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Evaluation of α-amylase and β-glucosidase inhibitory potential of *Hevea brasiliensis* and *Hevea spruceana* plants latex

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The present work aims to evaluate α-amylase and β-glucosidase inhibitory activity of two plants latex, extracted from *Hevea brasiliensis* and *Heva spruceana* based formulations. Results obtained showed that the extracted latex from *H. brasiliensis* and *H. spruceana* possess the highest α-amylase inhibitory activity with an IC_{50} value of 5.109 µg/ml and 6.427 µg/ml respectively. The β-glucosidase activity was also found to be higher in these samples with an IC_{50} value of 7.19 µg/ml and 7.537 µg/ml. Keeping in view the obtained results it is suggested that these plants can be considered as a multi-targeted antidiabetic drug and further work should be done on these plants for isolation and biological evaluation of the active compound.

Keywords: α-amylase, α-glucosidase, spectrophotometer, ELISA

INTRODUCTION

Diabetes mellitus, a cluster of disorders characterized by hyperglycemia. Notably, if chronically, it will much disturb the value of patients' lives. In 2011 the frequency of diabetic patients was up to 366 million and in 2030 will be risen to 552 million worldwide (Whiting et al. 2011). From 1991 to 2009, diabetes mellitus has also been increased from 2.3% to 7.7%, in Thailand (Deerochanawong, 2013). The most common diabetes is type 2 diabetes mellitus as about 90-95% and is generally caused due to insulin deficiency or by insulin resistance (Zhang et al. 2017). One therapeutic approach to treat diabetes is to retard the absorption of glucose via inhibition of enzymes, such as β-glucosidase, in the digestive organs (Holman et al. 1999; Kim et al. 2008). Particularly, after the meal (postprandial hyperglycemia) due to a carbohydrate diet, high blood glucose level or hyperglycemia is generally characterized clinical (Aryangat and Gerich, 2010; Campos, 2012). By disturbing carbohydrate digesting enzymes including α-glucosidase and α-amylase, the management of postprandial hyperglycemia can be attained (Ortiz-Andrade et al. 2007; Shang et al.2012). In the treatment of type 2 diabetes mellitus, α-amylase and β-glucosidase inhibitors are used. Miglitol, acarbose and voglibose are modern drugs, having β-glucosidase inhibitory activity (Hollander, 1992). The key enzyme which catalyzing the final step in the digestive process of carbohydrates is β-Glucosidase (Kumar et al. 2011). In animal tissues, in plants and microorganisms, an exo-type carbohydrase distributed widely, called β-Glucosidase (α-d-glucoside glucohydrolase)

(Kimura et al. 2004).Which catalyzes the liberation of α-glucose from the non-reducing end of the substrate. β-glucosidase is a membrane-bound enzyme that is present in the epithelium of the small intestine and works to make enable the absorption of glucose by the small intestine by catalyzing the hydrolytic cleavage of oligosaccharides into absorbable monosaccharides (Baig, 2002). The inhibition of β-glucosidase and α-amylase from medicinal plants have studied by many research groups, for example, the methanolic extracts of leaves of *Terminalia* species such as *T. microcarpa, T. kaerbachii, T. catapa, T. chebula, T. ballerica, T. arjuna*, and in vitro β-glucosidase activity was investigated (Anam et al. 2009). So, the present study was aimed to evaluate the α-amylase and β-glucosidase inhibitory activity of *H. Brasiliensis* and *H. Spruceana* extracted latex.

MATERIALS AND METHODS

Plant material and extraction of latex

Cool roots (250 g) of *H. Brasiliensis* and *H. Spruceana* were taken from the freezer and cut into rounded pieces 0.5 cm with a cutter. After 3 min of the first cut, instantly placed the pieces into 1 L flask that contained 500 mL of chilled extract buffer (0.1% Na_2SO_3, 0.2% NH_3, and 0.1% casein) and were mixed for about 30 min at room temperature. Then separated the homogenate and poured into a blank 2 L flask. The flask that contained root pieces was filled with 500 mL of fresh extraction buffer. Again, mixed the mixture for 30 min and transferred into a 2 L flask that already contained the homogenate. The process was repeated more than three times. Then the homogenate was centrifuged for 5 min in 30 mL centrifuge tubes at 18369g. By suction-drawing (using a pipet and rubber filler) separated the white layer of latex. The remaining mixture was again centrifuged and again a white layer of the latex was collected. The procedure was repeated 3 to 5 times until a good yield of latex was obtained. Latex quantification was done by coagulation with glacial acetic acid.

Quantification of latex

0.2 mL of glacial acetic acid was added to 4 mL of the latex, and the solution was centrifuge at 6140g for 5 min. The homogenate that is coagulated rubber was separated, and washed with deionized water. Then transferred using a paper and was allowed to kept overnight in an oven for drying at 45°C to get a constant weight (Buranov and Elmuradov, 2010).

α-amylase inhibitory activity

Firstly, plates were coated with 100 µL of purified PAb (polyclonal antibodies) or Mab (monoclonal antibodies) at 1 µg/well in 50 mM carbonate buffer of pH 9.6 for 16 hr at 20°C. All the following steps were done at 20°C. Washed the wells three times with PBST and non-specific binding sites were blocked with 1% BSA (bovine serum albumin) in PBS (phosphate-buffered saline) for 1 hr. Diluted the purified α-amylase in 1% BSA in PBST, and were added to the wells with an amount of 100 µL/well and then incubated for 1 hr. 100 µL of HRP-labeled PAb or Mab was added on all wells that were diluted in 1% BSA in PBST, and were incubated for 30 min. The process was done after washing three times with PBST. The ABTS substrate was added to all wells. After 20 min the reaction was stopped and absorbance was measured at 415 nm. Samples were analyzed three times. The control samples were prepared without the addition of α-amylase. The inhibition ratio of amylase activity was calculated by the following equation (Verity et al. 1999).

Inhibition ratio (%) = {(Ac – As)/Ac} × 100

Ac Absorbance of control

As Absorbance of sample

β-glucosidase inhibitory activity

β-Glucosidase inhibition assay was done by the method ELISA. For this purpose, a flask was taken and 49.5 µL phosphate buffer (pH 7.0) and 25 µL of 2 mM p-nitrophenyl α-D-glucopyranoside (pNPG) was added to the flask already contains 0.5 µL of sample and 5% dimethyl sulfoxide (DMSO) at several fractions (0.31-2.5 µg mL^{-1}). Then the reaction mixture was pre-incubated at 37°C for 5 min. After the addition of 25 µL β-glucosidase the reaction was started, and nonstop incubation was done for 30 min. By the addition of 1 mL of 0.01 M Na_2CO_3, the reaction was stopped. Calculating the release of p-nitrophenol at 400 nm determined the activity of β-glucosidase. The samples were analyzed three times. In the absence of sample increased the concentration of p-nitrophenyl α-D-glucopyranoside (substrate) at various fractions, by this β-glucosidase inhibition activity was determined. The control samples were prepared without the addition of the sample. The inhibition ratio of amylase activity was calculated by the following equation (Najib et al. 2019).

Inhibition ratio (%) = {(Ac – As)/Ac} × 100
Ac Absorbance of control
As Absorbance of sample

RESULTS AND DISCUSSION

In the present study, two amazon origin plants *H. brasiliensis* and *H. spruceana* latex were used. The extracted latex of both species was screened for their inhibitory effect on α-amylase and β-glucosidase enzymes.

α-Amylase inhibitory activity

The extracted latex of *H. brasiliensis* and *H. spruceana* were evaluated for their inhibitory effect on α-amylase enzyme by Serial Dilution method and both species showed significant α-amylase inhibitory effects (Table 1 and Figure 1) with 5.109 and 6.427 IC_{50} values respectively. Acarbose was used as a standard drug and showed an IC_{50} value of 8.01±0.009.

Table 1: α-amylase inhibitory potential of *H. brasiliensis* & *H. spruceana* latex

Concentration	*H. brasiliensis*			*H. spruceana*		
1000	0.512	0.509	0.506	0.954	0.958	0.956
500	0.499	0.496	0.494	0.892	0.887	0.882
250	0.486	0.483	0.479	0.878	0.874	0.865
125	0.475	0.472	0.468	0.863	0.862	0.858
62.5	0.455	0.452	0.449	0.786	0.783	0.781
25	0.391	0.389	0.372	0.589	0.585	0.583
12.5	0.298	0.296	0.287	0.539	0.534	0.532
6.25	0.278	0.273	0.268	0.498	0.495	0.491

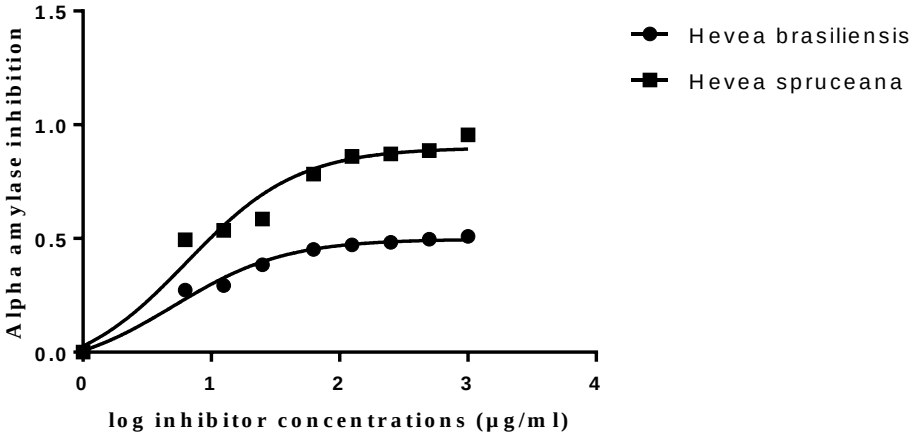

Figure 1: α-amylase inhibition of *H. brasiliensis* & *H. spruceana* latex

Table 2: α-Glucosidase inhibitory potential of *H. brasiliensis* & *H. spruceana* latex

Concentration	*H. brasiliensis*			*H. spruceana*		
1000	0.865	0.863	0.858	0.755	0.749	0.738
500	0.849	0.837	0.832	0.732	0.725	0.723
250	0.823	0.822	0.818	0.721	0.719	0.717
125	0.815	0.812	0.81	0.712	0.714	0.709
62.5	0.798	0.792	0.786	0.699	0.687	0.676
25	0.497	0.496	0.493	0.498	0.487	0.485
12.5	0.485	0.482	0.479	0.398	0.395	0.392
6.25	0.472	0.468	0.466	0.375	0.373	0.369

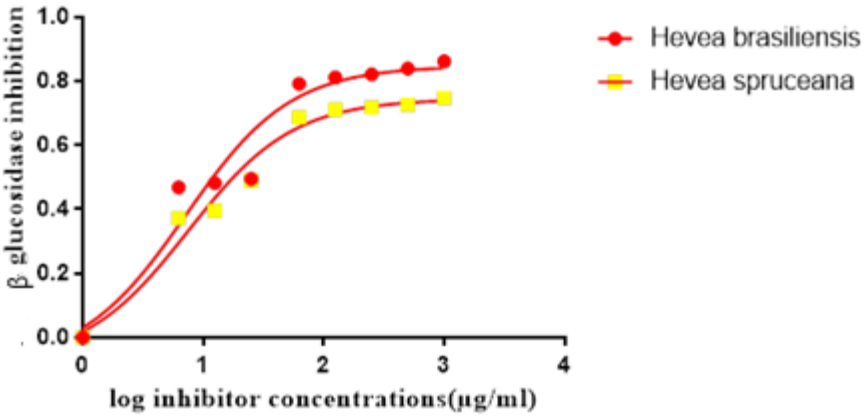

Figure 2: β-Glucosidase inhibition of *H. brasiliensis* & *H. spruceana* latex

β-Glucosidase inhibitory activity

β-Glucosidase inhibitory activity of both plants extracted latex was also evaluated. The absorbance and concentrations of both plants' latex are mentioned in (Table 2). Both species showed significant glucosidase inhibitory effects (Figure 2). The rapid increase of inhibitory activity is observed with a higher concentration of latex. *H. brasiliensis* and *H. spruceana* showed significant effects with 7.19 and 7.537 IC_{50} values respectively. Acarbose was used as a standard and showed IC_{50} value 8.01±0.009.

CONCLUSION

In conclusion, α-amylase and β-glucosidase inhibitory activity of the latex of the two plants *H. brasiliensis* and *H. spruceana* were evaluated. The biological investigation of the latex of selected plants showed significant activity. The results revealed that the extracted latex from *H. brasiliensis* and *H. spruceana* possess the highest α-amylase inhibitory activity with an IC_{50} value of 5.109 µg/ml and 6.427 µg/ml respectively, While the β-glucosidase activity was also found to be higher in these plants with an IC_{50} value of 7.19 µg/ml and 7.537 µg/ml. The results obtained exhibit that the extracted latex of these plant is very important from the medicinal point of view, and these latex needs further phytochemical exploitation to isolate phytochemical constituents having α-amylase and β-glucosidase inhibitory activity.

CONFLICT OF INTEREST

The authors declared that present study was Performed in the absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

All the author contributed equally

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