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IN VITRO ANTIDIABETIC ACTIVITY OF *PSIDIUM CATTLEIANUM* SABINE LEAVES EXTRACT

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ABSTRACT

The present investigation includes evaluation of an *in vitro* antidiabetic activity of *Psidium cattleianum* Sabine leaves extracts. Plant material was subjected to the extract preparation by soxhlet apparatus by using various solvents such as n-hexane, chloroform and 70% ethanol. The different phytochemical constituents present were detected. The antidiabetic activity of the plant were detected by using alpha amylase and alpha glucosidase enzyme in an *in vitro* model. The result suggests that the hydroalcoholic extract of *P. cattleianum* leaves exhibits a dose-dependent increase in percentage inhibitory activity on α-glucosidase enzymes (IC_{50} 143.49±10.14 µg/ml) and αamylase (IC_{50} of 120.2.3µg/ml). Acarbose was used as a standard drug. It is concluded that *P. cattleianum* leaves exhibits prominent antidiabetic activity.

KEYWORDS

Psidium cattleianum, α -amylase and α -glucosidase.

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INTRODUCTON

Diabetes mellitus (DM) is a prevelant metabolic disorder characterized by chronic hyperglycemia with disturbances in carbohydrate, fat and protein metabolism, glycosuria, hyperlipidemia, negative nitrogen balance and sometimes ketonaemia[1]. The currently available therapies which are mainly targeted towards reducing hyperglycemia, are not able to maintain the normoglycemic state in long run and they are more often associated with various side effects. Hence there is a need for an effective antidiabetic agent, which not only controls the hyperglycemia but also reduces diabetic complications[2]. The herbal medicines are widely

used for the treatment of disease because of their effectiveness, safety, affordability and acceptability.

Psidium cattleianum Sabine or strawberry guava is a shrub or small tree of Myrtaceae family. The plant is used as folk medicine in subtropical areas due to its pharmacological activities. The shoots, leaves and bark of the plant are extensively used in Brazilian traditional medicine to treat diseases such as diabetes, diarrhoea and also as a prophylactic hepatoprotective agent. The leaf extract of guava has been traditionally used for the treatment of diabetes in East Asia[3,4]. Several studies had already reported the chemical composition of *Psidium cattleianum* leaves and corroborated the presence of flavonoids, saponins, cardiac glycosides, phenolic compounds, anthraquinones, tannins and catechins in phytochemical screening. Many literatures claims that the antihyperglycemic potential of strawberry guava is mainly related to the phenolic compounds especially the catechins present in the plant. However, the antidiabetic activity of this plant have not been scientifically proved.

MATERIAL AND METHODS

Collection and authentication of plant material

The fresh leaves of *Psidium cattleianum* Sabine were collected in the month of December 2017 from Ambalavayal, Wayanad District, Kerala. The plant specimen (No: 148219) was authenticated by Dr. A. K. Pradeep, Assistant Professor and Head, Department of botany, University of Calicut, Kerala, India. The leaves were dried under shade and finally pulverised into coarse powder with the help of a mechanical grinder and then stored in a well closed container.

Extraction

The leaf powder was extracted by continuous hot percolation process (Successive solvent extraction) by using Soxhlet apparatus with different solvents of increasing order of polarity, started with a nonpolar solvent n-Hexane followed by mid-polar Chloroform and highly polar ethanol (70%)[5].

Phytochemical screening

The n-Hexane, Chloroform and 70% ethanol extracts of *Psidium cattleianum* Sabine were subjected to qualitative chemical test for the detection various plant constituents and which showed the presence of alkaloids, flavonoids, tannins, phenolic compounds, terpenoids, saponins, carbohydrates, proteins, amino acids, glycosides, vitamin C, starch, pectin and tryptophan.

In vitro antidiabetic studies

α – Amylase inhibition assay

1ml of 0.5% α - amylase was pre-incubated with 1ml of different concentrations of plant extract (25-150μg/ml) with 0.02M sodium phosphate buffer (pH 6.9) for 10 min at 37°C. 1ml of 05% starch was added as substrate to start the reaction and was incubated at 37°C for 5 min. The reaction was terminated by addition of 2ml of 3, 5-dinitrosalicilic acid (.5m g/ml) reagent. The reaction mixture was heated for 15 min in a boiling water bath and diluted with 10ml of distilled water in an ice bath. α - Amylase activity was determined by measuring the absorbance at 540nm by using UV-spectrophotometer. The experiments were repeated thrice. The percentage inhibition of α - amylase activity was calculated by using the formula;

% Inhibition = [(Abs of control - Abs of extract)/ Abs of control]× 100

The concentration of extract required to inhibit 50% of α – amylase activity under the assay conditions was calculated from the graph and defined as the IC_{50} value[6].

α – Glucosidase inhibition assay

The enzyme solution is prepared by dissolving 0.5mg α – glucosidase 10ml phosphate buffer (pH 7) containing 20mg BSA. It is diluted further to 1:10 with phosphate buffer. Different concentrations of sample (20-160μg/ml) are prepared and 5μl each of the solution or blank is then added to 250μl of 20mM p-nitrophenyl-α-D-glucopyranoside and 490μl of 1100mM phosphate buffer. It is then pre-incubated at 37°C for 5 min and the reaction started by addition of 250μl of enzyme solution and incubated at 37°C for 15 min. 250μl of phosphate buffer is added instead of enzyme for the blank. The reaction is then stopped by addition of 100μl of 200mM sodium carbonate solution and the amount of p-nitrophenol released is measured by reading the absorbance of sample against a blank at 400nm. The experiments were

repeated thrice. The percentage inhibition of α – glucosidase activity was calculated by using the formula;

% Inhibition = [(Abs of control – Abs of extract) / Abs of control] × 100

The concentration of extract required to inhibit 50% of α – glucosidase activity under the assay conditions was calculated from the graph and defined as the IC_{50} value[7].

Statistical Analysis

The data expressed as mean ± standard error mean (SEM). Different groups were assessed by One-way analysis of variance (ANOVA) for multiple comparisons followed by Dunnet's test (Graph Pad Prism 6 software, La Jolla CA. Trial version 5). The criterion for statistical significance set at $p < 0.05$.

RESULTS AND DISCUSSION

The preliminary phytochemical screening tests for various extarcts of *Psidium cattleianum* revealed the presence of alkaloids, flavonoids, tannins, phenolic compounds, terpenoids, saponins, carbohydrates, proteins, amino acids, glycosides, vitamin C, starch, pectin and tryptophan. The hydroalcohlic extract of *Psidium cattleianum* shows more biomarkers compared to the chloroform and hexane extracts. So the hydroalcoholic extract of *Psidium cattleianum* was selected for the *in vitro* antidiabetic potential.

α – Amylase inhibition assay

A dose-dependent increase in percentage inhibitory activity against α-amylase enzyme was observed. At a concentration 25μg/ml of extract showed a percentage inhibition of 17.38±0.24 and for 150μg/ml it was 51.78±0.24. The extract gave an IC_{50} value of 143.49μg/ml. The IC_{50} value of standard drug acarbose was found to be 122.0456μg/ml. The HAEPC extract showed a significant inhibition on α-amylase activity when compared to the control and standard drug acarbose. (Table No.1) (Figure No.1).

α – Glucosidase inhibition assay

The HAEPC revealed a significant inhibitory action on α-glucosidase enzyme. The percentage inhibition at 20-160μg/ml concentrations of extract showed a concentration dependent increase in percentage inhibition. The percentage inhibition varied from 3.92±0.14 to 63.23±0.22 for lowest concentration to the highest concentration. The concentration required for 50% inhibition (IC_{50}) was found to be 120.203μg/ml whereas α -glucosidase inhibitory activity of positive control acarbose produced percentage of 18.98±0.12 for 25μg/ml and 75.12±0.43 for 160μg/ml. The IC_{50} value of standard drug acarbose against α-glucosidase was found to be 91.208μg/ml The HAEPC showed a significant inhibition on α-glucosidase activity when compared to that of control and the standard drug acarbose. (Table No.2) (Figure No.2)

Diabetes Mellitus is a common metabolic disorder that occurs worldwide which is mainly due to the lack of insulin secretion or action. Alpha amylase and alpha glucosidase are two important the carbohydrate metabolizing enzymes that are mainly involved in the degradation of oligosaccharides and disaccharides into monosaccharides. The inhibition of these enzymes leads to increase in the carbohydrate digestion time and decrease in the glucose absorption. There are various drugs available such as acarbose, voglibose etc., to inhibit alpha amylase and alpha glucosidase enzymes but it causes various side effects such as diarrhoea, bloating etc[8].

The inhibitory effect of HAEPC on digestive enzymes α-amylase and α-glucosidase shows that it inhibits the formation of advanced glycation end products. The phenolic compounds like gallic acid and catechin and flavonoids from guava leaf may exhibit strong inhibitory activities against α-amylase and clear synergistic effect against α-glucosidase. It can also be possible with the presence of polysaccharides as the plant is reported with the presence of highly branched polysaccharides[9].

The values are mean ± SEM. The percentage inhibitions of HAEPC and acarbose were compared with control and the percentage inhibitions HAEPC were compared with that of standard by One-way ANOVA (Tukey test). The significant level was expressed as *.*** P<0.001.

The percentage inhibition of different concentrations of acarbose and HAEPC were

plotted against the concentration and the IC_{50} values were determined.

The values are mean ± SEM. The percentage inhibitions of HAEPC and acarbose were compared with control and the percentage inhibitions HAEPC were compared with that of standard by One-way ANOVA (Tukey test). The significant level was expressed as *.

*** P<0.001.

The percentage inhibitions of acarbose and HAEPC at different concentrations were plotted in a graph and the IC_{50} values were calculated from it.

Table No.1: α-Amylase inhibition of acarbose and HAEPC

S.No	Concentration (µg/ml)	Percentage inhibition (Mean ± SEM) (%)	
		Acarbose	HAEPC
1	25	26.92±0.24***	17.38±0.24***
2	50	30.44±0.34***	21.53±0.25***
3	75	37.70±0.25***	24.48±0.37***
4	100	44.65±0.27***	30.78±0.23***
5	125	50.74±0.34***	41.44±0.15***
6	150	60.34±0.43***	51.78±0.24***
IC_{50} value		122.04µg/ml	143.49µg/ml

Table No.2: α-Glucosidase inhibition of Acarbose and HAEPC

S.No	Concentration (µg/ml)	Percentage inhibition (Mean ± SEM) (%)	
		Acarbose	HAEPC
1	20	18.98±0.12***	3.92±0.14***
2	40	28.99±0.46***	10.19±0.18***
3	60	38.34±0.36***	16.41±0.19***
4	80	45.20±0.25***	23.17±0.16***
5	100	56.3±0.29***	38.22±0.28***
6	120	62.32±0.39***	49.05±0.48***
7	140	70.81±0.52***	58.62±0.27***
8	160	75.12±0.43***	63.23±0.22***
IC_{50} value		91.208µg/ml	120.203µg/ml

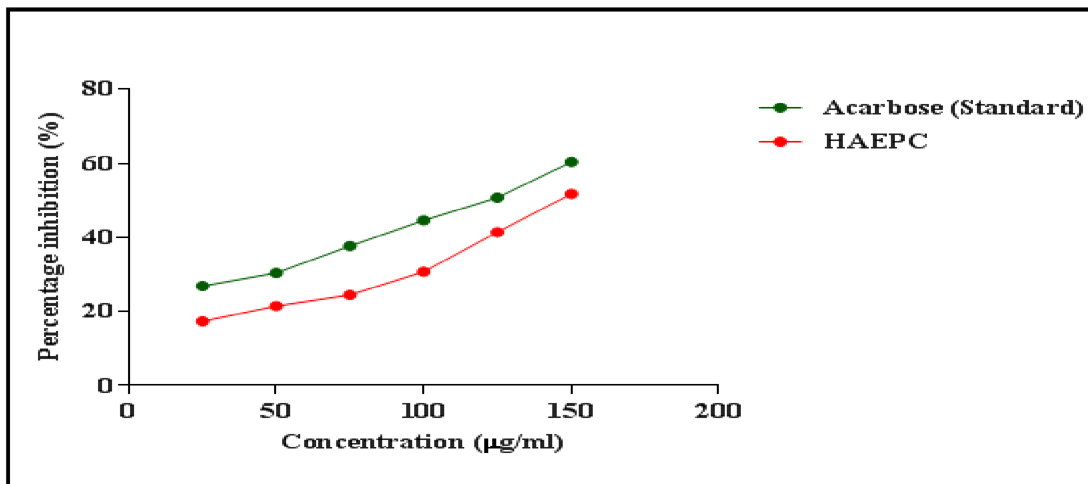

Figure No.1: α-Amylase inhibition of acarbose and HAEPC

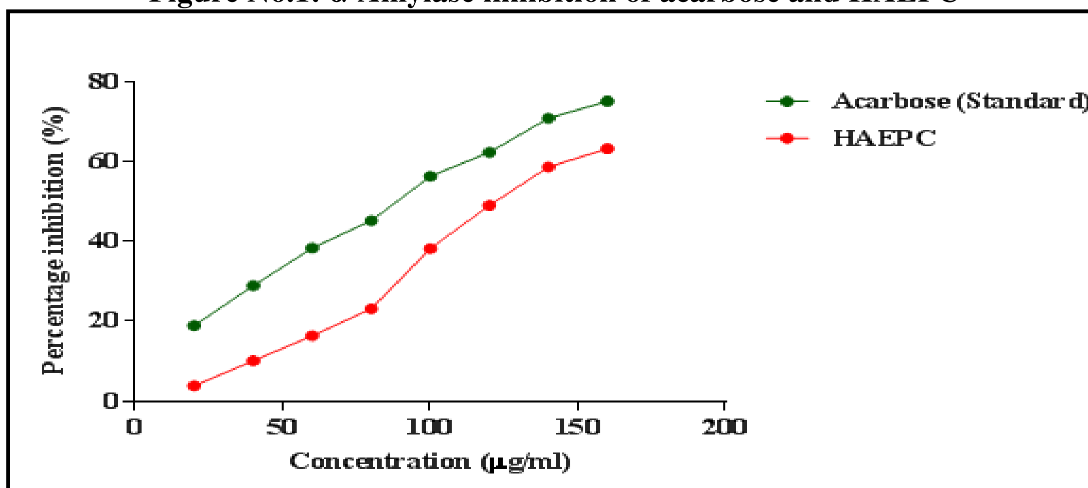

Figure No.2: α-Glucosidase inhibition of acarbose and HAEPC

CONCLUSION

In this present study, we evaluated *in vitro* alpha amylase and alpha glucosidase activity of crude hydroalcoholic extract of *Psidium cattleianum* sabine leaves. The plant showed significant inhibition activity which might be due the presence of phenolic compounds like gallic acid and catechin, flavonoids, polysaccharides etc.,

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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