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Research Article

An ethnopharmacology study of Indonesian medicinal plants in Gunung Sari village as dipeptidyl peptidase-IV inhibitor

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Abstract

During an ethnopharmacology study of traditional antidiabetic treatment in Gunung Sari village, Bogor region, Indonesia, fifteen traditional medicinal plants were selected, collected and prepared as crude extracts. Among fifteen plants, only three plants have previously been screened for dipeptidyl peptidase-IV (DPP-IV) inhibitors. Quantitative phytochemical analysis revealed total phenolics content (TPC) ranging from 2.27±0.16 to 5.39±0.05 mg GAE/g extract and total alkaloids content (TAC) from 1.07±0.02 to 4.33±0.07 mg QE/g extract. In-vitro DPP-IV inhibitory activity screening showed that *Piper ornatum* exhibited the highest inhibition (78.11±1.35 %) and the lowest activity by *Syzygium polyanthum* (34.30±1.57%) at a concentration of 250 µg/mL, respectively. Analysis of chemical constituents using liquid chromatography-high resolution mass spectrometry (LC-HRMS) indicated at least eleven compounds were present in the crude extract. Among them, several peaks were tentatively assigned as pipcrosides and crocatins, which have previously been isolated from *Piper crocatum*.

Keywords

ethnopharmacology study, dipeptidyl peptidase-IV, Pamijahan, *Piper ornatum*

Introduction

According to the statistics from International Diabetes Federation (IDF), there were 425 million diabetes mellitus (DM) patients in 2017 worldwide, and the number is predicted to increase to 693 million by 2045 (Cho et al. 2018). There are several types of diabetes, i.e., Type 1 and Type 2, including gestational diabetes, with diabetes mellitus Type 2 (T2DM), by far, is the most prevalent (Ansari et al. 2021). Several drugs with different mechanisms are available on the market and antidiabetic drugs targeting the incretin pathway are preferable to treat T2DM (Karagiannis et al. 2022). Glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1) are the predominant incretin hormones (Nauck and Meier 2016). GLP-1 stimulates insulin secretion and inhibits glucagon

secretion at pancreatic α cell. GLP-1 has a very short half-life (1–2 minutes), owing to rapid metabolic degradation by proteases like dipeptidyl peptidase-IV (DPP-IV) and neutral endopeptidase 24.11 (NEP 24.11). DPP-IV cleaves the peptide bond in Ala^{8}-Glu^{9}, resulting metabolite GLP-1(9–36)-NH_2 which was found to have 100-fold lower binding affinity compared to the intact peptide. (Manandhar and Ahn 2015; Fisman and Tenenbaum 2021). Therefore, antidiabetic drugs acting as DPP-IV inhibitors are relatively new drugs approved by the Federal Drugs Administration (FDA) (Karagiannis et al. 2012).

DPP-IVinhibitors represent a class of oral anti-hyperglycemic agents that inhibit the enzyme DPP-IV, thus augmenting the biological activity of the incretin hormones. Sitagliptin, vildagliptin, saxagliptin and alogliptin are several synthetic DPP-IV inhibitors available on the market (Chen et al. 2015). These synthetic drugs are expensive, especially for the term of long purposes therapy. Moreover, a recent study has shown that long treatment of these drugs may cause unacceptable adverse effects such as acute (but not chronic) pancreatitis (Knapen et al. 2017), angioedema (Byrd et al. 2011), severe joint pain (Douros et al. 2019) and inflammatory bowel disease (Abrahami et al. 2018). Thus, the development of antidiabetic agents, including natural products, is necessary to obtain drugs that are safe and have more efficient activity.

Complementary and or traditional medicine is a well-known practice and has a long history in eastern medicine, especially Ayurveda in India (Jaiswal and Williams 2017), Traditional Chinese Medicine (TCM) in China (Jin et al. 2019) and Jamu in Indonesia (Lim and Pranata 2020). Indonesia is the third largest country in terms of terrestrial biodiversity and has more than 30.000 plant species. Among that number, around 2500–7500 species are medicinal plants (Cahyaningsih et al. 2021). The Indonesian government, through The National Agency of Drug and Food Control (BPOM) and The Ministry of Health, encourages the scientific community to gather scientific evidence of Indonesian traditional medicines. Many medicinal plants in Indonesia remain unexplored and only a few studies have reported their use as DPP-IV inhibitors. In this study, screening of medicinal plants collected from Indonesia as DPP-IV inhibitors using an ethnopharmacology approach was conducted. The survey, collection of plant materials, phytochemistry and their activity as DPP-IV inhibitors were evaluated. Moreover the chemical constituents of the most active extract were also tentatively identified using liquid chromatography – high resolution mass spectrometry (LC-HRMS).

Materials and methods

Ethnopharmacology study

Study area

Gunung sari village is belong to Pamijahan district, Bogor region in west Java province, Indonesia (6°41'18.6"S, 106°40'33.8"E). This village is located around Mount Halimun Salak National Park (TNGHS), situated at an altitude of 1050–1200 meters above sea level. According to the Indonesian Statistics Bureau (Badan Pusat Statistik (BPS)), in 2019, the place was inhabited by 11.501 villagers, consisting of 6.142 males and 5.358 females. Rice (*Oryza sativa*), corn (*Zea mays*) and guava (*Psidium guajava*) are examples of plants giving a major contribution to the income of these communities. Gunung sari village has two seasons; dry and rainy. The rainy season spans the months of November–May, whereas the dry season spans from June to October.

Data collections

This study was authorized by the Research and Community Service of Pancasila University (LPPM – UP), Depok, Indonesia, with contract number 7915/LPPM/UP/XII/2021. The present study was conducted from January to February 2021. The sample size was determined using Slovin's formula with a 95% confidence level according to the following equation (Gudata et al. 2019).

$$\text{No}\,(\,\textit{number of samples}\,) = \frac{N}{(1 + Ne^2)}$$

where N = total population; e = tolerance level.

According to Slovin's formula, 387 respondents (312 females and 75 males) were selected as respondents. The gender gap among respondents between females and males at about 5:1 ratio was chosen based on the information from the community health center (Pusat Kesehatan Masyarakat – PusKesMas) as the lowest government healthcare system at the sub-district level. According to the provided information, the ratio of diabetic patients was in the ratio 3:1 (females to males). Prior to the interview process, verbal informed consent was obtained from each respondent. Data collection was conducted according to Jadid et al (2020), including the interview technique and selection of the informant. In addition, the informant's ages ranged from 20–65 years old. Where seven were between 20–25, sixty-eight ranging from 26–35, two hundred and three between 36–45, eighty-eight ranging from 46–55 and twenty-one between 56–65 years old. The criteria for the plant's selection were native to the region and traditionally used to treat antidiabetics as a complementary medicine.

In-vitro DPP-IV inhibition assay

General

The chemicals used in this experiment were purchased from Merck (Darmstadt, Germany) and Sigma-Aldrich (St. Louis, Missouri, USA). The *in-vitro* DPP-IV activity screening kit consisted of a DPP-IV assay buffer, substrate, enzyme and standard inhibitor (sitagliptin) purchased from BioVision (Waltham, Massachusetts, USA). The DPP-IV inhibitory assay was performed in Nunc F96 MicroWell Black and measured using Varioskan Flash (Thermo Scientific, Waltham, Massachusetts, USA). LC-HRMS

was performed on Waters Xevo-G2 XS QTof (Waters, Milford, Massachusetts, USA).

Plants material, determination and crude extracts preparation

During the ethnopharmacology study, various parts of plants were collected according to the survey results and the specimens were sent to Herbarium Bogoriense for determination. In addition, crude extracts were prepared by macerating the materials in 96% ethanol with 1:10 ratio (w/v) at room temperature for 16 hours. After sixteen hours, the mixture was separated using filter paper and the organic solvent was evaporated under reduced pressure to obtain thick crude extracts.

Qualitative and quantitative phytochemicals analysis

The presence of alkaloids, flavonoids, triterpenes/steroids, tannins, quinones and saponin in the crude extracts were identified using colorimetric methods following to the previous study (Dewi et al. 2022). The total phenolics content (TPC) was estimated using Folin-Ciocalteu method and total alkaloids (TAC) were determined according to the aluminum trichloride method with gallic acid and quercetin as the reference compounds. The value was expressed as gallic acid equivalent (mg GAE/g sample) and quercetin equivalent (mg QE/g sample) for total phenolics and alkaloids, respectively.

Tannins removal

The elimination of tannins from the crude extracts was conducted using the gelatin precipitation method developed by Prommajak et al. (Prommajak et al. 2018) and Setyaningsih et al. (Setyaningsih et al. 2019). In detail, one percent (1%) of gelatin solution was mixed with the test solution. The mixtures were shaken at 100 rpm for 10 min at 25 °C. The supernatant was dried and dissolved with dimethyl sulfoxide (DMSO) to exclude the false positive results.

DPP-IV inhibition assay

The inhibitory assay of DPP-IV was performed according to the manufacturer's protocol. In detail, two milligrams of each sample (after tannins removal) were accurately weighed and dissolved in DMSO to obtain a final concentration of 1000 µg/mL. The enzyme control activity was achieved by mixing assay buffer (49 µL), DPP-IV (1 µL), solvent (25 µL), inhibitor (0 µL) and substrate (25 µL). The pipetting summary for this experiment was tabulated in Table 1, with the final working concentration of samples being 250 µg/mL. The fluorescence-based assay was conducted on Nunc F96 MicroWell Black and measured using Varioskan Flash with an excitation wavelength of 360 nm and emission wavelength of 460 nm. The experiment was measured in kinetic mode for 30 minutes at 37 °C. Thermo Scientific SkanIt software version 2.4.5 was used for data processing and the inhibition was calculated using the following equation.

$$\%\ inhibition = \frac{(\text{Slope of enzyme control} - \text{Slope of sample})}{\text{Slope of enzyme control}} \times 100$$

Analysis of chemical constituents by LC-HRMS

The most active sample was prepared with a concentration of 1 mg/mL by accurately weighing 2.0 mg of sample, mixed with MeOH, sonicated for 10 minutes and then filtered through a 0.22 µm PTFE syringe filter (Waters, Milford, Massachusetts, USA). The LC-HRMS analysis was measured on Waters Xevo-G2 XS QTof. Separation was performed using Waters BEH C_{18} column (2.1 × 50 mm, 1.7 µm) as a stationary phase and the mobile phases consisted of acetonitrile (B) and Milli-Q water (A) supplemented with 0.1% formic acid. Gradient elution from 5% B in 1 min followed by increasing to 100% B in 10 minutes and hold at 100% B for 3 minutes and finally bring back to initial gradient for 3 min with a total run of 17 min and flow rate of 0.3 mL/min. Each run was compared to a blank sample and the injection volume was 1 µL. The UNIFI software version 1.5 was used to process data and tentatively determine molecular formulae by performing isotope abundance analysis and reporting the best-fitting empirical formula. Waters build-in library version 1.8 and Dictionary of Natural Products released version 29.2 were used as database searches. The results from databases searched were reviewed for compounds identified from the analyzed genera with molecular formulas or masses corresponding to the LC-HRMS data. Any matches were investigated by comparing the literature and the experimental data. Tentative compound assignments were made when matches were identified. The MS conditions were as follows: column temperature 40 °C, mass range: 100–1200 Da, cone voltage 30 V, capillary 2kV, source temperature 120 °C, desolvation temperature 500 °C, cone gas flow 50 L/h, desolvation gas flow 1000 L/h, collision energy (ramp: 10–40 eV). Leucine enkephaline was used as an internal mass correction, infused every 10 s during the whole run.

Statistical analysis

All experiments were performed in triplicate and expressed as mean ± standard deviation (s.d). The significance value (p<0.05) was calculated using Microsoft excel 2019.

Table 1. Pippeting summary of the DPP-IV activity screening.

	Assay buffer (µL)	DPP-IV (µL)	Solvent (µL)	Inhibitor (µL)	Substrate (µL)
Enzyme control	49	1	25	–	25
Background	50	–	25	–	25
Sitagliptin	49	1	–	25	25
Sample	49	1	–	25	25

Results and discussion

Ethnopharmacology study

The people of Gunung Sari received their knowledge of traditional medicines from their ancestors and passed it to their descendants through storytelling. According to the survey, we found fifteen traditional plants that are used for traditional antidiabetic remedies, including their mode of preparation (Table 2 and Fig. 1). Among fifteen plants, only *Psidium guajava* (leaves) (Setyaningsih et al. 2019), *Apium graveolens* (aerial part) (Amin et al. 2019) and *Momordica charantia* L. (seed) (Ansari et al. 2021) were recorded and screened for DPP-IV inhibitory activity. In addition, *Garcinia mangostana* pericarp was the most chosen plant by respondents to traditionally treat diabetes mellitus, which was proven to lower blood glucose levels in the streptozotocin-induced animal model (Taher et al. 2016).

Qualitative and quantitative phytochemical analysis

The various parts of the plants collected during the ethnopharmacology study were prepared as a crude extract

Table 2. Determination of selected Indonesian medicinal plants used for traditional antidiabetic remedies according to this study.

Plant family	Plant species (code)	Common name	Local name	Plant part used	Mode of preparation
Fabaceae	*Senna alexandrina* (DTc)	Egyptian senna	*Teh Jati*	leaves	decoction
Oxalidaceae	*Averrhoa bilimbi* (DBw)	Cucumber tree	*Belimbing wuluh*	fruits	decoction
Clusiaceae	*Garcinia mangostana* (KM)	Mangosteen	*Manggis*	inner pericarp	decoction
Piperaceae	*Piper ornatum* (DSM)	Celebes pepper	*Sirih Merah*	leaves	decoction
Myrtaceae	*Syzygium polyanthu*m (DSL)	Indonesian bay leaf	*Salam*	leaves	decoction
Cucurbitaceae	*Momordica charantia* (BPr)	Bitter melon	*Pare*	seeds	decoction
Moraceae	*Ficus carica* (DTi)	Fig	*Tin*	leaves	decoction
Acanthaceae	*Andrographis paniculate* (DSo)	Green chiretta	*Sambiloto*	leaves	decoction
Fabaceae	*Archidendron jiringa* (KJo)	Djenkol	*Jengkol*	eksokarp	decoction
Zingiberaceae	*Zingiber officinale* (RJ)	Ginger	*Jahe emprit*	rhizome	decoction
Apiaceae	*Apium graveolens* (DSel)	Celery	*Seledri*	aerial	decoction
Asphodelaceae	*Aloe vera* (DLB)	Aloe vera	*Lidah Buaya*	leaves	decoction
Lamiaceae	*Orthosiphon aristatus* (DKK)	Java tea	*Kumis Kucing*	leaves	decoction
Myrtaceae	*Psidium guajava* (DJu)	Guava	*Jambu biji*	leaves	decoction
Meliaceae	*Switenia macrophylla* (DMo)	Mahogany	*Mahoni*	leaves	decoction

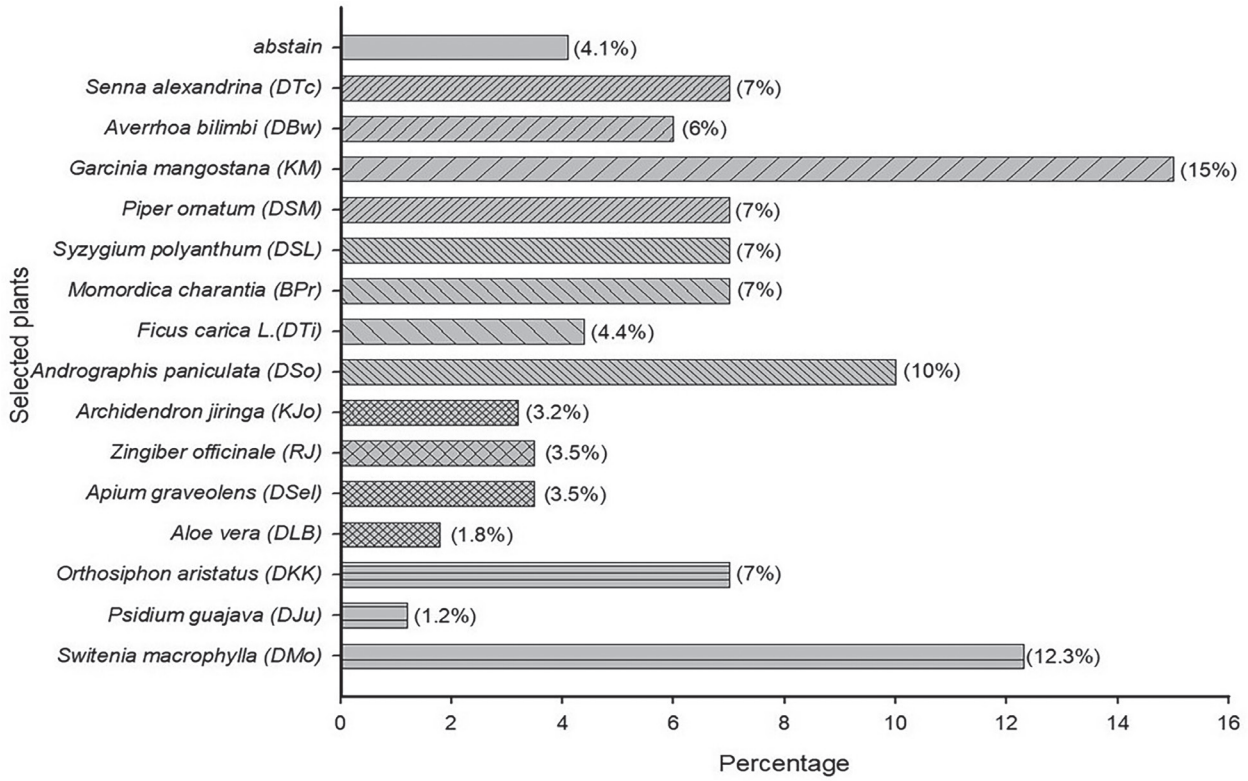

Figure 1. Distribution of medicinal plants of traditional antidiabetic treatment according to ethnopharmacology study in Gunung Sari village, Bogor, West Java. Parentheses represent the initial for corresponding plants.

Table 3. Qualitative and quantitative phytochemical screening of the crude extracts from the ethnophamacology study.

No.	Samples	alkaloids	flavonoids	saponins	tannins	quinones	steroids/ triterpenoids	total phenolics content (mg GAE/g extract)	total alkaloids content (mg QE/g extract)
1	*Senna alexandrina* (DTc)	+	+	+	+	+	+/+	5.39 ± 0.05	3.86 ± 0.04
2	*Averrhoa bilimbi* (DBw)	+	+	+	+	+	+/-	3.06 ± 0.05	1.23 ± 0.03
3	*Garcinia mangostana* (KM)	+	+	+	+	+	-/+	4.41 ± 0.08	2.91 ± 0.04
4	*Piper ornatum* (DSM)	+	+	+	+	+	+/-	5.81 ± 0.17	7.18 ± 0.09
5	*Syzygium polyanthum* (DSL)	+	+	-	+	+	+/-	2.96 ± 0.39	1.07 ± 0.02
6	*Momordica charantia* (BPr)	+	-	+	+	+	+/-	4.14 ± 0.12	1.73 ± 0.03
7	*Ficus carica* (DTi)	+	+	+	+	+	+/-	4.26 ± 0.10	4.06 ± 0.15
8	*Andrographis paniculate* (DSo)	+	+	+	+	+	+/-	4.96 ± 1.03	2.96 ± 0.03
9	*Archidendron jiringa* (KJo)	+	+	+	+	+	-/+	2.59 ± 0.05	1.42 ± 0.13
10	*Zingiber officinale* (RJ)	+	+	+	+	-	-/-	3.92 ± 0.90	2.26 ± 0.11
11	*Apium graveolens* (DSel)	+	+	+	+	-	+/-	2.64 ± 0.02	1.13 ± 0.02
12	*Aloe vera* (DLB)	+	-	+	+	-	+/-	4.88 ± 0.28	4.33 ± 0.07
12	*Orthosiphon aristatus* (DKK)	+	+	+	+	+	+/-	3.70 ± 0.15	1.39 ± 0.01
14	*Psidium guajava* (DJu)	+	+	+	+	+	+/-	4.48 ± 0.41	2.27 ± 0.01
15	*Switenia macrophylla* (DMo)	+	+	+	+	+	+/-	2.27 ± 0.16	1.85 ± 0.02

by macerating using 96% ethanol. It is widely known that different features of extraction (decoction, maceration, percolation, reflux and soxhlet) and solvents (water, ethanol and methanol) resulted in different class of extracted chemical composition (Zhang et al. 2018; Bitwell et al. 2023). For example an extraction study conducted on *Ocimum gratissimum* L. using different solvents (water, methanol and ethanol) revealed that the chemical constituent profiling was increased on methanol and ethanol crude extracts compared to water crude extract (Onyebuchi and Kavaz 2020). Furthermore, a qualitative phytochemical analysis was performed to provide a general overview of the chemical constituents in the crude extract. The result of phytochemical analysis is summarized in Table 3. Alkaloids and tannins were present in all samples, whereas other chemical constituents such as flavonoids, saponins, quinones and terpenoids/triterpenoids were varied among samples. Moreover, total phenolics content (TPC) and total alkaloids content (TAC) were determined according to the previous methods (Dewi et al. 2022). In this study, the lowest total phenolics content was showed by *Switenia macrophylla*, representing 2.27±0.16 mg/g extract gallic acid equivalent (GAE) and *Piper ornatum* contained the highest TPC with the value of 5.81±0.17 mg/g crude extract. In the other hand, *Syzygium polyanthum* (1.07±0.02) and *Piper ornatum* (7.18±0.09) showed the lowest and highest total alkaloids content, respectively.

In-vitro DPP-IV inhibitory activity screening

The potency of several Indonesian medicinal plants as DPP-IV inhibitors have been conducted previously. Ten medicinal plants were screened for DPP-IV inhibitor and the highest activity was achieved by *Caesalpinia sappan* (84.25%) at a concentration of 100 µg/mL (Setyaningsih et al. 2019). Twelve edible plants from Indonesia were also screened and it was found that *Ipomoea batatas* (L). have the highest inhibition (28.8±7.7%) at a concentration of 10 µg/mL (Amin et al. 2019). According to the previous report, the presence of tannins in the crude extract may give a positive result in DPP-IV inhibitory activity (Kim et al. 1998). Since qualitative phytochemical analysis indicated the presence of tannins in all crude extracts, tannin elimination was performed prior DPP-IV inhibitory activity screening using the gelatin precipitation method (Prommajak et al. 2018). In-vitro DPP-IV inhibitory activity on selected medicinal plants was presented in Fig. 2. According to our results, at a concentration of 250 µg/mL, crude extracts exhibited inhibition ranging from 34.30 to 78.33%, while the positive control, sitagliptin showed 100% inhibitory activity. The highest inhibition was achieved by crude extract of the leaves of *Piper ornatum* (78.33±1.35 %) and the lowest activity was exhibited by *Syzygium polyanthum* with an inhibition value of 34.30±1.57 %. In accordance, the leaves of *Psidium guajava*, which have previously been screened at a concentration of 100 µg/ml and exhibited 29.50% inhibition. Those results corroborated our finding that by using 2.5 fold higher concentration (250 µg/ml),

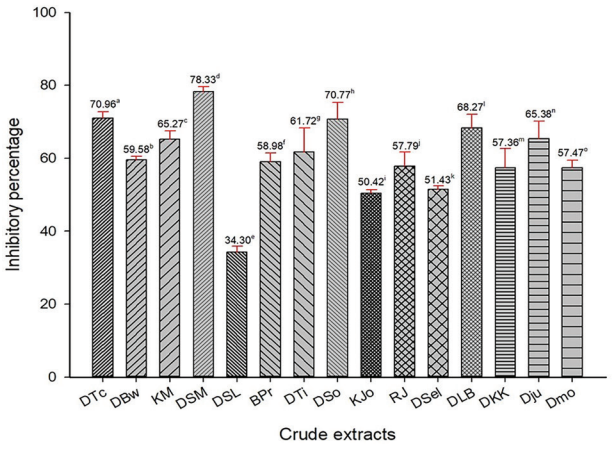

Figure 2. In-vitro DPP-IV inhibitory activity of crude extracts from selected Indonesian plants. Sitagliptin was used as the positive control and showed 100% inhibition at 250 µg/mL. All data presented as mean ± SD of triplicates (n=3) experiments. The different letters indicated significant differences compared to sitagliptin (p value = 0.05).

the inhibition was increased to 65.38 ± 4.88%. Compared to sitagliptin as a positive control, all crude extracts were statistically significant different.

Analysis of chemical constituent using LC-HRMS

Piper ornatum N.E.Br. is widely known as Celebes pepper and native to Sulawesi. According to the literature survey, no reported compounds have been isolated from *Piper ornatum*. Instead, thirty-six compounds have been isolated from *Piper crocatum* until the year 2021 (Heliawati et al. 2022). Interestingly, a specific search using a filtered keyword biological source "*Piper ornatum*" or "*Piper crocatum*" in the Dictionary of Natural Products on USB database version 29.2 gave no results. These indicated that 36 compounds isolated from *Piper ornatum* or *Piper crocatum* have previously been isolated from another source. Only crocatin A and B (Arbain et al. 2018), pipercroside A and B (Li et al. 2019a) and 5α,6β-dihydroxy-3β-(β-D-glucopyranosyloxy)-7*E*-megastigmen-9-one (Li et al. 2019b) were reported for the first time isolated from *Piper crocatum*. Furthernore, Piper ornatum crude extract analysis using LC-HRMS revealed that at least eleven compounds were present in the crude extract. Among those compounds, pipcroside B and crocatins were tentatively identified in the crude extract, which was isolated previously from *Piper crocatum*. (Figs 3, 4 and Table 4).

Several classes of compounds have been screened for DPP-IV inhibitor activity. Flavonoids compounds, i.e., kaempferol 7-O-α-L-rhamnoside (IC_{50}=20.81 μM), vitexin (IC_{50}=33.12 μM) and rutin (IC_{50}=32.93 μM) isolated from the leaves of *Smilax china* L. showed moderate DPP-IV inhibitory activities (Zhao et al. 2016). An isolated compound from *Rheum palmatum* L., emodin (phenolic) was also shown to have the capability to inhibit DPP-IV activity with an IC_{50} of 5.76 μM (Wang et al. 2017). In addition, stigmasterol isolated from *Fagonia cretica* L. was

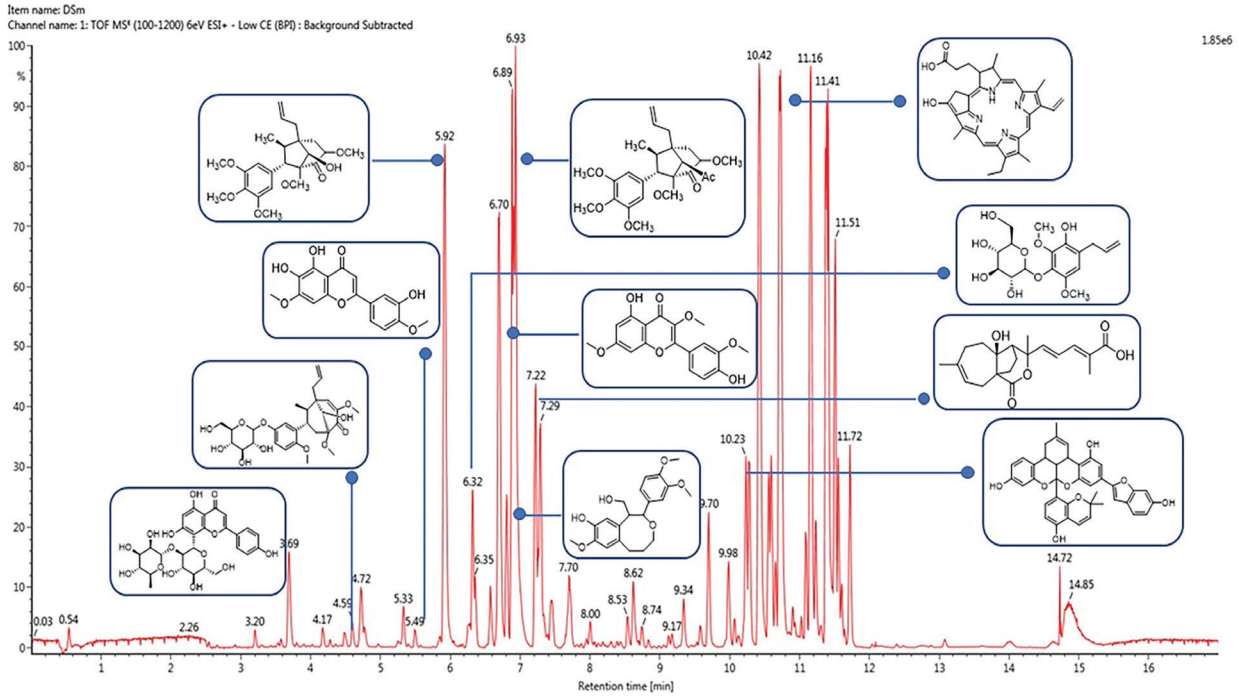

Figure 3. Chromatogram of *Piper ornatum* crude extract showed tentatively assigned compounds.

Table 4. Chemical constituents present in the crude extract of *Piper ornatum* (tentatively assigned).

Nr.	Compounds	Mol. formula	*m/z* (theoritical)	*m/z* (experimental)	*Δm* (mDa)	t_R (min)	Fragments (reference)	Relative intensity (%)
1	Vitexin 2"-O-rhamnoside	$C_{27}H_{30}O_{14}$	579.1708 $[M+H]^+$	579.1719	1.1	3.70	433, 313, 295 (Guo et al. 2021)	1.13
2	Pipcroside B	$C_{28}H_{38}O_{12}$	589.2255 $[M+Na]^+$	589.2238	1.7	4.48	393, 338, 282 (Chai et al. 2021)	0.15
3	3,5,6-trihydroxy-4',7-dimethoxyflavone	$C_{17}H_{14}O_7$	331.0812 $[M+H]^+$	331.0832	2.0	5.48	316, 301, 287 (Mohammadi and Kharazian 2022)	0.17
4	Crocatin B	$C_{23}H_{30}O_7$	441.1884 $[M+Na]^+$	441.1877	0.7	5.91	(Chai et al. 2021)	6.48
5	Pachypodol	$C_{18}H_{16}O_7$	345.0969 $[M+H]^+$	345. 0988	1.9	6.70	435.0974 (Ali et al. 2008)	5.66
6	Biodinin A	$C_{21}H_{26}O_6$	397.1622 $[M+Na]^+$	397.1626	0.5	6.81	356, 341, 165 (Ma et al. 1996)	1.38
7	Crocatins	$C_{25}H_{32}O_8$	483.1989 $[M+Na]^+$	483.1968	2.1	6.88	(Chai et al. 2021)	2.99
8	Crocatins	$C_{25}H_{32}O_8$	483.1989 $[M+Na]^+$	483.1968	2.1	6.93	(Chai et al. 2021)	5.78
9	Deacetylpseudolaric acid A	$C_{20}H_{26}O_5$	369.1672 $[M+Na]^+$	369.1698	2.6	7.22	–	2.52
10	Mulberofuran K	$C_{39}H_{32}O_8$	651.1989 $[M+Na]^+$	651.1970	2.0	10.23	(Forid et al. 2021)	1.76
11	Pyrophaeophorbide A	$C_{33}H_{34}N_4O_3$	535.2704 $[M+H]^+$	535.2703	1.0	10.71	535, 447 (R. Iwahori A. 1970)	3.90

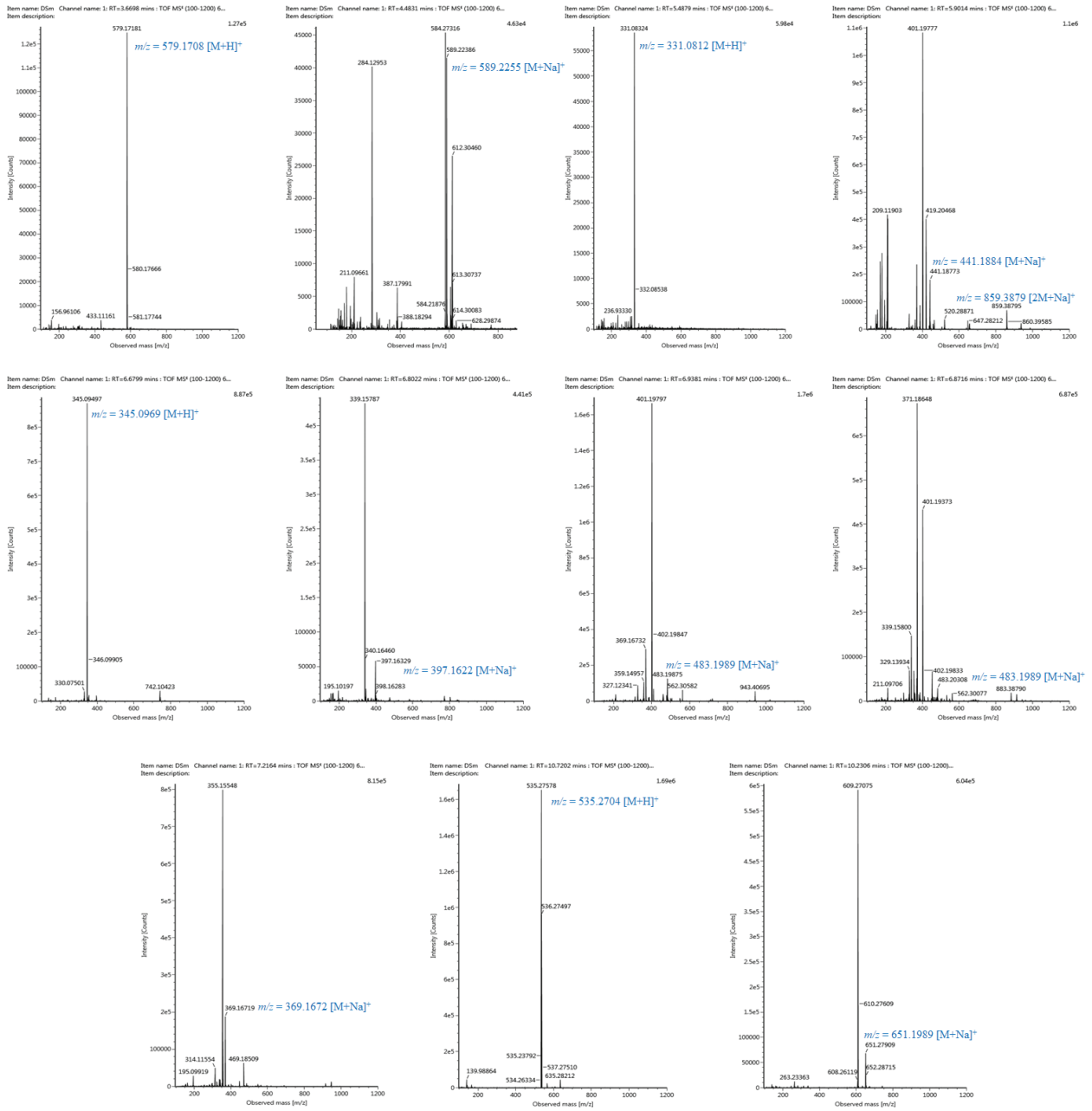

Figure 4. Mass spectrum of tentatively identified compounds.

found to have DPP-IV activity with IC_{50} >100 µM (Saleem et al. 2014). We found that the crude extract of *Piper ornatum* as the most active DPP-IV inhibitors in this study has an IC_{50} value of 192.67±1.53 µg/mL. The quantitative phytochemical screening and LC-HRMS analysis of our samples suggested that a combination of total phenolics and alkaloids content may affect the DPP-IV activity, which was indicated by the highest activity of *Piper ornatum* crude extract.

Conclusion

Ethnopharmacology is still an important approach among other methods in gathering information regarding complementary or traditional medicines. The scientific data recorded from this study may be used for further development in the preparation of standardized herbal medicines, which is still a common practice in Indonesia. Furthermore, an investigation on isolating secondary metabolites responsible for the bioactivity is presently ongoing and will be reported soon.

Author contribution

GFS: Conceptualization, methodology, investigation, formal analysis and writing original draft; GP: Methodology, investigation, formal analysis, writing-review; MEP: Validation, formal analysis, writing-review; NA: Methodology, investigation and formal analysis; DS: Methodology, formal analysis and supervision lab-

oratory work; MH: Methodology, formal analysis, supervision, reviewing and polishing the manuscript; SA: Conceptualization, methodology, formal analysis, supervision, reviewing and polishing the manuscript.

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