

Original Research Article

Effect of *Senna alata* (L) Roxb (Fabaceae) Leaf Extracts on alpha-Amylase, alpha-Glucosidase and Postprandial Hyperglycemia in Rats

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Abstract

Purpose: To investigate the inhibitory effect of *S. alata* leaf extracts on α -amylase and α -glucosidase, and its potential for reducing postprandial blood glucose level of rats.

Methods: The α -amylase and α -glucosidase inhibitory potentials of acetone, ethylacetate and hexane extracts of *S. alata* were investigated by reacting different concentrations (0.63 – 10.0 mg/mL) of the extracts with the enzymes and substrates while the mode(s) of inhibition of both enzymes were determined using the Lineweaver-Burk plot. The effect of oral administration of the hexane extract (with lowest IC_{50} for α -glucosidase) of the plant in sucrose-induced hyperglycemic rats was also determined by evaluating fasting blood glucose levels.

Results: The acetone extract of *S. alata* displayed the highest inhibitory activity against α -amylase (IC_{50} = 6.41 mg/mL) while hexane extract exhibited highest inhibitory effect on α -glucosidase (IC_{50} = 0.85 mg/mL). Both acetone and hexane extracts inhibited α -amylase and α -glucosidase in a competitive and uncompetitive manner respectively. The hexane extract of *S. alata* also caused significant reduction ($p < 0.05$) in the postprandial blood glucose level of sucrose-loaded rats within two hours.

Conclusions: It can be suggested that one of the mechanisms of anti-diabetic action of *S. alata* leaf is the inhibition of intestinal α -glucosidase.

Keywords: *Senna alata*, Diabetes, Postprandial hyperglycemia, Phytochemicals, α -glucosidase, α -amylase

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INTRODUCTION

Senna alata (Cassia alata) Linn. Roxb (Leguminosae), known as Ringworm plant, winged senna, craw-craw plant or king of the forest, is of great importance in traditional medical practice in many developing countries [1]. In Nigeria, it is called 'Asunwon oyinbo' and 'Nelkhi' in the Western and Eastern regions of the country respectively. This plant is indigenous to several regions of the world such as Africa and

South America. It is an erect, tropical, annual herb of 0.15 m high with leathery, compound yellowish-green leaves [2]. It thrives in sunny and moist areas and produces a characteristic offensive smell.

Ethnopharmacological studies have shown that the leaves of the plant (*S. alata*) have been used in the treatment of digestion-related ailments such as constipation, abdominal pain and liver diseases [3]. It has also enjoyed wide usage in

the management of dermatologic diseases like dermatitis, skin rash, athlete's foot, eczema and mycosis [4]. The whole plant is used in Brazil, Guatemala and Guinea in the treatment of malaria, flu and other infectious diseases [3]. Studies have also reported the antibacterial, antifungal and antiviral properties of *S. alata* leaf [1,6] as well as its laxative [3] and abortifacient properties in wistar rats [2]. Of particular interest is its use in the management of sugar-related disorders such as diabetes mellitus and hyperglycaemia [5-7].

Although *S. alata* has been validated scientifically to possess anti-diabetic property [6,7], there is no information on the possible mechanism by which this plant elicits its hypoglycemic effect. Therefore, the aim of this study was to determine the effect of leaf extracts of *S. alata* on α -amylase and α -glucosidase as well as its mode of inhibition of these enzymes, and to assess its effect on postprandial hyperglycemia.

EXPERIMENTAL

Sample collection

The leaves of *Senna alata* were obtained from Igando Area of Lagos in Nigeria in May 2013. The plant was identified and authenticated by the taxonomist Dr AB Kadiri of the Department of Botany, University of Lagos, Nigeria and voucher specimen (LUH 5603) was deposited in the University herbarium. The leaves were dried at 45 °C in the oven, pulverized and kept in air-tight plastic bags.

Experimental animals

Male wistar rats were obtained from the Animal House of the University of the Free State, South Africa. They were housed in plastic cages under a 12 h light/dark cycle at 20 – 25 °C and had free access to standard rat chow and tap water ad libitum. Ethical approval for the study was obtained from the Interfaculty Ethics Committee of the University of the Free State, South Africa with approval number NR 02/2013 and all experiments were performed according to the Guide for the Care and Use of Laboratory Animals [9].

Chemicals and reagents

Porcine pancreatic α -amylase, rat intestinal α -glucosidase and para nitrophenyl-glucopyranoside (pNPG) were products of Sigma-Adrich Co (St Louis, USA) while soluble

starch (extra pure) was obtained from JT Baker Inc. (Phillipsburg, USA). Other chemicals and reagents were of analytical grade and water used was glass-distilled.

Preparation of plant extracts

The powdered leaves were divided into three portions of 20 g each and these were extracted with acetone, ethylacetate or hexane. They were all left to steep in covered Erlenmeyer flask for 24 h and the resulting infusions were decanted, filtered with Whatman 150 mm filter paper and evaporated to dryness in a rotary evaporator (Cole Parmer SB 1100, Shangai, China).

α -Amylase inhibitory assay

This assay was carried out using a modified procedure of McCue and Shetty [10]. The extract (250 μ L, 0.63 – 10.0 mg/mL) was placed in a test tube and 250 μ L of 0.02 M sodium phosphate buffer (pH 6.9) containing α -amylase solution (0.5 mg/mL) was added. This solution was pre-incubated at 25 °C for 10 min, after which 250 μ L of 1 % starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added at timed intervals and then incubated at 25 °C for 10 min. The reaction was terminated by adding 500 μ L of dinitrosalicylic acid (DNS) reagent. The tubes were then incubated in boiling water for 5 min and cooled to room temperature and the reaction mixture was diluted with 5 mL distilled water. Absorbance then was measured at 540 nm in a spectrophotometer (Spectrumlab S23A, Globe Medical England). Distilled water and acarbose were used as control and standard respectively. The α -amylase inhibitory activity was calculated as in Eq 1.

$$\text{Inhibition (\%)} = \left\{ \frac{Ac - Ae}{Ac} \right\} 100 \dots\dots\dots (1)$$

where Ac and Ae are the absorbance value of the control and extract respectively.

Concentrations of extracts resulting in 50 % inhibition of enzyme activity (IC_{50}) were determined graphically using Microsoft Excel 2007.

Mode of α -amylase inhibition

The mode of inhibition of α -amylase by the leaf extract was conducted using the most potent extract according to the modified method described by Ali *et al* [11]. Briefly, 250 μ L of the extract (5 mg/mL) was pre-incubated with 250 μ L of α -amylase solution (0.5 mg/mL) for 10 min at 25 °C in one set of tubes. In another set of tubes, α -amylase was pre-incubated with 250 μ L of 0.02

M sodium phosphate buffer (pH 6.9). Starch solutions (250 μ L) at increasing concentrations (0.3–5.0 mg/mL) were added to both sets of reaction mixtures to start the reaction. Each mixture was then incubated for 10 min at 25 °C, and then boiled for 5 min in a water bath (TW20, Julabo GmbH, Germany) after addition of 500 μ L of DNS to stop the reaction. The amount of reducing sugars released was determined colorimetrically using a maltose standard curve and converted to reaction velocities (mM/min). A double reciprocal plot (Lineweaver-Burk) ($1/v$ against $1/[S]$) where v is reaction velocity and $[S]$ is substrate concentration was plotted to determine the mode of inhibition.

α -Glucosidase inhibitory assay

The effect of the plant extracts on α -glucosidase activity was determined according to the method described by Kim *et al* [12]. The substrate solution (3.0 mM), p-nitrophenyl glucopyranoside (pNPG) was prepared in 20 mM phosphate buffer, pH 6.9. 100 μ L of α -glucosidase (E.C. 3.2.1.20) (0.5 mg/mL) was pre-incubated with 50 μ L of the different concentrations of the extracts for 10 min. Then 50 μ L of 3.0 mM pNPG dissolved in 20 mM phosphate buffer (pH 6.9) was added to start the reaction. The reaction mixture was incubated at 37 °C for 20 min and stopped by adding 2 mL of 0.1 M Na_2CO_3 and the α -glucosidase activity was determined by measuring the yellow coloured para-nitrophenol released from pNPG at 405 nm using spectrophotometer (Spectrumlab S23A, Globe Medical England). Distilled water and acarbose were used as control and standard respectively. Percentage inhibition was calculated as in Eq 2.

$$\text{Inhibition (\%)} = \{(Ac-Ae)/Ac\}100 \dots\dots\dots (2)$$

where Ac and Ae are the absorbances of the control and extract respectively.

Concentrations of extracts resulting in 50 % inhibition of enzyme activity (IC_{50}) were determined graphically using Microsoft Excel.

Mode of α -glucosidase inhibition

The mode of inhibition of α -glucosidase by the extracts was determined using the extract with the lowest IC_{50} according to the modified method described by Ali *et al* [11]. Briefly, 50 μ L of the 5 mg/mL extract was pre-incubated with 100 μ L of α -glucosidase solution (0.5 mg/mL) for 10 min at 25 °C in one set of tubes. In another set of tubes, α -glucosidase was pre-incubated with 50 μ L of phosphate buffer (pH 6.9). At increasing

concentrations (0.63 – 2.0 mg/mL), 50 μ L of pNPG was added to both sets of reaction mixtures to start the reaction. The mixture was incubated for 10 min at 25 °C and 500 μ L of Na_2CO_3 was added to stop the reaction and absorbance was read at 405 nm using spectrophotometer (Spectrumlab S23A, Globe Medical England). The amount of para-nitrophenol released was determined colorimetrically using a para-nitrophenol calibration curve and converted to reaction velocities. A double reciprocal (Lineweaver-Burk) plot ($1/v$ against $1/[S]$) where v is reaction velocity and $[S]$ is substrate concentration was plotted to determine the mode of inhibition.

Sucrose-tolerance test

Ten normal rats were divided into two groups of five rats ($n = 5$) each: Group A was designated as the control rats while Group B was the hexane-extract treated rats. After an overnight fast (18 h), groups B rats were administered hexane extract of *S. alata* leaf (250 mg/kg body weight) orally by means of an orogastric tube while the rats in group A received distilled water (vehicle). Both groups were orally administered 3 g/kg body weight of sucrose solution 30 min after the administration of the extract. Postprandial blood glucose levels were then measured before (0 min) and at 30, 60, 90 and 120 min after oral administration of sucrose using Glucometer 4 Ames (Bayer Diagnostics, Germany). The postprandial blood glucose levels were plotted and compared with those of control rats.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 5 statistical package (GraphPad Software, USA). The data were analysed by one way analysis of variance (ANOVA) followed by Bonferroni test. All results were expressed as mean \pm SEM ($n = 3$).

RESULTS

Figure 1(a) shows the percentage inhibition of α -amylase activity by different extracts of *S. alata*. At all concentrations tested, the values obtained for the three extracts were significantly different ($p < 0.05$) from one another except at 10 mg/mL where the values for ethylacetate and hexane extract were similar. Acetone extract exhibited the highest percentage inhibition of the enzyme when compared to other extracts. The IC_{50} values generated from the percentage inhibition reveals that of all the extracts, acetone extract of *S. alata* has the lowest IC_{50} value (6.41 mg/mL)

but was higher than that of acarbose (5.19 mg/mL) (Table 1). Kinetic analysis of the α -amylase inhibition by the acetone extract of *S. alata* leaf using Lineweaver-Burk plot shows that it displayed a competitive mode of inhibition (Figure 1b).

The percentage inhibition of α -glucosidase activity by acetone, ethylacetate and hexane extracts of *S. alata* are presented in Figure 2(a). At lower concentrations (0.63 - 2.5 mg/mL), the values for hexane extract were significantly higher ($p < 0.05$) than the values for other

extracts. There were significant differences ($p < 0.05$) among all the extracts at 5 mg/mL but at the highest concentration (10 mg/mL), the values obtained for all the extracts were not significantly different from one another. The IC_{50} values generated from the percentage inhibition of the extracts reveals that hexane extract has the lowest value (0.85 mg/mL) compared to other extracts and acarbose (Table 1). Kinetic analysis of the α -glucosidase inhibition by the hexane extract of *S. alata* leaf using Lineweaver-Burk plot shows that it displayed an uncompetitive mode of inhibition (Figure 2b).

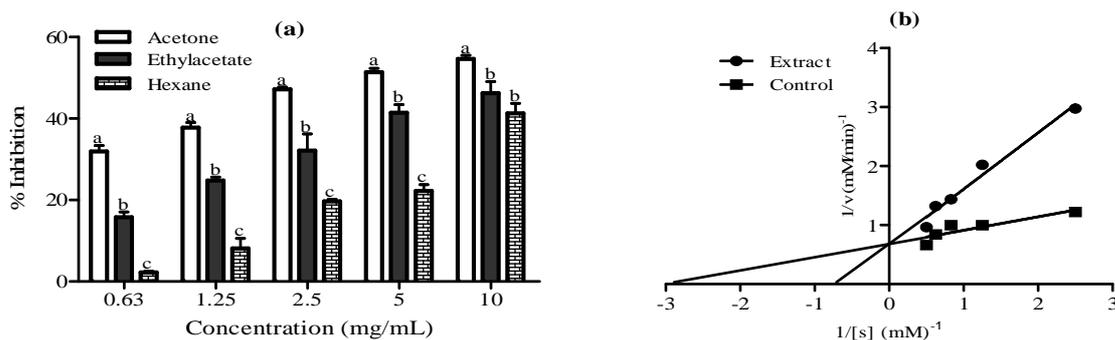


Figure 1: Inhibitory potency of *S. alata* leaf extracts against α -amylase activity (a). Mode of inhibition of α -amylase by acetone extract of *Senna alata* (b). Values are expressed as mean \pm SEM of triplicate determinations. Means not sharing a common letter at the same concentration are significantly different ($p < 0.05$)

Table 1: IC_{50} values for the inhibition of α -amylase and α -glucosidase by different extracts of *S. alata* leaf and acarbose

Extracts	IC_{50} (mg/mL)	
	α -Amylase	α -Glucosidase
Acarbose	5.19 \pm 0.15 ^a	1.26 \pm 0.02 ^a
Acetone	6.41 \pm 0.12 ^a	4.92 \pm 0.10 ^d
Ethylacetate	10.03 \pm 0.31 ^b	4.15 \pm 0.07 ^b
Hexane	12.05 \pm 0.38 ^b	0.85 \pm 0.02 ^a

The values are expressed as means \pm SEM of triplicate determinations. Means down vertical column not sharing common letter are significantly different ($P < 0.05$). Acarbose is the standard α -amylase and α -glucosidase inhibitor

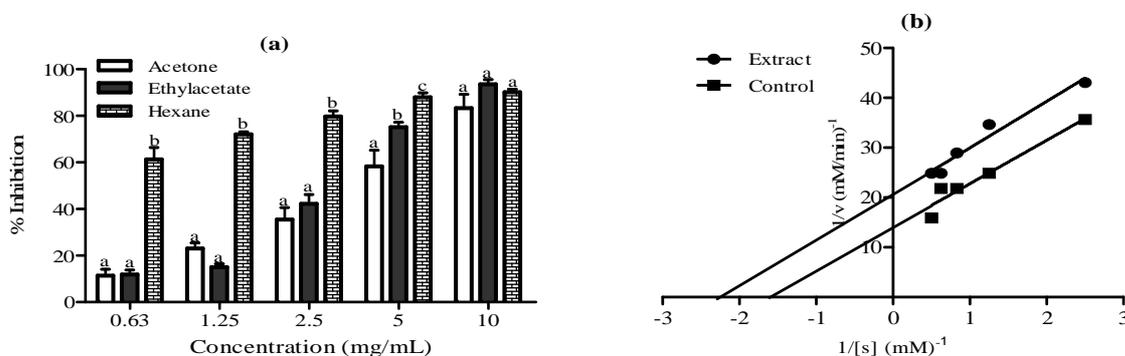


Figure 2: Inhibitory potency of *S. alata* leaf extracts against α -glucosidase activity (a), Mode of inhibition of α -glucosidase by hexane extract of *Senna alata* (b). Values are expressed as means \pm SEM of triplicate determinations. Means not sharing a common letter at the same concentration are significantly different ($p < 0.05$)

The effect of oral administration of the hexane extract of *S. alata* on sucrose-induced hyperglycemia is shown in Figure 3. The result shows that the rise in postprandial blood glucose level of the control and extract-treated rats were similar at the initial period after the administration of extract, until at 60 min when the extract significantly reduced ($p < 0.05$) the glucose level. This trend of reduction in the extract-treated hyperglycaemic animals continued but not significant by the end of the experiment. This is also corroborated by the significant difference between the area under curve (AUC) of the control and that of the hexane extract-treated animals (Table 2).

DISCUSSION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both [13]. It affects the metabolism of carbohydrates, fats and proteins in the body, leading to several complications such as nephropathy, neuropathy and retinopathy [14]. According to the International Diabetes Federation, the number of people living with diabetes in 2013 was 382 million worldwide and is projected to rise to 592 million by 2035 [15].

The management of hyperglycemia is the hallmark of treatment in diabetes. One of the therapeutic approaches for decreasing postprandial hyperglycemia is to retard the digestion and absorption of carbohydrates. This can be achieved by the inhibition of carbohydrate hydrolyzing enzymes, α -amylase and α -glucosidase, in the digestive tract [16]. Though

synthetic α -glucosidase inhibitors such as acarbose and voglibose are presently in use, their use is bedevilled by undesirable side effects such as nausea, diarrhoea and liver failure [17]. In this study, the low percentage inhibition of α -amylase by the extracts of *Senna alata* is a pointer to the fact that the plant is a mild inhibitor of the enzyme. This is desirable of antidiabetic medicinal plants in order to prevent some of the side effects produced by synthetic drugs [18]. The characteristic competitive inhibition of α -amylase exhibited by the acetone extract of *S. alata* suggests that the active component in the extract is structurally similar to the substrate. This suggests that the extract binds reversibly to the active site of the enzyme and occupies it in a mutually exclusive manner with the substrate [19].

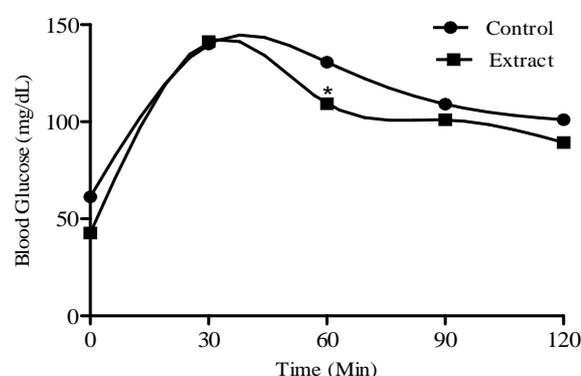


Figure 3: Effect of administration of hexane extract of *S. alata* on blood glucose level of sucrose-loaded normal rats. * Values are significantly lower than the control.

Table 2: Pharmacokinetic parameters of control and *S. alata* treated rats after sucrose ingestion

PK Parameters	Groups	
	Control	Extract
C_{max} (mg/dL)	140.00 \pm 2.50	141.30 \pm 3.10
T_{max} (min)	30.00 \pm 0.00	30.00 \pm 0.00
AUC (mg/dL.min)	13825.50 \pm 20.10	11530.10 \pm 16.70*

The values are expressed as means \pm SEM of triplicate determinations. *Mean values are significantly different from the control. (C_{max} is the maximum blood glucose level of the rats, T_{max} is the time at which the maximum blood glucose level was attained and AUC is the area under the curve)

The strong inhibition of α -glucosidase by hexane extract of *S. alata* as depicted by the lowest IC_{50} (0.85 mg/mL) suggests that it contains potent α -glucosidase inhibitor, indicating a potential role as an antidiabetic natural source. This is because any plant which is a strong inhibitor of α -glucosidase could serve as effective therapy for postprandial hyperglycemia with minimal side

effects [20]. Lineweaver-Burk plot showed that the hexane extract of this plant inhibited α -glucosidase in an uncompetitive manner. This suggests that the inhibitor has no structural similarity with the substrate and the inhibitor binds exclusively to the enzyme-substrate complex yielding an inactive enzyme-substrate-inhibitor complex [21].

In order to confirm the antihyperglycemic effect of the hexane extract of *S. alata in-vivo*, the effect of the oral administration of hexane extract of *S. alata* on postprandial hyperglycemia was tested. The reduction in the blood glucose level of the *S. alata* extract-treated rats compared to the control animals suggest that the hexane extract of the plant slowed down the digestion and absorption of sucrose in the animals.

CONCLUSION

Findings from this study show that of all the *S. alata* extracts tested, acetone and hexane extract provided the most effective inhibition of α -amylase and α -glucosidase, respectively, and that their mode of inhibiting both enzymes is competitive and uncompetitive inhibition, respectively. The hexane extract also reduces sucrose-induced postprandial hyperglycemia in rats. Therefore, it can be concluded that *S. alata* leaf extracts inhibit α -amylase as well as α -glucosidase and reduced postprandial hyperglycemia in rats.

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REFERENCES

- Adedayo O, Anderson WA, Moo-Young M, Snieckus V, Patil PA, Kolawole DO. *Phytochemistry and antibacterial activity of Senna alata flower*. *Pharm Biol* 2001; 39 (6): 408–412
- Yakubu MT, Adeshina AO, Oladiji AT, Akanji MA, Oloyede OB, Jimoh GA, Olatinwo AWO, Afolayan AJ. *Abortifacient potential of aqueous extract of Senna alata leaves in rats*. *J Reprod Contracep* 2010; 21(3):163-177
- Hennebelle T, Weniger B, Joseph H, Sahpaz S, Bailleul F. *Senna alata*. *Fitoterapia* 2009; 80: 385–393
- Ajibesin KK, Ekpo BA, Bala DN, Essien EE, Adesanya SA. *Ethnobotanical survey of Akwa Ibom State of Nigeria*. *J Ethnopharmacol* 2008; 115: 387–408
- Abo KA, Fred-Jaiyesimi AA, Jaiyesimi AEA. *Ethnobotanical studies of medicinal plants used in the management of diabetes mellitus in South Western Nigeria*. *J Ethnopharmacol* 2008; 115: 67–71
- Woradulayapinij W, Soonthornchareonnon N, Wiwat C. *In vitro HIV type 1 reverse transcriptase inhibitory activities of Thai medicinal plants and Canna indica L. rhizomes*. *J Ethnopharmacol* 2005; 101: 84–89
- Palanichamy S, Nagarajan S, Devasagayam M. *Effect of Cassia alata leaf extract on hyperglycemic rats*. *J Ethnopharmacol* 1988; 22: 81.
- Villasenor IM, Canlas AP, Pascua MPI, Sabando MN, Soliven LAP. *Bioactivity studies on Cassia alata Linn. leaf extracts*. *Phytother Res* 2002; 16, S93–S96.
- National Academy of Sciences. *Guide for the care and use of laboratory animals*, 8th Ed., Washington, USA, National Academic Press, 2011.
- McCue PP, Shetty K. *Inhibitory effects of rosmarinic acid extracts on porcine pancreatic α -amylase in vitro*. *Asia Pac J Clin Nutr* 2004; 13: 101–106.
- Ali H, Houghton PJ, Soumyanath A. *Alpha-amylase inhibitory activity of some Malaysian plants used to treat diabetes with particular reference to Phyllanthus amarus*. *J Ethnopharmacol* 2006; 107: 449-455.
- Kim YM, Jeong YK, Wang MH, Lee WY, Rhee HI. *Inhibitory effects of pine bark extract on alpha-glucosidase activity and postprandial hyperglycemia*. *Nutrition* 2005; 21: 756-761.
- Khan A, Zaman G, Anderson RA. *Bay leaves improve glucose and lipid profile of people with Type 2 diabetes*. *J Clin Biochem Nutr* 2009; 44: 52–56.
- Afolayan AJ, Sunmonu TO. *In vivo studies on antidiabetic plants used in South African Herbal Medicine*. *J Clin Biochem Nutr* 2010; 47: 98–106
- International Diabetes Federation (IDF). *IDF Diabetes Atlas, 6th ed.*, Brussels, Belgium, 2013.
- Matsui T, Ogunwande IA, Abesundara KJM, Matsumoto K. *Anti-hyperglycemic potential of natural products*. *Mini Rev Med Chem* 2006; 6 (3): 349–356.
- Ibrahim MA, Islam MS. *Butanol fraction of Khaya senegalensis root modulates β -cell function and ameliorates diabetes-related biochemical parameters in a type 2 diabetes rat model*. *J Ethnopharmacol* 2014; 154: 832–838.
- Dong H, Li M, Zhu F, Liu F, Huang J. *Inhibitory potential of trilobatin from Lithocarpus polystachyus Rehd against α -glucosidase and α -amylase linked to type 2 diabetes*. *Food Chem* 2012; 130: 261–266.
- Abesundara KJM, Matsui T, Matsumoto K. *α -Glucosidase inhibitory activity of some Sri Lanka plant extracts, one of which, Cassia auriculata, exerts a strong antihyperglycemic effect in rats comparable to the therapeutic drug acarbose*. *J Agric Food Chem* 2004; 52: 2541-2545.
- Kwon YI, Apostolidis E, Shetty K. *In vitro studies of eggplant (Solanum melongena) phenolics as inhibitors of key enzymes relevant for type 2 diabetes and hypertension*. *Bioresource Technol*. 2008; 99: 2981-2988.
- King JB, West MB, Cook PF, Hanigan MH. *A Novel, Species-specific class of uncompetitive inhibitors of γ -Glutamyl transpeptidase*. *J Biol Chem* 2009; 284(14): 9059 –9065.