloroform fraction can be separated from the FEA, FB, and FA by PC2 but not separated from EE. Fractions with medium polarity (FEA and FB) to high polarity (FA) do not separate. This indicates that some of the same compounds are present in the three fractions. The classification results with this score plot PCA are linear with the results of determining the content of total phenolic and total flavonoid compounds (Table 1), which shows that FEA, FB, and FA contain significantly higher amounts of phenolic and flavonoid compounds compared to FH and FC. Likewise, the antioxidant activity of FEA, FB, and FA produces strong to very strong antioxidant activity. This antioxidant activity results from the high phenolic and flavonoid compounds in the three fractions. Pindan *et al.*[8] in their study also stated that FEA from *P. canescens* has potent antioxidant activity and contains alkane, alkene, alcohol, and fatty acid compounds based on identification using GC-MS analysis. In addition, the results score plot PCA also shows that fraction with low polarity (FH) has linearity when determining the content of terpenoid compounds. This is in line with its activity, namely pancreatic lipase inhibition activity. Therefore, the chemical compounds separated and contained in FH may be the compounds responsible for pancreatic lipase inhibition activity. This clearly shows that the active compounds made from an extract or fraction are greatly affected by the type of plant and the polarity of the solvent [2]. This study, based on the PCA Biplot (Fig. 2) shows that FH contains several specific compounds that are well separated from other fractions, including compounds 7-Oxodehydroabietic acid, oleanonic acid, oleanolic acid, docosanamide, 9-Oxo-10(E), 12(E)-octadecadienoic acid, eleostearic acid, 1-stearoylglycerol, ethyl linolenate, and 12-Oxo-phytodienoic acid. While FEA, FB, and FA contain several of the same compounds and are not clearly separated, these compounds are grouped in one quadrant near the coordinate point 0.0. The chemical compound in FH was a compound from the terpenoid and fatty acid groups. Several research results confirm that terpenoid compounds have lipase-inhibitory activity [107].

Sosnowska *et al.* [111] reported in their study that strawberry and raspberry fruit extracts, which have high fatty acid concentrations, strongly inhibit the effect on pancreatic lipase enzymes. Fatty acid components that bind to bile acids can inhibit pancreatic lipase enzymes. The binding of fatty acids to bile acids will affect the surface properties of the substrate emulsion so that lipase cannot bind to the substrate and will be absorbed in the oil-water interface [112].

Furthermore, analysis using PCA also showed the clustering results of compounds by the heatmap of the top 32 phytochemical compounds (Fig. 3); it shows that diosmetin (trihydroxyflavone group) and pinoquercetin (pentahydroxyflavone group) compounds are found in high amounts in FEA. These findings are consistent with the investigation conducted by Latief *et al.* [18], which states that flavonoid compounds (5.7 dihydroxy isoflavones) can be isolated from the ethyl acetate extract of *P. canescens*. Flavonoid compounds are a class renowned for their decisive antioxidant action. Various mechanisms, such as the direct scavenging of ROS, the stimulation of antioxidant enzymes, the enhancement of metal chelation activity, the elevation of α-tocopherol radicals, the suppression of NADPH oxidase, the alleviation of oxidative stress induced by NO, the augmentation of uric acid levels, and the reinforcement of the antioxidant capacity of small-molecule antioxidants, are used by flavonoids to exhibit antioxidant activity *in vitro* [113]. Flavonoids with trihydroxyflavone and pentahydroxyflavone functional groups work as antioxidants by directly capturing reactive oxygen by structural flavonoid molecules [114].

CONCLUSION

The study presented that the ethyl acetate fractions of *P. canescens* had the highest amounts of phenolic and flavonoid compounds and produced very strong antioxidant activity. The compounds diosmetin and pinoquercetin were identified as compounds that may contribute to the antioxidant activity of the ethyl acetate fraction of *P. canescens*. In addition, the *n*-hexane fraction showed significant terpenoid content and a strong inhibitory effect on the lipase enzyme. The compounds 7-Oxodehydroabietic acid, oleanonic acid, oleanolic acid, docosanamide, 9-Oxo-10 (E), 12 (E) -octadecadienoic acid, eleostearic acid, 1-stearoylglycerol, ethyl linolenate, and 12-oxo-phytodienoic acid have been identified from the *n*-hexane fraction as compounds that play an important role in pancreatic lipase inhibitory activity. Further research to determine the correlation between the amount of active compound content and the bioactivity of the extract and fraction of *P. canescens* leaves can be done to complement this study's limitations. Enzyme kinetics analysis and molecular mechanisms of bioactive compounds can be used to validate the data obtained in this study. In addition, further research using experimental animals and optimization of safer solvents will provide more comprehensive bioactivity information and increase our knowledge of ethnomedicine based on *P. canescens* herbal

preparations. The alternative solvents to replace *n*-hexane and chloroform that are less toxic suggested the use of bio-based solvents, such as sunflower oil, as a substitute for *n*-hexane and dimethyl carbonate as an alternative to chloroform replacement.

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AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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CONFLICT OF INTEREST

We officially affirm that no conflicts of interest arise from any financial, personal, or other affiliations with individuals or organizations related to the subject matter addressed in the journal.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

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SUPPLEMENTARY MATERIAL

The supplementary material can be accessed at the link here: [https://japsonline.com/admin/php/uploadss/4515_pdf.pdf]