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IN-VITRO EVALUATION OF ANTIDIABETIC ACTIVITY ON METHANOL AND PETROLEUM ETHER EXTRACT OF *IPOMOEA SEPIARIA* LEAVES

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ABSTRACT

Preparation of plant extract was done, the collected fresh leaves were thoroughly cleaned with distilled water, dried well and powdered. It was soaked in absolute ethanol in cold (72 hrs). After three days, the extract was filtered, and then it was evaporated at 40^0C in cylindrical water bath for the elimination of solvent. A semisolid extract (40g) was obtained after complete elimination of alcohol under reduced pressure. It was stored in refrigerator until used. Evaluate the methanol and petroleum ether extract of *Ipomoea sepiaria* leaves from Alpha-amylase activity through *in-vitro* method by hydrolysis of starch in presence of α-amylase enzyme. This process was quantified by using iodine, which gives blue colour with starch. Acarbose showed the best when compared to extracts, among all concentration of extracts, the methanol extract of 500mg/ml showed the superior anti-diabetic activity when compared to rest of the concentrations of methanol extracts and petroleum ether extracts. **RESULT**: Acarbose showed the best anti-diabetic activity when compared to all concentration of extracts, followed by the methanolic extract of 500mg/ml showed less significant anti-diabetic activity. **CONCLUSION**: From the *Ipomoea sepiaria* leaves extract finally find out the antidiabetic activity at the dose of 500mg/ml when compared to Acarbose.

KEYWORDS: Diabetes mellitus (DM), anti-diabetic, Acarbose, alpha-amylase inhibition method.

1. INTRODUCTION

Diabetes mellitus.[1] (DM) prevalence is increasing with ageing of the population and life style changes associated with rapid urbanization, the genus *Ipomoea*.[2,3,4] since time immemorial has been in continuous use for medicinal uses. *Ipomoea* species is to treat constipation diabetes, aphrodisiac, astringent, Immunodeficiency Syndrome (AIDS) and hypertension. From the review of literature identified that folk-lore anti-diabetic activity.[5,6] was examined. So we have made an attempt for *in-vitro* anti-diabetic activity. Methanol and petroleum ether extract of *Ipomoea sepiaria* was studied for the *in-vitro* anti-diabetic activity by the alpha-amylase inhibition method.

2. MATERIALS AND METHODS

2.1. Plant materials

Fresh leaves of *Ipomoea sepiaria* were collected and botanically identified. The leaves were washed with distilled water, shade dried, powdered, and stored in an air tight container until future use. *Preparation of methanolic extract*- Preparation of plant extract.[7] was done the collected fresh leaves were thoroughly cleaned with distilled water, dried well and powdered. It was soaked in absolute methanol in cold (72 hrs). After three days, the extract was filtered, and then it was evaporated at 40^0C in cylindrical water bath for the elimination of solvent. A semisolid extract (40g) was obtained after complete elimination of alcohol under reduced pressure. It was stored in refrigerator until used. *Preparation of Petroleum Ether Extract*- This extract.[7] was prepared by using Soxhlet apparatus. About 150gm of dried leaves powder was taken in a muslin cloth bag. The purified pet.ether was passed through the tube where the powder bag was kept. The petroleum ether was passed through siphon tube to reach the round bottom flask in which porcelain chips were provided. The vapor's containing the constituents pass through the condenser and reach the tube containing powder bag and the process was repeated. This was continued for 24hrs. Then the round bottom flask containing extract was transferred to a beaker and was allowed to evaporate in a water bath. This concentrated petroleum ether extract was used for further studies.

2.2. Alpha-amylase inhibition assay

Importance Alpha-amylase enzyme in the body in humans, the digestion of starch involves several stages. Initially, partial digestion by the salivary amylase results in the degradation of polymeric substrates into shorter oligomers. Later on in the gut these are further hydrolyzed by pancreatic α-amylases into maltose, maltotriose and small malto-oligosaccharides. The

digestive enzyme (α-amylase) is responsible for hydrolysing dietary starch (maltose), which breaks down into glucose prior to absorption. Inhibition of α-amylase can lead to reduction in post prandial hyperglycaemia in diabetic condition.

2.3. MATERIALS AND METHODS

The selected methanol and petroleum ether extract of *Ipomoea sepiaria* were serially diluted to get a required concentration to perform alpha amylase assay.

2.4. Alpha- amylase inhibition assay.[8, 9] principle and procedure.

Alpha-amylase activity was measured in-vitro by hydrolysis of starch in presence of α-amylase enzyme. This process was quantified by using iodine, which gives blue colour with starch. The reduced intensity of blue colour indicates the enzyme-induced hydrolysis of starch in to monosaccharides. If the substance/extract possesses α-amylase inhibitory activity, the intensity of blue colour will be more. In other words, the intensity of blue colour in test sample is directly proportional to α-amylase inhibitory activity.

Enzyme: (Type VI B: From porcine pancreas, 5, 00,000 U),[otto-A2366] [15.8 U/mg solid at pH 6.9] - Stored at 2-8^{0}C.

Substrate: Starch 1%.

Positive Control: Acarbose- Stored at RT-Glucobay (Bayer pharma, India).

Sodium dihydrogen orthophosphate (NaHat RT

Disodium hydrogen phosphate (NaIndicator: Iodine solution 1% Instrument- UV- Visible Spectrometer.

Preparation of working solution

Phosphate Buffer (40 mM, pH 7, 25^{0}C):

Solution A: 6.24 g of NaH_2PO_4 -1L

Solution B: 7.12 g of Na_2HPO_4 -1L

Enzyme (0.5128 U/ml)- 3.246 mg α-amylase ----- 100 ml of 40 mM Phosphate Buffer

NaCl solution (0.006 M)

Positive control

Stock- 50 mg of Acarbose in 50 ml of 40 mM Phosphate buffer

Working stock: Take 25 µl of stock, made upto 10 ml (2.5 µg/ml) with 40 mM Phosphate buffer

Procedure

Alpha-amylase activity was carried out by starch-iodine method. 10 µL of a-amylase solution (0.025 mg/mL) was mixed with 390 µL of phosphate buffer (0.02 M containing 0.006 M NaCl, pH 7.0) containing different concentration of extracts. After incubation at 37 °C for 10 min, 100 µL of starch solution (1%) was added, and the mixture was re-incubated for 1 h. Next, 0.1 mL of 1% iodine solution was added, and after adding 5 mL distilled water, the absorbance was taken at 565 nm. Sample, substrate and a-amylase blank determinations were carried out under the same reaction conditions. Inhibition of enzyme activity was calculated as (%) = (A-C) X100/ (B-C), where, A= absorbance of the sample, B= absorbance of blank (without a-amylase), and C= absorbance of control (without starch).

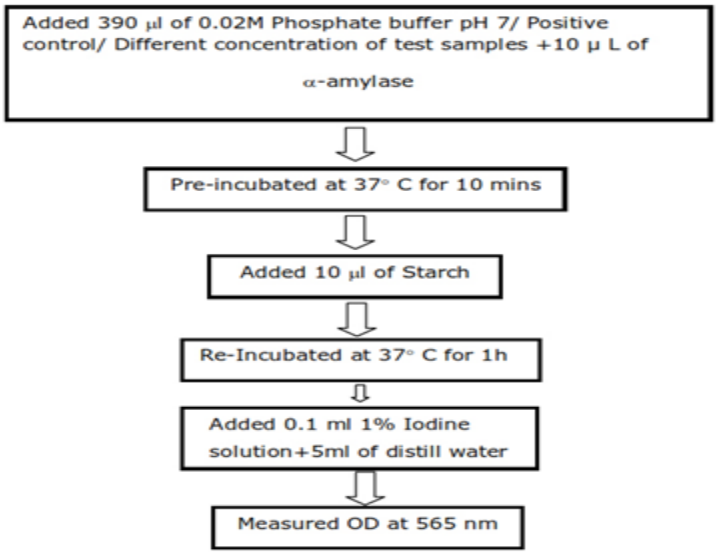

Fig.1. Schematic flow chart of α-amylase enzyme inhibition[10] assay procedure

2.5. Alpha- amylase inhibition assay

The below table shows inhibition of α-amylase activity for the methanol and pet.ether extracts and are compared with standard Acarbose. All the tested extracts showed dose dependent inhibition of enzyme. The extracts exhibited IC_{50}µg/mL will be considered active in comparison with other tested extracts showed enzyme inhibition activity with IC_{50} 135.33,123.83,90.14,61.22,28.43&147.52,139.64,120.45,101.93,47.26 and standard shows 0.59 µg/mL respectively.

Table-1: Comparison of compounds with Acarbose IC_{50} in µg/ml against α-amaylase activity

SAMPLE	CONCENTRACTION	IC_{50}(µg/ml)
Methanol	100(mg/ml)	135.33±1.8
Methanol	200(mg/ml)	123.83±1.2
Methanol	300(mg/ml)	90.00±0.6
Methanol	400(mg/ml)	61.22±1.4
Methanol	500(mg/ml)	28.43±0.8
Petroleum ether	100(mg/ml)	147.52±1.2
Petroleum ether	200(mg/ml)	139.64±1.5
Petroleum ether	300(mg/ml)	120.45±1.2
Petroleum ether	400(mg/ml)	101.93±0.8
Petroleum ether	500(mg/ml)	47.26±1.2
Acarbose	2.5(µg/ml)	0.59±0.12

All values reported as Mean ± S.E.M (n=3)

3. RESULTS AND DISCUSSION

Preparation of plant extract was done, the collected fresh leaves were thoroughly cleaned with distilled water, dried well and powdered. It was soaked in absolute ethanol in cold (72 hrs). After three days, the extract was filtered, and then it was evaporated at 40^0C in cylindrical water bath for the elimination of solvent.

A semisolid extract (40g) was obtained after complete elimination of alcohol under reduced pressure. It was stored in refrigerator until used. The methanol and petroleum ether extract of *Ipomoea sepiaria* leaves showed the better antidiabetic activity and it is also confirmed from the ethnobotanical and folklore data so further investigation to be carried out in order to identify a novel chemical moiety for better anti-diabetic activity.

Alpha-amylase activity was measured in-vitro by hydrolysis of starch in presence of α-amylase enzyme. This process was quantified by using iodine, which gives blue colour with starch. The reduced intensity of blue colour indicates the enzyme-induced hydrolysis of starch in to monosaccharides. If the substance/extract possesses α-amylase inhibitory activity, the intensity of blue colour will be more. In other words, the intensity of blue colour in test sample is directly proportional to α-amylase inhibitory activity.

Acarbose shows the best when compared to extracts, among all concentration of extracts, the methanol extract of 500mg/ml showed the superior anti-diabetic activity when compared to rest of the concentrations of methanol extracts and pet.ether extracts.

4. CONCLUSION

Acarbose showed the best when compared to extracts, among all concentration of extracts, the methanolic extract of 500mg/ml showed the superior anti-diabetic activity when compared to rest of the concentrations of methanol and petroleum ether extracts.

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