Pre-print manuscript of following article.

Journal of Agricultural and Food Chemistry 2014, 62 (33), p8411-8414.

DOI: 10.1021/jf502667z; http://pubs.acs.org/doi/abs/10.1021/jf502667z

An α-Amylase Inhibitory Triterpene from *Abrus precatorius* Leaves

Ryuta Yonemoto, Miyuki Shimada, Maria D. P. T. Gunawan-Puteri, Eisuke Kato, and Jun Kawabata*

Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan

*Corresponding author:

Jun Kawabata, e-mail: junk@chem.agr.hokudai.ac.jp, Tel/Fax: +81-11-706-2496.

Running header : α-amylase inhibitors from *Abrus precatorius*

ABSTRACT: In the screening experiments for porcine pancreatic α-amylase inhibitors in 18 plants obtained from Indonesia, a potent inhibitory activity was detected in the extract of leaves of *Abrus precatorius*. The enzyme assay-guided fractionation of the extract led to the isolation of a triterpene ketone, lupenone (**1**) as a potent α-amylase inhibitor together with 24-methylenecycloartenone (**2**) and luteolin (**3**). The mode of inhibition of **1** against porcine pancreatic α-amylase was a mixed inhibition. This is the first report that describes the potent α-amylase-inhibitory activity of the low polar triterpene ketone similar to **1**. Comparison of the activities of the isolate and related compounds indicated the importance of C-3 ketone and the lupane skeleton in the α-amylase-inhibitory activity.

KEYWORDS: *Abrus precatorius, α-amylase inhibitor, triterpene ketone*

INTRODUCTION

Diabetes mellitus is a typical chronic disease related to both obesity and ageing and has thus become a serious global health problem.[1] One of the therapeutic approaches for preventing diabetes is the suppression of intestinal digestion and absorption of dietary carbohydrates through the inhibition of carbohydrate hydrolyzing enzymes, α-amylase and α-glucosidase, in the digestive organs.[2] In this context, several research efforts have screened for effective α-amylase and α-glucosidase inhibitors from natural sources to develop a physiological functional food or lead compounds for the use of antidiabetic medicines.[3, 4]

In the course of our continuing search for rat intestinal α-glucosidase-inhibitory principles from plants, we have isolated and identified several active compounds from a variety of plants.[5-9] However, the search for α-amylase inhibitors from natural sources have led to relatively scarce results.[10-12] It is well known that α-amylase recognizes a consecutive glucose chain as a substrate by using its subsite.[13] In fact, acarbose, a typical α-amylase inhibitor, has a strong affinity for the enzyme because of its pseudotetrasaccharide structure.[14] This is partly the reason for the limited number of cases involved with the identification of small molecule α-amylase inhibitors from natural sources. Most of these are polyphenols with low enzyme specificity.[15-19] Recently, a triterpene glycoside has been isolated as a specific inhibitor by high through-put screening,[20] and more surprisingly, a simple low polar ketone, chalcone, has been found to possess considerable α-amylase inhibitory activity.[21] The molecular docking study revealed that the low polar small molecule could interact with hydrophobic amino acid residues near the active site of the enzyme. From these results, we have thus focused on searching for non-polyphenolic low polar α-amylase inhibitors

from plant origin.

In this paper, we present the results of a study on α-amylase inhibition and identification of active principles from the plant extract of indigenous plants obtained from Indonesia that show a potent inhibitory activity. In the screening experiments for porcine pancreatic α-amylase (PPA) inhibitors in 18 plants obtained from Indonesia, the potent α-amylase-inhibitory activity was found in a tannin-free low polar extract of the leaves of *Abrus precatorius*. *A. precatorius* (Fabaceae) is a perennial climber found in tropical and subtropical regions. It is famous for its beans, which contain a proteinaceous toxin.[22, 23] In addition, several other biological activities have been reported,[24-26] although there have been no reports on α-amylase and α-glucosidase inhibitory activity of this plant.

MATERIALS AND METHODS

Materials. Eighteen Indonesian plants including *A. precatorius* leaves were purchased from Merapi Farma Traditional Herbs Distributor, Yogyakarta, Indonesia, in January 2007. PPA (A3176) was obtained from Sigma-Aldrich Co. (Tokyo, Japan). All chemicals used in the present study were of reagent grade and were purchased from Wako Pure Chem. Co. (Osaka, Japan) unless otherwise stated.

General procedure. NMR spectra were recorded on Bruker AMX500 (^{1}H, 500 MHz; ^{13}C, 125 MHz) and Jeol EX-270 (^{1}H, 270 MHz) instruments. Chemical shifts were determined relative to a reference signal of tetramethylsilane in chloroform-*d* (δ_H 0.00 ppm, δ_C 0.0 ppm) or a residual solvent signal of methanol-d_4 (δ_H 3.30 ppm). Field desorption mass spectra (FD-MS) were determined by a Jeol T100GCV instrument.

PPA inhibitory activity determination. The PPA-inhibitory activity was

78 determined by using the method described by Ali *et al.*[27] with a slight modification. PPA
79 was dissolved in sodium phosphate buffer (20 mM, pH 6.9) containing 6.7 mM of NaCl
80 to give a concentration of 0.5 unit/mL solution. Potato starch (0.01 g/mL) in the same
81 buffer was used as a substrate solution. A coloring reagent (DNS) was prepared by
82 mixing a 4.8 M 3,5-dinitrosalicylic acid solution (20 mL) and a solution of (+)-sodium
83 potassium tartrate tetrahydrate (12 g) in 0.4 M of NaOH (40 mL). A sample solution
84 (100 μL) in 50% dimethyl sulfoxide (DMSO) and the PPA solution (150 μL) were
85 mixed in a micro tube (1.5 mL). The tube was stoppered and pre-incubated at 37 º C for
86 15 min. The starch solution (250 μL) was then added and the mixture was incubated at
87 37 º C for 15 min. To terminate the reaction, the tube was dipped in boiling water for 1
88 min. After cooling, the contents of the micro tube were directly passed through a small
89 ODS (Cosmosil 75C_{18}-OPN, Nacalai Tesque Co., Kyoto, Japan) column (3 mL) to
90 remove any sample constituents that may interfere with the following color reaction.
91 The reaction mixture (100 μL) was then mixed with DNS reagent (50 μL) in a micro
92 tube, stoppered and heated in boiling water for 15 min. The ice-cooled mixture was
93 diluted with water (450 μL), the solution (200 μL) was transferred into 96-well micro
94 plate, and the optical density was determined at a wavelength of 540 nm. The control
95 experiment was performed using 50% DMSO in place of the sample solution. Blank
96 experiments for sample and control were performed using the sodium phosphate buffer
97 in place of the enzyme solution and each blank value was subtracted from the sample
98 and the control values, respectively. The inhibitory activity (%) was calculated as
99 [1-OD_{540} (sample)/OD_{540} (control)] × 100.

100 **Screening experiment.** The screening experiments for PPA inhibition were
101 performed with extracts of 18 plant species. Each dried plant was extracted with 50%

aqueous methanol. The extracts were evaporated and partitioned with chloroform-methanol-water (4:1:5). The organic phase was washed with 1% saline to give a tannin-free extract,[28] which was then evaporated, re-dissolved in 50% aqueous DMSO, and subjected as the test sample to the assay for PPA inhibitory activity at the final concentration of the extractable constituents obtained from 0.3 g of plant material in 1 mL of solution.

Isolation of lupenone (1), 24-methylenecycloartenone (2), and luteolin (3) from *A. precatorius* leaves. Dried leaves (40 g) of *A. precatorius* were extracted with 50% aqueous MeOH. The extract was concentrated and partitioned with chloroform-methanol-water (4:1:5). The organic phase was washed with 1% saline and evaporated to give a tannin-free chloroform-soluble part (1.5 g). This fraction was charged onto a silica gel column and eluted successively with hexane-ethyl acetate (9:1, F1), hexane-ethyl acetate (3:1, F2), hexane-ethyl acetate (1:1) containing 0.1% formic acid (F3), ethyl acetate containing 0.1% formic acid (F4) and methanol containing 0.1% formic acid (F5). The PPA inhibitory activity was observed in F1, F4 and F5.

The less polar F1 (114 mg) was further fractionated by silica gel column chromatography with a hexane-ethyl acetate gradient. The PPA inhibitory activity was eluted mainly in a hexane-ethyl acetate (30:1) eluate (F1-2, 78 mg). F1-2 was further purified by silica gel preparative TLC [hexane-ethyl acetate (11:1)] to give an active band (F1-2-2, R_f = 0.50, 11 mg). F1-2-2 was finally purified by preparative HPLC (column: Inertsil ODS-3, 4.6 × 250 mm, GL-Science Co., Tokyo, Japan; mobile phase: MeOH; flow rate: 1.0 mL/min; detection: UV 210 nm). Two major peaks were collected and identified as lupenone (**1**, t_R = 21.0 min, 0.7 mg) and 24-methylenecycloartenone (**2**, t_R = 16.0 min, 0.3 mg).

126 The more polar active fraction (F4, 200 mg) of the first silica gel column was
127 re-chromatographed by a silica gel column (hexane-ethyl acetate-methanol). The most
128 polar fraction (F4-4, 110 mg) eluted by hexane-ethyl acetate (1:5) and methanol showed
129 PPA inhibitory activity. The final polar fraction (F5, 480 mg) of the first column was
130 expected to contain the similar active constituents to F4-4, and thus, F4-4 and F-5 were
131 merged (F45) and subjected to further fractionation. F45 was chromatographed on a
132 silica gel column with a chloroform-methanol gradient. The PPA inhibitory activity was
133 eluted in a chloroform-methanol (10:1 and 7:1) eluate (F45-5, 75 mg). F45-5 gave a
134 single peak (t_R = 41.3 min) by HPLC analysis (column: Inertsil ODS-3, 4.6 × 250 mm;
135 mobile phase: 10-100% MeOH in water containing 0.1% formic acid (0-60 min); flow
136 rate: 1.0 mL/min; detection: UV 254 nm) and was identified as luteolin by comparison
137 with an authentic sample.
138 *Lupenone (**1**).* FD-MS *m*/*z* 424 ($[M]^+$); ^{1}H-NMR (500 MHz, chloroform-*d*) δ: 0.80
139 (3H, s), 0.93 (3H, s), 0.96 (3H, s), 1.00-1.16 (3H, m), 1.02 (3H, s), 1.07 (3H, s), 1.07
140 (3H, s), 1.17-1.52 (15H, m), 1.66-1.72 (2H, m), 1.68 (3H, s), 1.86-1.98 (2H, m),
141 2.33-2.54 (2H, m), 2.41 (1H, m), 4.57 (1H, m), 4.69 (1H, m); ^{13}C-NMR (125 MHz,
142 chloroform-*d*) δ: 14.5, 15.8, 16.0, 18.0, 19.3, 19.7, 21.0, 21.5, 25.2, 26.7, 27.4, 29.8,
143 33.6, 34.2, 35.5, 36.9, 38.2, 39.6, 40.0, 40.8, 42.9, 42.9, 47.3, 48.0, 48.3, 49.8, 55.0,
144 109.4, 150.9, 218.2.
145 *24-Methylenecycloartenone (**2**).* FD-MS *m*/*z* 438 ($[M]^+$); ^{1}H-NMR (500 MHz,
146 chloroform-*d*) δ: 0.58 (1H, d, *J*= 4.4 Hz), 0.79 (1H, d, *J*= 4.4 Hz), 0.90 (3H, d, *J*= 5.6
147 Hz), 0.91 (3H, s), 1.00 (3H, s), 1.03 (3H, d, *J*= 6.8 Hz), 1.04 (3H, d, *J*=6.8 Hz), 1.05
148 (3H, s), 1.10 (1H, m), 1.10 (3H, s), 1.13 (2H, m), 1.14 (1H, m), 1.31 (1H, m), 1.32 (2H,
149 m), 1.40 (1H, m), 1.41 (1H, m), 1.54 (1H, m), 1.55 (2H, m), 1.59 (1H, m), 1.64 (1H, m),

1.67 (2H, m), 1.71 (1H, dd, *J*=12.0 and 4.4 Hz), 1.86 (1H, dt, *J*=14.0 (t) and 5.6 (d) Hz), 1.89 (1H, m), 1.92 (1H, m), 2.05 (1H, m), 2.13 (1H, m), 2.24 (1H, m), 2.30 (1H, m), 2.71 (1H, dt, *J*=14.0 (t) and 5.6 (d) Hz), 4.67 (1H, brs), 4.72 (1H, brs); ^{13}C-NMR (125 MHz, chloroform-*d*)) δ: 18.1, 18.3, 19.3, 20.8, 21.1, 21.5, 21.9, 22.0, 22.2, 25.9, 26.0, 26.8, 28.2, 29.6, 31.3, 32.8, 33.4, 33.8, 35.0, 36.1, 37.5, 45.4, 47.9, 48.5, 48.8, 50.2, 52.3, 106.0, 156.9, 216.6.

*Luteolin (**3**).* FD-MS *m*/*z* 286 ([M]$^+$); ^{1}H-NMR (270 MHz, methanol-d_4) δ: 6.20 (1H, d, *J*=2.1 Hz), 6.43 (1H, d, *J*=2.1 Hz), 6.54 (1H, s), 6.90 (1H, d, *J*= 8.7 Hz), 7.38 (2H, m).

RESULTS AND DISCUSSION

In the screening experiment, tannin-free extracts from 2 out of 18 Indonesian plants, namely *A. precatorius* leaves (73%) and *Baeckea frutescens* bark (58%), out of 18 showed >50% PPA inhibitory activity (**Table 1**). Based on this result, we chose extracts of *A. precatorius* for identifying active principles.

The tannin-free organic phase of *A. precatorius* leaves was chromatographed on a silica gel column and PPA-inhibitory activity was observed in two discrete fractions. The less polar active fraction was further chromatographed on a silica gel column followed by silica gel preparative TLC and reverse phase HPLC to afford two major peaks in the final active fraction. These two constituents were identified as lupenone (**1**)[29] and 24-methylenecycloartenone (**2**)[30] by comparison of their analytical data with those reported in the references and an authentic specimen of lupenone. The more polar active fraction of the first column was also chromatographed on silica gel and the resultant active eluate showed a single peak in the reverse phase HPLC analysis. The

active constituent of the final fraction was identified as luteolin (**3**) by comparison with an authentic specimen (**Figure 1**).

The PPA-inhibitory assay of the isolates showed a potent activity of **1** (IC_{50}=31 μM) and a weaker activity of **3** (IC_{50}=3.1 mM) (**Table 2**). The activity was not observed in **2** (0%) at 0.6 mM, although a further experiment using a higher concentration could not be performed due to limited sample amount. The potent inhibitory activity of **1** against PPA has not ever been reported, whereas **3** has been known as a moderate PPA inhibitor.[15, 19] The inhibition mode of **1** was determined to be mixed-inhibition type by the double reciprocal plot experiment (**Figure 2**).

It is interesting that a low polar triterpene such as **1** showed significant PPA inhibition, because only few studies have investigated the PPA-inhibitory activity of triterpenes. Ali *et al.* reported triterpenic inhibitors, ursolic acid, oreanolic acid, and lupeol, from a Malaysian plant, *Phyllanthus amarus*, and ursolic acid showed the highest activity among the isolates.[27] The structural similarity of those triterpenes and **1** prompted us to perform a brief activity comparison experiments. The PPA-inhibitory assay of commercially available lupenone (**1**), lupeol (**4**) and ursolic acid (**5**) (**Figure 1**) at a concentration of 50 μM showed the strongest activity of **1** (84%) followed by **5** (32%) and the lowest **4** (8%) (**Table 2**). These results together with the low inhibitory activity of **2** show that both the lupane skeleton and a ketone at C-3 would be essential for exerting a potent PPA inhibition. To the best of our knowledge, this is the first finding of the potent PPA inhibitory activity of lupenone, although some other biological activities[29, 31] including α-glucosidase inhibition[32] have been reported for **1**. There are few examples of non-polyphenolic low polar small molecular inhibitor against PPA. It should be interesting to determine how efficiently a low polar molecule

198 such as **1** could associate with the PPA enzyme.

199 In conclusion, the enzyme assay-guided fractionation of the extract from the dried
200 leaves of *A. precatorius* led to the isolation of a triterpene ketone, lupenone (**1**), as a
201 potent PPA inhibitor together with lower active luteolin (**3**). The relatively higher
202 activity of **1** and the larger amount of **3** present in *A. precatorius* leaves suggest that
203 these compounds are the major inhibitory components in this plant that could be useful
204 for the treatment of diabetes, although *in vivo* experiments are warranted.

205

206 **Notes**

207 The authors declare no competing financial interest.

208

209 **ACKNOWLEDGMENTS**

210 The authors thank Mr. Yusuke Takata and Dr. Eri Fukushi of the GC-MS and NMR
211 Laboratory, Faculty of Agriculture, Hokkaido University for their skillful measurements
212 of mass spectra.

213

REFERENCES

(1) King, H.; Aubert, R. E.; Herman, W. H. Global burden of diabetes, 1995-2025; prevalence, numerical estimates, and projections. *Diabetes Care* **1998**, *21*, 1414-1431.

(2) Puls, W.; Keup, U.; Krause, H. P.; Thomas, G.; Hofmeister, F. Glucosidase inhibition: a new approach to the treatment of diabetes, obesity, and hyperlipoproteinaemia. *Naturwissenschaften* **1977**, *64*, 536-537.

(3) Matsui, T.; Ogunwande, I. A.; Abesundara, K. J. M.; Matsumoto, K. Anti-hyperglycemic potential of natural products. *Mini-Rev. Med. Chem.* **2006**, *6*, 109-120.

(4) Kumar, S.; Narwal, S.; Kumar, V.; Prakash, O. α-Glucosidase inhibitors from plants: A natural approach to treat diabetes. *Pharmacogn. Rev.* **2011**, *5*, 19-29.

(5) Jong-Anurakkun, N.; Bhandari, M. R.; Kawabata, J. α-Glucosidase inhibitors from devil tree (*Alstonia scholaris*). *Food Chem.* **2007**, *103*, 1319-1323.

(6) Gao, H.; Huang, Y.-N.; Gao, B.; Xu, P.-Y.; Inagaki, C; Kawabata, J. α-Glucosidase inhibitory effect by the flower buds of *Tussilago farfara* L. *Food Chem.* **2008**, *106*, 1195-1201.

(7) Yoshida, K.; Hishida, A.; Iida, O.; Hosokawa, K.; Kawabata, J. Flavonol caffeoylglycosides as α-glucosidase inhibitors from *Spiraea cantoniensis* flower. *J. Agric. Food Chem.* **2008**, *56*, 4367-4371.

(8) Takahashi, K.; Yoshioka, Y.; Kato, E.; Katsuki, S.; Iida, O.; Hosokawa, K; Kawabata, J. Methyl caffeate as an α-glucosidase inhibitor from *Solanum torvum* fruits and the activity of its related compounds. *Biosci. Biotechnol. Biochem.* **2010**, *74*, 741-745.

(9) Gunawan-Puteri, M. D. P. T.; Kawabata, J. Novel α-glucosidase inhibitors from

Macaranga tanarius leaves. *Food Chem.* **2010**, *123*, 384-389.

(10) Hansawasdi, C.; Kawabata, J.; Kasai, T. α-Amylase inhibitors from roselle (*Hibiscus sabdariffa* Linn.) tea. *Biosci. Biotechnol. Biochem.* **2000**, *64*, 1041-1043.

(11) Bhandari, M. R.; Jong-Anurakkun, N.; Gao, H.; Kawabata, J. α-Glucosidase and α-amylase inhibitory activities of Nepalese medicinal herb Pakhanbhed (*Bergenia ciliata* Haw.). *Food Chem.* **2008**, *106*, 247-252.

(12) Gunawan-Puteri, M. D. P. T.; Kato, E.; Kawabata, J. α-Amylase inhibitors from an Indonesian medicinal herb, *Phyllanthus urinaria*. *J. Sci. Food Agric.* **2012**, *92*, 606-609.

(13) Brayer, G. D.; Sidhu, G.; Maurus, R.; Rydberg, E. H.; Braun, C.; Wang, Y.; Nguyen, N. T.; Overall, C. M.; Withers, S. G. Subsite mapping of the human pancreatic α-amylase active site through structural, kinetic, and mutagenesis techniques. *Biochemistry* **2000**, *39*, 4778-4791.

(14) Li, C.; Begum, A.; Numao, S.; Park, K. H.; Withers, S. G.; Brayer, G. D. Acarbose rearrangement mechanism implied by the kinetic and structural analysis of human pancreatic α-amylase in complex with analogues and their elongated counterparts. *Biochemistry* **2005**, *44*, 3347-3357.

(15) Kim, J. -S.; Kwon, C. -S.; Son, K. H. Inhibition of alpha-glucosidase and amylase by luteolin, a flavonoid. *Biosci. Biotechnol. Biochem.* **2000**, *64*, 2458-2461.

(16) McDougall, G. J.; Shpiro, F.; Dobson, P.; Smith, P.; Blake, A.; Stewart, D. Different polyphenolic components of soft fruits inhibit α-amylase and α-glucosidase. *J. Agric. Food Chem.* **2005**, *53*, 2760-2766.

(17) He, Q.; Lv, Y.; Yao, K. Effects of tea polyphenols on the activities of α-amylase, pepsin, trypsin and lipase. *Food Chem.* **2006**, *101*, 1178-1182.

(18) Li, H.; Tanaka, T.; Zhang, Y. -J.; Yang, C. -R.; Kouno, I. Rubusuaviins A-F, monomeric and oligomeric ellagitannins from Chinese sweet tea and their α-amylase inhibitory activity. *Chem. Pharm. Bull.* **2007**, *55*, 1325-1331.

(19) Piparo, E. L.; Scheib, H.; Frei, N.; Williamson, G.; Grigorov, M.; Chou, C. J. Flavonoids for controlling starch digestion: Structural requirements for inhibiting human α-amylase. *J. Med. Chem.* **2008**, *51*, 3555-3561.

(20) Tarling, C. A.; Woods, K.; Zhang, R.; Brastianos, H. C.; Brayer, G. D.; Andersen, R. J.; Withers, S. G. The search for novel human pancreatic α-amylase inhibitors: high-throughput screening of terrestrial and marine natural product extracts. *ChemBioChem* **2008**, *9*, 433-438.

(21) Najafian, M.; Ebrahim-Habibi, A.; Hezareh, N.; Yaghmaei, P.; Parivar, K.; Larijani, B. Trans-chalcone: a novel small molecule inhibitor of mammalian alpha-amylase. *Mol. Biol. Rep.* **2011**, *38*, 1617-1620.

(22) D'Silva, I.; Vaidyanathan, C. S.; Podder, S. K. Ribosome-inactivating proteins and agglutinins from callus and suspension cultures of *Ricinus communis* L. and *Abrus precatorius* L. *Plant Sci.* **1993**, *94*, 161-172.

(23) Olsnes, S. The history of ricin, abrin and related toxins. *Toxicon* **2004**, *44*, 361-370.

(24) Attal, A. R.; Otari, K. V.; Shete, R. V.; Upasani, C. D.; Nandgude, T. D. *Abrus precatorius* Linnaeus: a phytopharmacological review. *J. Pharm. Res.* **2010**, *3*, 2585-2587.

(25) De Britto, A. J.; Kumar, P. B. J. R.; Gracelin, D. H. S. *Abrus precatorius* L. : a medicinal plant with potential as antibacterial agent. *J. Pharm. Res.* **2012**, *5*, 1207-1209.

(26) Shenoy, A.; Varghese, B. P.; Rajan, M. S.; Koshy, S.; Joshi, M.; Shabaraya, A. R.

Anticonvulsant activity of ethanolic extract of *Abrus precatorius* leaves. *Adv. Res. Pharmaceut. Biol.* **2012**, *2*, 53-61.

(27) Ali, H.; Houghton, P. J.; Soumyanath, A. α-Amylase inhibitory activity of some Malaysian plants used treat diabetes; with particular reference to *Phyllanthus amarus*. *J. Ethnopharmacol.* **2006**, *107*, 449-455.

(28) Wall, M. E.; Wan, M. C.; Brown, D. M.; Fullas, F.; Olwald, J. B.; Josephson, F. F.; Thornton, N. M.; Pezzuto, J. M.; Beecher, C. W. W.; Farnsworth, N. R.; Cordell, G. A.; Kinghorn, A. D. Effect of tannins on screening of plant extracts for enzyme inhibitory activity and techniques for their removal. *Phytomedicine* **1996**, *3*, 281-285.

(29) Na, M.; Kim, B. Y.; Osada, H.; Ahn, J. S. Inhibition of protein tyrosine phosphatase 1B by lupeol and lupenone isolated from *Sorbus commixta*. *J. Enzyme Inhib. Med. Chem.* **2009**, *24*, 1056-1059.

(30) Jayasinghe, U. L. B.; Vithana, H. S. K.; Wannigama, G. P.; Fujimoto, Y. 24-Methylenecycloartenone from *Bhesa nitidissima*. *Fitoterapia* **2001**, *72*, 594-595.

(31) Ahn, E. -K.; Oh, J. S. Lupenone isolated from *Adenophora triphylla* var. *japonica* extract inhibits adipogenic differentiation through the downregulation of PPARγ in 3T3-L1 cells. *Phytother. Res.* **2013**, *27*, 761-766.

(32) Mohamed, I. E.; El Nur, E. B. E., Choudhary, M. I.; Khan, S. N. Bioactive natural products from two Sudanese medicinal plants *Diospyros mespiliformis* and *Croton zambesicus*. *Rec. Nat. Prod.* **2009**, *3*, 198-203.

Figure captions

Figure 1. Structures of **1**-**5**.

Figure 2. Double reciprocal plot (Lineweaver-Burk) for the α-amylase inhibition by lupenone (**1**)

314 **Table 1. PPA inhibitory activity of tannin-free extracts of Indonesian plants.**

Plant	Part	Inhibitory activity (%)*
Abrus precatorius	leaf	73
Carica papaya	leaf	33
Gynura procumbens	aerial part	6
Menta arvensis	leaf	15
Ruella napifera	leaf	15
Sida rhombifolia	aerial part	12
Tribulus terrestris	aerial part	0
Caesalpinia sappan	bark	0
Ruellia tuberosa	fruit	23
Helicteres isora	leaf	4
Sonchus arvensis	fruit	5
Cryptocarya massoy	bark	0
Piper nigrum	seed	18
Bruceal javanica	seed	2
Alyxia stellata	bark	39
Baeckea frutescens	bark	58
Murraya paniculata	aerial part	22
Borreria hispida	leaf	21

315 * The concentration was adjusted to the extractable constituents obtained from 0.3 g of
316 plant material in 1 mL solution.

317

318

319 **Table 2. PPA inhibitory activity of isolated and related compounds**

	Inhibitory activity (%)	
	50 μM	100 μM
1	84	87
2	-	0*
3	-	-**
4	8	16
5	32	45

320 -: not tested; * 0% at 0.6 mM; ** IC_{50} = 3.1 mM

321

322

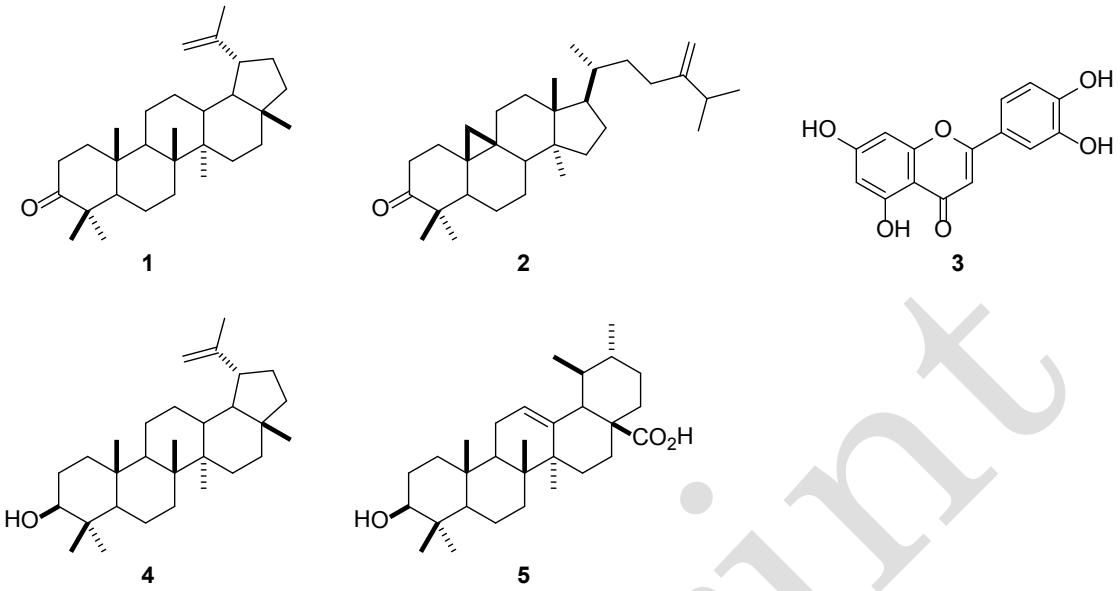
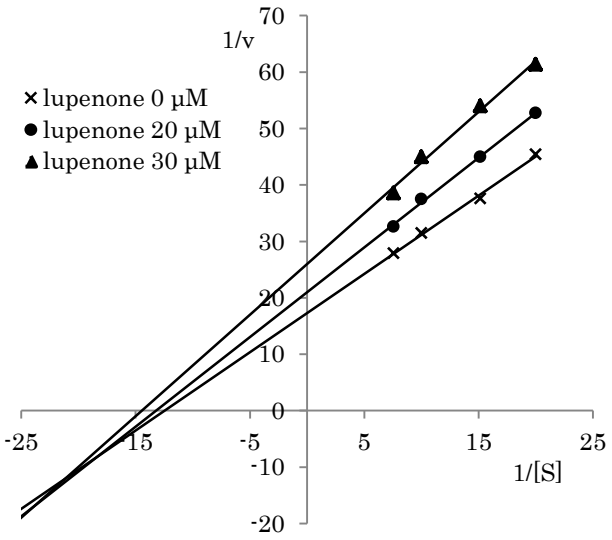


Figure 1. Structures of **1**-**5**.



326

327

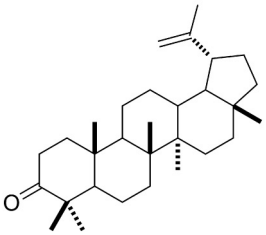
328 **Figure 2.** Double reciprocal plot (Lineweaver-Burk) for the α-amylase inhibition by

329 lupenone (**1**)

330

331 TOC Graphic.

332



α-amylase inhibitor

333 Photo courtesy of the U.S. Geological Survey