HAYATI
Journal of Biosciences
Vol. 33 No. 1, January 2026 153-166
DOI:10.4308/hjb.33.1.153-166
ISSN: 1978-3019
EISSN: 2086-4094

Research Article

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Anti-glucosidase Activity and Antioxidant Capacity of *Premna serratifolia* from Sintang, Kapuas Hulu, and Sambas District, West Kalimantan, Indonesia

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ARTICLE INFO

Article history:
Received February 27, 2025
Received in revised form July 19, 2025
Accepted September 4, 2025
Available Online October 16, 2025

KEYWORDS:
Antioxidant,
α-glucosidase activity,
Coumpond,
Functional Group,
Premna serratifolia

ABSTRACT

The secondary metabolites in plants such as *Premna serratifolia* (*P. serratifolia*) are influenced by their growing locations, which in turn affects their bioactivity. This study aimed to compare the antioxidant activity and α-glucosidase inhibition of *P. serratifolia* leaf extracts from the Sintang, Kapuas Hulu, and Sambas districts in West Kalimantan. Antioxidant capacity was evaluated using the DPPH method, while the reduction of α-glucosidase activity was assessed using the PNPG method. Functional groups and active compounds were identified using FTIR and UHPLC-Q-Orbitrap HRMS. The findings indicated that the extract from Kapuas Hulu, obtained through maceration (KHM), exhibited the highest antioxidant activity (IC_{50} 18.39) and contained the most total phenolic content (TPC). The best α-glucosidase inhibition activity (IC_{50} 4194.14) was found in the extract from Sambas obtained through Soxhlet extraction (SSI), which had the highest total flavonoid content (TFC). Principal Component Analysis (PCA) revealed that the functional groups and active compounds of *P. serratifolia* from Kapuas Hulu and Sintang were similar, as both regions are located in eastern West Kalimantan. Additionally, Partial Least Squares (PLS) analysis revealed that the C-H alkane, O-H, and isoferulic acid were the most influential compounds in determining antioxidant activity. At the same time, the C=O group and NP-000308 predominantly influenced α-glucosidase inhibition.

1. Introduction

West Kalimantan, one of the provinces in Indonesia located at 2°05'N-3°05'S and 108°30'-114°10'E, is home to more than 208 medicinal plants, divided across coastal and archipelago regions, inland areas, and international borders (Figure 1). Sambas Regency, located in the coastal region bordering the South China Sea, is the northernmost part of West Kalimantan. In contrast, Kapuas Hulu and Sintang Regencies, located in the inland area bordering Malaysia, are intersected by the Kapuas River in the eastern part of the province (Department of Communication and Information of West Kalimantan Province 2024). Medicinal plants growing in these regions produce distinct secondary metabolites due to topographical factors, such as altitude (Wen *et al*. 2020), which influence their bioactivity. For instance, *Cydonia*

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Figure 1. Location of sampling areas of *P. serratifolia* in the province of West Kalimantan 1. Sambas, 2. Sintang, and 3. Kapuas Hulu district

oblonga, *Tuber indicum*, and *Coffea arabica* from Santa Lucia, Yunnan, and Brazil demonstrate higher antioxidant activity than those from other regions (Li *et al*. 2019; Baroni *et al*. 2022; Liao *et al*. 2022). Significant α-glucosidase inhibition activity has been observed in *Andrographis paniculata* from the Bogor area (Rafi *et al*. 2021). One notable medicinal plant from West Kalimantan known for its antioxidant and α-glucosidase inhibitory properties is Buas-buas (*Premna serratifolia*).

The antioxidant properties of *P. serratifolia* leaves have been reported by many researchers (Puspita *et al*. 2020; Simamora *et al*. 2020; Hadiarti and Qurbaniah 2022; Lolita 2022). *P. serratifolia* leaves from Pontianak, obtained through maceration, successfully reduced 50% of DPPH free radicals at a concentration of 35 µg/mL (Hadiarti and Qurbaniah 2022). Infusion and decoction extracts of *P. serratifolia* leaves from Central Sulawesi exhibited free radical protection with IC_{50} values of 6.82 and 7.28 µg/mL, respectively (Timotius *et al*. 2018). Meanwhile, ethanol and its macerate demonstrated IC_{50} values of 50.63 and 66.83 µg/mL, respectively (Simamora *et al*. 2020). *P. serratifolia* leaves collected from Rasau Jaya, Kubu Raya, produced oxidative stress defense with IC_{50} values of 532.24 µg/mL and 24.40 µg/mL when soaked in wasbenzene and water, respectively (Isnindar *et al*. 2016). Additionally, leaves subjected to Soxhlet extraction with 80% ethanol from Sungai Raya Subdistrict in the same regency exhibited antioxidant efficacy with an IC_{50} value of 63.93 mg/mL (Purwanti *et al*. 2018). In another study, *P. serratifolia* leaves from Melawi Regency macerated with ethanol demonstrated an IC_{50} value of 20.66 µg/mL (Puspita *et al*. 2020). Soxhlet extraction of *P. serratifolia* from Western Ghat, India, showed DPPH radical reduction at an IC_{50} concentration of 101.20 µg/mL (Selvam *et al*. 2012). Dried leaves of *P. serratifolia* from Chikkaballapura district, Karnataka, India, extracted with chloroform using Soxhlet, yielded an IC_{50} value of 219.08 µg/mL (Lolita 2022). Moreover, leaves collected from Universiti Putra Malaysia in Bintulu, Sarawak, showed 73.5% free radical inhibition (Chua *et al*. 2015).

Aside from antioxidant properties, *P. serratifolia* has also been proven to inhibit α-glucosidase (Hadiarti 2017; Timotius *et al*. 2018; Simamora *et al*. 2020; Hadiarti *et al*. 2021, 2023; Fitriarni *et al*. 2022). For example, leaves collected from Central Sulawesi and macerated with ethanol and water exhibited IC_{50} values of 151.91 and 558.15 µg/mL, respectively (Timotius *et al*. 2018). However, extraction through percolation

and decoction with water resulted in lower IC_{50} values of 4.27 and 0.046 µg Gallic Acid Equivalent/mL, respectively (Simamora *et al*. 2020). Soxhlet extraction of *P. serratifolia* from Pontianak demonstrated 91.03% inhibition against α-glucosidase at a concentration of 2% (Hadiarti 2017). The α-glucosidase inhibition activity produced by ethanol extract and ethyl acetate fraction of *P. serratifolia* from Pontianak reached 77.63 and 50.915 mmol Acarbose Equivalent per 100 g (Hadiarti *et al*. 2021, 2023). Extract from Pelang Village, Ketapang Regency, boiled in water, reduced 50% α-glucosidase suppression at a concentration of 77.80 µg/mL (Fitriarni *et al*. 2022).

Although these studies demonstrate the antioxidant activity and anti-α-glucosidase enzyme activity of *P. serratifolia* from various regions using different extraction methods and solvents, there is limited research comparing the chemical fingerprint and bioactivity of *P. serratifolia* grown specifically in West Kalimantan, Indonesia. Fourier-transform infrared (FTIR) and Ultra-High Performance Liquid Chromatography (UHPLC) are necessary for fingerprint analysis to evaluate the quality of *P. serratifolia* extract raw materials before they are developed into Standardized Herbal Medicine (OHT). Developing OHT poses a challenge due to difficulties in controlling the active compounds within raw materials in terms of quality and quantity (Irwan and Junaidi 2020; Karimi *et al.* 2020; Wen *et al*. 2020). Therefore, this study compares the radical scavenging ability and α-glucosidase inhibition activity of *P. serratifolia* obtained from the Sintang, Kapuas Hulu, and Sambas districts in West Kalimantan.

2. Materials and Methods

2.1. Preparation of *P. serratifolia*

The study collected *P. serratifolia* leaves from three districts in West Kalimantan Province: Kapuas Hulu, Sintang, and Sambas. The plant specimens were identified by botanists at Universitas Muhammadiyah Pontianak. The leaf extracts were obtained using ethanol pro-analysis through maceration with a ratio of 1:3 and Soxhlet extraction temperature of 60°C of the dried leaves. The extracts were preserved in a refrigerator freezer.

2.2. Photochemistry Quantitative Analysis

Qualitative phytochemical identification of the extracts was conducted following a previously established methodology (Rubianti *et al.* 2022). The total phytochemical content was determined for flavonoids (TFC) and phenolics (TPC). TFC was measured using the $AlCl_3$ method, while TPC was assessed using the Folin-Ciocalteu method. The measurements were conducted using a UV-Vis spectrophotometer, with quercetin (TFC) and gallic acid (TPC) as standard references. The results were expressed in quercetin equivalent (QE) and gallic acid equivalent (GAE) per 100 g of *P. serratifolia* extract (Hadiarti *et al*. 2021).

2.3. The Functional Group Analysis with FTIR

The functional group analysis was performed on *P. serratifolia* extract using FTIR (Shimadzu IR Prestige-21) in triplicate for each extract. To prepare for analysis, 2 mg of the extract was blended with 180 mg of KBr and compressed at a pressure of 8 tons for 15 minutes to form a pellet. The plate was then analyzed three times for each extract within the wavelength range of 4000-400 cm^{-1}, with a resolution of 4 cm^{-1}, and at a scanning rate of 45 scans per minute (Hadiarti *et al*. 2021).

2.4. Identified Compound by UHPLC HRMS

The UHPLC-Q-Orbitrap HRMS (ThermoScientific) analysis was conducted by weighing 10 mg of *P. serratifolia* extract, dissolving it in 4 mL of methanol: water (50:50), sonicated for 30 minutes, and left overnight at room temperature. Subsequently, the dissolved isolate is filtered with a 0.2 µm PTFE membrane. A 1 µL aliquot of each sample filtrate is injected into the UHPLC-Q-Orbitrap HRMS. The instrument used in this study is a UPLC Vanquish Tandem Q Exactive Plus Orbitrap HRMS ThermoScientific with an m/z range of 50-4000 (García-Reyes *et al*. 2017). The ESI parameters include a source voltage of +4.5 kV, a capillary temperature of 200ºC, and a nebulizer gas flow rate of 1.5 L/min. The mass spectrometer operates in positive and negative ion modes with an m/z range of 200 to 2000. The column used is an Accucore C18, 100×2.1 mm, 1.5 µm (ThermoScientific) with a column temperature set at 30°C. The mobile phase consists of a binary solvent system comprising H_2O + 0.1% formic acid (A) and acetonitrile + 0.1% formic acid (B). The gradient method for these solvents is as follows: 0-1 minute (5% B), 1-25 minutes (5-95% B), 25-28 minutes (95% B), and 28-30 minutes (5% B). The solvent is injected with a volume of 5 µL and a flow rate of 0.2 mL/min. Data interpretation, including spectrum selection, retention time (tR) alignment, and comparison with scientific databases, is

processed by Compound Discoverer version 2.2 (Farooq *et al*. 2020; Rafi *et al*. 2021; Umar *et al*. 2021).

2.5. Evaluation of α-Glucosidase Activity Reduction

The inactivation of α-glucosidase activity was assessed using acarbose as a standard control and PNPG as the substrate. *P. serratifolia* extract (10 µL from 30 mg/mL), phosphate buffer (50 µL), α-glucosidase (25 µL) in phosphate buffer (pH 7), and PNPG (25 µL) were mixed with the help of a vortexer. A blank was prepared with the extract or fraction solution in all reagents without adding the enzyme and incubated at 37°C for 30 minutes. Then, 15 µL of 0.2 M sodium carbonate was added to stop the reaction, and the absorbance was measured with a spectrophotometer at λ = 400 nm to determine the absorbance. The inhibition activity was expressed using the IC_{50} value, which was calculated by linear regression analysis of the inhibition percentage versus the sample concentration curve. (Hadiarti *et al*. 2023).

2.6. Analysis of Antioxidant Activity

The assessment of antioxidant activity was executed by introducing 100 µL of the extract solution to 0.5 mL of the DPPH solution. Ascorbic acid, as the DPPH standard solution, was prepared by dissolving 4 mg in 10 mL of ethanol, and its absorbance was quantified at a wavelength of 517 nm. The mixture was then incubated at 37 °C for 30 minutes, and the absorbance was measured at 517 nm using a *UV-Vis* spectrophotometer (Hadiarti and Qurbaniah 2022).

2.7. Data Analysis

One-way ANOVA and Tukey's test ($p<0.05$) were employed to appraise the dissimilarities in yield data, TPC, TFC, inactivation of α-glucosidase, and antioxidant activity. PCA was used to examine the differences and similarities in functional groups of *P. serratifolia* extracts obtained through maceration and Soxhlet extraction from three districts: Sintang, Kapuas Hulu, and Sambas. Meanwhile, PLS validated by the cross-validation method was employed to identify the functional groups contributing to radical scavenging ability and inhibiting α-glucosidase. All data analysis steps were conducted using The Unscrambler X software (Sajak *et al*. 2016; Umar *et al*. 2021).

3. Results

3.1. Yield Extraction of *P. serratifolia* Leaves

The extraction of *P. serratifolia* from the Kapuas Hulu, Sintang, and Sambas districts was conducted using maceration and Soxhlet extraction with ethanol pro-analysis solvent. As shown in Table 1, Soxhlet extraction yielded the highest efficiency compared to maceration.

3.2. Phenolic, Flavonoid, Antioxidant, and α-Glucosidase Inhibition Activity in *P. serratifolia*

Table 2 shows the TPC, TFC, and IC_{50} of antioxidant and α-glucosidase inhibitory activity of *P. serratifolia* leaves from the Kapuas Hulu, Sintang, and Sambas districts. The extract obtained from maceration in Kapuas Hulu (KHM) exhibited the highest total phenolic content

Table 1. Yield extraction of *P. serratifolia* leaves from West Kalimantan

Area code	District	Coordinate	Collection time	Season	Extraction method	Sample code	Yield (%)
2	Sintang	0°4′26″S	Juny 2023	Dry	Maceration	STM	7.064
		111°29′51″E			Soxhlation	STI	15.678
	Kapuas	0°28′23″S	Juny 2023	Dry	Maceration	KHM	6.223
3	Hulu	112°42′9″E			Soxhlation	KHI	20.562
1	Sambas	1°12′05″S	July 2023	Dry	Maceration	SSM	7.645
		109°6′46″E			Soxhlation	SSI	23.011

Table 2. TPC, TFC, IC_{50} of antioxidant and α-glucosidase inhibitory of *P. serratifolia* leaves

Sample code	Total of phenolic content (mg GAE/g)	Total of flavonoid content (mg QE/g)	IC_{50} (mg/ml extract)	
			Antioxidant (DPPH)	α-glucosidase inhibitory
STM	68.75	13.63	59.30	8828.66
STI	45.85	10.79	74.79	10312.45
KHM	141.78	14.59	18.39	9650.71
KHI	43.01	8.21	52.16	8739.43
SSM	74.68	12.68	53.79	5486.80
SSI	82.89	15.23	45.52	4194.14

(TPC), while the Soxhlet extract from Sambas (SSI) had the highest total flavonoid content (TFC).

3.3. Identified Functional Groups of *P. serratifolia* From Different Districts

The FTIR analysis identified eight functional groups in *P. serratifolia* extracts, as shown in Table 3 and Figure 2A and B.

3.4. The PCA of FTIR Spectra of *P. serratifolia* Extract

Principal Component Analysis (PCA) of the functional groups in the extracts from Kapuas Hulu, Sintang, and Sambas revealed distinct clusters (Figure 3).

3.5. PLS Analysis of Identified Functional Groups of *P. serratifolia* vs Antioxidant Activity and α-glucosidase Inhibitory Potential

According to the PLS analysis depicted in Figure 4, peaks A and D, representing C-H alkanes and O-H stretching, respectively, make significant contributions to antioxidant activity, with regression coefficients of -0.038 and -0.036. Additionally, C=O, O-H bending, C-O-C ring, C-O-H, and C=C aromatic groups also influence antioxidant activity, with regression coefficients ranging from -0.026 to -0.003. The functional group with the least impact on antioxidant activity is peak B, representing =C-H alkenes, with a stretching vibration mode and a regression coefficient of 1.014. In contrast to the findings shown in Figure 5, the C=O group is the most effective in inhibiting α-glucosidase, with a regression coefficient of -18.881. Additionally, C-H alkanes, O-H stretching and bending, as well as C-O-H (represented by A, E, G, and D, respectively), also contribute to α-glucosidase inhibition. In contrast, the C-H alkenes show a positive value, with a regression coefficient of 8.139, indicating the least significant effect on α-glucosidase inhibition. The antioxidant model achieved an R^2 of 0.735 and an RMSE of 9.64, while the α-glucosidase inhibition model had an R^2 of 0.723 and an RMSE of 1371.12.

3.6. Tentative Identification of Compounds in *P. serratifolia* of Diverse Locations

According to Table 4, thirteen flavonoid and phenolic compounds were successfully identified from *P. serratifolia* extracts based on UHPLC-Q-Orbitrap HRMS analysis, as shown in Figure 6.

3.7. The PCA Plot of The Identified Compound of *P. serratifolia* Extract

The similarities and differences in the compounds identified using UPLC-Q-Orbitrap HRMS from *P. serratifolia* extracts from Sintang, Kapuas Hulu, and Sambas regions are illustrated in the PCA plot. Figure 7 shows a clear separation of compounds based on geographic origin.

3.8. The PLS Analysis of Tentative Identification of Compounds of *P. serratifolia* vs Antioxidant Activity and α-glucosidase Inhibitory Potential

According to Figure 8, isoferulic acid is the phenolic compound in the *P. serratifolia* extract that predominantly influences antioxidant activity, with a regression coefficient of -1.5603e-07. Additionally, NP-015559, salicylic acid, and isorhamnetin are identified as contributing to free radical reduction, with regression coefficients of -7.6161e-08, -5.5525e-08, and -5.2107e-08, respectively. The compound with the least impact on antioxidant activity is apigenin, represented by peak E, with a stretching vibration mode and a regression coefficient of 1.2425e-07. Based on the data shown in Figure 9, the most efficient flavonoid in reducing α-glucosidase concentration is NP-000308, which has a regression coefficient of -3.9533. Isorhamnetin also plays a significant role as an α-glucosidase inhibitor,

Table 3. Identified functional groups from *P. serratifolia* extract

Functional group	Reference wavenumber (cm^{-1})	Detected wavenumber (cm^{-1})	Vibration mode	Code	STM	STI	KHM	KHI	SSM	SSI
C-H alkanes	2990 -2850	2939	Stretching	A	√	√	√	√		√
=C-H alkenes	995-685	879	Bending	B	√	√	√	√		
C=C aromatic	1625-1440	1512	Stretching	C	√	√	√	√		
O-H	3550-3200	3410-3201	Stretching	D	√	√	√	√		√
O-H	1390-1310	1388-1334	Bending	E	√	√	√	√	√	√
C=O	1820-1640	1645-1635	Stretching	F		√	√		√	√
C-O-H	1045-1087	1257	Stretching	G	√	√	√	√	√	√
C-O-C ring	1100-1170	1165	Stretching	H	√	√	√		√	

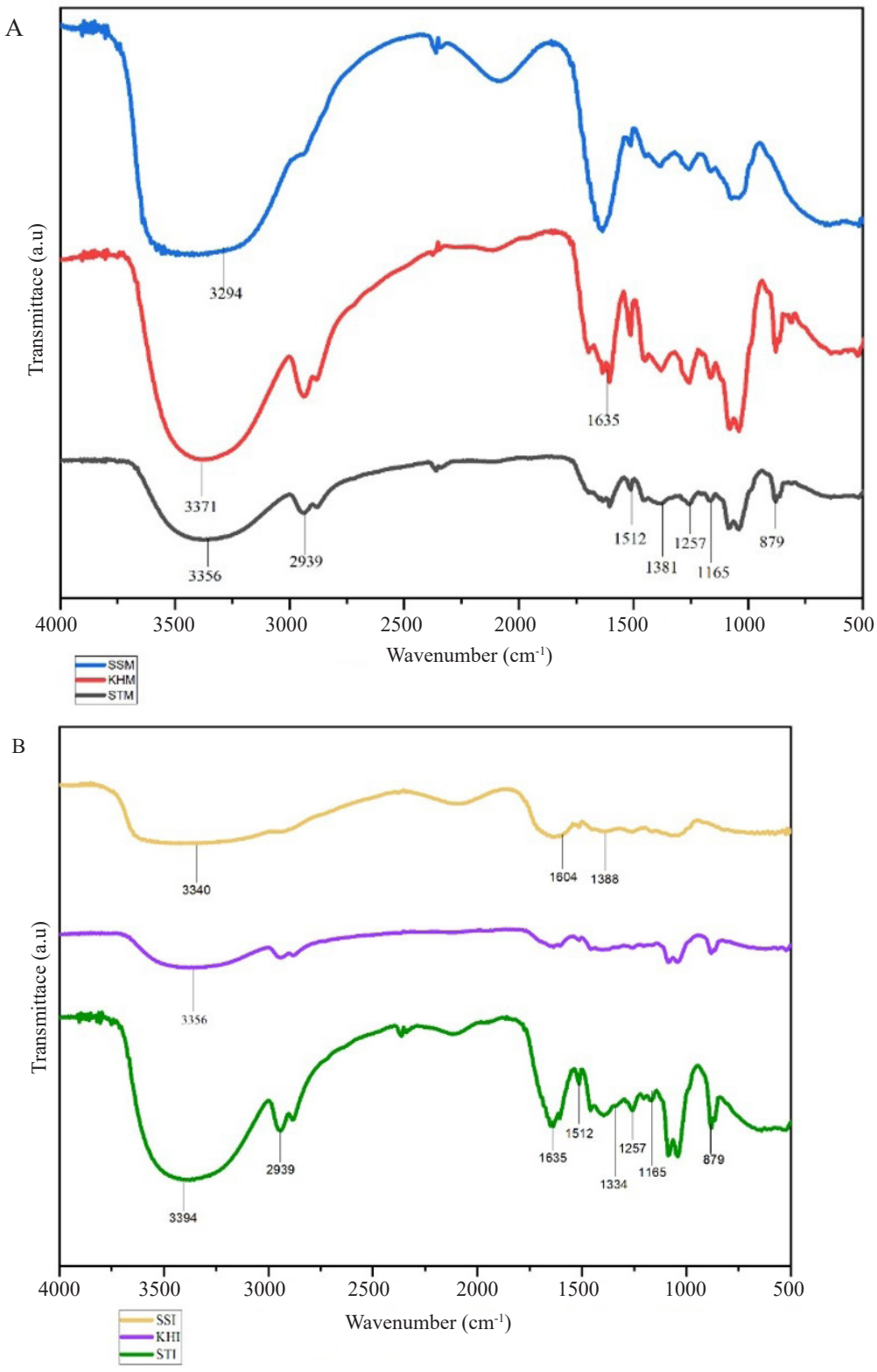

Figure 2. The FTIR spectra of *P. serratifolia* extracts from maceration (A: SSM, KHM, STM) and soxhlet extraction (B: SSI, KHI, STI) were obtained from Sambas, Kapuas Hulu, and Sintang

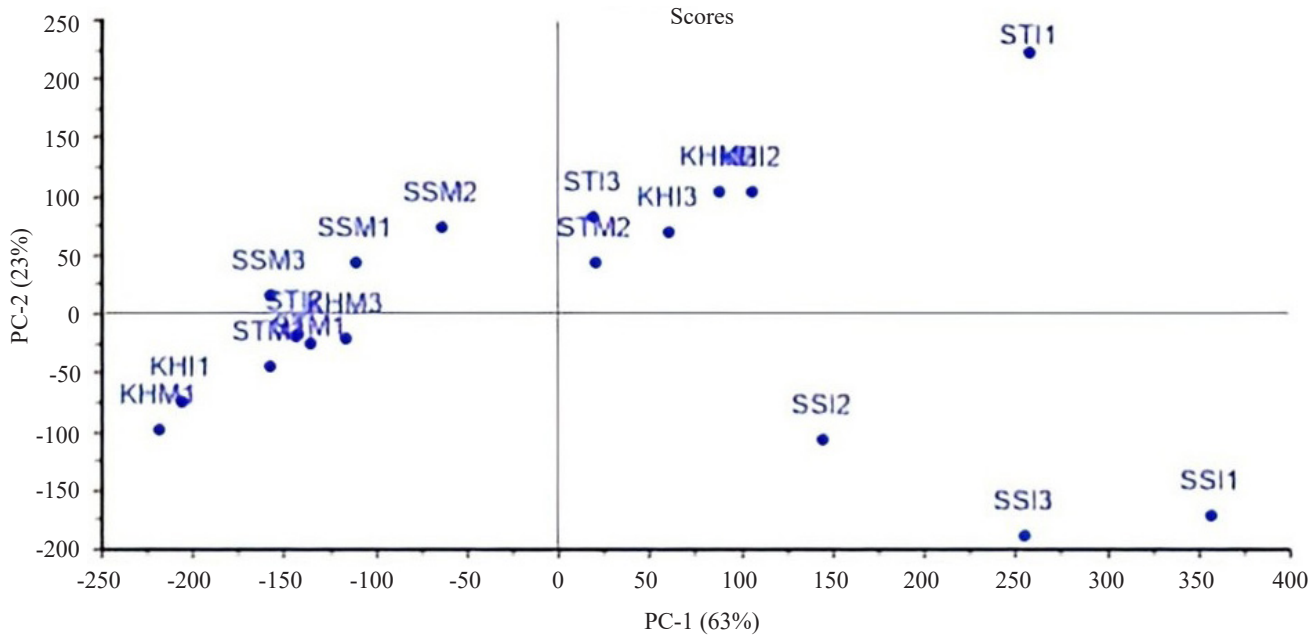

Figure 3. The PCA plot of FTIR spectra of *P. serratifolia* extracts: SSM, KHM, and STM (maceration from Sambas, Kapuas Hulu, and Sintang); SSI, KHI, and STI (soxhlation from the same regions)

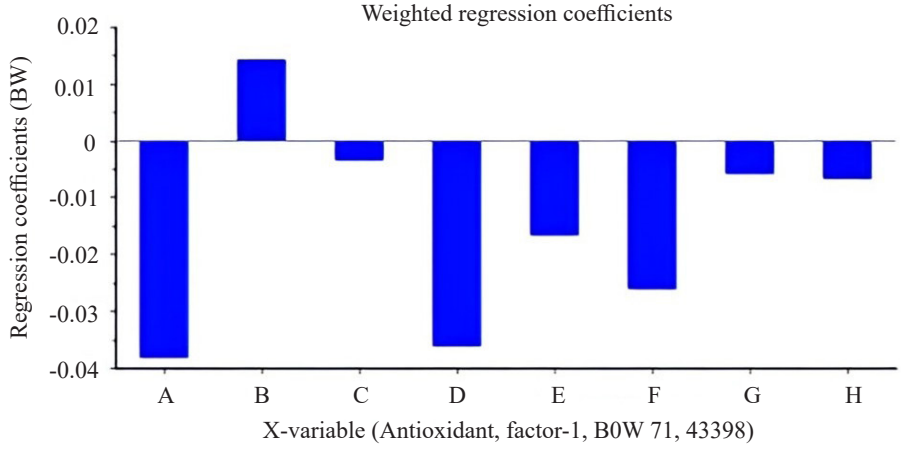

Figure 4. The PLS plot of FTIR spectra of *P. serratifolia* extract vs antioxidant activity

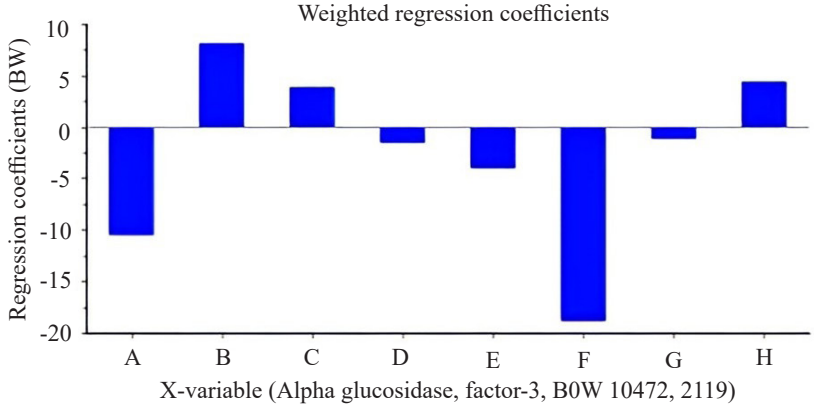

Figure 5. The PLS plot of FTIR spectra of *P. serratifolia* extract vs anti-α-glucosidase capacity

Table 4. Identified Compounds from *P. serratifolia* Extracts by UHPLC-Q-Orbitrap HRMS

Code	Compound	Experiment mass (m/z)	Accuracy (ppm)	[M-H]-/ [M+H]+	Molecular Formula	STM	STI	KHM	KHI	SSM	SSI
A	NP-005013	284.06898	1.777740385	283.06104	$C_{16}H_{12}O_5$	√	√	√	√	√	√
B	Isorhamnetin	316.05904	2.325520287	315.05110	$C_{16}H_{12}O_7$	√	√	√	√	√	√
C	Sinapinic acid	224.06862	0.647123608	223.06068	$C_{11}H_{12}O_5$	√	√	√	√	√	√
D	Isoferulic acid	194.05802	0.566841104	193.05008	$C_{10}H_{10}O_4$			√			√
E	Apigenin	270.05344	2.277332222	269.04550	$C_{15}H_{10}O_5$	√	√	√	√	√	√
F	5-O-methyl embelin	308.19956	2.595727510	307.19162	$C_{18}H_{28}O_4$	√			√	√	√
G	Tricin	330.07480	2.560032342	329.06686	$C_{17}H_{14}O_7$	√				√	√
H	NP-000308	300.06403	2.132882655	299.05609	$C_{16}H_{12}O_6$	√	√	√		√	√
I	Salicylic acid	138.03153	-1.195377627	137.02359	$C_7H_6O_3$	√		√	√	√	√
J	NP-011548	298.25141	2.062023003	297.24347	$C_{18}H_{34}O_3$	√	√	√	√	√	√
K	NP-015559	330.07471	2.287366175	329.06677	$C_{17}H_{14}O_7$	√		√		√	√
L	Luteolin	286.04847	2.552021561	285.04053	$C_{15}H_{10}O_6$					√	√
M	Quercetin	302.04334	2.267891600	301.03543	$C_{15}H_{10}O_7$						√

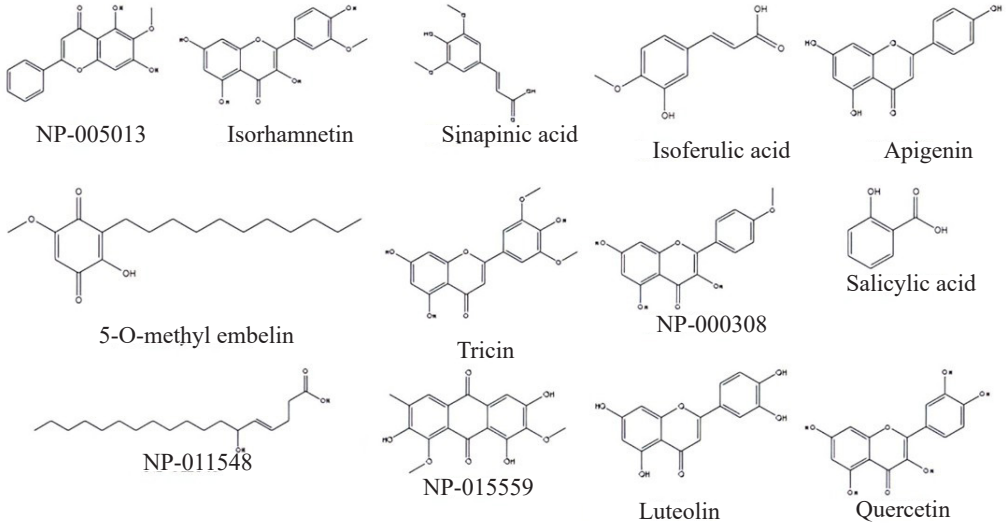

Figure 6: Structure of identified compounds from *P. serratifolia* extract

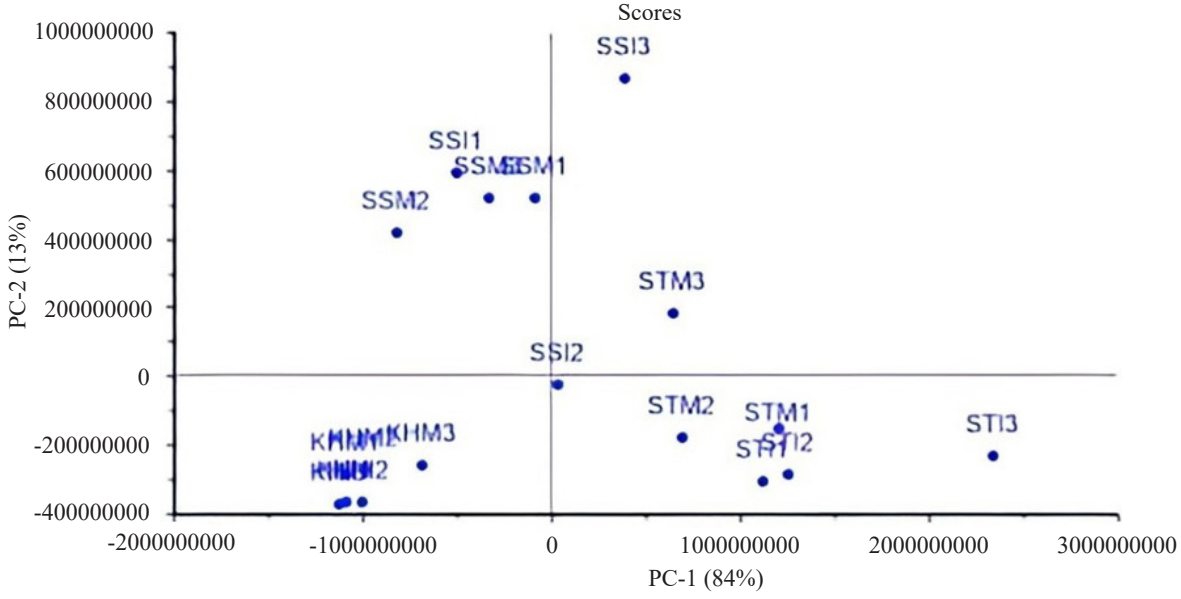

Figure 7. The PCA plot of HRMS spectra of *P. serratifolia* extract

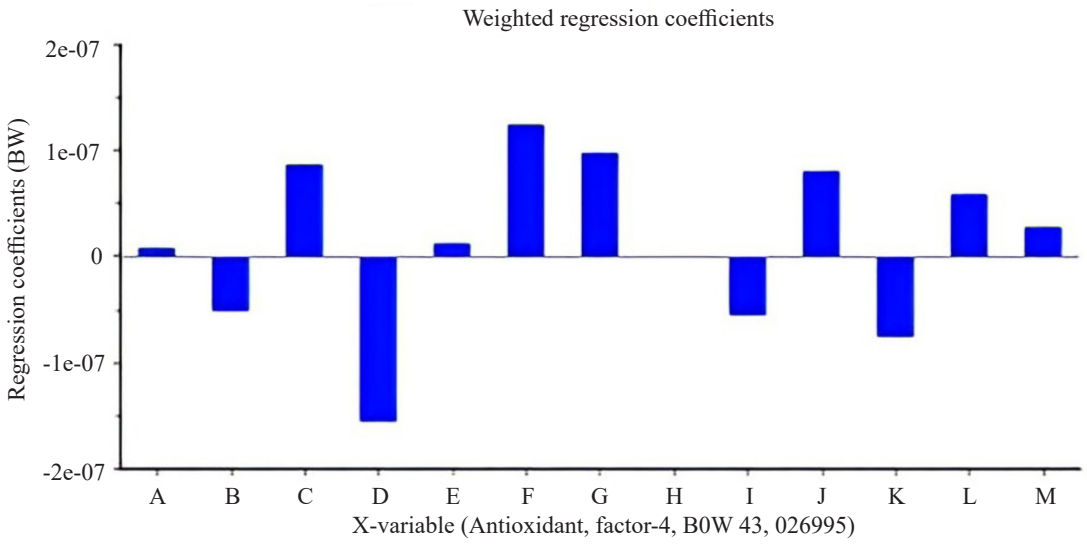

Figure 8. The PLS plot of HRMS spectra of *P. serratifolia* extract vs antioxidant activity

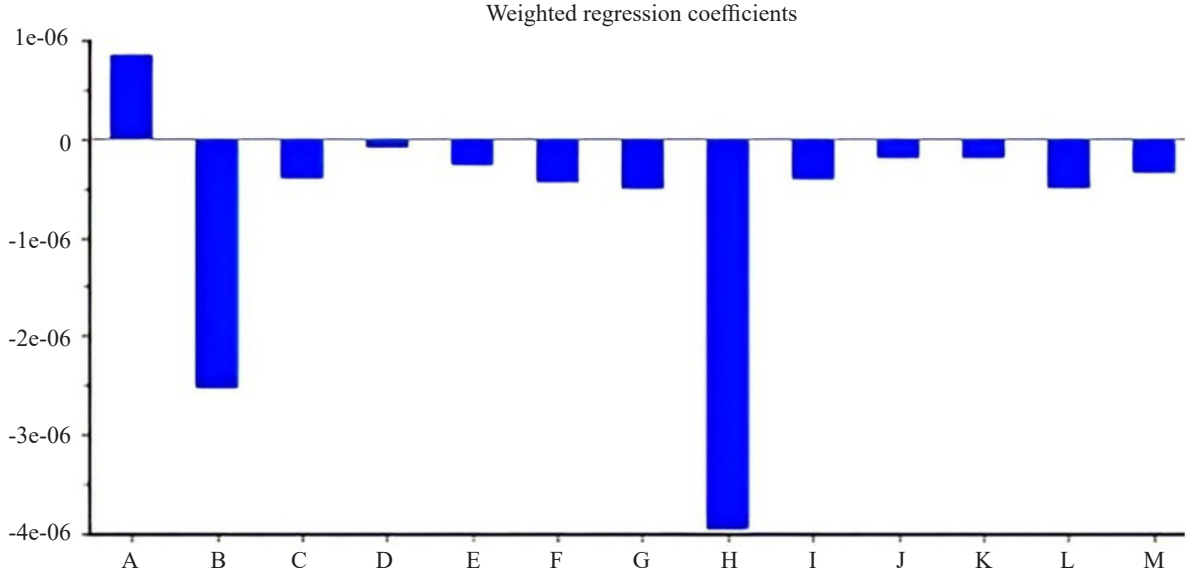

Figure 9. The PLS plot of HRMS spectra of *P. serratifolia* extract vs anti-α-glucosidase capacity

with a regression coefficient of -2.5232. Conversely, NP-005013 demonstrates a positive value, with a regression coefficient of 8.5594, indicating the least significant effect on α-glucosidase inhibition.

4. Discussion

The high efficiency of Soxhlet extraction of *P. serratifolia* leaves can be attributed to continuous extraction with fresh solvent at elevated temperatures, which enhances the extraction process (Zhang *et al*. 2018). Although all the *P. serratifolia* leaves were extracted using ethanol, the yields obtained were different. Leaves from the Sambas district had the highest yield in both extraction methods. This variation may be due to geographical factors such as altitude, soil, and climate affecting the plant's chemical composition (Li *et al*. 2019; Rafi *et al*. 2021; Jahangir *et al*. 2023). *P. serratifolia* from Sambas may have higher levels of polar compounds, leading to a higher yield when extracted using ethanol.

Not only does the yield vary, but differences in growing areas also influence the phenolic and flavonoid content in the leaf extracts of *P. serratifolia*. A decrease in TPC was observed in the Soxhlet extracts from Sintang (STI) and Kapuas Hulu (KHI), likely due to the heat applied

during Soxhlet extraction, which may degrade, oxidize, or polymerize phenolic compounds (Maghsoudlou *et al*. 2019). The high TPC in Kapuas Hulu and high TFC in Sambas are predicted to contribute to their antioxidant and α-glucosidase inhibition activities. This observation is consistent with previous studies, which show that TPC has a significant impact on antioxidant activity (Hadiarti and Qurbaniah 2022), while TFC is a major contributor to α-glucosidase inhibition (Hadiarti *et al*. 2021). Additionally, the antioxidant activity and TPC of the maceration extracts were generally superior to the Soxhlet extracts, a result also supported by earlier findings (Das *et al*. 2019). In addition to the variation in extraction yield and phenolic content, differences in geographical origin may also lead to distinct secondary metabolite profiles even within the same species.

These include O-H stretching and bending, as well as C-O-H, which were present in all extracts of *P. serratifolia* leaves, according to FTIR spectra. However, some functional groups, such as =C-H alkenes, C=C aromatic, and C-O-C rings, were absent in the extracts from Sambas. Additionally, the C=O group was not detected in extracts from Sintang (STM) and Kapuas Hulu (KHI). These findings are consistent with the idea that similar species growing in different geographical areas can exhibit variations in the intensities of their functional groups, which can impact bioactivity (Rafi *et al.* 2021). The variation in functional group intensities in *P. serratifolia* sourced from Sintang, Sambas, and Kapuas Hulu results in differing antioxidant and α-glucosidase inhibition activities. The 3200-3400 cm^{-1} band mainly reflects O-H stretching with minimal N–H overlap, supported by the absence of nitrogenous alkaloids, while the 879 cm^{-1} band indicates aromatic C-H bending that may overlap with the 3000-3100 cm^{-1} region. Similar absorption bands such as -OH stretching (3435 cm^{-1} and 3378 cm^{-1}), C-H alkanes (2921 cm^{-1} and 2983-2831 cm^{-1}), C-O-O ring (1155-1269 cm^{-1}), C=C aromatic (1421 cm^{-1}), =C-H (995-675 cm^{-1}) have been reported on Martynia annua (Duraisamy *et al*. 2022), *Smallanthus sonchifolius* (Aziz *et al*. 2021, 2020), *Amphoricarpos autariatus*, *Phaseolus vulgaris*, *Arachis hypogaea*, *Plukenetia volubilis* (Thummajitsakul *et al*. 2023), *Jatropha curcas*, and *Jatropha multifida* (Umar *et al*. 2023).

The extracts from Sintang (STM) and Kapuas Hulu (KHM) of *P. serratifolia* leaves are closely situated, likely due to the geographical proximity of these districts in eastern West Kalimantan, as indicated by the PCA plot. In contrast, Sambas (SSM and SSI) extracts are separated from those from Kapuas Hulu and Sintang, as Sambas is located in northern West Kalimantan. Additionally, the clustering of functional groups reflects the different extraction methods (maceration and Soxhlet) employed for each sample. This pattern of clustering by region and extraction method is also observed in other species, such as *Jatropha curcas* and *J. multifida* (Umar *et al*. 2023).

In this study, the detection of functional groups of *P. serratifolia* leaves within metabolites that significantly contribute to antioxidant activity was determined through the examination of the regression coefficient plot in the PLS analysis. A negative regression coefficient is associated with the absorption of functional groups that actively contribute to antioxidant activity and α-glucosidase inhibition, as indicated by IC_{50} values (Guo *et al*. 2017; Murugesu *et al*. 2018). Based on PLS analysis, the C-H alkanes, O-H stretching, C=O, O-H bending, C-O-C ring, C-O-H, and C=C aromatic groups are influencing antioxidant activity. The impact of these active functional groups on antioxidant activity is also observed in extracts from *Syzygium polyanthum* (Rohaeti *et al*. 2020), *Curculigo orchioides*, and *C. latifolia* (Umar *et al*. 2021). Meanwhile, the α-glucosidase inhibition was contributed by C=O C-H alkanes, O-H stretching and bending, as well as C-O-H. The carbonyl, C-O-H, and C-H alkane groups in extracts from *C. orchioides*, *C. latifolia*, and *Smallanthus sonchifolius* have similarly been reported to influence α-glucosidase inhibition activity (Aziz *et al*. 2021; Umar *et al*. 2021).

According to UHPLC-Q-Orbitrap HRMS spectra of *P. serratifolia* leaf extracts, several flavonoids were identified, including NP-005013, isorhamnetin, apigenin, tricin, NP-000308, scopoletin, NP-015559, luteolin, and quercetin. Interestingly, some of these flavonoids were also found in *P. serratifolia* samples from other locations, such as Pontianak, Central Sulawesi, and Allahabad (India) (Dianita and Jantan 2017; Simamora *et al*. 2019; Singh *et al*. 2021; Hadiarti *et al*. 2023). As for the identified phenolic compounds, they include sinapinic acid, isoferulic acid, 5-O-methyl embelin, salicylic acid, and NP-011548. To our knowledge, these phenolics have not been previously reported in *P. serratifolia*; however, additional studies and isolation work are needed to verify their distinctiveness and biological relevance.

The PCA plot indicates that *P. serratifolia* from Sambas (both SSM and SSI) is located in a different cluster compared to KHM, KHI, STM, and STI, which originate from Kapuas Hulu and Sintang. This divergence can be attributed to the geographical proximity of Kapuas Hulu and Sintang in the eastern part of West Kalimantan, in contrast with Sambas, which is located in the northern

region. Notably, despite using different extraction methods (maceration and Soxhlet) for *P. serratifolia* from each area, the chemical composition of the extracted compounds remains consistent. The proximity of samples from the same area on the plot evidences this. A similar pattern is observed in *Andrographis paniculata* obtained from West, Central, and East Java, where each region exhibits specific compound differences (Rafi *et al*. 2021).

The impact of various compounds on antioxidant activity and α-glucosidase inhibition was analyzed using PLS analysis. The negative coefficients of some compounds indicate their potential to act as free radical scavengers or α-glucosidase inhibitors, based on the areas measured in the UHPLC-Q-Orbitrap HRMS spectra. Isoferulic acid, NP-015559, salicylic acid, and isorhamnetin are identified as contributing to free radical reduction. The ability of isoferulic acid and salicylic acid to inhibit free radicals is also supported by evidence from Cimicifugae Rhizoma and Abelmoschus esculentus extracts (Esan *et al*. 2017; Liu *et al*. 2024). While phenolic compounds significantly influence antioxidant activity, α-glucosidase inhibition in the *P. serratifolia* extract is primarily influenced by flavonoids. NP-000308 and sorhamnetin show a significant role as an α-glucosidase inhibitor. Extracts of *Oenanthe javanica*, *Hippophae rhamnoides*, and *Ginkgo biloba* containing isorhamnetin have been shown to inhibit α-glucosidase (Alqudah *et al*. 2023).

The KHM extract, derived from the maceration of *P. serratifolia* from Kapuas Hulu, demonstrated superior antioxidant properties by exhibiting the highest TPC. The presence of C-H alkane groups and O-H stretching in phenolic compounds significantly contributed to its antioxidant activity, as indicated by PLS analysis of the FTIR spectra. Furthermore, PLS analysis of the phenolic compounds detected by UHPLC-Q-Orbitrap revealed that isoferulic acid had the most substantial influence on antioxidant activity. Notably, the structure of isoferulic acid, which contains three C-H alkane groups and two hydroxyl groups, underpins its crucial role in antioxidant activity. This highlights how the isoferulic acid groups in KHM, containing phenolic compounds, facilitate free radical reduction.

The SSI extract, identified as the best α-glucosidase inhibitor with the highest TFC, was obtained from Sambas using Soxhlet extraction. According to PLS analysis of the FTIR spectra, flavonoids containing a C=O group are the most effective in inhibiting α-glucosidase. Quercetin, exclusively identified in SSI, is the flavonoid influencing α-glucosidase inhibitory activity, based on PLS analysis of UHPLC-Q-Orbitrap HRMS spectra. The carbonyl group in quercetin plays the most significant role in reducing α-glucosidase enzyme activity.

The variations in antioxidant and α-glucosidase inhibitory activities observed among *P. serratifolia* from Sambas, Sintang, and Kapuas Hulu are unlikely to be explained solely by differences in extraction methods. Instead, these differences may reflect ecological and environmental heterogeneity across the collection sites, which can influence metabolic pathways and alter both the composition and levels of secondary metabolites. As a result, the biological activities of *P. serratifolia* extracts vary among regions, highlighting the close relationship between environmental conditions and secondary metabolism. Nevertheless, this interpretation remains a hypothesis that requires further experimental validation to confirm the specific influence of environmental factors on metabolite pathways and bioactivity of *P. serratifolia*.

In conclusion, the KHM extract of *P. serratifolia* from Kapuas Hulu, obtained via maceration, exhibited the strongest antioxidant activity, supported by its highest TPC and the presence of functional groups such as C-H alkanes and O-H phenolics as revealed by PLS analysis of FTIR spectra. Isoferulic acid, identified through UHPLC-Q-Orbitrap and possessing three C-H alkane and two hydroxyl groups, was determined to be the key contributor to this activity. Meanwhile, the SSI extract from Sambas, obtained via Soxhlet extraction, demonstrated the most potent α-glucosidase inhibitory activity, associated with its highest TFC and the presence of flavonoids containing C=O groups. Quercetin, uniquely found in SSI, emerged as the primary compound responsible for this inhibition, with its carbonyl group playing a pivotal role. These findings emphasize the importance of specific phenolic and flavonoid compounds in determining the bioactivity of *P. serratifolia* extracts from different regions. The research findings can provide a basis for standardizing raw materials of antioxidant herbal medicine derived from *P. serratifolia*, which can be further evaluated through in vivo testing.

Acknowledgements

Thanks to the Ministry of Education, Culture, Research, and Technology for funding the research, as stated in decree number 0557/E5.5/AL.04/2023. Special thanks to the head of the Advanced Research Laboratory and Biofarmaka at IPB University, as well as the Organic

Laboratory at Universitas Gadjah Mada, for facilitating the in vitro testing, analysis using UHPLC-Q-Orbitrap HRMS, and FTIR.

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