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Alpha-glucosidase inhibition assay of *Lawsonia inermis* Linnaeus leaf ethanol and water extracts

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Abstract: Background : *Lawsonia inermis* Linnaeus (*L. inermis* L.), commonly known as daun inai has been used to treat diabetes among diabetic patients in Medan, Indonesia.
Method : This study is conducted to identify the chemical compound of *L. inermis* L. ethanol (EE) and aquaous (EA) extract and evaluate its alpha-glucosidase inhibition activity. The dried powdered leaves of *L. inermis* L. were extracted to to obtain two extracts namely EE and WE. Qualitative phytochemical screening was conducted to identify chemical compounds in both extracts. Overnight-fasted normal rats were divided into four groups and received the treatment orally. Group I: acarbose (10 mg/kg); groups II, III and IV: EE (1 g/kg), WE (1 g/kg) and distilled water (10 ml/kg). Ten minutes later, the rats were challenged with starch (3 g/kg). Blood glucose levels (BGL) were measured at 0 , 30, 60 and 120 min. Areas under the curve (AUC) were determined. The similar procedure were applied to oral glucose challenge test at dose 2 g/kg.
Result : EE and WE of *L. inermis* L consist of tannin, alkaloid, steroid, triterpenoid, flavonoid and saponin. Both extracts have no significant effect to inhibit alpha-glucosidase activity.
Conclusion : EE and EA of *L. inermis* L have no alpha-glucosidase inhibition activity. Other mechanism of actions as antidiabetic should be investigated.
Keywords : alpha-glucosidase inhibition, *Lawsonia inermis* Linnaeus leaf, ethanol extract, water extract.

1.Introduction

Diabetes mellitus is a common group of metabolic disorders[1] that is characterized by hyperglycaemia[2-5]. Postprandial hyperglycemia is one of the earliest condition to detect type 2 diabetes mellitus[6].Therefore, hyperglycemia after meal management is one of crucial approaches in DM treatment. Acarbose, a known alpha glucosidase and alpha amylase inhibitor, was used as standard in post prandial hyperglycaemia management[7] . By inhibiting alpha glucosidase and alpha amylase, the convertion of carbohydrate into glucose will be delayed that resulting the slow of rising the blood glucose level after meals.

It is well known that plants have been used in traditional herbal medicine for many year[8] , one of which *Lawsonia inermis* as antidiabetic[3]. Previous study by Abdillah[9] showed antihyperglycemic activity of *L. inermis* L. leaf ethanol extract in alloxan-induced diabetic mice. However, there is no data to support its mechanims of action as antihyperglycemic. The present study was conducted to investigate the possible of its mechanisms of action through alpha-glucosidase inhibition.

2.Materials and Methods

2.1.Plant material collection and preparation of extracts:

Lawsonia inermis Linnaeus leaves were collected from Titi Kuning, Medan, Indonesia. The plant was identified at the School of Biological Sciences, Herbarium Medanense MEDA, University of Sumatera Utara, Medan, Indonesia and given a herbarium identification number - No.672/MEDA/2016. The fresh leaves were dried under shade and ground into powder. The ethanol and water extract were prepared by by macerated in ethanol (EE) and infused in water (WE). The freeze-dried extracts were kept in the freezer (-20°C) until used for animal experimentation. Prior to treatment, the extracts were dissolved in distilled water.

2.2.Phytochemical Identification

Phytochemical qualitative analysis was performed using the general method for phytochemical screening of alkaloids, tannins, glycosides, flavonoids, alkaloids and saponins with modification [10-12]

2.3. Animals:

Healthy male Wistar rats weighing between 180-250 g (n=48) were obtained from the Animal House, Universitas Sumatera Utara (USU), Medan, Indonesia. There were housed in the transit animal room, School of Pharmacy, USU and allowed to acclimatized (25 – 30C) for about a week before the experiments. The animals had *ad libitum* access to water and food (standard laboratory chow) a 12 h light-12 h dark cycle and room temperature (25 – 30°C). The procedures approved by the Animal Ethics Committee, FMIPA USU, Medan, Indonesia (Approval number: 184/KEPH-FMIPA/2016).

2.4. In vivo enzyme inhibition studies

Four groups of six normal rats were fasted for 16 hours and treated as follows:

Group I : Acarbose 10 mg/kg (positive control) Group II: EE 1 g/kg
Group III: WE 1 g/kg
Group IV: DW 10 ml/kg

2.4.1. Oral starch tolerance test in normal rats

The rats were treated with starch 3 g/kg (R & M Chemicals, Essex, UK) orally, ten min after giving the above treatments.

2.4.2. Oral glucose tolerance test in normal rats

The rats were treated with glucose 2 g/kg (R & M Chemicals, Essex, UK) orally, ten min after giving the above treatments.

Blood specimens (one drop) were obtained by pricking the rat's tail vein, and BGL were measured using an Easy Touch (Chiuan Rwey Enterprise Co., Ltd, Taiwan) at the following time points: 0 (before treatment), 30, 60 and 120 min after starch and glucose administration, respectively. The area under the curve (AUC) was determined using the formula below:

AUC (mmol/hr) = 0.25(BGL0 + BGL30) +0.25(BGL30 + BGL60) + 0.5(30/2 (BGL60 + BGL120) where BGL0, BGL30, BGL60 and BGL 120 represent BGL at 0, 30, 60 and 120 min.

2.5. Statistical analysis

The data was expressed as mean ± standard error of the mean (SEM). Statistical significance was determined by IBM-SPSS statistical program version 21 (IBM Corp., Armonk, NY). One-way ANOVA was used followed by Dunnet as a *post hoc* test. Differences were considered significant with the p-value was less than 0.05.

3.Results

Extraction of *L.inermis* powder leaves yielded 12,2 % of EE and 22,5% of EA.

3.1.Phytochemical screening

The phytchemical screening of the extracts showed the presence of alkaloids, steroids, triterpenoids, tannins, flavonoids, saponins and glycosides in ethanol extract (EE); whereas tannins, flavonoids, saponins and glycosides were identified in water extract (WE) (Table 3.1).

Table 3.1. Phytochemcal constituents of *Lawsonia inermis* L leaf ethanol extract (EE) and water ekstract (WE)

Test	Ethanol Extract	Water Extract
Alkaloids	(+)	(-)
Steroids	(+)	(-)
Triterpenoids	(+)	(-)
Tannins	(+)	(+)
Flavonoids	(+)	(+)
Saponins	(+)	(+)
Glycosides	(+)	(+)

3.2. Enzyme inibition *in vivo*

3.2.1. Oral starch tolerance test in normal rats

Table 3.2.showed that the blood glucose levels (BGL) peak of all groups were stated at 30 min. The highest BGLrising was found in DW-treated groups at 23% ;72.0 ± 5.09 to 88.7 ± 9.09; whereas acarbose showed the lowest at 5.3% (71.3 ± 3.34 to75.2 ±2.46). However, only acarbose-treated group as positive control showed statistically significant at 30 and 60 min compared to DW-treated group ($p<0.05$ respectively).

Tabel 3.2. Effect of acarbose (10 mg/kg), ethanol extract (EE) (1 g/kg), water extract (WE) (1 g/kg) and Distilled Water (DW) on the blood glucose levels of normal rats after starch loading (3 g/kg).

Group	Blood Gluscose Level (mg/dl)			
	0’	30’	60’	120’
Acarbose (10 mg/kg)	71.3 ± 3.34	75.2 ±2.46*	59.8 ± 2.56*	56.7 ± 2.23
EE (1 g/kg)	69.7 ± 2.09	77.3 ± 3.32	68.3 ± 2.80	67.2 ± 1.78
WE (1 g/kg)	68.0 ± 9.16	80.3 ± 9.52	68.5 ± 3.80	65.5 ± 2.76
DW (10 ml/kg)	72.0 ± 5.09	88.7 ± 9.09	77.5 ± 5.94	67.8 ± 6.21

Data were analysed using one way ANOVA and Dunnett’s test as a *post hoc* (*$p<0,05$)

Area under the curve (AUC) of acarbose-, EE and W E-treated groups were lower than DW-treated group. However, only acarbose demonstrated significant different compared to DW-treated group ($p<0.05$) (Table 3.3).

Tabel 3.3. Effect of acarbose (10 mg/kg), ethanol extract (EE) (1 g/kg), water extract (WE) (1 g/kg) and distilled water (DW) on the Area under the curve (AUC) of normal rats after starch loading (3 g/kg).

Grup	AUC (x ± SEM)
Acarbose	128.6 ± 3.08*
EE	140.9 ± 3.73
WE	141.2 ± 5.89
DW	154.3 ± 8.57

Data were analysed using one way ANOVA and Dunnett’s test as a *post hoc* (*$p<0,05$)

3.2.1. Oral glucose tolerance test in normal rats

The BGL peak of all groups were shown at 30 min. EE-treated group showed the highest value of BGL increasing 118% (61.7 ± 2.60 to 132.3 ± 11.62) while acarbose-treated group showed the lowest level at 83% (62.0 ± 4.30 to 117.2 ±6.68). However, no statistically different found within groups for 120 min observation (Table 3.4).

Table 3.4. Effect of acarbose (10 mg/kg), ethanol extract (EE) (1 g/kg), water extract (WE) (1 g/kg) and Distilled Water (DW) on the blood glucose level (BGL) of normal rats after glucose loading (3 g/kg).

Group	Blood Glucose Level (mg/dl)			
	0'	30'	60'	120'
Acarbose	62.0 ± 4.30	117.2 ±6.68	98.7 ± 7.73	65.8 ± 5.43
EE	61.7 ± 2.60	132.3 ± 11.62	113.2 ± 11.3	85.2 ± 7.96
WE	64.3 ± 4.06	116.7 ± 8.94	103.0 ± 6.15	69.7 ± 4.45
DW	60.5 ± 2.64	122.7 ± 8.89	118.4 ± 5.02	93.0 ± 12.74

The AUC of acarbose-, EE- and WE-treated groups were lower than DW-treated groups. However, compared to DW-treated group, no statistically different were found on both extracts- and acarbose-treated group .

Table 3.5. Effect of acarbose (10 mg/kg), ethanol extract (EE) (1 g/kg), water extract (WE) (1 g/kg) and Distilled Water (DW) on the Area under the curve (AUC) of normal rats after glucose loading (3 g/kg).

Group	AUC (x ± SEM)
Acarbose	181,0 ± 11,80
EE	208,4 ± 14,75
WE	186,7 ± 6,52
DW	211,7 ± 12,66

4. Discussion

Phytochemical screening revealed the presence of tannins, flavonoids, saponins and glycosides in both EE and WE of *L.inermis* L leaf. This results supported the previous study by Kamal & Jawaid] and Li et al. [13-14] . Alkaloids, steroids, triterpenoids[15], tannins, flavonoids, saponins[16] and glycosides were have antidiabetic properties[17-18] by different mechanisms of action. Plants contain compounds that are rich in polyphenols, which are known can inhibit enzyme activity[19] . Tannins were investigated to inhibit alpha-glucosidase and alpha-amylase[20].

Area Under the Curve (AUC) is the area under a plot of plasma concentration for a drug against time after drug administration (Katzung, 2004). AUC reflects the bioavailability of a drug. An increased AUC value means that more of a drug was absorbed. Conversely, a decreased AUC value means that a smaller amount of the drug was absorbed. Starch is a polysaccharide, while glucose is a monosaccharide. After starch loading, these carbohydrates should be converted by the carbohydrate digestive enzymes (alpha-glucosidase and alpha-amylase) into monosaccharides (glucose) to be absorbed. In the presence of an alpha-glucosidase inhibitor, such as acarbose[7], the convertion of complex carbohydrates (starch) into disaccarides and monosaccharides (glucose) is inhibited. Therefore, the amount of glucose that can be absorbed is decreased.

In the oral starch and glucose tolerance tests, oral administration of the extracts (EE and WE) as expected to inhibit the enzyme activity (if any), resulting in lower glucose absorption rates, and subsequently, lower AUC values. The present study showed that both extracts (1 g/kg, respectively) tended to inhibit the rise of BGL after starch loading, however no statistically significant compared to DW as negative control. This result suggested that both extracts have no action through alpha-glucosidase enzyme activity. It is accepted that after

glucose loading no significant different to all treated groups, since the alpha glucosidase enzyme have role in carbohydrate convertion to glucose only but not on glucose itself.

Conclusion

EE and WE of *L. inermis* L have no alpha-glucosidase inhibition activity. Other mechanisms of action as antidiabetic should be investigated.

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