JJBS

Volume 15, Number 5,December 2022
ISSN 1995-6673
Pages 897 – 904
https://doi.org/10.54319/jjbs/150520

Jordan Journal of Biological Sciences

Chemical Compounds, Antioxidant Properties, and Enzyme Inhibitory Activities of Kitolod Leaf and Fruit Hexane Extracts as Antidiabetic

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Received: Oct 5, 2022; Revised: Dec 17, 2022; Accepted Dec 23, 2022

Abstract

Diabetes (DMT 2) is one of the most common free radicals-incited conditions, and its treatment calls for natural antioxidants fit for daily administration with minimum burden to the bodily system. Traditionally used to treat eye disease in Indonesia, kitolod [*Isotoma longiflora* (L) Presl.] was studied for its antidiabetic prospect. Hexane extracts from the leaves and fruit were analyzed for their non-polar chemical compounds, antioxidant properties, and α-amylase and α-glucosidase enzyme inhibition activities. Liquid chromatography (LC)-mass spectrometry (MS) identified 29 compounds out of the leaf and 36 compounds out of the fruit. The extracts showed moderate antioxidant activity with IC_{50} values of (90.586 ± 0.663) μg mL^{-1} and (92.832 ± 1.042) μg mL^{-1}. Their inhibitory against α-amylase was very strong, with IC_{50} values of (38.511 ± 0.068) mL^{-1} and (39.790 ± 0.233) mL^{-1}, while ones against α-glucosidase were strong to moderate with IC_{50} value of leaf extract (40.833 ± 0.571) μg mL^{-1} and of fruit extract (65.383 ± 0.511) μg mL^{-1}. Seeing the high potential in kitolod extracts to inhibit α-amylase and α-glucosidase enzymes, they should correspond with functional food for people with diabetes.

Keywords: Antioxidant, Diabetes herbal medicine, Environmentally friendly, Functional food, *Isotoma longiflora* (L) Presl., Medicinal plant, Phytochemical, Sangkobak

1. Introduction

While technology improves human welfare, its practices emit free radicals into the environment. Add them up with ones naturally released by living organisms, and their levels can be too high to tolerate (Kaewseejan and Siriamornpun, 2015). In such cases of environmental imbalance, free radicals can interfere with the work of various cellular elements, such as proteins, lipids, carbohydrates, and nucleic acids (Sies, 2015). Moreover, at the cellular level, excessive free radicals can cause oxidative stress (Dando *et al*., 2015). Such activities, in the long run, can affect human physiological conditions (Damat *et al.*, 2021a; Ma, 2014) by triggering ailments like inflammation, diabetes, cardiovascular, a neuro degenerative disorder, and cancer (Elmakawy *et al*., 2019; Pingitore *et al.,* 2015; Setyobudi *et al*., 2019 and 2021).

With reported prevalence rate of diabetes in adults in 2021 to reach 537 × 10^6 (IDF, 2021), Diabetes mellitus type 2 (DMT 2) is a global issue. Indonesia is ranked fourth place with 21 000 000 – still higher than Mexico at 13 100 000 – yet lower than China at 89 500 000, India at 67 800 000 and USA at 30 700 000 (Lin *et al*., 2020). As one of the most common free radicals-incited conditions, DMT 2 treatment calls for natural antioxidants fit for daily administration with minimum burden to the bodily system.

Antioxidants can protect cells from the damage caused by free radicals by developing safe interactions with them (Gupta and Gupta, 2015; Setyobudi *et al*., 2022) and terminating any reactions before the molecular interference stage (Elmakawy, 2019). Chemically-processed medicine containing synthetic antioxidants works fast in neutralizing free radicals, but long-term consumption is detrimental to health. Besides, the production process itself discharges more free radicals into the environment. Therefore, plant-based supplements and functional foods containing phytochemicals and secondary metabolites should help improve human health and prevent the forming of problems due to free radicals (Abdel-Mawgoud *et al*., 2019; Ahmed *et al*., 2015; Damat *et al*., 2019). Furthermore, natural means are environmentally friendly

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since additional free radicals from the production process will be low.

People with diabetes need regular antioxidant intake to keep free radicals at bay. Based on the results of epidemiological studies, it is known that antioxidants can reduce diabetes-related complications and restore insulin sensitivity (Rajendiran *et al*., 2018). This situation calls for herbal medicine fit for daily administration with minimum burden to the bodily system. Easy to grow and cultivate, Kitolod – Sangkobak, Javanese local name [*Isotoma longiflora* (L) Presl.] is a medicinal plant traditionally used to treat eye disease in Indonesia that has been known to contain secondary metabolites. Previous studies had screened alkaloid, terpenoid, and phenol contents (which showed antioxidant activity and worked as an anticancer against the MCF7 breast cancer cell line) in the leaf, stem, and fruit (Egarani *et al*., 2020). Terpenoid compounds are found in many organisms, especially in green and flowering plants. The leaves, fruit, flowers, stems, and roots of the kitolod plant contained flavonoid compounds of (10.48 ± 0.10) mg kg^{-1}, (2.27 ± 0.23) mg kg^{-1} , (1.10 ± 0.11) mg kg^{-1}, (0.72 ± 0.12) mg kg^{-1}, and (0.53 ± 0.13) mg kg^{-1}, respectively (Enggarani *et al*., 2020). Fruits and flowers are known to have very strong antioxidant activity. Kitolod leaf parts have strong antioxidant activity, while the stem and roots have weak antioxidant activity.

Plants containing terpenoids have been used for medicine in the Middle East (Pichersky and Raguso., 2016). Furthermore, Hapsari *et al*. (2016) underlined its ability to treat respiratory diseases like bronchitis and asthma due to its anti-inflammatory and analgesic functions. Furthermore, its ethanol extract inhibits the growth of several microorganisms, such as candida fungus and tuberculosis bacteria (*Mycobacterium tuberculosis*). In order to see the plant's prospect as a source of natural antioxidants to treat diabetics, this research explores the bioactive components in kitolod hexane extracts. It identifies their antioxidant properties and their α-amylase and α-glucosidase enzyme inhibitory through an *in-vitro* assay.

2. Materials and Methods

2.1. Plant extraction

Leaves and fruit of kitolod were collected from natural populations in Karangploso, Pendem, Batu, East Java, Indonesia. Preparatory steps involved washing under running water, draining for 2 h, and drying in a dryer cabinet (GETRA, FJ-15C, China) at 50 °C for 36 h. Voucher specimens have been deposited at the Herbarium of Bali Botanic Garden, National Research and Innovation Agency, Bedugul, Bali, Indonesia. The dried leaves and fruit were each ground to powder, sifted with 40 mesh filters, packaged in a closed glass beaker, and stored at -20 °C (LG, GN 304SL, South Korea) in the dark prior to use.

Powdered leaves (200 g) were macerated with 1 L hexane (Merck, USA) for 48 h at room temperature (on temperature 27 ºC) and separated from its filtrate in a Buchner funnel layered with Whatman No.2 filter paper connected to a vacuum pump. The fruit specimen also received the same treatments. Ethanol 99.9 %, Merck USA filtrates were fractionated successively with n-hexane and ethyl acetate with purity ≥ 99 %. Each fraction was then concentrated using a vacuum rotary evaporator (Heidolph RE-502, Germany) at a temperature of 40 °C until one-fifth of the volume was reached and transferred into a clean bottle. The solvent is separated by flowing nitrogen gas into the erlenmeyer. The process will end when the solvent is no longer smelled.

2.2. Liquid chromatography (LC)-mass spectrometry (MS)

LC/MS analysis to identify bioactive compounds employed Shimadzu LCMS-8040 LC/MS, Shimadzu Column, and FC-ODS Shim Pack (2 mm × 150 mm, 3 μm). The column temperature was maintained at 35 °C, and the injection volume was fixed at 1 μL. The flow gradients were programmed on 0/0 or 0 min, 15/85 at 5 min, 20/80 at 20 min, and 90/10 at 24 min. The flow rate was 0.5 mL min^{-1} (90 % methanol in water) with disolvation at 350 °C. Low-energy CID induced fragmentation (Salih *et al*., 2017). Other chemicals and reagents used in this research were classified as an analytical grade, purchased from Sigma Aldrich Chemical Co. (St Louis, Mo, USA).

2.3. Antioxidant activity test

2.3.1. DPPH (1, 1-diphenyl-2-picryl-hydroxyl) radical scavenging activity

IC_{50} method (Damat *et al*., 2021b; Liu *et al*., 2014) was brought to determine the level of extracts regarding the ability to inhibit 50 % of free radicals added. The hexane (Merck, Canada) extracts were prepared in six different concentrations [(0, 20, 40, 60, 80, 100) μg mL^{-1})]. Each kitolod leaf and fruit sample in methanol (100 μL) and DPPH in ethanol (1.9 mL of 0.1 mM) was mixed and homogenized, then stored in a tightly-lidded container and allowed to stand for 30 min at room temperature in the dark. The positive control comprised quercetin. The blanks included all the reaction reagents except the extract and the positive control substances. The purple to yellow colour formed in the test solution was measured at 517 nm. The tests were carried out in triplicates. IC_{50} was determined from percent inhibition vs. concentration plots. The capacity to scavenge the DPPH radical was based on Equation (1):

$$\text{DPPH}^{\bullet}\text{ scavenging activity \%} = [(A_C - A_S) / A_C] \times 100 \quad (1)$$

(A_C) is the absorbance of the negative control reaction, and (A_S) is the absorbance in the presence of plant extracts. The results were conveyed as IC_{50} [the concentration (μg mL^{-1}) of the hexane extract that scavenges 50 % of $DPPH^{\bullet}$ radical].

2.3.2. Inhibition of α-amylase assay

Following the standard method, α-amylase inhibitory activities of kitolod hexane extracts were performed (Chelladurai and Chinnachamy, 2018). The substrate was of starch solution (0.5 % w v^{-1}), gained after boiling potato starch in distilled water for 15 min. The enzyme solution was 1 mg of porcine pancreatic α-amylase dissolved in 20 mM phosphate buffer (100 mL, pH 6.9). The sample solutions prepared in Dimethyl sulfoxide (Merck, USA) were of various concentrations (10 to 100) mg mL^{-1}. DNS solution (Merck, USA) (20 mL of 96 mM 3,5-dinitro salicylic acid, 12 g sodium potassium tartrate (Merck,

USA) in 8 mL of 2 M NaOH (Merck, USA), and 12 mL deionized water) served as the colouring reagent.

Three experiments– test, blank, and control – were conducted. For tests, enzyme solutions were added in 1 mL of each test in a test tube and incubated at 25 °C for 30 min. 1 mL taken from each mixture was then mixed with 1 mL starch solution and incubated at 25 °C for 3 min. After that, 1 mL of DNS solution was added. The tube was then covered and heated in a water bath at 85 °C for 15 min. Once the tube cooled, the reaction mixture was diluted with distilled water (9 mL). The absorbance was finally recorded at 540 nm. For blanks, DNS solution was added prior to starch solution. As of control, the procedure was the same as tests outside of replacing plant extracts with 1 mL Dimethyl Sulfoxide. Acarbose (Glyco AC 50 acarbose, Mumbai India), a well-known antidiabetic medicine, was selected as the positive control. The percentage of inhibition was calculated as per Equation (2):

$$\text{Inhibition (\%)} = [(A_C - A_S) / A_C] \times 100 \quad (2)$$

Ac was the absorbance of the control, and AS was the absorbance in the presence of the plant extracts. The results were recorded as IC_{50} [the hexane extract concentration (µg mL^{-1}) inhibiting 50 % of α-amylase activity].

2.3.3. Inhibition of α-glucosidase assay

Samples were prepared in varying concentrations from (2 to 200) µg mL^{-1} of hexane extract dissolved in Dimethyl Sulfoxide. In a microplate with a 30 µL sample, quasi-phosphate 36 µL phosphate buffer of pH 6.9 and 17 µl 4 mM substrate of p-nitrophenyl-α-D-glucopyranose (p-NPG) were added (Sigma-Aldrich, Switzerland). After incubation for 5 min at 39 °C, 25 µL enzyme solution α-glucosidase (Saccharomyces cerevisiae, Sigma-Aldrich-Germany) of 0.8 unit L^{-1} was added and incubated again for 15 min at 39 °C to reduce the substrate. The reaction was then stopped by adding 100 µL of Na_2CO_3 200 mM solution (Merck, USA). To measure the amount of p-nitrophenol released from p-NPG, a microplate reader (Versamax ELISA Microplate Reader, USA) at 400 nm was utilized to determine the inhibition rates of alpha-glucosidase. The reading of the results was carried out three times. Acarbose became the positive control. Substrate + enzyme solution (no extract added) served as blank, and substrate (no enzyme added) acted as control (Mahayasih *et al*., 2017). The inhibition percentage was calculated referring to Equation (3):

$$\text{Inhibition (\%)} = [(\text{blank absorption} - \text{sample absorption}) / \text{blank absorption}] \times 100 \quad (3)$$

The results were expressed as IC_{50} [the concentration (µg mL^{-1}) of the hexane extracts that inhibited 50 % of α-glucosidase activity].

2.4. Data analysis

All experiments were performed in triplicate, of which data were then analysed on Microsoft Excel, noted as mean ± standard deviation (n = 3). The IC_{50} values were computed using GraphPad Prism 7 for Windows, GraphPad Software, La Jola, California, USA. Differences were regarded as significant when the rates hit $P < 0.05$ (Adinurani, 2016, 2022).

3. Results and Discussion

3.1. Chemical Compounds

Samples were of 200 g kitolod leaf powder and fruit powder in hexane macerations for 72 h. LC-MS discovered 29 compounds out of 22.064 g hexane extract from the leaf and 36 compounds out of 37.126 g from the fruit. The results of compound spectrum analysis are displayed in Table 1 and Table 2, respectively. The components contained in both hexane extracts were grouped into three: (i) ones of > 5.000 %, (ii) between 3.000 % and 5.000 %, and (iii) lower than 3.000 %.

Components in the leaf sample that belong to Group 1 are β Caryophyllene (7.296 %), Chrysandiol (7.175 %), Chrysartemin B (6.499 %), Chrysartemin A (6.337 %), and 5 Ethylidene 5,6 dihydro 3,6,6 trimethyl 2 pyranone (6.329 %); Santamarine (5.764 %) is in sesquiterpene category. Components in Group 2 are Myrcene (3.690 %), Thymol (4.118 %), Capelin (3.929 %), β Farnesene (3.238 %), α-Cardinal (3.562 %), Nerolidol (4.374 %) and Stigmasterol (4.864 %). Components listed in Group 3 include Benzaldehyde, Benzoicacid, Sabinene, Camphene, Nojigiku alcohol, Germacrene D, cis Spiro ketalenol etherpolyne, Kikkanol B, Clovane 2 β.9 α diols, Kikkanol C, Kikkanol D, Chrysartemin A, Arteglasin A, β Cyclopyrethrosin, and Flavoxanthin.

As of the fruit sample, Apigenin (10.081 %), Chrysetunone (6.387 %), and Chrysandiol (5.297 %) are incorporated in Group 1. Group 2 consists of Chrysanthenone, Thymol, Linalool,5 Ethylidene 5.6 dihydro 3,6,6 trimethyl 2 pyranones, Bornylacetate, β Caryophyllene, Chrysartemin A, Chrysartemin B, and β Sitosterol. The remaining 27 compounds are gathered in Group 3.

A total of 80.343 % of chemical components found in the leaf extract and 72.830 % in the fruit extract are classified as sesquiterpenes – or those that have shown antioxidant activities. Yu *et al*. (2013) pointed out that β-farnesene had the potential to control plant-angels ticks and influence genetic engineering. Francomano *et al*. (2019) stated that β-caryophyllene proved anti-inflammatory activity by inhibiting major inflammatory mediators such as nitrite oxide synthase. Su *et al*. (2015) reported that α-cardinal contained in *Diospyros discolor* Willd essential oil exhibited cytotoxic activity against human colon, liver, and lung cancer cells. An alcoholic sesquiterpene, Nerolidol, was indicative of anti-inflammatory, antioxidant, and anticancer properties (Ni *et al*., 2019). Chrysandiol belongs to a sesquiterpene group widely found in flowers and leaves with antidiabetic activity (Jiang *et al*., 2021). Santamarine was also classified as a sesquiterpene that has antioxidant activity (Oh *et al*., 2021). Betha-sitosterol is widely found in traditional medicines since many plants produce it; despite its role in prostate enlargement, the compound showed antioxidant activities (Vo *et al*., 2020).

3.2. Antioxidant properties

Antioxidant potential can be measured by various methods, including hydrogen atom transfer, single electron transfer, or targeted scavenging activity (Christodoulou *et*

al., 2022). The antioxidant activity test method for DPPH radicals is widely used to analyze the antioxidants of extracts of natural ingredients (Mishra *et al*., 2019). Antioxidant activity capacity test (DPPH) was carried out on hexane extracts from kitolod fruit and leaves and compared with quercetin standards.

The results of DPPH radical scavenging assay towards leaf hexane extract, fruit hexane extract, and quercetin are summarized in Table 3. The differences in activity were due to the amount of hydrogen or electron thrown to free radicals (Makasana *et al*., 2017). A smaller IC_{50} value indicates a higher radical scavenging capability, and that means a higher effectiveness (Promprom and Chatan, 2017). It is therefore conclusive that kitolod hexane extracts – containing potent antioxidants Myrcene and β-caryophyllene (Noriega *et al*., 2019) – have better performance compared to the controls. Moreover, nerolidol, a sesquiterpene also contained in kitolod, had been recommended by Chan *et al*. (2016) to prevent oxidation of unsaturated fatty acids.

Table 1. Phyto components of kitolod leaf identified by LC-MS

Peak No.	RT (min)	Exact Mass (g mol^{-1})	Molecular Weight (g mol^{-1})	Compound	Chemical Formula	Composition (%)
1	1.230	106.041	106.124	Benzaldehyde	C_7H_6O	1.923
2	1.289	122.036	122.123	Benzoic acid	$C_7H_6O_2$	1.165
3	1.483	136.125	136.238	Sabinene	$C_{10}H_{16}$	1.314
4	1.497	136.125	136.238	Camphene	$C_{10}H_{16}$	1.913
5	1.500	136.125	136.238	Myrcene	$C_{10}H_{16}$	3.690
6	1.611	150.104	150.221	Thymol	$C_{10}H_{14}O$	4.118
7	1.626	152.120	152.237	Nojigiku alcohol	$C_{10}H_{16}O$	1.314
8	2.800	166.099	166.220	5 Ethylidene 5,6 dihydro 3,6,6 trimethyl two pyranone	$C_{10}H_{14}O_2$	6.329
9	2.812	168.057	168.195	Capelin B-Caryophyllene	$C_{12}H_8O$	3.929
10	5.494	204.187	204.357	B-Farnesene	$C_{15}H_{24}$	7.296
11	5.498	204.187	204.357	Germacrene D	$C_{15}H_{24}$	3.238
12	5.507	204.187	204.357	cis Spiroketalenolether polyyne	$C_{15}H_{24}$	2.643
13	5.852	214.099	214.264	α-Cardinal	$C_{14}H_{14}O_2$	2.643
14	6.947	222.198	222.372	Nerolidol	$C_{15}H_{26}O$	3.562
15	6.950	222.198	222.372	Kikkanol B	$C_{15}H_{26}O$	4.374
16	7.979	236.177	236.355	Chrysandiol	$C_{15}H_{24}O_2$	1.904
17	8.001	252.172	252.354	Clovane 2 β,9 α diol	$C_{15}H_{24}O_3$	7.175
18	8.007	238.193	238.371	Santamarine	$C_{15}H_{26}O_2$	2.513
19	8.018	248.141	248.322	Kikkanol C	$C_{15}H_{20}O_3$	5.764
20	8.245	252.172	252.354	Kikkanol A	$C_{15}H_{24}O_3$	2.188
21	8.298	254.188	254.370	Kikkanol D	$C_{15}H_{26}O_3$	2.498
22	8.303	254.188	254.370	Chrysartemin A	$C_{15}H_{26}O_2$	1.283
23	9.938	278.115	278.304	Chrysartemin B	$C_{15}H_{18}O_5$	6.337
24	9.940	278.115	278.304	Arteglasin A	$C_{15}H_{18}O_5$	6.499
25	11.496	304.131	304.342	β Cyclopyrethrosin	$C_{17}H_{20}O_5$	3.535
26	11.525	306.146	306.358	Stigmasterol	$C_{17}H_{22}O_5$	1.927
27	15.048	412,370	412.702	β Sitosterol	$C_{29}H_{48}O$	2.643
28	15.638	414.386	414.718	Flavoxanthin	$C_{29}H_{50}O$	4.864
29	33.604	584.422	584.885		$C_{40}H_{56}O_3$	1.419

RT: Retention Time

Table 2. Phyto components of kitolod fruit identified by LC-MS

Peak No	RT (min)	Exact Mass (g mol^{-1})	Molecular Weight (g mol^{-1})	Compound	Chemical Formula	**Composition (%)**
1	1.230	106.042	106.124	Benzaldehyde	C_7H_6O	1.309
2	1.289	122.037	122.123	Benzoic acid	$C_7H_6O_2$	0.757
3	1.471	134.110	134.222	p Cymene	$C_{10}H_{14}$	0.492
4	1.476	136.125	136.238	α Terpinene	$C_{10}H_{16}$	2.067
5	1.477	136.125	136.238	α Pinene	$C_{10}H_{16}$	1.631
6	1.479	136.125	136.238	β Pinene	$C_{10}H_{16}$	2.401
7	1.483	136.125	136.238	Sabinene	$C_{10}H_{16}$	4.588
8	1.497	136.125	136.238	Camphene	$C_{10}H_{16}$	3.238
9	1.500	136.125	136.238	Myrcene	$C_{10}H_{16}$	2.726
10	1.599	150.105	150.221	Chrysanthenone	$C_{10}H_{14}O$	4.319
11	1.611	150.105	150.221	Thymol	$C_{10}H_{14}O$	3.247
12	1.635	154.136	154.253	Borneol	$C_{10}H_{18}O$	1.958
13	1.637	154.136	154.253	Linalool	$C_{10}H_{18}O$	3.248
14	2.800	166.099	166.220	5 Ethylidene 5,6 dihydro 3,6,6 trimethyl 2 pyranone	$C_{10}H_{14}O_2$	0.973
15	2.810	168.042	168.148	2,6 Dimethoxy p benzoquinone	$C_8H_8O_4$	1.082
				Bornyl acetate		
16	5.156	196.146	196.290	Calacorene	$C_{12}H_{20}O_2$	2.708
17	5.233	200.157	200.325	Calamine	$C_{15}H_{20}$	4.331
18	5.284	202.172	202.341	α Copaene	$C_{15}H_{22}$	1.851
19	5.488	204.188	204.357	β Caryophyllene	$C_{15}H_{24}$	2.646
20	5.494	204.188	204.357	β Farnesene	$C_{15}H_{24}$	2.709
21	5.498	204.188	204.357	α Selinene	$C_{15}H_{24}$	1.476
22	5.500	204.188	204.357	β Element	$C_{15}H_{24}$	2.646
23	5.504	204,188	204.357	Germacrene D	$C_{15}H_{24}$ $C_{15}H_{24}$	2.709
24	5.507	204,188	204.357	α Cardinal	$C_{15}H_{26}O$	1.957
25	6.947	222.198	222.372	t Muurolol	$C_{15}H_{26}O$	1.742
26	6.956	222.198	222.372	Kikkanol B	$C_{15}H_{24}O_2$	0.973
27	7.979	236.178	236.355	Chrysandiol	$C_{15}H_{24}O_3$	5.297
28	8.001	252.173	252.354	Santamarina	$C_{15}H_{20}O_3$	3.237
29	8.018	248.141	248.322	Chrysetunone	$C_{15}H_{24}O_3$	6.387
30	8.243	252.173	252.354	Kikkanol A	$C_{15}H_{26}O_3$	1.082
31	8.298	254.188	254.370	Apigenin	$C_{15}H_{10}O_5$	1.082
32	9.365	270.053	270.240	Chrysartemin A	$C_{15}H_{18}O_5$	10.081
33	9.938	278.115	278.304	Chrysartemin B	$C_{15}H_{10}O_5$	3.248
34	9.940	278,115	278.304	Stigmasterol	$C_{29}H_{48}O$	4.253
35	15.048	412.371	412.702	β Sitosterol	$C_{29}H_{50}O$	2.836
36	15.638	414.386	414.718			3.734

RT: Retention Time

Table 3. Radical scavenging activity, α-amylase activity, andα-glucosidase activity inhibitions of kitolod leaf and fruit hexaneextract

Materials	Radical scavenging activity	Inhibition of α-amylase activity	Inhibition of α-glucosidase activity
	IC_{50} (μg mL^{-1})	IC_{50} (mg mL^{-1})	IC_{50} (μg mL^{-1})
Leaf	92.832 ± 1.042[a]	39.790 ± 0.233[a]	40.833 ± 0.571[b]
Fruit	90.586 ± 0.663[a]	38.511 ± 0.068[a]	65.383 ± 0.511[c]
Quercetin	111.923 ± 0.676[b]	184.677 ± 1.480[b]	87.051 ± 0.430[d]
Acarbose	-	231.647 ± 0.593[c]	14.289 ± 0.112[a]

Note: The above value is mean ± sd. Subcritp shows a meaningful difference with $P < 0.05$

3.3. Enzyme inhibitory

3.3.1. α-amylase inhibitory activity

The inhibitory activity of the alpha-amylase enzyme was carried out in vitro. As a comparison, inhibition uses acarbose and quercetin. The α-amylase enzyme has a role in inhibiting the breakdown of oligosaccharides and disaccharides into monosaccharides so that it will delay glucose absorption (Jemaa *et al*., 2017). This mechanism will efficiently reduce postprandial sugar levels (Duarte *et al*., 2020).

Referring to Table 3, since the extracts came out with lower IC_{50} values – (39.790 ± 0.233) μg mL^{-1} and (38.511 ± 0.068) μg mL^{-1} for leaf and fruit correspondingly – than acarbose and quercetin, their α-amylase inhibitory activities are equally proven higher than the latter two. The potential of the hexane extract in inhibiting α-amylase is more likely due to the presence of β-sitosterol. The fact that α-amylase inhibition plays a role in the process of starch and glycogen digestion is the basic idea to treat carbohydrate absorption disorders in people with diabetes (Sales *et al*., 2012). The active compounds in both extracts are of the same type of inhibitory activity and only differ in concentrations. Rufino *et al*. (2015) said that myrcene had an antidiabetic effect, and Sales *et al.* (2012) supported the finding that terpenoids had high bioactivity against hyperglycemia. The reason behind choosing hexane extraction is due to previous discoveries that hexane extractfrom plants (*Cinnamomum zeylanicum* Blume, *Crataegus oxyacantha* (Ram Tulsi), *Hibiscus sabdariffa* L.*, Morus alba* L.*, Portulaca oleracea* L., *Rubus fruticosus, Syzygium aromaticum* (L.) Merrill and Perry, *Teucrium polium* L., *Trigonella foenum-graecum* L., and *Vaccinium arctostaphylos* L.) are able to inhibit α-amylase enzyme (Salehi *et al.*, 2013) and that bioactive compounds in hexane fraction are able to inhibit the activity of α-amylase hydrolyzed polysaccharides into end products containing mixtures of maltose, malt triose, and oligosaccharides (6-8 glucose units) (Sales *et al*., 2012).

3.3.2. α-glucosidaseinhibitory activity

The enzyme α-glucosidase hydrolyzes oligosaccharides into monosaccharides. Furthermore, monosaccharides are absorbed by the small intestine to increase blood glucose levels (Sallau *et al*., 2018). The activity of the α-glucosidase enzyme can be inhibited by the hexane extract of kitolod leaves and fruit *in vitro*. Positive comparators were from acarbose and quercetin. Terpene compounds found in various medicinal plants contribute to the inhibition of alpha-glucosidase enzyme activity (Al Kury *et al*., 2022). Terpenes obtained from plants show inhibition of α-glucosidase and prove their potential in the management of diabetes (Panigrahy *et al*., 2020).

Signification from Table 3 shows that the IC value of$_{50}$ acarbose drugs is smaller compared to hexane extracts from kitolod leaves and fruits as well as quercetin positive standards. This suggests that the inhibitory activity of the enzyme alpha-glucosidase acarbose > hexane extract from the leaves > the hexane extract from the fruit > quercetin. The content of sesquiterpene (capelin) has a role in inhibiting the activity of the enzyme alpha-glucosidase. Capelin, a component in the hexane extract, apparently plays an active role in antidiabetics (Islam *et al.*, 2016). Myrcene, one of the terpenoids predominant components that serves as an antioxidant (Wang *et al*., 2019), should support the process. Capelinand capillinol have shown the ability to inhibit α-glucosidase, so Islam *et al*. (2016) agreed that the keto group in capelin should be a significant determinant on anti-diabetes potential.

4. Conclusion

In conclusion, spanning over 38 substances combined, the main chemical compounds in kitolod leaf and kitolod fruit hexane extracts are predominated by sesquiterpenes. Their lowest IC_{50} values of antioxidant inhibitory activity, lowest IC_{50} values of α-amylase inhibitory activity, and fairly low IC_{50} values of α-glucosidase inhibitory activity should prove that kitolod leaf and kitolod fruit hexane extract showed potential as antioxidants and that they can function as antidiabetic.

Acknowledgments

The authors wish to thank the Ministry of Research and Technology, Republic of Indonesia, for funding this research (E.5c/092/DPPM/L/IV/2019).

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