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IN-VITRO ANTIDIABETIC INVESTIGATION OF *PSOPHOCARPUS TETRAGONALOBUS* HYDROALCOHOLIC POD EXTRACT

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ABSTRACT
Medicinally important herbal plants are one of the primary assets of remedial specialists. Undoubtedly, 80% of the total populace utilizes plants in medical care. There is an expanding interest in utilizing therapeutic plants and their phytoconstituents as normal sources due to their notable pharmacological effects in the body. *Psophocarpus tetragonalobus* of the Fabaceae commonly known as winged bean is well known for its high nutritional value but remains as an underutilized crop. The intention of this study was to explore the scientific basis of traditional usage of *Psophocarpus tetragonalobus* as an anti-diabetic agent in hyperglycemic patients. Hydroalcoholic extract from its dried pods were prepared and were analyzed both qualitatively and quantitatively for evaluation of phytochemical agents followed by anti-diabetic study in vitro.The results of phytochemical investigation indicated the presence of carbohydrates, proteins, phenols, flavonoids, glycosides, amino acids and fatty acids in crude extract. The prepared hydroalcoholic extract of the pods were analysed for its α-amylase and alpha glucosidase inhibition assay using the 3, 5- dinitrosalicylic acid method. The IC_{50} values of α amylase and α- glucosidase inhibitory activity of PTPH were 722.65±0.29 and 302.47 ±0.34 respectively and were closer to that of the standard drug Acarbose which exhibited the IC_{50} values of 548.74±0.38 and 231.22 ±0.93 respectively. The pod extracts of *P.tetragonalobus* exhibit notable α-amylase and glucosidase inhibitory activity in the crude hydroalcoholic extract and hence can be used as a regular green vegetable and also be investigated further in isolating pure compounds with anti-diabetic activity.

KEYWORDS: *Psophocarpus tetragonalobus,* anti-diabetic, hydroalcoholic extract, Amylase, Glucosidase

INTRODUCTION
Herbal medicine is the oldest and still the most widely used system of medicine in the world today. It is medicine made exclusively from plants. It is used in all societies and is common to all cultures. It is the art or practice of using herbs and herbal preparations to maintain health and to prevent, alleviate, or cure diseases. Herbal medicine is increasingly being validated by scientific investigation which seeks to understand the active chemistry of the plant. Many modern pharmaceuticals have been modeled on, or derived from chemicals found in plants.[1]

The therapeutic activity of a plant is due to its complex chemical nature associated with different parts of the plant. Indian populations mainly rely on herbal medicines due to their effectiveness, availability low cost, less toxic effects and also shortage of doctors in rural areas. The therapeutic activity or medicinal actions are due the presence of active constituents within the plants. These classes of constituents are mainly terpenoids (such as sesquiterpenes, saponins, iridoids, carotenoids and steroids), phenolics (such as tannins, quinones, salicylates and lignins), and their glycosides (such as flavonoids, glucosinolates and cyanogens), alkaloids, polysaccharides (such as gums and mucilages) peptides, essential oils and resins.[2]

PLANT PROFILE
Psophocarpus tetragonolobus
Family : leguminosae
Synonyms : winged bean, Goa bean, four-angled and dragon bean.
Kingdom : plantea
Order : fabales
Family : fabaceae, leguminosae
Genus : Psophocarpus

Plant Morphology

It is a herbaceous perennial climber with 3 - 5 m in height cultivated at an altitudes ranging from 0 - 2000 m and grows mainly in tropical environments. Fruits are in the form of elongated pods (15 – 30 cm long, 3 cm wide) square or rectangular in shape. Flowers: Pea-like flowers classified as papilionaceous are usually light blue, but occasionally white (2.5 - 3.5 cm wide).[3]

Figure1: *Psophocarpus tetragonalobus* pods and plant.

Ethnomedical uses

The entire winged bean plant is edible. The leaves, flowers, roots, and bean pods can be eaten raw or cooked. Each of these parts contains vitamin A and C, calcium and iron among other nutrients. The young leaves can be prepared similar to spinach. The nutrient-rich, tuberous roots have a nutty flavour. They are about 20% protein; winged bean roots have more protein than many other vegetables. The leaves and flowers are also high in protein (10–15%). The seeds are about 35% protein and 18% fat. They can be eaten dried or roasted. Dried and ground seeds make useful flour and can be brewed to make a coffee -like drink.[4, 5, 6]

MATERIALS AND METHODS

Collection and authentification of the plant

The plant parts were collected in the month of August from Kerala, Kannur district by adopting proper collection method. The collected material was shade dried, crushed and stored.

Hydro-Alcoholic (70%) extraction

The dried pods were subjected to exhaustive extraction with 70% v/v hydro alcohol using cold maceration method. On completion of the extraction process the solvent from the filtered extract was concentrated to get *P.tetragonalobus pod* hydro alcoholic extract (PTPH) and the yield obtained were tabulated.

Preliminary Phytochemical Screening

Plants are enriched with different variety of chemical compounds together with primary metabolites and secondary metabolites. Extracts were treated with various reagents for understanding of the chemical constituents of plants. These secondary metabolites hold many pharmacological activities and play a crucial role in curing many human diseases.[7,8, 9]

EVALUATION OF IN VITRO HYPOGLYCEMIC ACTIVITY OF PTPH

Inhibition of Alpha-Amylase enzyme activity by PT extracts

Chemicals

- α-glucosidase , α-amylase.
- 3, 5, di-nitro salicylic acid (DNS), Phosphate buffer, starch solution.
- Acarbose standard.

Preparation of standard solution

A stock solution of 10 mg/ml acarbose was made ready using distilled water. By using the stock many concentrations ranging from, 50, 100, 150, 200 and 250µg/ml of acarbose were organized.

Preparation of test samples: 10mg of the total extract is dissolved in distilled water and a stock solution of 100mg/ml was setup. From this variety of concentrations starting from 100, 150, 200, 250 and 300 µg/ml of the extracts were arranged using distilled water.

Procedure

A starch arrangement was set up in sodium phosphate buffer, sodium chloride and kept in a water bath previously kept for boiling. The α-amylase arrangement was set up by blending α-amylase in a similar buffer. An aggregate of 500 µl of test extracts and standard acarbose (100-1000µg/ml) were added to 500 µl of 0.20 mm phosphate buffer containing α-amylase (0.5mg/ml) arrangement and were made to incubate at 25°C for 10 min. After these, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube and incubated at 25°C for 10 min. The stopping of reaction was done with 1.0 ml of 3, 5 dinitrosalicylic acid (DNS) shading reagent. The test tubes were then kept in a water vessel for 5 min, cooled to room temperature. They were then diluted subsequently with 10 ml distilled water and absorbance was estimated at 540 nm. Blank determination was also conducted in same manner and percentage inhibitions and IC_{50} of both were compared to get the results using the equation.[10] (Tania Paul et.al, 2013).

% Inhibition = (Ac-As)/Ac X 100

Inhibition of alpha-glucosidase enzyme activity by PT extracts

Reagents required

- Sodium carbonate ($Na_2 CO_3$), Sodium dihydrogen phosphate, di-sodium hydrogen phosphate, p-nitrophenyl-α-D-glucopyranoside , α-glucosidase, Acarbose

Preparation of standard solution:

From the 10 mg/ml stock in distilled water, 2, 4, 6, 8 and 10 µg/ml of varied concentrations of acarbose were prepared.

Preparation of test extract samples: From the stock solution of 100mg/ml of the extracts in distilled water 100, 150, 200, 250 and 300 µg/ml of various concentrations of extract were created.

Theory

The inhibitory potential of α-glucosidase enzyme was performed by incubating phosphate buffer enzyme solution with phosphate buffer which contains test samples of different concentrates at 37°C for 1 hr in maltose solution. The mixture was allowed to be in the boiling water for few min and cooled. Upon addition of glucose reagent measurement of its absorbance at 540 nm gives the amount of liberated glucose from maltose by the action of α-glucosidase enzyme.[11](Javad Ahamed et.al, 2011)

Procedure

The inhibitory activity was determined by incubating a solution of starch substrate (2 % w/v maltose or sucrose) 1ml with 0.2 M Tris buffer pH 8.0 and various concentration of plant extract for 5 min at 37°C. The reaction was initiated by adding 1ml of α-glucosidase enzyme (1U/ml) to it followed by incubation for 10 min at 37°C. Then, the reaction mixture was heated for 2 min in boiling water bath to stop the reaction. The amount of liberated glucose is measured by glucose oxidase peroxidase method. Percentage inhibition (I %) was calculated by

% Inhibition = (Ac-As)/Ac X 100

RESULTS AND DISCUSSION

Collection and authentication of Plant material

The plant parts comprising of pods were collected in the month of August from Kerala, Kannur district by adopting proper collection method. The pods were properly sorted out and dried separately. Plant identification and authentification was done by Prof. Dr. Sreeja, Department of Biology, Sir Syed College, Kannur. The collected material was shade dried, crushed and stored.

Extraction of plant material

The plant material was subjected to cold maceration using hydroalcohol and the extract was collected for further analysis.

Phytochemical investigation

The phytochemical analysis of all the extracts were performed according to the procedures mentioned in reference books. The results showed the occurence of carbohydrates, proteins, phenols, flavonoids, glycosides, amino acids and fatty acids in crude extract.

1. Alpha amylase inhibitory action of PT extracts

The *in-vitro* α-amylase inhibitory studies demonstrated that PTPH had comparable α-amylase inhibitory activity. The IC_{50} of PTPH was found to be 722.76µg/ml which can be contrasted with the standard acarbose whose IC_{50} value was found to be 548.74µg/ml.

2. Alpha glucosidase inhibitory action of PT extracts

PTPH gave a satisfactory result with an IC_{50} value of 302.47±0.29µg/ml when put side by side with acarbose which is a potent enzyme inhibitor. At a concentration 100µg/ml of PTPH extract showed a percentage inhibition of 35.45±0.35 and for 1000 µg/ml it was 79.34±0.35. The table given below gives the data regarding the assay.

Table 1: Inhibition of alpha amylase activity by PTPH extracts.

Conc (µg/ml)	Hydro-alcoholic	Standard Acarbose
100	22.18±0.34	29.76±0.24
200	29.88±0.10	35.23±0.37
400	34.89±0.22	41.20±0.28
600	45.87±0.30	51.96±0.32
800	55.74±0.19	63.22±0.14
1000	59.54±0.25	69.97±0.33
IC_{50}	**722.65±0.29**	**548.74±0.38**

Table 2: Inhibition of alpha glucosidase activity by PTPH extract.

Conc (µg/ml)	Hydro-alcoholic	Standard Acarbose
100	35.45±0.35	39.73±1.35
200	42.93±0.25	45.43±1.04
400	61.45±1.05	65.48±0.91
600	73.32±0.21	78.84±1.21
800	75.04±0.33	83.58±0.91
1000	79.34±0.35	91.67±0.91
IC_{50}	**302.47 ±0.34**	**231.22±0.93**

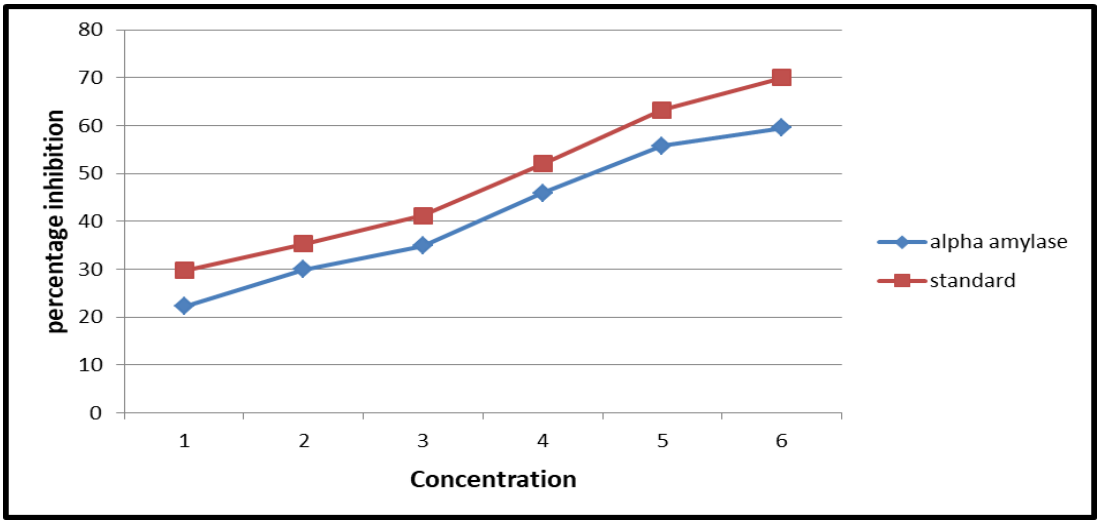

Figure 2: Graph showing the inhibition of PTPH extract on Alpha amylase enzyme.

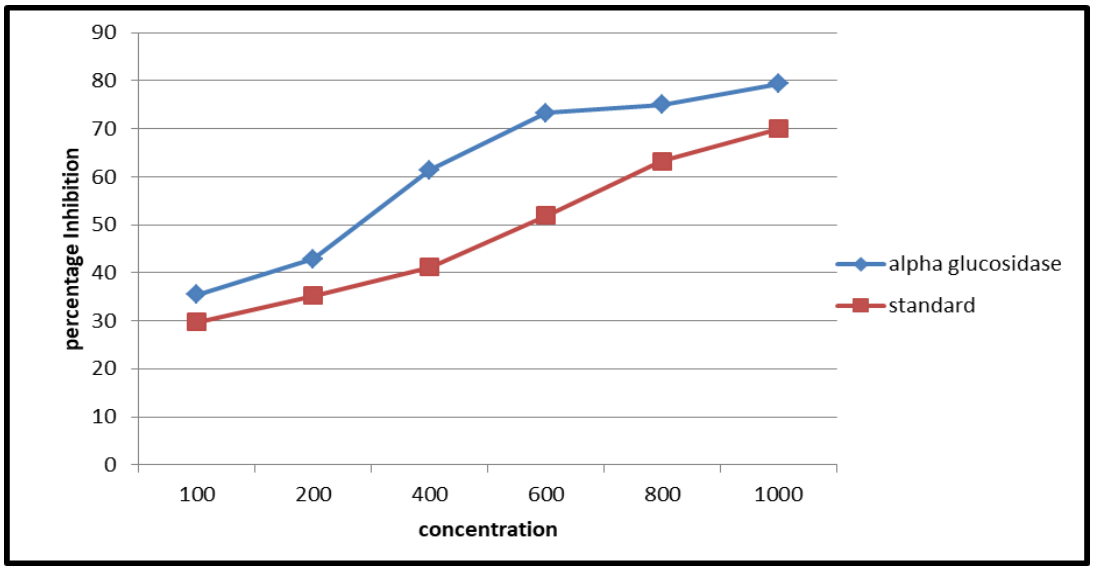

FIGURE 3: Graph showing the inhibition of PTPH extract on Alpha glucosidase enzyme.

DISCUSSION

Plants had been utilized for restorative purposes some time before written history. Antiquated Chinese and Egyptian papyrus compositions portray therapeutic uses for plants. Indigenous societies (such as African and Native American) utilized spices in their mending ceremonies, while others created conventional clinical frameworks, (for example, Ayurveda and Traditional Chinese Medicine) in which natural treatments were utilized. Separation and portrayal of pharmacologically dynamic mixes from restorative plants proceeds with today. All the more as of late, drug disclosure strategies have been applied to the normalization of natural prescriptions dependent on the marker idea. In late years, the connecting of the customary information on restorative plants to current exploration exercises gives another methodology and makes the pace of disclosure of medications more powerful when contrasted with irregular assortments.[12]

In the present study, *Psophocarpus tetragonalobus* were selected based on their ethnomedicinal and nutritional values and after authentication the plant was studied for its phytochemical and anti-diabetic activity.

IN *VITRO* HYPOGLYCAEMIC ACTIVITY

In-Vitro Inhibition of α–Glucosidase and α-Amylase

α-glucosidase and amylase are digestive enzymes situated in the brush border surface film of intestinal cells and is a vital chemical of sugar metabolism.[13] α-glucosidase inhibitory movement block the activities of α-glucosidase protein in the digestive system which is the rate restricting advance in the change of Oligosaccharide and disaccharide to monosaccharide, vital for gastro intestinal assimilation which would thus cause a decline in the retention of glucose and thusly the decrease of postprandial blood glucose level elevation.[14] The Glucosidase inhibitor acarbose which forestalls the rise in post prandial blood glucose level is a first line drug in treatment of type 2 diabetes which cannot be controlled through eating regimen alone. Consequently

one of the remedial approaches for diminishing postprandial (PP) blood glucose levels in quiet with diabetes mellitus is to forestall assimilation of sugar after food admission. Hindrance of these proteins (α-amylase and α-glucosidases) diminished the high postprandial (PP) blood glucose tops in diabetes.[15] In the current work it is observed that the PT extract has inhibitory activity on these enzymes .It also showed that the alpha glucosidase has been inhibited more when compared to alpha amylase.

Natural polyphenols have been reported to inhibit the activity of carbohydrate hydrolyzing enzymes.[16] Phytochemical investigation carried out identified the occurrence of many active metabolites including phenolic compounds and vitamins. The results suggest that hydroalcohol extract of PT efficiently inhibits α-glucosidase enzymes *in vitro* and thus reduce the rate of digestion and absorption of carbohydrates. Thus the anti-diabetic action of *PT* can also be attributed to the intestinal α-glucosidases inhibitory activity.

CONCLUSION

The aqueous alcoholic fraction of the *PT* pods had the superior antidiabetic activity focusing on the inhibitory effects on *α*-Glucosidase and *α*-amylase. Our study is the initial work that reports a potential mode of action of *PT* and suggests that the effect of this plant is due to the inhibition of digestive enzymes. On the other hand, the presence of active constituents like flavonoids and phenols along with vitamins, glycosides, fatty acids concludes that this herb has multiple biological properties. Further detailed studies using diabetic animal models are been made to proceed and steps are taken to isolate the active ingredients of this plant, identify them, and study their bioactivity.

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