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In vitro antidiabetic activities of Myanmar medicinal plants: *Cassia siamea* Lam. and *Butea monosperma* Roxb.

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This study aimed to evaluate the antidiabetic potentials of *Cassia siamea* and *Butea monosperma*. Cytotoxic activity of test extracts was performed by a hemolytic assay. Estimation of the antidiabetic properties was explored by α-glucosidase and DPP-IV inhibition assays. The glucose transportation activity of test extracts across the yeast cells was expressed by a glucose uptake assay**.** Non-hemolytic effects of test extracts were shown as lysis per cent less than 15 on RBCs. The inhibition potential of test extracts on α-glucosidase enzyme illustrates that IC_{50} values (µg/mL) of *C. siamea* was 76.33±12.2 and *B. monosperma* was 77.28±2.02. While the IC_{50} values of the acarbose was 36.76±1.55 µg/mL. In addition, the tested extracts showed the ability to inhibit DPP-IV enzyme activity in a concentration-dependent manner. The IC_{50} (µg/mL) values of *C. siamea, B. monosperma,* and the sitagliptin were 117.02±9.73, 103±8.5, and 144.85±13.43, respectively in DPP-IV inhibitory assay. Moreover, the test extracts could transport glucose in yeast cells representing the glucose uptake effectively especially in *B. monosperma* with a concentration-dependent manner in all tested glucose concentrations. This study provided that the tested extracts promise to possess the antidiabetic potential with non-hemolytic properties, diabetic-enzymes suppressing potency and glucose utilizing ability.

Keywords: Cytotoxic activity, Diabetes mellitus, Dipeptidyl peptidase IV (DPP-IV), Glucose uptake, Insulin.

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Introduction

Insulin regulates the blood sugar to maintain a normal by converting glucose into energy which stores in muscle, fat cells, and liver to use when the body needs it[1]. In sensitivity or lack of insulin makes the cell unable to metabolize sugar regularly and hence develops diabetes. Insulin resistance initiated known as Type 2 diabetes mellitus (T2DM) is characterized by the cells in the body not responding to the insulin and causes the lack of insulin secretion. Resistant of target tissues to normal circulating levels of insulin cause non-insulin-dependent diabetes (T2DM)[2].

The hemolytic effect of the plant extracts is crucial to determine in the treatment to an administration of the plant derivatives[3]. The hemolytic activity of plant extracts should preferably confirm their cytotoxic effect in drug–membrane interaction. The cytotoxic compound causes a loss of membrane integrity and cell damage due to cell lysis[4].

The action of the α-glucosidase enzyme is to enhance the blood glucose level by catalyzing the release of α-glucose. The blood glucose level is reduced by retarding the digestion and absorption of carbohydrates via the inhibition of α-amylase and α-glucosidases enzymes[5]. In non-insulin-dependent diabetes mellitus patients, the inhibition of these enzymes lowers blood glucose levels and enhances glucose uptake and insulin sensitivity[6]. GLP-1 (glucagon-like peptide -1), stimulated the insulin secretion that regulated blood glucose levels. GLP-1 is deactivated by the proteolytic enzyme named Dipeptidyl peptidase IV (DPP-IV). Hence, DPP-IV inhibitor induces the activation of GLP-1 to maintain the glucose level in the blood. Therefore, inhibiting the DPP-IV action has been recognized as the therapeutic target in the treatment of T2DM[7]. Glucose intolerance T2DM is characterized by an impaired capacity for glucose transport into muscle or glucose uptake in muscle and adipose tissue. Insulin reduces the concentration of blood glucose levels by increasing glucose uptake in muscle and adipose tissue[8]. Some edible medicinal plants having dual effects are used for hyperglycemic action and are also used to control diseases and their complications[9]. However, there are no reports in synthetic medicine that possess both of these properties[10].

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It has been reported that the genus *Cassia* has nutritional, medicinal, economical importance around the world. *Cassia siamea* consist of alkaloids, glycosides, coumarins, chromones, terpenenoids, tannin, sterols and polyphenols[11]. *C. siamea* has also been reported to have ethnopharmacological benefits for the treatment of a variety of ailments, including fever, malaria, diabetes, hypertension, asthma, constipation, and diuresis[12].

Butea monosperma, widely distributed in South East Asian and it has been used in traditional medicine purposes. Some study indicated that *B. monosperma* possesses anti-diabetic, anti-cancer, anti-inflammatory, anti-asthmatic, anti-oxidant, anti-convulsant, anti-microbial, anti-viral and hepatoprotective properties[13]. Determination for the leaves and stem bark of *B. monosperma* by different *in vitro* techniques exhibited significant anti-diabetic activity[14].

Despite the fact that different studied of *C. Siamea* and *B. monosperma* extracts from different parts of the plants have been examined for a variety of purposes, there is still need for a few *in vitro* investigations of these plants from different locations. Furthermore, there aren't many investigations that have been done on the bioactivity of *B. monosperma* flower extract. This study was aimed to fulfill this gap by investigating the cytotoxic and antidiabetic properties using *in vitro* methods such as alpha-glucosidase inhibition, DPP-IV inhibition and glucose uptake assays of *C. siamea* and *B. monosperma*.

Materials and Methods

Plant sample collection and identification

Tested plant samples (Table 1) were collected from Ta-soe, Katae village with geographical location 21°35'41.9" N, 96°0707.7" E, Kyaukse Township, Mandalay Region, Myanmar. *C. siamea* Lam. (leave) (DBR.CC.059) was collected in October, 2020 and *B. monosperma* Roxb (flower) (DBR.CC.061) was collected in March, 2020. The plant samples were identified by authorized botanist from Botany Department, Mandalay University, Mandalay, Myanmar.

Table 1 — The collected test plant

Botanical name	Myanmar name	Family	Part of uses
Cassia siamea Lam.	Mae-zali	Caesalpiniaceae	Leaves
Butea monosperma Roxb.	Paukpwint	Fabaceae	Flower

Preparation of plant extracts

The plant samples were cleaned, air-dried, powdered, and stored in air-tight containers for further use. Each of air dried powdered samples was soaked in 95% ethanol for 1 month. The solvent were filtered and filtrates were concentrated by a rotary evaporator (IKA RV 10, Germany) to get the crude extracts. The concentrated plant extracts were dried and evaporated at room temperature, then freeze-dried and stored in the refrigerator for further experiment.

Hemolysis assay

Comparison of hemolytic activity between tested-extracts: *C. siamea*, *B. monosperma*, positive control: Triton X-100 and negative control: Phosphate buffer saline (PBS) was carried out by method of Muhammad Riaz, *et al.*[15]. The 'O' The blood sample was collected from healthy volunteer after taking the consent form to draw the blood sample and placed in heparinized tubes to avoid coagulation. Five milliliter of blood cells was added into a 15 mL sterile Falcon tube and centrifuged at 850 × g for 5 min. Then, the supernatant was gently removed, and the remaining RBCs were washed with 5 mL of chilled PBS for three times. After washing three time with PBS, RBCs were re-suspended by cool PBS, and then 2% erythrocyte suspension was prepared in sterile phosphate buffer saline used for further hemolytic assay. RBCs count were need to maintain at 7.068 ×108 cells mL−1 for each assay. Triton X-100 (0·1%) was used as a positive control (100% lysis) and PBS for the negative (0% lysis) control in each experiment. Briefly, 20 µL (1 mg/mL) of sample extracts were placed in 1.5 mL microtubes and gently mixed with 180 µL of blood suspension, and then incubated at 37°C for 30 minutes. After incubation, sample tubes were immediately agitation for 10 minutes and placed on ice for 5 minutes and centrifuged again for 5 minutes at 1310 g. Added 100 µL of supernatant to 900 µL cooled PBS and then kept on ice and each sample was placed in 96-well to measure at 576 nm (BMG Labtech, SPECTRO Star Nano). Hemolysis (%) was calculated as follows:

$$\%\ \text{Lysis} = \frac{\text{OD of sample}}{\text{OD of positive control}} \times 100$$

Alpha glucosidase inhibition assay

Ranilla *et al.*, method was used with a minor modification in the α-glucosidase inhibition experiment[16]. Comparison of α-glucosidase inhibition (%) activity and IC_{50} (µg/mL) value between tested-

extracts: *C. siamea, B. monosperma* and standard drug: Acarbose was carried out. Solutions of the substrate, enzymes, and reagents were diluted with 0.1 M phosphate buffer at pH 6.9. Briefly, 10 µL of test samples and acarbose (standard drug) at various concentrations were mixed with phosphate buffer (0.1 M, pH 6.9) and pre-measured at 405 nm. After that, added 20 µL of the α-glucosidase enzyme to the reaction mixture and incubated at 37°C for 15 minutes. And then, 20 µL of substrate [5 mM p-nitrophenyl-α-D-glucopyranoside] solution was mixed to initiate the reaction and placed at 37°C for another 15 minutes. After incubation, 80 µL of sodium carbonate solution (0.2 M) was added to the test solution to stop the reaction. After that, the absorbent was recorded at 405 nm as the final read. Inhibition per cent was calculated by the following equation:

$$\% \text{ Inhibition} = \left(1 - \frac{\text{OD of sample}}{\text{OD of control}}\right) \times 100$$

where, OD of control is the reaction mixture without plant extracts.

Dipeptidyl peptidase IV (DPP-IV) inhibition assay

Experiment was done by Konrad *et al.*, method with slight modification[17]. Comparison of DPP-IV inhibition (%) activity and IC_{50} (µg/mL) value between tested-extracts: *C. siamea*, *B. monosperma* and standard drug: Sitagliptin was carried out. All tested samples with different concentrations, reagents, and enzymes were prepared with 100 mM Tris buffer (pH 8.0). The reaction mixture consisting 25 µL of tested samples, 75 µL of Tris buffer (pH 8.0), and 25 µL of (1.59 mM Gly- Pro-p-nitroanilide). The reaction mixture was stored at 37°C for 20 minutes. After incubation, 50 µL of DPP-IV (0.01 units/mL) solution was added and incubated at 37°C for 60 minutes. 100 µL of 1M sodium acetate buffer (pH 4.0) was added to the reaction mixture to stop the reaction and measured at 385 nm with a microplate reader. Per cent inhibition was calculated by the following equation:

$$\% \text{ Inhibition} = \left(1 - \frac{\text{OD of sample}}{\text{OD of control}}\right) \times 100$$

where, OD of control is the reaction mixture without plant extracts.

Glucose uptake by yeast cells

Glucose uptake activity of test samples across yeast cells was carried out by the method of Pitchaipillai R and Ponniah T[18]. Briefly, commercial baker's yeast was mixed with distilled water and incubated overnight. The cells were centrifuged at 3000 × g for 5 minutes and repeated until the clear supernatant by the addition of distilled water to the pallet. The yeast suspension was prepared (10%) in distilled water for further use. Five concentrations of test extract (0.1 – 1 mg) were mixed with 1 mL of glucose solution (5–25 mM), then incubated at 37°C for 10 minutes. After that, 100 µL of yeast suspension was adding to the reaction mixture, vortexed and incubated again at 37°C for 60 minutes. After incubation, the test tubes were centrifuged at 2500 × g for 5 minutes. The supernatant was measured for glucose estimation at 540 nm. Metformin was used as the standard drug. The glucose utilization percentage was calculated by the following formula:

$$\text{Increase in glucose uptake (\%)} = \left(\frac{\text{OD of control} - \text{OD of sample}}{\text{OD of control}}\right) \times 100$$

Where, OD of control is the absorbance of the control reaction (containing all reagents except the test sample) and OD of sample is the absorbance of the test sample.

Statistical analysis

The experimental results were performed in triplicate and the data were expressed as the mean±SD. Values for each sample were subjected to one-way analysis of variance (ANOVA) followed by Dunnett's or Tukey's multiple comparison tests ($P \leq 0.05$) using GraphPad Prism ver. 7.00 (GraphPad Software, La Jolla, California, USA).

Results

Phytochemical examination of plant samples

Phytochemical determination of tested samples was confirmed by Trease and Evans[19]. In this study, tested samples consisted of active constituents especially glycosides, phenolic compound, alkaloid, flavonoids, and tannin in phytochemical screening (Table 2).

Table 2 — Phytochemical examination of test plant

Tested sample	Alkaloid	Glycoside	Phenolic	Flavonoid	Tannin	Carbohydrate	Saponin	Reducing suger	Cyanogenic
Cassia siamea	+	+	+	+	+	+	+	+	-
Butea monosperma	+	+	+	+	+	+	+	+	-

(+) present, (-) absent

Table 4 — α-glucosidase enzyme inhibition (%) and IC_{50} (µg/mL) value by test extracts and acarbose

Concentration (µg/mL)	*Cassia siamea* Lam.		*Butea monosperma* Roxb.		Acarbose	
	Inhibition (%)	IC_{50} (µg/mL)	Inhibition (%)	IC_{50} (µg/mL)	Inhibition (%)	IC_{50} (µg/mL)
500	83.37±2.55[b]	76.33±12.20[b]	93.88±2.40	77.28±2.02[b]	97.26±0.73	36.76±1.55
250	76.79±4.49[c]		70.22±2.14[b]		91.08±4.23	
125	66.65±7.01[c]		60.35±1.47[c]		84.99±4.76	
62.5	44.32±3.67[b]		45.33±1.43[b]		73.92±4.6	
31.25	17.50±6.98[c]		23.29±3.5[c]		42.65±2.46	
15.625	6.59±0.5[a]		13.94±0.2[b]		20.91±1.50	

Values are expressed as mean±standard deviation (n = 3). Different letter ([a-c]) indicated difference (P <0.05).

Table 5 — DPP-IV enzyme inhibition (%) and IC_{50} (µg/mL) value by test extracts and sitagliptin conc. (µg/mL)

Concentration (µg/mL)	*Cassia siamea* Lam.		*Butea monosperma* Roxb.		Sitagliptin	
	Inhibition (%)	IC_{50} (µg/mL)	Inhibition (%)	IC_{50} (µg/mL)	Inhibition (%)	IC_{50} (µg/mL)
500	77.31±8.03[c]	117.02±9.73[d]	84.56±3.88[c]	103±8.5[b]	89.89±1.42	144.85±13.43
250	70.94±6.64		67.69±1.63[c]		68.18±6.42	
125	52.32±3.07		60.77±8.15[c]		44.18±2.09	
62.5	30.53±1.08		25.49±5.83		24.24±1.96	
31.25	28.9±2.17		21.92±2.72		22.22±4.28	
15.625	9.09±10[a]		15.33±1.17[d]		19.19±1.42	

Values are expressed as mean±SD, (n = 3). Different letter ([a-c]) indicated difference (P <0.05)

Fig. 3 — Comparison of the glucose uptake effects of tested samples and metformin (standard drug) in glucose concentration at, a) 5 mM; b) 10 mM; c) 25 mM. Values are expressed as mean±standard deviation (n = 3). Different letter ([a-d]) indicated difference (P <0.05).

enzyme inhibitor[22]. This study indicated that both of the tested extracts showed potent inhibition activity on the DPP-IV enzyme. Table 5 demonstrated the inhibitory activity of *C. siamea*, *B. monosperma* and sitagliptin for the DPP-IV enzyme. In the investigation of inhibitory potency on DPP-IV enzyme, *C. siamea* and *B. monosperma* described good inhibition with their respective IC_{50} values of 117.025±9.75 and 103.01±8.5 µg/mL were significantly (P <0.05). However, Sitagliptin, the standard drug exhibited at 144.85±13.43 µg/mL. Therefore, the extracts of *C. siamea* and *B. monosperma* had more inhibitory effects than the sitagliptin, indicating that both of the tested extracts possess the DPP-IV inhibitory potential in a dose-dependent manner (Fig. 2b).

Glucose uptake/transport effect

The amount of glucose remaining in the medium after a specific time serves as an indicator of glucose uptake by the yeast cells[23]. In this study, the glucose uptake per cent in the yeast cells was indicated the amount of glucose transported across the yeast cells' membrane by the extracts of *C. siamea*, *B. monosperma*, and metformin. The increased glucose transport rate across the cell membrane in the yeast cells system is described in Fig. 3. From this data, the concentration of test samples and metformin ranges (0.1-1 mg/mL) is directly proportional to the per cent of glucose utilization, which means that the ability of glucose transport across yeast cells in a concentration-dependent manner. The highest concentration of *C. siamea* showed increased of glucose uptake ability

and the maximum per cent of glucose uptake were 75, 70.1, and 71.4% at 5, 10 and 25 mM, respectively. For the glucose uptake ability of *B. monosperma* expressed significantly (P <0.05) as 87.86, 84.2, and 83% at 5, 10, and 25 mM, respectively. In addition, the uptakes of glucose per cent in yeast cells by test samples showed increased glucose uptake capacity in all test glucose concentrations. On the other hand, metformin indicated the highest percentage of glucose utilization in per cent of 89, 86, and 86% in tested glucose concentrations, 5, 10, and 25 mM, respectively (Fig. 3). This study confirmed that the tested extract possess the ability to enhance glucose utilization across the yeast cells, especially in *B. monosperma* compared to the standard drug.

Discussion

The main constituents of plant extract responsible for antidiabetic action that has been reported are alkaloids, phenolic acids, flavonoids, glycosides, saponins, polysaccharides, stilbenes, and tannins. The single active compound or combining phytochemicals complex had antidiabetic property through different mechanisms described as; insulin secretion, glucose uptake by the cells, stimulating β cells, inhibition of gluconeogenic enzymes and protection of reactive oxygen species (ROS). It is revealed that some herbs could regenerate of ß-cells, maintain normal sugar levels in the blood, possess antioxidant potency and reduce the cholesterol level[24].

C. siamea (Fabaceae family) has several ethno biological and ethno medicinal activities like anti-hyperglycemic, antidiabetic and anti-lipemic effects[25]. Chromone alkaloids (barakol, cassiarin A-B), anthraquinones, bianthraquinones, flavonoids and phenolic are the main constituents in *C. siamea* and the most active compound is Cassiamin A. The methanol extracts for flowers and leaves of *C. siamea* had anti-hyperglycemic activity due to the presence of flavonoids such as quercetin, luteolin and D-pinitol[25]. The leaf extract 500 mg/kg of *C. siamea* significantly reduced the blood glucose levels and decreased lipid parameters such as total cholesterol and triglycerides in streptozotocin-induced diabetic rats[26]. It was reported for the fruit extract of *B. monosperma* has similar activity to metformin (standard anti-diabetes drug), indicating to increase the glycolysis and uptake of glucose in muscles and to reduce the gluconeogenesis in the liver[27]. The antihyperglycemic action of *B. monosperma* includes enhanced insulin secretion and hepatic glycogen formation in treated type 2 diabetic rats[28]. The flowers extract of *B. monosperma* reduced blood glucose and serum cholesterol, improved HDL-cholesterol and increased the antioxidant enzymes activities in a high-fat diet and streptozotocin-induced diabetes in rats. Similarly, flower extract of *B. monosperma* n-butanolic fraction significantly decreased the dexamethasone-induced hyperglycemia and hyperlipidemia in mice. Moreover, *B. monosperma* leaves extract elevated the blood insulin levels, stimulated insulin secretion in isolated rat islets, and enhanced hepatic glycogen formation in type 2 diabetic rats[29]. There are many reports for the antidiabetic actions of *C. siamea* and *B. monosperma* with different potential mechanisms. However, they are still needed to evaluated for their hemolytic effect, inhibition potency on α-glucosidase and DPP-IV enzymes and responsible for the glucose uptake ability from the indigenous extracts of *C. siamea* leave, and *B. monosperma* flower. This study intended to study the ability of plant extract to inhibit and reverse the actions of α-glucosidase and DPP-IV enzymes, which could develop α-glucosidase and DPP-IV inhibitors and their glucose uptake ability in the treatment of diabetes to overcome the burden of the undesirable effects of synthetic one.

In a previous study by the authors, ethanolic extracts of *C. siamea* and *B. monosperma* showed a significant inhibitory activity on advanced glycation end products (AGEs) formation due to the considerable antioxidant activity[30]. The most useful model of cytotoxicity is erythrocyte hemolytic study for the responsibility of a direct indication of toxicity and a general indication of membrane toxicity[31]. In this investigation, both extracts expressed a non-hemolytic activity on HRBCs with less than 15% range of cell lysis effects compared to completed cell lysis control, Triton x-100 (Table 3, Fig. 1). This result confirmed that tested-extracts could be safe to use as an antidiabetic therapeutic agent in diabetic mellitus.

The effective therapy for the disturbing of glucose absorption after a meal in T2DM is to develop the inhibition activity of α –glucosidase enzyme. Recently, α –glucosidase inhibitors from indigenous plants are still benefits for the treatment of diabetes due to their pharmacological potency. The inhibitory potential of the DPP-IV enzyme is the effective way for lowering blood glucose levels by enhancing glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) to secret the insulin. The high levels of AGEs promote the

expression of DPP-IV under diabetes conditions[32]. In this study, the inhibition activity of glucosidase of *C. siamea* and *B. monosperma* extracts expressed with IC_{50} values as 76.33±12.2 and 77.28±2.02 µg/mL, respectively, significantly (P <0.05) while the IC_{50} values of the acarbose showed the activity of 36.76±1.55 µg/mL (Table 4). Therefore, both test extracts exhibited a higher inhibitory potency on α-glucosidase Fig. 2a. A previous study by the authors indicated that *C. siamea* and *B. monosperma* had potent inhibition on AGE products, which could reduce the rate of DPP-IV enzyme action. The current investigation provided that both tested extracts possess the DPP-IV inhibitory potential in a dose-dependent manner. The 50% inhibitory activity of *C. siamea* and *B. monosperma* on DPP-IV enzyme at 117.02±9. 73 and 103±8.5 µg/mL, respectively, were significantly (P <0.05). On the other hand, the standard drug sitagliptin exhibited at 144.85±13.43 µg/mL (Table 5). According to this results, the extracts of *C. siamea* and *B. monosperma* had a higher potential inhibitory effect than the sitagliptin (Fig. 2b).

The effective stimulating efficacy on uptake of glucose through facilitated diffusion in yeast cell model system is need to be observed in management of diabetes mellitus[33]. In this study, the per cent increase in glucose uptake occurs in the tested samples concentration. This observation provided that the plant extract is capable of enhancing glucose uptake effectively especially in *B. monosperma*. Therefore, test extracts had glucose utilizing ability by promoting the transportation of glucose across the yeast cell membrane. Thus, the intracellular glucose level is reduced when glucose transportation occurs.

Conclusion

The present study demonstrated that the extracts of *C. siamea* and *B. monosperma* produce antidiabetic action through α-glucosidase and DPP-IV enzymes inhibitory activity with a non-hemolytic effect. In addition, both of tested extracts can attribute to enhancing the glucose uptake ability, which transports the glucose across the yeast cells' membrane. In conclusion, the hypoglycemic activities of tested extracts may possess the active constituents responsible for their antioxidant potency. Therefore, tested extracts provided the possible mechanism of glucose reducing action via inhibiting the α-glucosidase and DPP-IV enzymes and stimulating the glucose transport across in cells. Further studies are required to confirm for an effective diabetic therapeutic compound of these tested extracts by using cell lines and animal models.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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