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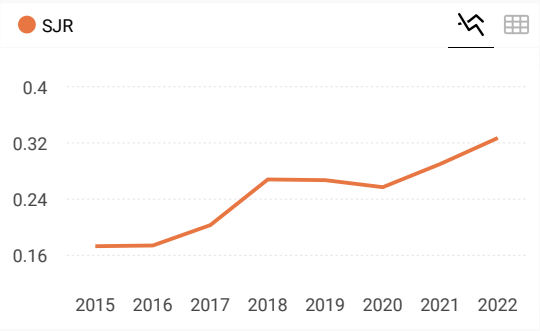

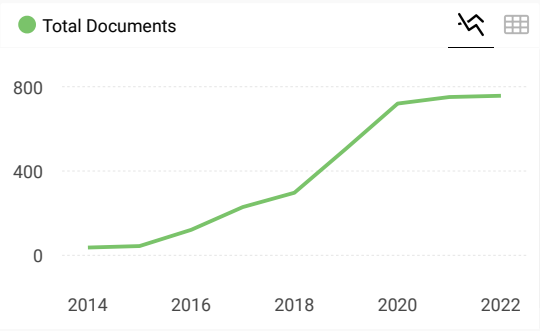

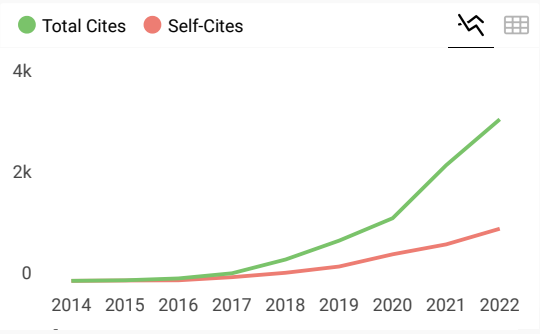

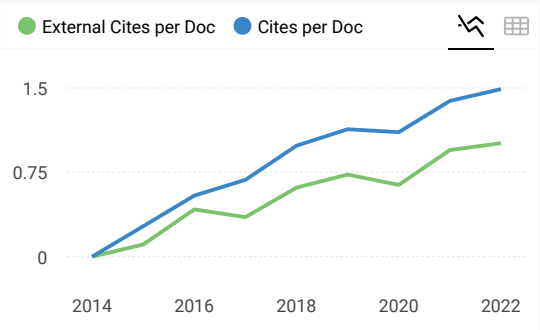

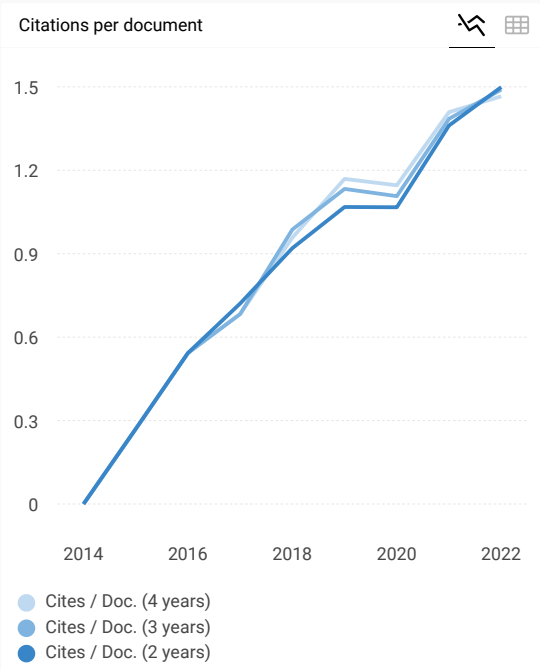

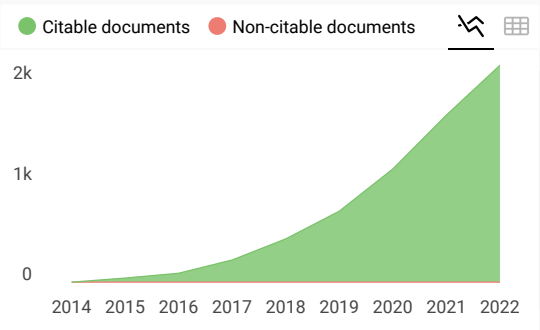

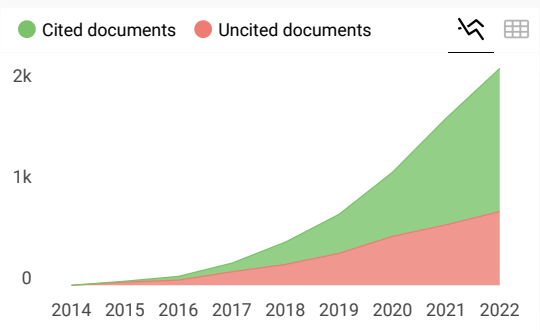

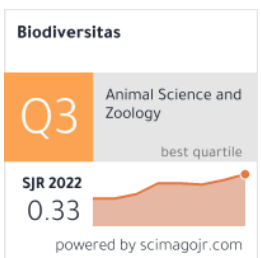

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Information from the internet: Balagadde FK, Song H, Ozaki J, Collins CH, Barnet M, Arnold FH, Quake SR, You L. 2008. A synthetic *Escherichia coli* predator-prey ecosystem. Mol Syst Biol 4:187. www.molecularsystembiology.com

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α-amylase and α-glucosidase inhibitory effects of four *Piper* species and GC-MS analysis of *Piper crocatum*

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Abstract. *Hartini YS, Setyaningsih D. 2023. α-amylase and α-glucosidase inhibitory effects of four* Piper *species and GC-MS analysis of Piper* crocatum*. Biodiversitas 24: 1313-1319.* Antidiabetic activity can be determined by measuring the level of inhibition of α-amylase and/or α-glucosidase enzymes. Our previous study reported the activity of *Piper crocatum* as an inhibitor of α-amylase and α-glucosidase enzymes; hence, this study reported the same activity of three different *Piper* species: *Piper betle, Piper aduncum,* and *Piper retrofractum.* It was also reported that the GC-MS chromatogram profile of *P. crocatum*. The *Piper* species was tested against α-amylase and α-glucosidase enzymes; activity measurements were carried out using UV-Vis spectrophotometry. Analysis of compound content in *P. crocatum* was carried out through GC-MS spectra and its library. The *Piper* species showed inhibitory activity against both α-amylase and α-glucosidase. Compared to *P. betle*, *P. aduncum*, and *P. retrofractum*, *P. crocatum* had the highest activity against both enzymes, with IC_{50} of 8,463 ± 0.318 mg/mL and 10,013 ± 0.070 mg/mL, respectively. The GC-MS profile of *P. crocatum* leaf methanol extract showed that there were 69 peaks, with the highest peak at RT 49,007 (20.49%), followed by RT 48,495 (11.00%), RT 6,984 (8.6%), RT 30.356 (4.51 %), RT 8,654 (3.44%), while the other peaks were below 3%. Based on the GC-MS dictionary, *P. crocatum* contains the main compounds: trans-isoelemicin/alpha-asarone, patchulane, myrcene, 1-octadecyne, and trans-ocimene. Therefore, *Piper crocatum* extract is considered to be developed as an antidiabetic agent.

Keywords: α-amylase, α-glucosidase, GC-MS, *Piper*, *P. crocatum*, species

INTRODUCTION

A particular plant species, which has the same pharmacological activity as other species in the same genus among these species, can have different levels and types of mechanisms of action. The *Piper* plants genus has several pharmacological activities, one of which is antidiabetic. The genus *Piper* includes about 4,226 species spread over various regions, particularly in tropical and sub-tropical (The Plant List 2018). In addition to Indonesia, species of the genus *Piper* are also used in traditional medicine systems in several countries, namely the medical system in China, ayurvedic medicine in India, as well as in Latin America and the West Indies. The active compounds in the *Piper* plant are responsible for the type of activity caused, especially the secondary metabolite compounds produced by the plant. Several studies have reported the content of secondary plant metabolites of the genus *Piper* (Parmar et al. 1997; Mgbeahuruike et al. 2017; Nugroho et al. 2020). In Indonesia, some communities still use several species of *Piper*, such as betel leaf (*Piper betle* L.) to treat nosebleeds, Javanese chili (*Piper retrofractum* Vahl) for various traditional medicinal ingredients, and red betel *(Piper crocatum* Ruiz & Pav.) for the treatment of diabetes mellitus/hyperglycemia (Nugroho and Hartini 2020). Antimicrobial, antiulcer, anti-inflammatory, anticancer, antimutagenic, antioxidant, and antidiabetic properties are the crucial pharmacological properties of *P. betle* (Gupta et al. 2022). Our previous study reported the activity of *P. crocatum* as an inhibitor of α-amylase and α-glucosidase enzymes (Hartini and Setyaningsih 2021). The antidiabetic activity of ethanolic extract of *P. betl*e leaves in catfish, *Clarias gariepinus* have been reported. The enzyme inhibition activity of *P. betle* was excellent compared to the acarbose (Perumal and Saravanabhavan 2018). In vivo study showed that forest betel leaf (*Piper aduncum* L.) extract had an antidiabetic activity (Sitinjak et al. 2016), but there have been no reports on the antidiabetic activity of *P. retrofractum*. Several pharmacological activities of *P. retrofractum* have been reported, including antimicrobial, antioxidant, antihyperurychemia, antihyperlipidemia, antileishmanial, antiproliferative, antiaging, dan antiobesity (Taufik and Soleha 2020).

In pharmaceutical industries, high-quality final products should be supported by clinical studies. Before performing clinical studies, there is a need to conduct a series of pre-clinical studies about using betle leaves medically (Nayaka et al. 2021). Furthermore, testing the antidiabetic activity of a plant was carried out to find the most effective blood glucose-lowering drugs. These tests can be carried out in vivo, in vitro, and in silico. α-amylase and α-glucosidase enzymes are significant targets in type 2 diabetes mellitus. In vitro drug activity testing against the two enzymes was carried out to determine the potential of the tested material as an antidiabetic. Both enzymes catalyze the hydrolysis of the glycosidic bonds of amylase starch so that glucose is released. Hyperglycemia increases because of reduced insulin which causes blood glucose to be unable to enter

muscle cells, adipose tissue, or the liver, thus disrupting the metabolism. The increase in blood glucose is due to the activity of the α-amylase and/or α-glucosidase enzymes that convert starch that enters the body into glucose. Plants of the *Piper* genus are expected to be able to inhibit the work of α-amylase and/or α-glucosidase enzymes, thereby inhibiting the conversion of starch into glucose. It will control blood glucose. The stronger the activity of *Piper* sp. in inhibiting the two enzymes, the stronger the potential of the plant as an antidiabetic. This study compared the antidiabetic activity of four species of the genus *Piper*, namely *P. betle*, *P. aduncum*, *P. retrofractum*, and *P. crocatum*. Gas Chromatography-Mass Spectrometry (GC-MS) analysis effectively identifies and quantifies chemicals in a complex mixture (Al-Rubaye et al. 2017). Gas Chromatography and Mass Spectrometry provide distinct but complementary results; while GC separates mixture components, MS can analyze and identify these components (Simon-Manso et al. 2013). This study confirmed the inhibitory activity of three different *Piper* species, namely *P. betle*, *P. aduncum*, and *P. retrofractum*, against the α-amylase and α-glucosidase activity. Due to the highest antidiabetic potential provided by *P. crocatum*, as found in this study, GC-MS chromatography was further conducted to figure out the phytocomponents of the extract. Among the four species, *P. crocatum*, the *Piper* species with the highest antidiabetic potential, was analyzed for its compound content using GC-MS chromatography.

MATERIALS AND METHODS

Materials

For this study, the fresh leaves of four species of the genus *Piper*, consisting of *P. betle*, *P. aduncum*, *P. retrofractum*, and *P. crocatum*, were received from PT Merapi Farma Herbal, Yogyakarta, Indonesia. The plant was identified by a Taxonomist (Mr. Yohanes Dwiatmaka, M.Si.). Meanwhile, the chemicals materials used in this research were amylase enzyme (SIGMA Aldrich), α-glucosidase (SIGMA Aldrich), ethanol pro analysis (E. Merck), double distilled water, dimethyl sulfoxide pro analysis (E. Merck), iodine iodide reagent, potato starch, 1N HCl, and acarbose.

Methods

Extraction

The *Piper* leaves were extracted with methanol using the maceration method. First, *Piper* leaves were washed under running water, then dried in an oven at 50°C until dry (moisture content<10%). Next, the dried leaves were made into powder, weighed, put into the macerator, added methanol until all the powder was submerged, then left overnight with stirring. Finally, the extracts from 3 times maceration were collected, then evaporated using a rotary evaporator to obtain a thick methanol *Piper* sp. extract.

In vitro α-amylase and α-glucosidase activity test on Piper sp.

The α-amylase enzyme inhibitory activity test was followed by Ononamadu et al. (2020). First, the potato powder (1% w/v), 1.0 mL extract/1 mL acarbose, 1.0 mL -amylase enzyme (1% w/v), and 2.0 mL acetate buffer (0.1M, 7.2 pH) were combined. Next, the inhibitory effect of the sample blank solution measurement was done by pouring 1.0 mL of 0.5% (w/v) potato starch solution into a test tube. After incubating the mixture for an hour, a 0.1 ml iodine-iodide was added. Next, the α-glucosidase enzyme inhibitory activity test was performed based on Pandithurai et al. (2015) with a slight modification. In a test tube, 100.0 µL of extract and standard solutions from each series of test solutions were mixed with 400.0 µL of phosphate buffer and 250.0 µL of maltose substrate solution and pre-incubated at 37°C for 5 minutes. Following pre-incubation, 250.0 µL of phosphate buffer solution pH 7.0 was added and homogenized. The solution was then incubated for 30 minutes at 37°C before 0.3 mL was taken and 0.3 mL of DNS reagent was added to the test tube. This solution was then homogenized and heated in boiling water for 5 minutes. Finally, the solution was mixed, and 3.0 mL of distilled water was added. A UV-vis spectrophotometer set to 536 nm was used to measure absorbance. Equation (1) was used to calculate the percentage of activity reduction. Symbol Ac expresses the absorbance provided by the control experiment, while the As symbol is the tested sample absorbance:

$$\% \text{ inhibition} = (As\text{-}Ac/As) \times 100 \quad (1)$$

The results were statistically evaluated for ANOVA ($p < 0.05$) and continued by Tukey's posthoc test (α-=0.05) using an IBM SPSS version.

GC-MS analysis of P. crocatum

The chromatography *P. crocatum* extract analysis used an Agilent GC 6890N 59765 B MSD connected with MS. The capillary column was Agilent 19091S-433 HP-5ms 5% phenyl methyl siloxane. The maximum temperature was 325°C with a nominal length of 30.0 m, a diameter of 250 µm, a film thickness of 0.25 µm, an initial flow of 1.0 mL/min, an initial pressure of 8.65 psi, and an average velocity of 37 cm/sec. Finally, the GC chromatogram peak data were analyzed descriptively using MS library data from each chromatogram peak.

RESULTS AND DISCUSSION

The test results for the inhibitory activity of α-amylase and α-glucosidase enzymes are shown in Figures 1 and 2. The three *Piper* species tested (*P. betle*, *P. aduncum*, and *P. retrofractum*) showed inhibitory activity against the α-amylase enzyme. In Figure 1, the activity inhibitory profile of the three *Piper* species showed a similar activity pattern between *P. retrofractum*, *P. aduncum*, *and P. betle*. The increased levels of *Piper* extract would increase the inhibitory activity of the α-amylase enzyme. *P. retrofractum* showed the highest α-

amylase inhibitory activity, followed by *P. aduncum* and *P. betle.* However, there was a slight difference in *P. betle* at a 5 mg/mL level, where an increased activity that exceeded the activity of *P. aduncum* was observed.

The three *Piper* species tested (*P. betle, P. aduncum,* and *P. retrofractum*) inhibited the activity of the α-glucosidase enzyme (Figure 2). At the levels of 2.5 mg/mL, 5 mg/mL, and 10 mg/mL, the activity inhibitory profile of the three *Piper* species showed a linear equation of activity levels; yet, the difference only occurred at the levels of 1.25mg/mL and 10 mg/mL. At 1.25 mg/mL, *P. betle* showed the highest α-glucosidase inhibitory activity, while *P. aduncum* had the highest activity at 10 mg/mL. In our previous research, it was reported that *P. crocatum* has the potential to inhibit the activity of α-amylase and α-glucosidase enzymes (Hartini and Setyaningsih 2021). Figure 1 shows that the inhibitory activity of *P. crocatum* against the α-amylase enzyme was higher than the other 3 *Piper* species (*P. betle, P. aduncum, P. retrofractum*), as well as the inhibitory activity of *P. crocatum* against the α-glucosidase enzyme as shown in Figure 2.

Figure 3 shows the inhibitory potential of the four *Piper* species (*P. aduncum, P. betle, P. retrofractum,* and *P. crocatum*) against both α-amylase and α-glucosidase enzymes. The four *Piper* species have the potential for inhibition of α-amylase and α-glucosidase enzymes with IC_{50} values ranging from 8,463-10,317 mg/mL for activity against α-amylase enzymes and IC_{50} values ranging from 10,013-11,567 mg/mL for inhibitory activity against α-glucosidase. The inhibitory activities of the α-amylase enzyme *P. crocatum* were not different from that of *P. retrofractum.* However, it was different in the α-glucosidase enzyme inhibition. Inhibition of *P. crocatum* to the α-glucosidase enzyme was not different from that of *P. aduncum* ($P<0.05$). Therefore, all *Piper* species can be measured for their activity against the α-amylase enzyme and their inhibitory activity against the α-glucosidase enzyme. Therefore, testing the antidiabetic potential of *Piper* species can be carried out on one of these enzymes. In addition, compared to the three *Piper* species tested, *P. crocatum* showed the lowest IC_{50} values for both α-amylase and α-glucosidase enzyme inhibition, with IC_{50} values of 8,463 mg/mL and 10,013 mg/mL, respectively. Acarbose, as a positive control, produced a better α-amylase (IC (IC_{50} 0.837 ± 0.076 mg/mL) and α-glucosidase ((IC_{50} 0.690 ± 0.124 mg/mL) enzyme inhibition than *P. crocatum* extract. Ibrahim et al. (2017) reported that the IC_{50} value of acarbose against α-amylase and α-glucosidase was 200,19 ± 7,29 mg/mL and 182,26 ± 1,05 mg/mL, respectively. Vadivelan et al. (2019) reported that the IC_{50} value of acarbose against α-amylase and α-glucosidase was 11,84 ± 0,19 mg/mL and 13,39 ± 0,11 mg/mL, respectively. Several researchers reported different IC_{50} values but concluded that acarbose is more powerful in inhibiting α-amylase and α-glucosidase. That shows natural enzyme inhibitors can be used as an alternative for treating people with diabetes with minimal side effects.

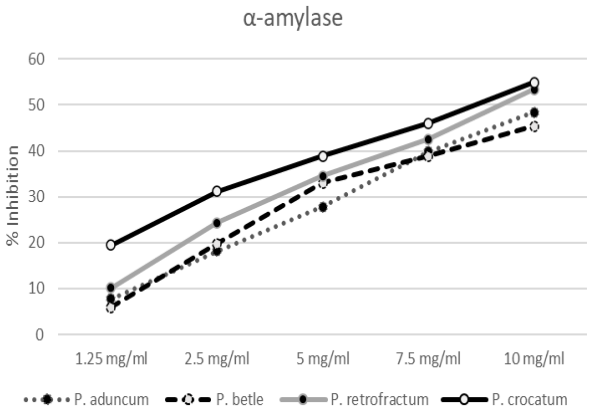

Figure 1. The α-amylase enzymes inhibitory activity of four *Piper* species

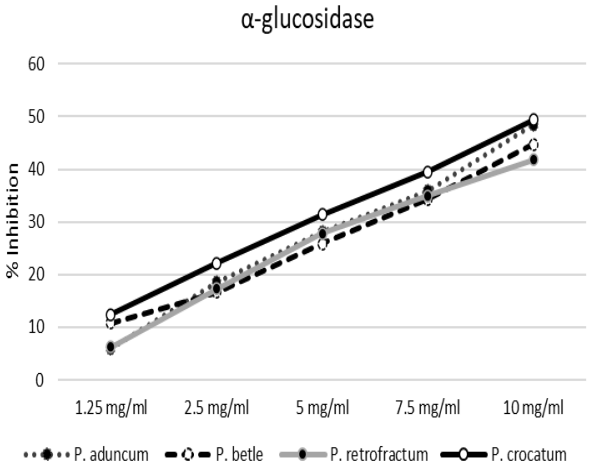

Figure 2. The α-glucosidase enzymes inhibitory activity of four *Piper* species

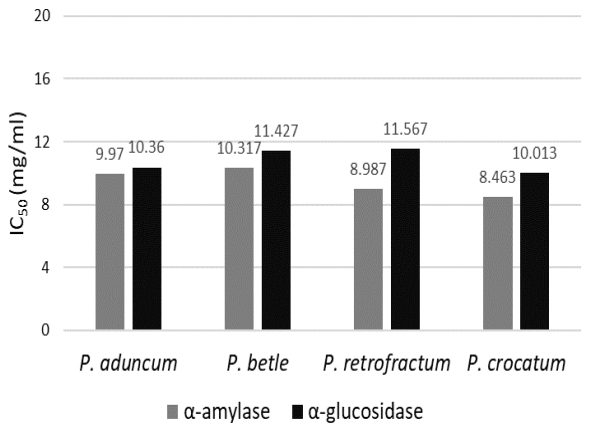

Figure 3. The potency of α-amylase and α-glucosidase enzymes inhibitory activity of four *Piper* species

Compared to the other three species, *P. crocatum* showed the highest potential to inhibit the activity of both enzymes. The GM-MS chromatogram profile showed that *P. crocatum* contained compounds with 69 different peaks. Based on the MS library data, these compound types are presented in Table 1. Of the 69 peaks that appeared, the highest peak was at peak number 56, at Retention Time/RT 49,007, with a peak area of 20.49%, followed by peak number 55 at RT 48,495, with a peak area of 11.00%; peak number 1 at RT 6,984, with a peak area of 8.6 %; peak number 33 at RT 30,356, with a peak area of 4.51%; peak number 6 at RT 8,654, with a peak area of 3.44%. Meanwhile, the other peaks had a peak area of below 3%. GC-MS library data showed that *P. crocatum* contains 58 suspected types of compounds along with their molecular formulas and weights. Based on the GC-MS dictionary, *P. crocatum* contains the main compounds: trans-isoelemicin/alpha-asarone, patchulane, myrcene, 1-octadecyne, and trans-ocimene. Some of the same compounds that appeared at different peaks were beta-caryophyllene, caryophyllene oxide, 1 octadecyne, 2-carene 2-acetyl, and neophytadiene.

Terpenoids represent a promising source of biologically active natural compounds, which have the potential for research and development of new substances with pharmacologic activity. Several studies have confirmed terpene-group compounds responsible for the hydrolysis activity of the alpha glycosidic chain on the amylase enzyme. Some plants are reported to contain terpenes that have α-amylase and α-glucosidase inhibitory activities, including *Potentilla fulgens* (Kumar et al. 2013), *Hertia cheirifolia* (Majouli et al. 2016), *Smallantus macroscyphus* (Serra-Barcellona et al. 2014), *Hedychium coronarium* (Panigrahy et al. 2018). The content of terpenoid and flavonoid compounds in *Asparagus racemosus* Willd extract showed inhibitory activity of alpha-amylase and alpha-glucosidase enzymes (Vadivelan et al. 2019). According to Chelladurai and Chinnachamy (2018), flavonoids, terpenoids, and tannins were responsible for antidiabetic activity. Most studies have focused on phenolic compounds, in which the highest inhibitory activities with the potential of inhibition are related to the number of hydroxyl groups in the compound molecule. The inhibitory potential of flavonoids was stated to be correlated with the number of hydroxyl groups in ring B of the flavonoid framework that forms hydrogen bonds between the hydroxyl groups of polyphenol ligands and catalytic residues from the enzyme binding site (Michelle de Sales et al. 2012; Shah et al. 2018). In this finding, activity against α-amylase and α-glucosidase was related to the terpenoid content in *P. crocatum.* Although triterpenoids are distributed widely in plants, the inhibitory activity of α-amylase was only related to oleanane, ursane, and lupane types. Still, the mechanism by which this activity occurs is unknown. Several terpenoids that are similar to *P. crocatum*, including the monoterpene group (Myrcene, Limonene, and Ocimene) and the sesquiterpene group (Caryophyllene, Nerolidol/Peruviol/Penetrol), are also reported to be responsible to the antidiabetic activity (Mahnashi et al. 2022). Some terpenes present an inhibitory activity on α-amylase at 30% to 98% (Michelle de Sales et al. 2012). The therapeutic agents available for diabetes have not been satisfactory for all patients. Terpenes from various sources have been reported to reduce plasma glucose levels and oxidative stress, thereby preventing diabetes complications. Terpenes also reduce the side effects that arise from conventional antidiabetic drugs.

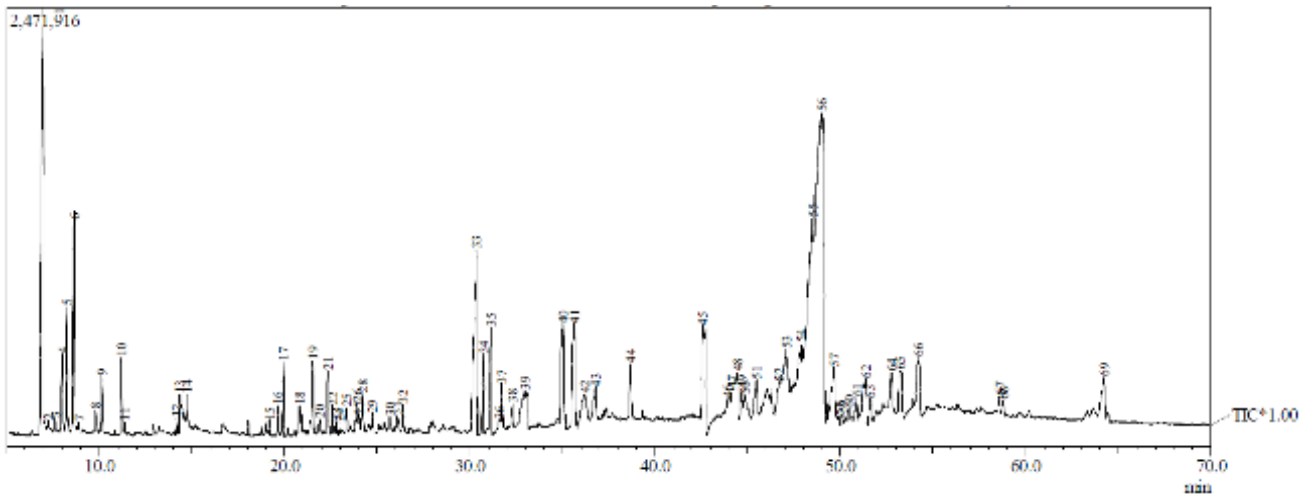

Figure 4. Gas chromatography-mass spectrum of *P. crocatum* leaf extract

Table 1. Identified phytocomponents in *P. crocatum* leaf extract by GC-MS analysis

No	RT	Peak area%	Name of compound	Molecular formula	Mol. weight
1	6.984	8.60	Myrcene	$C_{10}H_{16}$	136
2	7.256	0.26	Sabinene	$C_{10}H_{16}$	126
3	7.622	0.31	alpha-Terpinene	$C_{10}H_{16}$	136
4	8.006	1.06	Limonene	$C_{10}H_{16}$	136
5	8.255	1.56	trans-beta-Ocimene	$C_{10}H_{16}$	136
6	8.654	3.44	Trans-Ocimene	$C_{10}H_{16}$	136
7	8.892	0.10	Gamma-Terpinene	$C_{10}H_{16}$	136
8	9.817	0.22	Terpinolene	$C_{10}H_{16}$	136
9	10.147	0.62	Linalool	$C_{10}H_{18}O$	154
10	11.187	0.77	Beta-Ocimene	$C_{10}H_{16}$	136
11	11.375	0.12	Nerol	$C_{10}H_{18}O$	154
12	14.164	0.17	Trans-Geraniol	$C_{10}H_{18}O$	154
13	14.353	1.14	Coumarin	C_8H_8O	120
14	14.732	0.53	1,6-Octadiene, 3,5-dimethyl-trans	$C_{10}H_{18}$	138
15	19.188	0.16	Beta-Elemene	$C_{15}H_{24}$	204
16	19.642	0.26	Trans-alpha-Bergamotene	$C_{15}H_{24}$	204
17	19.952	0.91	Trans-Caryophyllene	$C_{15}H_{24}$	204
18	20.810	0.49	Beta-Selinene	$C_{15}H_{24}$	204
19	21.534	0.99	Beta- Caryophyllene	$C_{15}H_{24}$	204
20	21.913	0.34	Beta- Caryophyllene	$C_{15}H_{24}$	204
21	22.343	0.84	Alpha-Longipinene	$C_{15}H_{24}$	204
22	22.597	0.37	Gamma-Cadinene	$C_{15}H_{24}$	204
23	22.825	0.11	Alpha-Copaene	$C_{15}H_{24}$	204
24	22.975	0.10	Gamma-Gurjunene	$C_{15}H_{24}$	204
25	23.342	0.30	Alpha-Bisabolol	$C_{15}H_{26}O$	222
26	23.937	0.43	Caryophyllene oxide	$C_{15}H_{24}O$	220
27	24.042	0.18	Alpha-Farnesene	$C_{15}H_{24}$	204
28	24.230	0.48	Alpha-Bisabolol	$C_{15}H_{26}O$	222
29	24.738	0.0	Alpha-Farnesene	$C_{15}H_{24}$	204
30	25.670	0.28	Caryophyllene oxide	$C_{15}H_{24}O$	220
31	26.094	0.42	Myrcenol	$C_{10}H_{18}O$	154
32	26.402	0.40	Caryophyllene oxide	$C_{15}H_{24}O$	220
33	30.356	4.51	1-Octadecyne	$C_{18}H_{34}$	250
34	30.743	1.04	1-Octadecyne	$C_{18}H_{34}$	250
35	31.153	1.90	1-Octadecyne	$C_{18}H_{34}$	250
36	31.575	0.15	Methyl cis-6-octadecenoate	$C_{19}H_{36}O_2$	296
37	31.741	0.46	Methyl hexadecanoate	$C_{17}H_{34}O_2$	270
38	32.303	0.29	Nerolidol	$C_{15}H_{26}O$	222
39	32.974	2.14	Octadecanoic acid	$C_{18}H_{36}O_2$	284
40	35.036	2.63	Ethol	$C_{16}H_{34}O$	242
41	35.648	2.62	Phytol	$C_{20}H_{40}O$	296
42	36.211	1.66	Linolelaidic acid, methyl ester	$C_{19}H_{34}O_2$	294
43	36.788	0.96	Vanicol	$C_{18}H_{36}O_2$	284
44	38.674	0.83	1-Eicosanol	$C_{20}H_{42}O$	298
45	42.581	3.43	Di-n-octyl-phtalate	$C_{24}H_{38}O_4$	390
46	43.908	050	Patchulane	$C_{15}H_{26}$	206
47	44.158	1.22	Phenylchroman	$C_{15}H_{14}O$	210
48	44.449	0.61	Alpha-Asarone	$C_{12}H_{16}O_3$	208
49	44.694	0.51	2-Carene, 2-acetyl	$C_{12}H_{18}O$	178
50	44.867	0.56	2-Carene, 2-acetyl	$C_{12}H_{18}O$	178
51	45.581	1.07	Calarene	$C_{15}H_{24}$	204
52	46.692	1.37	2-Phenylpropane-1,1-Dioldiethanoate	$C_{13}H_{16}O_4$	236
53	47.075	1.58	2-Carene, 2-acetyl	$C_{12}H_{18}O$	178
54	47.855	1.32	Edulan III	$C_{13}H_{20}O$	192
55	48.495	11.00	Patchulane,	$C_{15}H_{26}$	206
56	49.007	20.49	Trans-Isoelemicin, alpha-asarone	$C_{12}H_{16}O_3$	208
57	49.649	1.63	2-Carene, 2-Acetyl	$C_{12}H_{18}O$	178
58	49.942	0.13	Valerenal	$C_{15}H_{22}O$	218
59	50.123	0.18	Venucarol	$C_{15}H_{22}O_4$	266
60	50.454	0.43	4,7-Octadecadiynoic acid, methyl ester (CAS) Methyl 4,7-Octadecadiynoate	$C_{19}H_{30}O_2$	290
61	50.893	0.7	Spathulenol	$C_{15}H_{24}O$	220
62	51.372	1.75	Vitamin E	$C_{29}H_{50}O_2$	430
63	51.631	0.47	Alpha-Neoclovene	$C_{15}H_{24}$	204
64	52.761	0.93	Dicholesteryl succinate	$C_{58}H_{94}O_4$	854
65	53.241	1.41	Trans-Stigmasta-5,22-dien-3.beta-ol	$C_{29}H_{48}O$	412
66	54.231	1.48	Dicholesteryl succinate	$C_{58}H_{94}O_4$	854
67	58.588	0.28	Neophytadiene	$C_{20}H_{38}$	278
68	58.791	0.22	Octadecyl hexadecanoate	$C_{34}H_{68}O_2$	509
69	64.235	1.32	Neophytadiene	$C_{20}H_{38}$	278
Total		100%			

In addition to terpenes as α-glucosidase and α-amylase inhibitory compounds, Panigrahy et al. (2020) describe various mechanistic actions of terpenes as antidiabetic agents. Those mechanistic actions, namely, the insulin-mimetic action of terpene compounds, reduction of oxidative stress by terpene compounds, the anti-hyperglycemic activity of terpene compounds, hypolipidemic activity of terpenes, reduction of blood glucose level by terpenes, terpenes as aldose reductase inhibitor, terpenes against some novel therapeutic targets, effect of terpenes on other complications of diabetes, and miscellaneous effects of terpenes. Although there have been reports of in vitro and in vivo tests, terpenes as antidiabetics have not been investigated in clinical studies (Panigrahy et al. 2020). Furthermore, Chen et al. (2019) reported recent advances in developing sesquiterpenoids in treating type 2 diabetes.

The four *Piper* species tested for inhibitory activity against starch hydrolyzing enzymes showed activity against α-amylase and α-glucosidase. Elya et al. (2015), Pandithurai et al. (2015), and Ibrahim et al. (2017) also reported the inhibitory activity of plant extracts against both enzymes. It indicates that the potential antidiabetic test can choose one of them. This finding aligns with Kazeem et al. (2013), who analyzed the target of action of starch hydrolyzing enzyme inhibitor compounds. They suggested that one of the mechanisms by which *Morinda lucida* exhibited its hypoglycemic potential was by inhibiting pancreatic amylase and intestinal glucosidase of the animals used in that study.

In conclusion, *P. betle*, *P. aduncum*, *P. retrofractum* and *P. crocatum* have inhibitory activity against α-amylase enzymes with IC_{50} respectively 10,317mg/mL; 9,970 mg/mL; 8,987mg/mL; and 8,463 mg/mL. *P. betle*, *P. aduncum*, *P. retrofractum* and *P. crocatum* also inhibit the α-glucosidase enzyme respectively with IC_{50} values 11,427mg/mL; 10,360mg/mL; 11,567mg/mL; and 10,013 mg/mL. *P. crocatum* has the highest inhibitory activity compared to the other three *Piper* species, both against α-amylase and α-glucosidase. The terpene group of compounds, particularly monoterpenes, and sesquiterpenes in *P. crocatum*, may be responsible for the anti-α-amylase and α-glucosidase activities. The researchers used acarbose for positive control, and it showed that it had an IC_{50} value of 0.837 ± 0.076 mg/mL for the α-amylase inhibitory and 0.690 ± 0.124 mg/mL for the α-glucosidase inhibitory activities. Although the IC_{50} value of *P. crocatum*, among other *Piper* species, is much higher than acarbose, this species is more recommended for diabetes treatment. That suggests considering its action mechanism to inhibit the conversion of starch/polysaccharides into glucose/ monosaccharides.

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