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Ethnopharmacological Investigation of Medicinal Plants from Gunung Sari Village, Indonesia, as Potential Dipeptidyl Peptidase-IV Inhibitors

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ABSTRACT

A study on traditional antidiabetic remedies in Gunung Sari village, Bogor, Indonesia, investigated fifteen medicinal plants, which were collected and processed into crude extracts. Only three of these plants had been previously examined for their potential as dipeptidyl peptidase-IV (DPP-IV) inhibitors. Phytochemical quantification revealed total phenolic content (TPC) between 2.27±0.16 and 5.39±0.05 mg GAE/g extract, while total alkaloid content (TAC) ranged from 1.07±0.02 to 4.33±0.07 mg QE/g extract. Screening for DPP-IV inhibitory activity in vitro indicated that Piper ornatum demonstrated the strongest inhibition at 78.11±1.35%, whereas Syzygium polyanthum exhibited the weakest activity at 34.30±1.57% at 250 µg/mL. LC-HRMS analysis of the extracts identified at least eleven chemical constituents, including peaks tentatively corresponding to pipcrosides and crocatins, compounds previously isolated from Piper crocatum.

Keywords: Piper ornatum, Ethnopharmacology study, Pamijahan, Dipeptidyl peptidase-IV

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Introduction

Diabetes mellitus (DM) is a major global health issue, affecting an estimated 425 million people in 2017, a figure expected to rise to 693 million by 2045 [1]. The disease manifests in various forms, including Type 1, Type 2, and gestational diabetes, with Type 2 diabetes mellitus (T2DM) being the most widespread [2]. A range of therapeutic options exists, among which agents targeting the incretin pathway have gained prominence for T2DM management [3]. The principal incretin hormones, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), enhance insulin secretion and suppress glucagon release from pancreatic α-cells [4]. GLP-1, however, is rapidly degraded within 1–2 minutes by enzymes such as dipeptidyl peptidase-IV (DPP-IV) and neutral endopeptidase 24.11 (NEP 24.11), producing a metabolite, GLP-1(9–36)-NH2, which exhibits roughly 100-fold lower receptor binding than the intact peptide [5, 6]. As a result, DPP-IV inhibitors have emerged as an important class of FDA-approved antidiabetic medications [7].

By inhibiting DPP-IV, these drugs prolong the activity of incretin hormones, thereby improving glucose regulation. Commonly prescribed synthetic inhibitors include sitagliptin, vildagliptin, saxagliptin, and alogliptin [8]. Despite their effectiveness, these agents are costly over long-term use and may be associated with side effects such as acute pancreatitis [9], angioedema [10], severe joint pain [11], and inflammatory bowel disease [12]. This underscores the need for alternative antidiabetic agents, particularly from natural sources, that offer better safety profiles and efficacy.

Traditional medicine has long played a central role in healthcare systems across Asia, including Ayurveda in India [13], Traditional Chinese Medicine (TCM) in China [14], and Jamu in Indonesia [15]. Indonesia, the third most biodiverse country worldwide, harbors over 30,000 plant species, of which approximately 2,500–7,500 are recognized for medicinal purposes [16]. To support the scientific validation of these plants, the Indonesian

government, through BPOM and the Ministry of Health, encourages research into traditional remedies. Nonetheless, a majority of Indonesian medicinal plants remain unexamined, and only a few have been investigated for DPP-IV inhibitory activity. In this context, the present study applied an ethnopharmacological approach to screen Indonesian plants for DPP-IV inhibition, incorporating field surveys, phytochemical analyses, and in vitro activity assessment, with chemical profiling of the most potent extracts using LC-HRMS.

Materials and Methods

Study area

The study was conducted in Gunung Sari village, located in the Pamijahan district of Bogor, West Java, Indonesia (6°41'18.6"S, 106°40'33.8"E). Nestled near Mount Halimun Salak National Park (TNGHS) at 1050–1200 meters above sea level, Gunung Sari had a population of 11,501 in 2019 (6,142 males and 5,358 females) according to BPS. The local economy relies heavily on agricultural products such as rice (Oryza sativa), corn (Zea mays), and guava (Psidium guajava). The village experiences a tropical climate with a rainy season from November to May and a dry season from June to October.

Ethnopharmacological data collection

This research received approval from the Research and Community Service Office of Pancasila University (LPPM–UP), Depok, Indonesia (Contract No. 7915/LPPM/UP/XII/2021) and was conducted between January and February 2021. Sample size was calculated using Slovin's formula with a 95% confidence interval [17].

$$\%\ inhibition\ = \frac{(\text{Slope of enzyme control}\ -\ \text{Slope of sample})}{\text{Slope of enzyme control}} \times 100 \quad (1)$$

where N = total population; e = tolerance level.

Using Slovin's formula, a total of 387 individuals were recruited for the study, comprising 312 females and 75 males. This 5:1 female-to-male distribution reflected data obtained from the local sub-district health center (Pusat Kesehatan Masyarakat – PusKesMas), which indicated a higher prevalence of diabetes among women, roughly in a 3:1 female-to-male ratio. Prior to data collection, all participants provided verbal informed consent. Interviews followed the approach described by Jadid *et al*. (2020) [18], including selection criteria for informants. Participant ages spanned from 20 to 65 years, with seven individuals aged 20–25, sixty-eight aged 26–35, 203 aged 36–45, eighty-eight aged 46–55, and twenty-one aged 56–65. Plants included in this study were chosen based on their native status and traditional use for managing diabetes as complementary remedies.

In-vitro DPP-IV inhibitory assay

Reagents and equipment

All reagents were obtained from Merck (Darmstadt, Germany) and Sigma-Aldrich (St. Louis, Missouri, USA). The DPP-IV screening kit, containing assay buffer, substrate, enzyme, and sitagliptin as a reference inhibitor, was purchased from BioVision (Waltham, Massachusetts, USA). Assays were conducted in black 96-well Nunc MicroWell plates, and fluorescence readings were recorded with a Varioskan Flash spectrophotometer (Thermo Scientific, Waltham, Massachusetts, USA). LC-HRMS profiling was performed on a Waters Xevo-G2 XS QTof system (Waters, Milford, Massachusetts, USA).

Plant collection and extract preparation

Plant parts were collected based on ethnopharmacological surveys and authenticated at Herbarium Bogoriense. Crude extracts were obtained by macerating plant materials in 96% ethanol at a 1:10 ratio (w/v) for 16 hours at room temperature. Following filtration, the solvent was removed under reduced pressure to yield concentrated crude extracts.

Phytochemical profiling

Qualitative analysis for alkaloids, flavonoids, triterpenes/steroids, tannins, quinones, and saponins was performed using colorimetric methods [19]. Total phenolic content (TPC) was measured with the Folin–Ciocalteu assay, and total alkaloid content (TAC) was determined using the aluminum chloride method, with gallic acid and quercetin as calibration standards, respectively. Values were expressed as mg gallic acid equivalent per gram (mg GAE/g) for phenolics and mg quercetin equivalent per gram (mg QE/g) for alkaloids.

Tannin removal

To prevent false positives, tannins were removed using a gelatin precipitation method adapted from Prommajak *et al*. (2018) [20] and Setyaningsih *et al*. (2019) [21]. Briefly, a 1% gelatin solution was added to the extract, followed by shaking at 100 rpm for 10 minutes at 25 °C. The supernatant was dried and re-dissolved in DMSO before DPP-IV testing.

DPP-IV enzyme assay

DPP-IV inhibition was assessed according to the kit protocol. Extracts (2 mg) were dissolved in DMSO to 1000 µg/mL, then applied at a final working concentration of 250 µg/mL. Enzyme control reactions included assay buffer (49 µL), DPP-IV (1 µL), solvent (25 µL), no inhibitor, and substrate (25 µL). Fluorescence was recorded on a Varioskan Flash at 360 nm excitation and 460 nm emission for 30 minutes at 37 °C in kinetic mode. Thermo Scientific SkanIt software version 2.4.5 was used for data processing, and the inhibition was calculated using the following equation.

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

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

Chemical constituents analysis by LC-HRMS

The sample exhibiting the strongest activity was prepared at a 1 mg/mL concentration by weighing 2.0 mg of material, dissolving it in methanol, sonicated for 10 minutes, and filtering using a 0.22 µm PTFE syringe filter (Waters, Milford, MA, USA). LC-HRMS measurements were carried out on a Waters Xevo-G2 XS QTof system. Chromatographic separation was employed using a Waters BEH C18 column (2.1 × 50 mm, 1.7 µm) with acetonitrile (B) and Milli-Q water containing 0.1% formic acid (A) as mobile phases. The gradient started at 5% B for 1 minute, increased to 100% B over 10 minutes, maintained for 3 minutes, and returned to the starting conditions over 3 minutes, giving a total runtime of 17 minutes at a flow of 0.3 mL/min. Each injection was 1 µL, and blank samples were run for baseline comparison. Data were processed using UNIFI software v1.5, which estimated molecular formulas via isotope pattern analysis. Tentative compound identifications were cross-checked against Waters' library v1.8 and the Dictionary of Natural Products v29.2, with literature verification of matches. MS parameters included a column temperature of 40 °C, mass range 100–1200 Da, cone voltage 30 V, capillary 2 kV, source temperature 120 °C, desolvation temperature 500 °C, cone gas 50 L/h, desolvation gas 1000 L/h, and collision energy ramp of 10–40 eV. Leucine enkephaline was infused every 10 seconds as an internal calibrant throughout the analysis.

Statistical analysis

All measurements were carried out in triplicate, with results presented as mean ± standard deviation. Statistical significance was determined at $p < 0.05$ using Microsoft Excel 2019.

Ethnopharmacological survey

Traditional medicinal knowledge in Gunung Sari is inherited through generations via oral storytelling. The survey revealed fifteen plant species traditionally used for managing diabetes, with details of preparation documented

(Table 2, Figure 1). Among these, Psidium guajava leaves [21], Apium graveolens aerial parts [22], and Momordica charantia seeds [2] were selected for DPP-IV inhibition screening. Garcinia mangostana pericarp was the most frequently reported plant for traditional diabetes treatment, and its efficacy in reducing blood glucose was confirmed in streptozotocin-induced animal models [23].

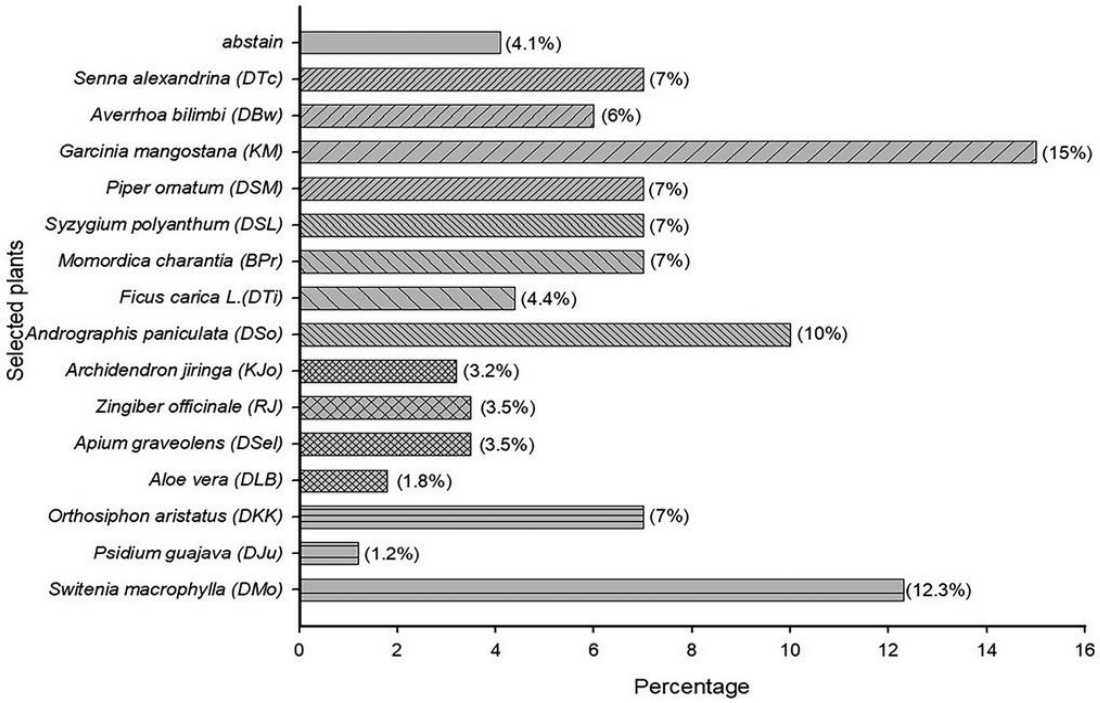


Figure 1. Ethnopharmacological survey showing the distribution of medicinal plants used for traditional antidiabetic remedies in Gunung Sari village, Bogor, West Java, with the initials of each plant indicated in parentheses.

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 polyanthum* (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

Qualitative and quantitative phytochemical analysis

Crude extracts were prepared from various plant parts collected during the ethnopharmacological survey by maceration in 96% ethanol. It is well-established that both the extraction method (e.g., maceration, decoction, percolation, reflux, or Soxhlet) and the solvent used (water, ethanol, methanol) can influence the types and quantities of chemical constituents obtained [24, 25]. For instance, a study on Ocimum gratissimum L. demonstrated that methanol and ethanol extracts yielded a richer chemical profile compared to aqueous extracts [26]. A qualitative phytochemical screening was conducted to provide an overview of the major constituents in the extracts, and the findings are summarized in **Table 3**. Alkaloids and tannins were detected in all samples, while other constituents such as flavonoids, saponins, quinones, and terpenoids/triterpenoids varied across the extracts. Additionally, total phenolic content (TPC) and total alkaloid content (TAC) were quantified following established protocols [19]. Among the studied plants, Swietenia macrophylla exhibited the lowest TPC at 2.27 ± 0.16 mg GAE/g extract, whereas Piper ornatum showed the highest TPC at 5.81 ± 0.17 mg/g extract. Regarding total alkaloids, Syzygium polyanthum had the lowest content (1.07 ± 0.02 mg/g), while Piper ornatum contained the highest (7.18 ± 0.09 mg/g).

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

In vitro screening of DPP-IV inhibitory activity

Several Indonesian medicinal plants have previously been investigated for their potential as DPP-IV inhibitors. In earlier screenings, Caesalpinia sappan demonstrated the strongest inhibition at 84.25% when tested at 100 µg/mL [21], while Ipomoea batatas (L.) among twelve edible species showed 28.8 ± 7.7% inhibition at 10 µg/mL [22]. It has been suggested that tannins present in crude extracts can contribute to DPP-IV inhibition [27]. Since all extracts were found to contain tannins during phytochemical analysis, tannins were removed using the gelatin precipitation technique prior to testing [20].

In our study **(Figure 2)**, DPP-IV inhibition of the selected plant extracts at 250 µg/mL ranged from 34.30% to 78.33%, whereas the pharmaceutical control, sitagliptin, achieved complete inhibition (100%). The extract from Piper ornatum leaves exhibited the strongest inhibitory effect (78.33 ± 1.35%), while Syzygium polyanthum showed the lowest (34.30 ± 1.57%). Notably, Psidium guajava leaves, previously reported to inhibit 29.50% at 100 µg/mL, showed an increased activity of 65.38 ± 4.88% at the higher concentration used here. All plant extracts differed significantly from the positive control, indicating statistically meaningful inhibition.

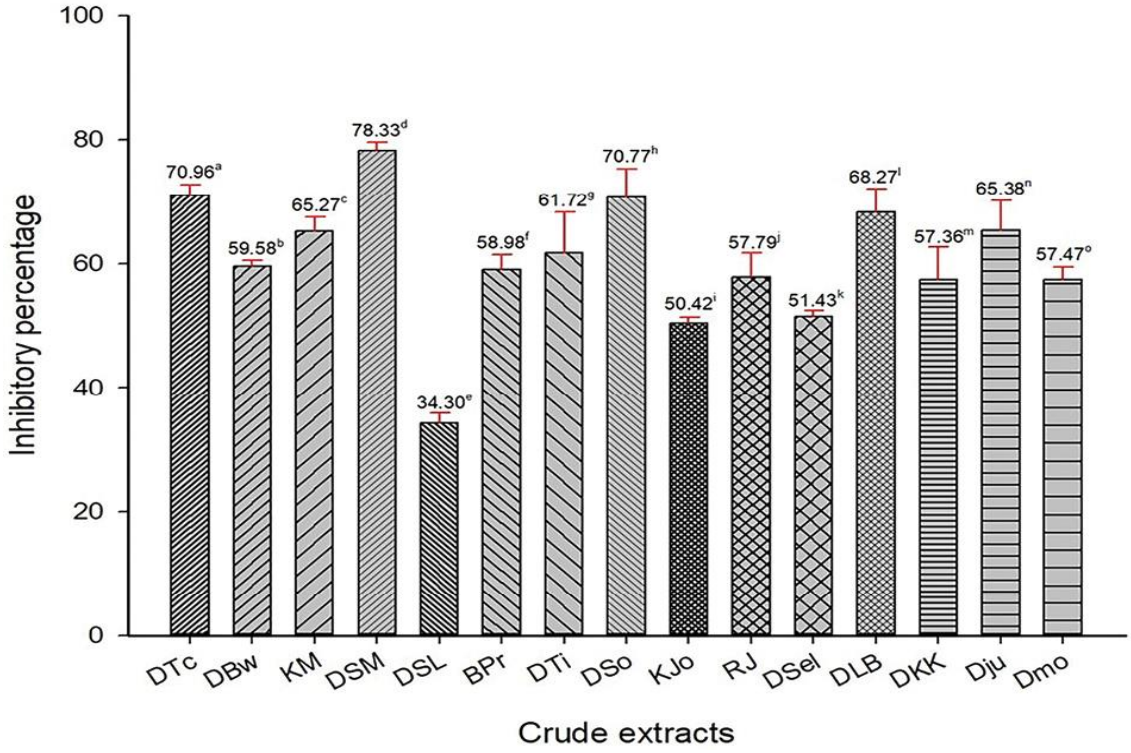


Figure 2. DPP-IV inhibitory effects of crude extracts from selected Indonesian plants measured in vitro. Sitagliptin was used as a reference standard and achieved full inhibition (100%) at 250 µg/mL. Values represent the mean ± SD of three independent experiments (n = 3), and differing letters indicate significant differences relative to sitagliptin (p = 0.05).

LC-HRMS profiling of chemical constituents

Piper ornatum N.E.Br., also known as Celebes pepper and native to Sulawesi, has not been reported in the literature as a source of isolated compounds. In contrast, thirty-six compounds have been described from Piper crocatum up to 2021 [28]. A targeted search in the Dictionary of Natural Products (USB database, version 29.2) using the biological source filters “Piper ornatum” or “Piper crocatum” returned no matches, indicating that these compounds had originally been isolated from other plant sources. Only crocatin A and B [29], pipercroside A and B [30], and 5α,6β-dihydroxy-3β-(β-D-glucopyranosyloxy)-7E-megastigmen-9-one [31] were documented as first-time isolates from Piper crocatum.

Analysis of the Piper ornatum crude extract via LC-HRMS revealed at least eleven chemical constituents. Among these, pipercroside B and crocatins were tentatively identified, compounds previously reported in Piper crocatum **(Figures 3 and 4; Table 4).**

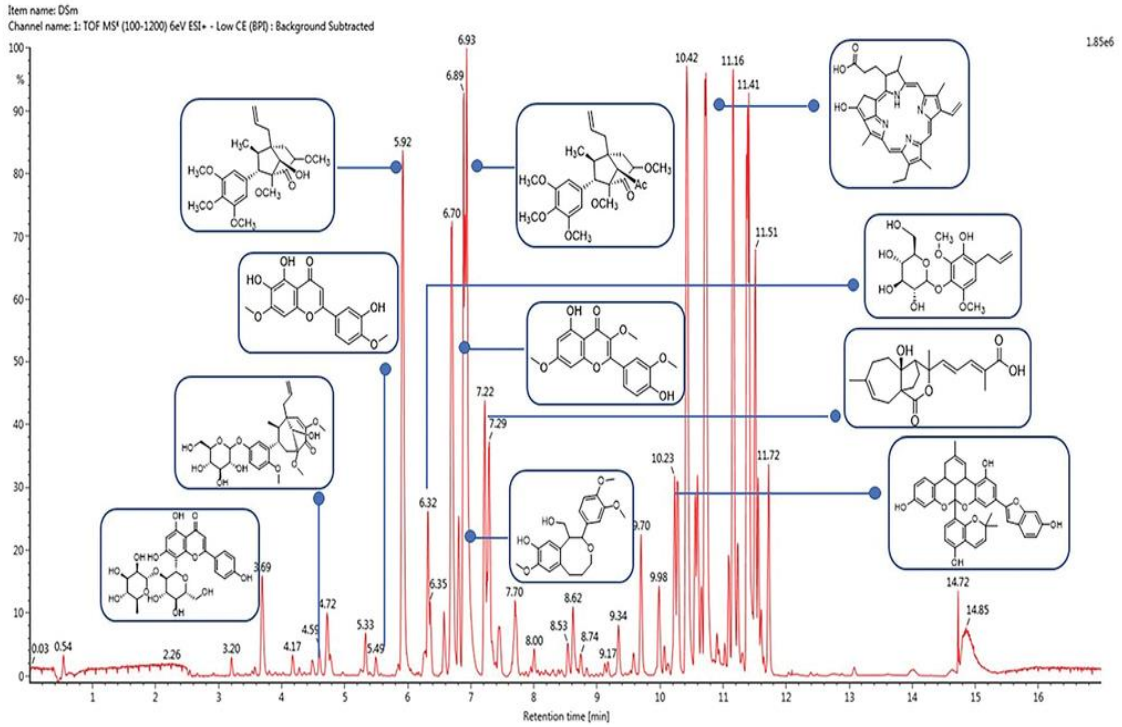
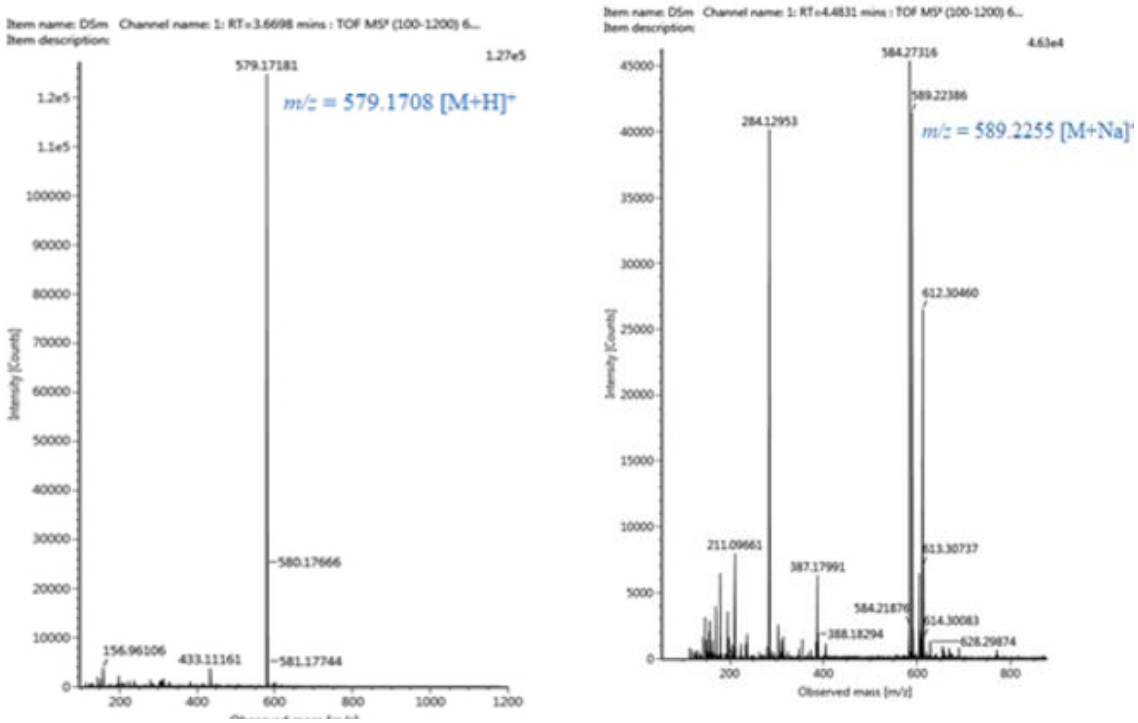
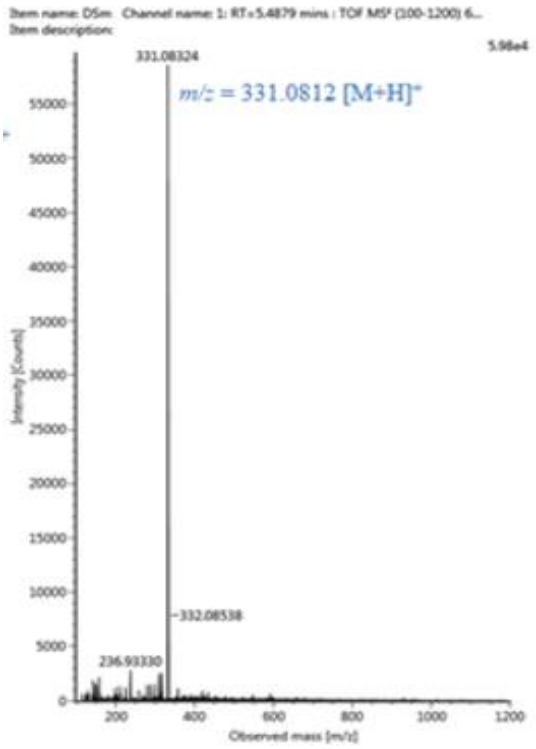


Figure 3. Chromatogram of the piper ornatum crude extract highlighting the compounds tentatively identified.



a) b)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5.98e4
331.08324
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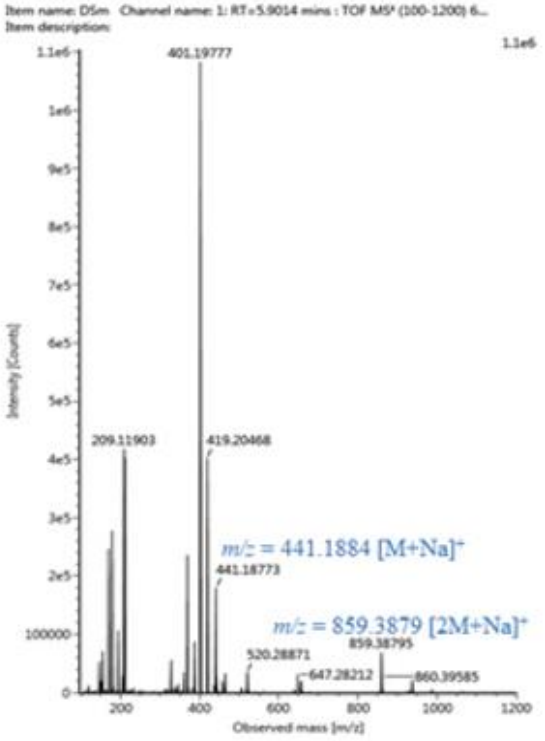
332.08538
236.93330
Intensity [Counts]
Observed mass [m/z]

c)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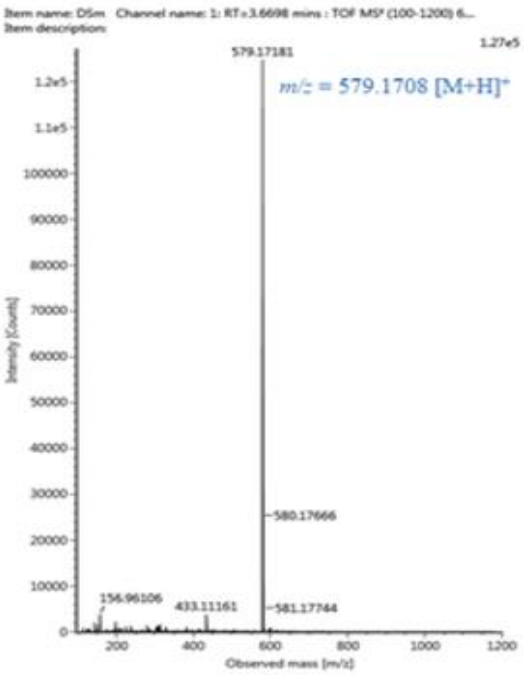
Item description:
1.1e6
401.19777
209.11903
419.20468
m/z = 441.1884 [M+Na]⁺
441.18773
m/z = 859.3879 [2M+Na]⁺
859.38795
520.28871
647.28212
860.39585
Intensity [Counts]
Observed mass [m/z]

d)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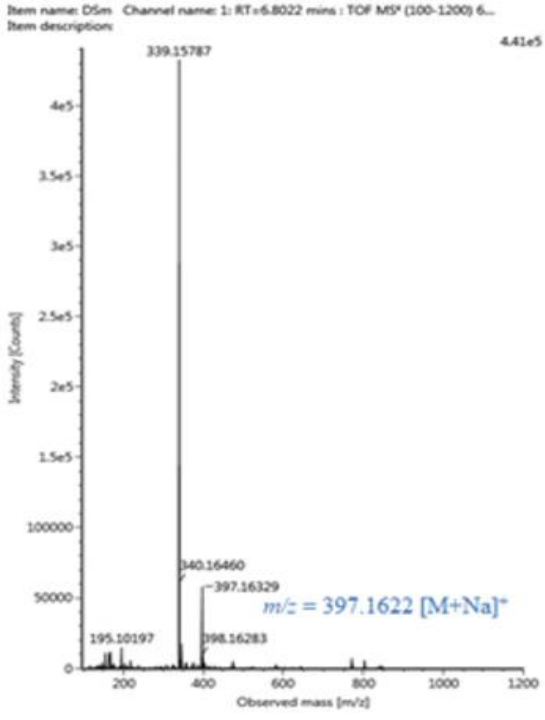
579.17181
m/z = 579.1708 [M+H]⁺
580.17666
156.96106
433.11161
581.17744
Intensity [Counts]
Observed mass [m/z]

e)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Item description:
4.41e5
339.15787
340.16460
397.16329
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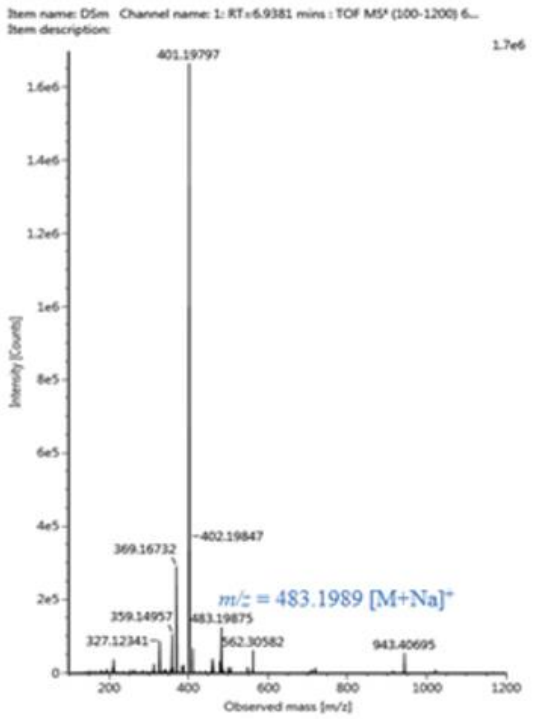
Intensity [Counts]
Observed mass [m/z]

f)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359.14957
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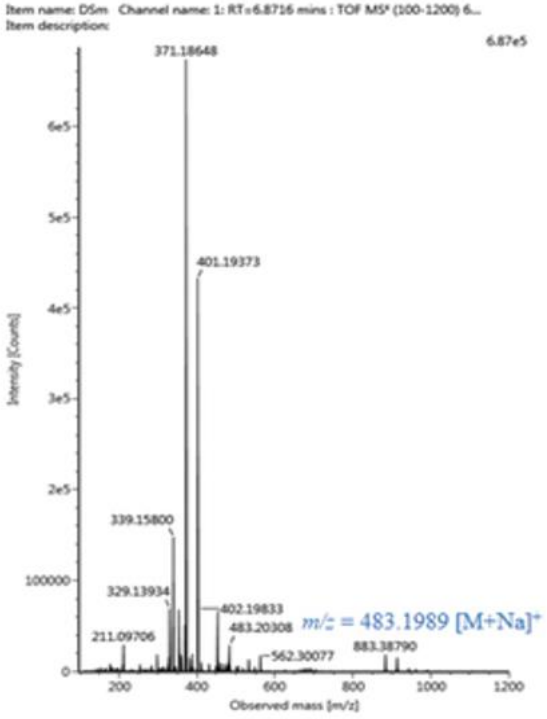
Intensity [Counts]
Observed mass [m/z]

g)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Item description:
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402.19833
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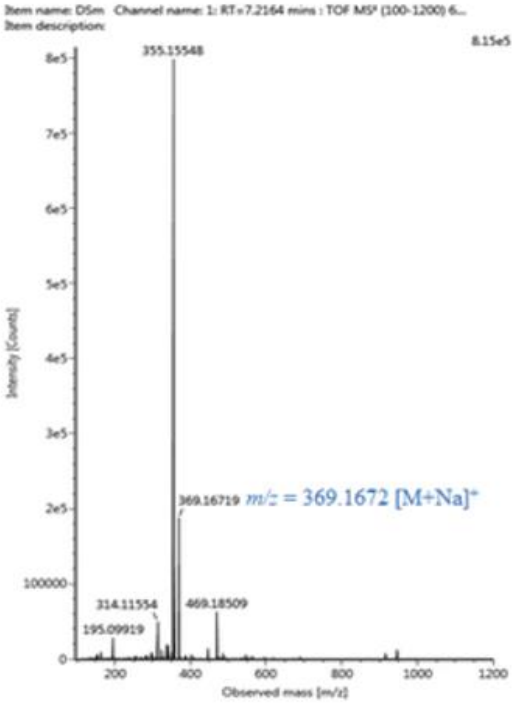
m/z = 483.1989 [M+Na]⁺
Intensity [Counts]
Observed mass [m/z]

h)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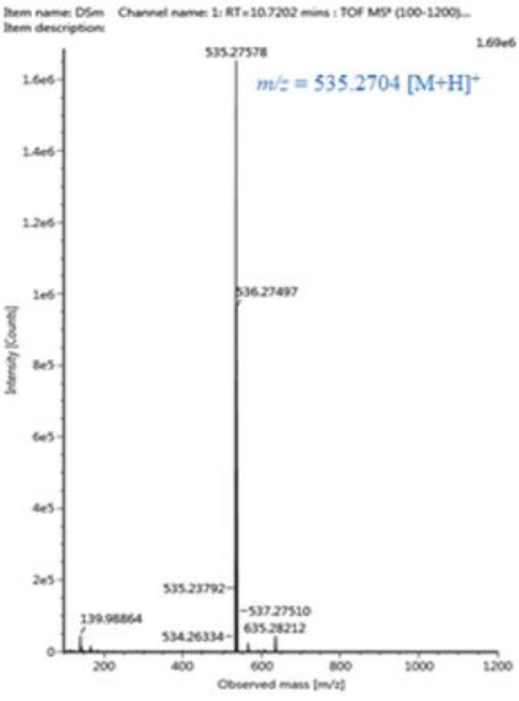
314.11554
469.18509
195.09919
Intensity [Counts]
Observed mass [m/z]

i)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Item description:
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m/z = 535.2704 [M+H]⁺
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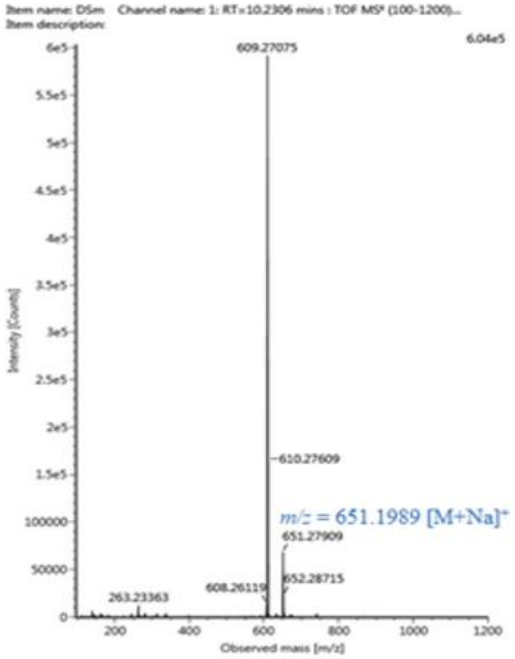
535.23792
537.27510
139.98864
534.26334
635.28212
Intensity [Counts]
Observed mass [m/z]

j)



k)

Figure 4. Mass spectra of the compounds tentatively identified.

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 [32]	1.13
2	Pipcroside B	$C_{28}H_{38}O_{12}$	589.2255 $[M+Na]^+$	589.2238	1.7	4.48	393, 338, 282 [33]	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 [34]	0.17
4	Crocatin B	$C_{23}H_{30}O_7$	441.1884 $[M+Na]^+$	441.1877	0.7	5.91	[33]	6.48
5	Pachypodol	$C_{18}H_{16}O_7$	345.0969 $[M+H]^+$	345. 0988	1.9	6.70	435.0974 [35]	5.66
6	Biodinin A	$C_{21}H_{26}O_6$	397.1622 $[M+Na]^+$	397.1626	0.5	6.81	356, 341, 165 [36]	1.38
7	Crocatins	$C_{25}H_{32}O_8$	483.1989 $[M+Na]^+$	483.1968	2.1	6.88	[33]	2.99
8	Crocatins	$C_{25}H_{32}O_8$	483.1989 $[M+Na]^+$	483.1968	2.1	6.93	[33]	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	[37]	1.76
11	Pyrophaeophorbide A	$C_{33}H_{34}N_4O_3$	535.2704 $[M+H]^+$	535.2703	1.0	10.71	535, 447 [38]	3.90

Various classes of natural compounds have been evaluated for their DPP-IV inhibitory potential. For example, flavonoids such as kaempferol 7-O-α-L-rhamnoside (IC50 = 20.81 μM), vitexin (IC50 = 33.12 μM), and rutin (IC50 = 32.93 μM), all isolated from Smilax china L. leaves, demonstrated moderate DPP-IV inhibition [39]. Emodin, a phenolic compound from Rheum palmatum L., also exhibited DPP-IV inhibitory activity with an IC50 of 5.76 μM [40]. Additionally, stigmasterol from Fagonia cretica L. showed weak inhibition with an IC50 greater than 100 μM [41]. In this study, the crude extract of Piper ornatum, identified as the most potent DPP-IV inhibitor, displayed an IC50 of 192.67 ± 1.53 μg/mL. The quantitative phytochemical analysis combined with LC-HRMS profiling suggests that the observed activity may be influenced by the total phenolic and alkaloid content, as indicated by the pronounced activity of the Piper ornatum extract.

Conclusion

Ethnopharmacological approaches remain valuable for collecting information on traditional and complementary medicinal practices. The findings of this study provide scientific evidence that may support the development of standardized herbal medicines, which continue to be widely practiced in Indonesia. Ongoing work is focused on isolating the specific secondary metabolites responsible for the observed bioactivity, and results from these investigations will be reported in future studies.

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