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ORIGINAL ARTICLE

Revealing anti-diabetic potency of medicinal plants of Meru Betiri National Park, Jember – Indonesia



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Anti-diabetic;
Meru Betiri National Park;
Indonesian medicinal plants

Abstract Eighteen extracts of six medicinal plants of Meru Betiri National Park (Jember, Indonesia) were studied for their anti-diabetic potency as well as their antioxidant activities (DPPH, superoxide anion and hydroxyl radical scavenging). Total phenolic and flavonoid content were also determined. Total phenols and flavonoid content increased with polarity order, from hexane, ethyl acetate to methanol extracts. Similar pattern resulted in antioxidant activity testing. Ethyl acetate extract of *Antidesma bunius*, methanol extract of *Lagerstroemia speciosa* and *Lunasia amara* possessed higher α -amilase inhibition compared to standard inhibitor, acarbose. Moreover, ethyl acetate extract of *Antidesma bunius*, methanol extract of *Arcangelisia flava*, *Lagerstroemia speciosa*, *Lunasia amara* and hexane fraction of *Merremia mamosa* indicated higher α -glucosidase inhibition compared to standard anti-diabetic drug, acarbose. The extracts with higher phenolic and flavonoid content did not always showed higher inhibition activities α -amilase and α -glucosidase. Overall, the six medicinal plants exhibited potent sources for diabetic mellitus medication.

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1. Introduction

Despite Indonesian economic growth has benefited to standard of living improvement, the expansion has caused life style changes among Indonesian into poor diets which contributed to increasing metabolic syndromes cases including diabetic mellitus. International diabetes federation estimated around 415 million people are living with diabetes in 2015 which the number did not include the untreated and yet undiagnosed. In Indonesia, the diseases became a serious problem in which

epidemiologic studies indicates the number of diabetic mellitus cases was 10 million and has increased in productive age (Federation, 2016).

Indonesian archipelago is occupied by 40,000 endemic plants including over 7000 recorded medicinal plants. This has made the archipelago becomes the second largest biodiversity in the world. The indigenous people of Indonesia has relied on medicinal plants for their health need through generations (Nugraha and Keller, 2011). However, only around 200 Indonesian medicinal plants have been studied which include medicinal plants used in diabetic therapy.

Located in East Java, the Meru Betiri National Park is home for enormous number of flora yet few species have been identified. These diverse flora are part of diverse vegetation including mangrove, swamp and dense tropical forest (Darmadja et al., 2012). Initial medicinal plant research in Meru Betiri National Park have identified 266 medicinal plants. Our studies selected six medicinal plants which are traditional used by the indigenous people of Indonesia in diabetic therapy (*Antidesma bunius* (L) Spreng, *Antidesma montanum* Blume, *Arcangelisia flava* (L) Merr, *Lagerstroemia speciosa* (L) Pers, *Lunasia amara* Blanco, and *Merremia mammosa* Hall f. This paper reported inhibition activities of extracts fractions (hexane, ethyl acetate, methanol) against α -amylase, α -glucosidase as well as their antioxidant potency.

2. Materials and methods

2.1. Plant samples

The leaves of *A. bunius*, *A. montanum*, *A. flava*, *L. speciosa*, *L. amara*, and *M. mammosa* were collected from Meru Betiri National Park and identified by a botanist at Purwodadi Botanical Garden (Pasuruhan-Indonesia). Voucher specimens were deposited at Centre for Development of Advance Science and Technology (CDAST), University of Jember (Jember-Indonesia) under accession number AB, AM, AF, LS, LA MM, respectively.

2.2. Extraction

Dried leaves (50 g) was powdered and transferred into conical flask (500 ml). Subsequent extractions was employed from hexane (250 ml), ethyl acetate (250 ml) and methanol (250 ml). Each fractions were separately vacuum dried to obtain hexane, ethyl acetate and methanol extracts. Percentage yield were calculated using formula below.

$$\% \text{yield} = \frac{\text{weight of crude extract}}{\text{weight of raw material}} \times 100 \quad (1)$$

2.3. Total phenolic and flavonoid content analysis

Total phenolic concentration was determined using gallic acid as a standard, adapted from a published method (Taga et al., 1984). Sample solutions (50 μ l) were loaded into separate tubes (4 replicates). Solution of Na_2CO_3 (2% w/v, 1 ml) was added and the tubes were incubated for 2 min. Folin-ciocalteu (50% v/v, 50 μ l) was added and the mixture were again incubated for 30 min. Absorbance was read at λ 750 nm and the total phenolic value was calculated in gallic acid

equivalent (GAE) against gallic acid standard curve. The solution of extract was then used as working solution for the next experiments.

The flavonoid content was also determined using quercetin as a standard based on published method (Choi et al., 2002). Sample solutions (150 μ l) were loaded into separate tubes (4 replicates). Distilled water (400 μ l) and NaNO_2 (5% w/v, 30 μ l) were added and mixture were incubated for 5 min. AlCl_3 (10%, w/v, 30 μ l) were added and the mixture were again incubated for 6 min. NaOH (1M, 200 μ l) and distilled water (240 μ l) were added and the mixture were incubated for 6 min. Absorbance was read at 415 nm and the total flavonoids was calculated in quercetin equivalent against quercetin standard curve.

2.4. Antioxidant activity

2.4.1. 2,2-diphenyl-1-picrylhydrazyl (DPPH)

The ability of the extract fractions to scavenge free radicals was determined using a DPPH microplate assay adapted from a published method (Soler-Rivas et al., 2000). Sample solutions (2 μ g GAE/mL, 200 μ l) were loaded into a 96-well plate (in replicates of 4) and serially diluted. A separate set of wells were loaded with 200 μ l of either MeOH or vitamin C (1 mM, 50 μ l) for the negative and positive controls, respectively. To the first three rows of sample solution, DPPH (90 μ M, 100 μ l) was added and thoroughly mixed, allowing the last row to be used to correct for background absorbance (Abs blank). Plates were incubated for 30 min in the absence of light before the absorbance at 515 nm was measured using a microplate reader. Lower absorbances signified higher scavenging activities. Free radical-scavenging activities were determined using Eq. (2). These percentage activities were graphed against logarithmic sample concentrations and the sample concentrations that sequestered 50% of the DPPH free radicals (i.e. loss of purple colour) were interpolated and are presented as IC_{50} mean \pm s.d. values. All antioxidant activity data were tested for significant differences between each pair using student's t-tests ($\alpha = 0.05$).

$$\text{Percentage activity} = \left[\frac{A_0 - A_1}{A_0} \right] \times 100\% \quad (2)$$

2.4.2. Superoxide anion radical scavenging

Free radical scavenging activity was also determined using pirogalol based on a published method (Tang et al., 2010). To sample solutions (2 μ g GAE/ml, 200 μ l) in tube, tris-HCl buffer (pH 8.2, 50 mM, 1.7 ml) was added and the mixture was then incubated for 10 min. Pirogalol (10 mM in HCl 10 mM, 100 μ l) was added. Absorbance was read at 320 nm using spectrophotometry UV-Vis. The percentage activities were graphed against logarithmic sample concentrations (Eq. (2)). Vitamin C (1 mM, 50 μ l) was used as a standard.

2.4.3. Hydroxyl radical scavenging

The activity was determined using a standard published method (Halliwell et al., 1987). A solution of extract (2 μ g GAE/ml, 150 μ l), 2-deoxy-D-ribose (28 mM, 50 μ l), in phosphate buffer (20 mM, pH 7.4), EDTA (1 mM, 100 μ l), FeCl_3 (10 mM, 100 μ l), H_2O_2 (11 mM, 50 μ l) and ascorbic acid (1 mM, 50 μ l) was loaded into eppendorf tube. Solution of

2-tiobarbiturat (ATB) (1%, 500 µl) and ATC (2.8%, 500 µl) were added and the mixture was incubated at 37 °C for 1 h. Mixture was then vortexed and re-incubated at 100 °C for 20 min to produce pink coloration. The solution was then cooled to room temperature and its absorbance was measured using spectrophotometry UV-Vis at λ 532 nm. The scavenging percentage was calculated using Eq. (2). Ascorbic acid (1 mM, 50 µl) was used as a standard compound.

2.5. α-Amylase and α-glucosidase inhibition activity

2.5.1. α-Amylase inhibition

The capability to inhibit α-amylase was determined using a published method (Hashim et al., 2013). Sample (25 µg GAE/ml, 100 µl) was loaded into eppendorf tubes and labelled with S⁺ and S⁻. DMSO (100 µl) was loaded into separate eppendorf tubes as control with label C⁺ and C⁻. Enzym α-amylase (0.1 unit/ml, 150 µl) was loaded to the eppendorf labelled S⁺ and C⁺ while phosphate buffer (pH 6.9, 150 µl) was loaded to the eppendorf labelled S⁻ and C⁻. Solutions were then incubated at 37 °C for 15 min. Starch (1% w/v in water, 250 µl) were added to the tubes and incubated at 37 °C for 15 min. The enzymatic reaction was stopped by boiling the tubes for 1 min using water bath. A portion of solution (160 µl) were taken from each eppendorf and transferred into empty eppendorf with similar label and boiled for 15 min. Reagent 3, 5-dinitrosalisilat (80 µl) and distilled water (720 µl) were added. Solution (200 µl) was taken and loaded into 96-well plates. A standard anti-diabetic, acarbose, was used as comparison. Percentage α-amylase inhibition of extracts were calculated using Eq. (3).

$$\text{Percentage inhibition} = \left[\frac{(C^+ - C^-) - (S^+ - S^-)}{(C^+ - C^-)} \right] \times 100\% \quad (3)$$

2.5.2. α-Glucosidase inhibition

The α-glucosidase inhibition was determined by using adapted method from published protocol (Miyazawa et al., 2005). Maltose (0.125 nM, 100 µl) was loaded into eppendorf tubes and labelled with C⁺, C⁻, S⁺, S⁻. Sample solution (25 µg GAE/ml, 100 µl) was added into eppendorf tubes S⁺ and S⁻ while DMSO (100 µl) was added into C⁺ and C⁻. Phosphate buffer (pH 7, 190 µl) was added into each tubes. The tubes were vortexed and α-glucosidase (1 unit/µL, 10 µl) was added into tubes C⁻ and S⁺ while aqua (10 µl) was added into tubes C⁺ and S⁻. The tubes were then incubated at 37 °C for 1 h. Reaction was quenched by boiling the tubes at water bath for 3 min. Phenol buffer (pH 7, 5 µl) and peroxides (0.5 unit/µl, 5 µl) aminoantipyrine (4 mg/ml, 5 µl) and glucose oxidase (0.8 unit/µl, 5 µl) were added in each tubes. Mixture were then incubated at 37 °C for 10 min. A portion of solution (200 µl) was loaded into well plates and the absorbance was recorded at 500 nm using microplate reader. Percentage inhibition was calculated based on formula 3.

2.5.3. The kinetic of α-amylase inhibition

The kinetic was determined by using method described in Section 2.5.1 with serial concentrations starch 15, 0.5%, 0.25%, 0.125%, 0.0625% with incubation period of 0, 3, 6, 9, 12,

18, 21 min for each starch concentration. Lineweaver-Burk curve was obtained using the formula 4 below.

$$\frac{1}{v_0} = \frac{K_M}{V_{Max}} \frac{1}{[S]} + \frac{1}{V_{Max}} \quad (4)$$

2.5.4. The kinetic of α-glucosidase inhibition

The determination was used the same protocol described in Section 2.5.3. with serial concentration of maltose 1 M, 0.5 M, 0.25 M, 0.125 M, and 0.0625 M with incubation time frame of 0, 10, 20, 40, 60 and 80 min.

3. Results and discussion

Separately, six medicinal plants were sequentially extracted using hexane, ethyl acetate and methanol which methanol extract majored the highest yield (Fig. 1).

Phenols is common molecules of bioactive secondary metabolites was distributed more in the extracts in line with solvent (extraction) polarity order. From eighteen extracts, methanol extract of *Lagerstroemia speciosa* has the highest phenolic content with value of 425 ± 1.16 mg GAE/g (Table 1).

The similar pattern was showed in the distribution of flavonoid contents in the different solvent extracts in which methanol extract *Lagerstroemia speciosa* possessed the highest flavonoid content with a value of 647.96 ± 7.12 mg QE/g.

Antioxidant molecules involves in protective mechanism of living cells against oxidative stress which could occur due to native metabolism activities or harsh environment. Prolonged oxidative stress can cause cell components malfunction, cell necrosis and lead to organ failure. Antioxidant active medicinal plants intake might suppress the oxidative stress preventing from pathologic condition (Fang et al., 2002; Lobo et al., 2010).

The three different antioxidant experiments showed the activities were proportional with phenolic and flavonoid content of the extracts (Tables 2 and 3). In the DPPH experiment, the methanol of *Arcangelisia flava* and *Merremia mammosa* had higher activity against standard antioxidant, vitamin C with IC₅₀ value of 0.57 ± 0.01, 0.79 ± 0.10 and 0.92 ± 0.19 µg GAE/ml, respectively.

In the hydroxyl radical scavenging activity, methanol extracts of *Arcangelisia flava* and *Merremia mammosa*

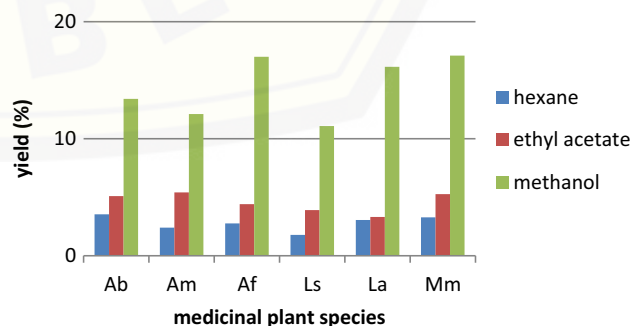


Fig. 1 Extraction yield of *Antidesma bunius* (Ab), *Antidesma montanum* (Am), *Arcangelisia flava* (Af), *Lagerstroemia speciosa* (Ls), *Lunasia amara* (La), and *Merremia mammosa* (Mm).

Table 1 Phenolic and flavonoid equivalent number of extracts.

Plant species	Phenolic total (mg GAE/g)			Flavonoid total (mg QE/g)		
	Hexane	Ethyl acetate	Methanol	Hexane	Ethyl acetate	Methanol
<i>Antidesma bunius</i>	26.11 ± 2.44	79.26 ± 5.73	106.55 ± 3.84	102.17 ± 1.62	175.94 ± 3.53	396.70 ± 14.90
<i>Antidesma montanum</i>	38.44 ± 2.48	41.43 ± 2.22	52.44 ± 0.72	110.27 ± 5.28	207.74 ± 2.80	209.19 ± 16.43
<i>Arcangelisia flava</i>	23.36 ± 0.68	114.99 ± 4.83	135.25 ± 6.02	56.70 ± 3.78	141.04 ± 3.59	267.61 ± 1.92
<i>Lagerstroemia speciosa</i>	6.68 ± 0.33	161.17 ± 3.19	425.51 ± 1.16	69.75 ± 1.88	211.35 ± 3.33	647.92 ± 7.12
<i>Lunasia amara</i> Blanco	18.90 ± 0.47	33.18 ± 0.95	42.74 ± 0.70	63.45 ± 2.73	127.14 ± 5.36	105.13 ± 3.80
<i>Merremia mammosa</i>	23.12 ± 0.92	157.05 ± 3.11	359.92 ± 10.77	215.62 ± 6.67	160.88 ± 2.00	449.46 ± 4.30

Note: GAE: Gallic acid equivalent; Quercetin equivalent.

Table 2 DPPH scavenging activity of plant extracts.

Plant species	Antioxidant activity (IC ₅₀ , µg GAE/ml)		
	Hexane	Ethyl acetate	Methanol
<i>Antidesma bunius</i>	2.93 ± 0.31	2.70 ± 0.21	1.40 ± 0.04
<i>Antidesma montanum</i>	2.50 ± 0.17	4.76 ± 1.20	3.01 ± 0.43
<i>Arcangelisia flava</i>	1.41 ± 0.02	0.80 ± 0.02	0.57 ± 0.01
<i>Lagerstroemia speciosa</i>	4.90 ± 0.50	1.42 ± 0.07	0.79 ± 0.10
<i>Lunasia amara</i> Blanco	13.60 ± 0.40	3.33 ± 0.13	3.32 ± 0.19
<i>Merremia mammosa</i>	1.36 ± 0.05	0.70 ± 0.01	0.69 ± 0.02
Asam Askorbat	0.92 ± 0.19		

Table 3 Percentage inhibition of hydroxyl and superoxide anion radical scavenging of plant extracts.

Plant species	Hydroxyl radical scavenging (%)			Superoxide anion radical scavenging (%)		
	Hexane	Ethyl acetate	Methanol	Hexane	Ethyl acetate	Methanol
<i>Antidesma bunius</i>	41.81 ± 2.28	46.11 ± 0.80	48.55 ± 0.80	22.81 ± 2.78	10.81 ± 1.75	47.71 ± 1.46
<i>Antidesma montanum</i>	26.95 ± 2.01	19.16 ± 2.28	34.73 ± 0.88	1.87 ± 0.41	5.31 ± 1.70	27.46 ± 2.28
<i>Arcangelisia flava</i>	59.25 ± 0.25	73.07 ± 0.96	90.51 ± 0.08	16.74 ± 0.04	22.16 ± 0.73	12.17 ± 0.11
<i>Lagerstroemia speciosa</i>	18.12 ± 0.35	48.08 ± 0.00	50.64 ± 0.73	11.03 ± 4.20	33.03 ± 4.76	36.56 ± 6.51
<i>Lunasia amara</i> Blanco	1.74 ± 0.70	38.68 ± 0.00	40.07 ± 0.70	8.76 ± 2.80	28.20 ± 5.88	27.54 ± 2.87
<i>Merremia mammosa</i>	64.55 ± 0.22	82.66 ± 2.88	91.76 ± 0.38	20.61 ± 0.84	18.67 ± 1.35	25.44 ± 1.21
Ascorbic acid		88.78 ± 0.08			20.43 ± 0.57	

indicated higher inhibition than the standard antioxidant, vitamin C with value of 90.51 ± 0.08%, 91.76 ± 0.38%, 88.78 ± 0.08%, respectively. Compared to the less polar extracts (hexane and ethyl acetate extracts), methanol extracts of the six medicinal plants possessed higher antioxidant activity against superoxide anion radicals.

Acarbose is a standard drugs in diabetes mellitus therapy which is able to reduce the level of sugar in the bloods by inhibiting metabolic decomposition of carbohydrate. The acarbose treatment is commonly used in diabetes therapy related with cells response failure to insulin's property.

Six medicinal plants (*Antidesma bunius*, *Antidesma montanum*, *Arcangelisia flava*, *Lagerstroemia speciosa*, *Lunasia amara* and *Merremia mammosa*), were reported to have anti-diabetic activity. Eighteen extract showed inhibition activity against α -amylase and α -glucosidase (Table 4). Ethyl acetate extract of *Antidesma bunius* indicated the highest inhibition among the other extract. Moreover, the extract had higher inhibition compared to standard drug, acarbose, with inhibition value of 95.39 ± 4.27%, 91.06 ± 2.15%, respectively.

Further experiment showed 10 extracts to have α -glucosidase inhibition with percentage inhibition value higher than acarbose (Table 4).

Interestingly, the α -amylase and α -glucosidase inhibition activity of the extracts did not always agree with total phenol and flavonoid content (Table 4). For example, ethyl acetate extract of *Antidesma bunius*, *Arcangelisia flava* and *Lunasia amara* gave higher inhibition than the standard (acarbose). Moreover, hexane extract of *Antidesma montanum* showed higher α -glucosidase than acarbose. These suggested that α -amylase and α -glucosidase inhibition activity might be correlated with non-phenolic or non-flavonoid bioactive constituents.

The enzyme inhibition was kinetically studied on selected extracts, methanol extract of *Lagerstroemia speciosa*, ethyl acetate extracts of *Arcangelisia flava* and *Antidesma bunius* (Fig. 2 and Table 5). The Lineweaver-Burk curve of *Arcangelisia flava* extract showed the cross section at value of $x < 0$ and $y > 0$ indicated non-competitive enzyme inhibition. From the graph, the extract of *Lagerstroemia speciosa* and *Antidesma*

Table 4 α -amilase and α -glukosidase inhibition.

Plant sepecies	Inhibition (%)					
	α -amilase			α -glukosidase		
	Hexane	Ethyl acetate	Methanol	Hexane	Ethyl acetate	Methanol
<i>Antidesma bunius</i>	82.45 ± 1.84	95.39 ± 4.27	41.31 ± 4.83	53.01 ± 3.10	93.17 ± 4.95	5.04 ± 1.49
<i>Antidesma montanum</i>	16.84 ± 0.31	46.45 ± 4.03	15.25 ± 1.63	79.84 ± 3.46	17.89 ± 1.57	25.37 ± 1.76
<i>Arcangelisia flava</i>	43.18 ± 0.79	64.24 ± 3.53	3.48 ± 1.60	61.23 ± 3.20	78.96 ± 2.28	95.04 ± 3.55
<i>Lagerstroemia speciosa</i>	54.42 ± 2.36	58.50 ± 11.19	90.82 ± 2.70	79.77 ± 0.65	27.64 ± 1.31	94.44 ± 0.43
<i>Lunasia amara</i>	69.73 ± 4.71	86.39 ± 2.57	90.49 ± 2.95	32.62 ± 1.31	66.10 ± 0.89	83.33 ± 3.20
<i>Merremia mammosa</i>	47.73 ± 1.20	43.03 ± 2.15	11.82 ± 1.20	66.19 ± 0.41	52.25 ± 2.87	12.77 ± 2.56
Acarbose	91.06 ± 2.15			54.85 ± 1.48		

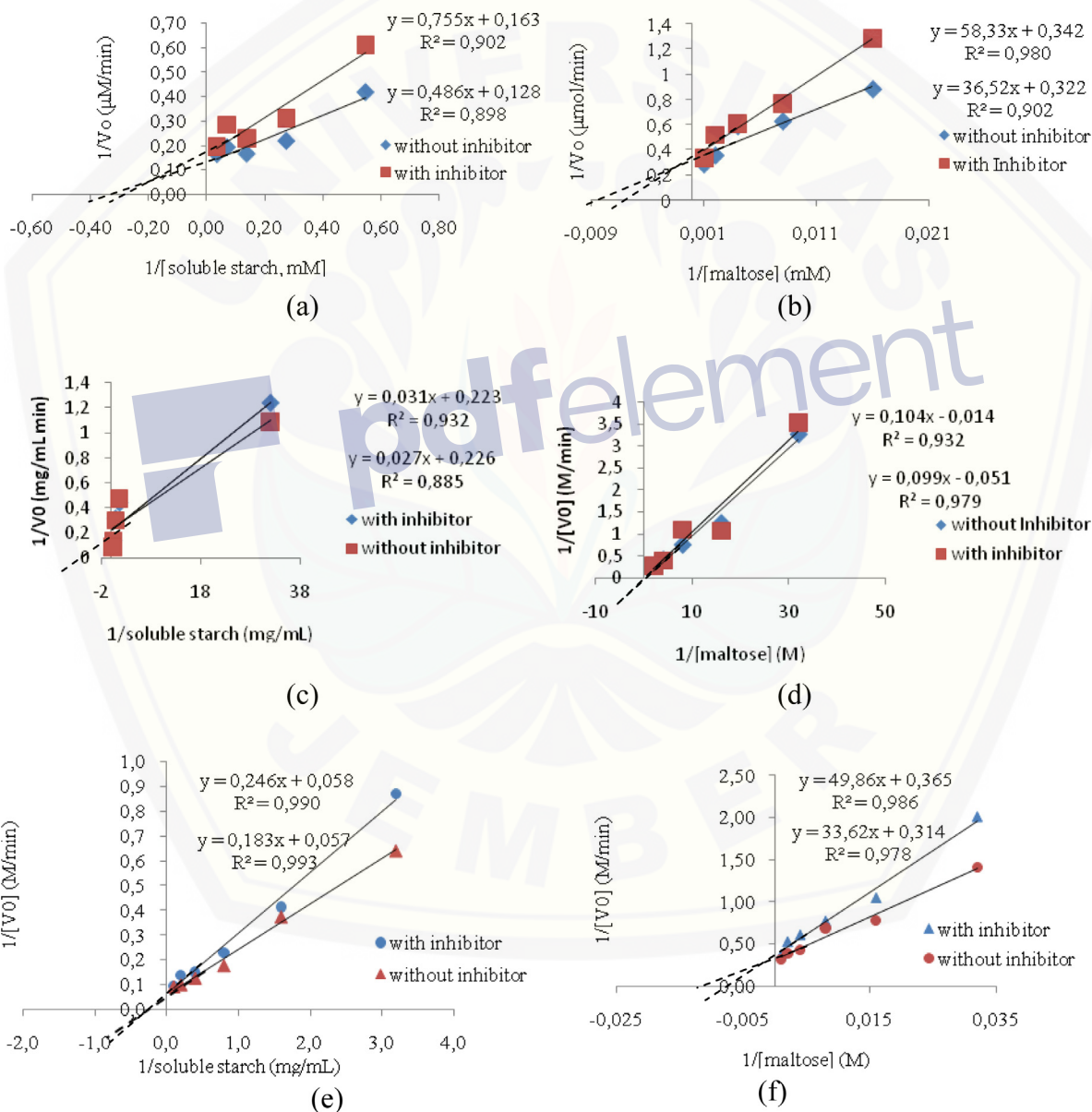


Fig. 2 Lineweaver-Burk curve of α -amilase and α -glukosidase inhibition of *Arcangelisia flava* (a, b), *Antidesma bunius* (c, d), *Lagerstroemia speciosa* (e, f).

Table 5 Kinetic study of α -amilase and α -glukosidase.

Plant species		α -Amilase		α -Glukosidase	
		Control	Extract	Control	Extract
Af	V_{max} (μ M/min)	7.84 \pm 0.29	6.17 \pm 0.29	3.12 \pm 0.28	2.93 \pm 0.20
	K_M (mM)	3.81 \pm 0.01	4.66 \pm 0.47	114.24 \pm 15.69	170.90 \pm 10.28
Ls	V_{max} (μ M/min)	17.49 \pm 1.01	17.18 \pm 5.98	3.18 \pm 0.06	2.74 \pm 0.04
	K_M (mM)	3.19 \pm 0.31	4.77 \pm 2.21	106.63 \pm 5.91	136.64 \pm 8,88
Ab	V_{max} (μ M/min)	13.24 \pm 0.18	13.34 \pm 0.38	22.96 \pm 1.35	67.64 \pm 1.40
	K_M (mM)	0.36 \pm 0.01	0.43 \pm 0.01	2.251 \pm 0.03	7.08 \pm 0.22

Note: Af: *Arcangelisia flava*; Ls: *Lagerstroemia speciosa*; Ab: *Antidesma bunius*.

bunius showed competitive inhibition which referred to selective interaction of the extract with active site of enzymes. Inhibition interaction of extracts and enzyme interaction was clearly indicated from decreasing of K_M values compared to the control (Table 5).

4. Conclusions

To summarise, the six medicinal plants showed a potent activity for α -amylase and α -glucosidase inhibition agents which confirmed the traditional uses of the plants in diabetes mellitus therapy.

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Conflict of interest

The authors declared that there is no conflict of interest.

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