Journal of Pharmaceutical and Biological Research

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Research Article

Evaluation of *In-Vitro* Anti-Diabetic Activity of *Pulsatilla Nuttalliana* (mill.) Leaves extract

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ABSTRACT

The present study is to evaluate the anti-diabetic activity of *Pulsatilla nuttalliana* leaves extract Diabetes, often referred to by doctors as diabetes mellitus, describes a group of metabolic diseases in which the person has high blood glucose (blood sugar), either because insulin production is inadequate, or because the body's cells do not respond properly to insulin, or both. In recent years there has been a tremendous increase in demand for herbal drugs due to its safety, efficacy and better therapeutic results Due to its safety, efficacy and better therapeutic results. Due to its economic pricing as compared to synthetic or allopathic drugs which have several therapeutic complication. As we know that everything in this world change time by time since thousands of year the ear was of ayurveda or herbal origin drug. But last few decades it was replaced by allopathic system of medicine, which was rapidly accepted work wide but latter due to its lots of adverse effect and safety profile and the people are more believing in natural origin drug. The leaf extraction has been performed by sequential extraction method using the solvent with increasing polarity order (petroleum ether, ethyl acetate and ethanol) and the active extract was tested by *in-vitro* antidiabetic screening method. The *in-vitro* antidiabetic studies have been performed based on the α-amylase inhibition assay. Each extracts were tested for α-amylase inhibition and the extracts with minimum IC50 have been undergone phytochemical screening. The preliminary phytochemical tests was performed to identify the active phytochemicals present in the ethanolic extract of *Pulsatilla nuttalliana* and showed the presence of Phenols, Flavanoids, Alkaloids, Glycosides, Saponins and Terpenoids. The present study suggested that the isolation of active constituents from ethanolic extract of *Pulsatilla nuttalliana* leaf and characterize the compounds by using preliminary phytochemical studies.

Keywords: *Pulsatilla nuttalliana*, *in-vitro* antidiabetic, Flavanoids, Alkaloids, Glycosides.

ARTICLE INFO

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Journal QR-Code

ARTICLE HISTORY: Received 15 June 2023, **Accepted** 31 July 2023, **Available Online** 12 Sept 2023

Citation: *Nalamala Kavya, et al. Evaluation of In-Vitro Anti-Diabetic Activity of Pulsatilla Nuttalliana (mill.) Leaves extract. J. Pharm. Bio. Res., 2023, 11(1): 15-19.*

CONTENTS

1. Introduction

Diabetes mellitus is the collective name of metabolic abnormalities, primarily caused by the defect in secretion of insulin hormone by the pancreatic islets. It is chiefly manifested in the form of elevated levels of blood glucose (hyperglycemia). The reduced action of insulin on target tissues leads to a group of abnormalities, affecting the biochemistry and physiology of carbohydrate, fat, and protein [1, 2]. According to the reports of International Diabetes Foundation 2014, the estimated global prevalence of diabetes among adults is 8.3% (387 million), which will reach an estimated value of 53% (592 million) by the year 2035. Diabetes mellitus stands 5th among the diseases that can lead to death around the world. Approximately, 4.9 million deaths were recorded in 2014, and 8 per 20 died persons were of old age (≥79 years of age). In the technologically advanced countries, huge amount of sum is spent on the prevention and treatment of diabetes as well as on the discovery of new synthetic or natural drugs. In 2014, the global health management expenditures on diabetes reached USD 612 billion, which represented 11% of total worldwide healthcare expenditures [3]. Certainly, a large number of synthetic drugs have been discovered in the past, but these drugs were found to have side effects. Therefore, researchers were focusing to develop new drugs from natural sources that are safe without having any side effects. One of the recent developments in the field of natural products is the exploration of a potent plant species, such as Pulsatilla nuttalliana.

As mentioned earlier, diabetes mellitus has a close association with other metabolic abnormalities; one of the core abnormalities is oxidative stress. Biochemical studies have revealed an increased generation of Reactive Oxygen Species (ROS) in the cells and tissues of diabetic patients [3]. To tackle the ROS, the presence of potent antioxidant in the body of a patient is necessary because an antioxidant has the capacity of retarding or completely inhibiting the oxidation of other substances. In this regard, DPPH radical scavenging assay is one of the popular antioxidant assays, originally introduced by Marsden Blois of Stanford University in 1958. Several workers have used this method to investigate the antioxidant potential of synthetic drugs and natural products. Brand-Williams and his colleagues have introduced a modified version of Blois method in 1995, which is used as a reference by various group of researchers in recent days [4]. Similarly, indicators of the possible antidiabetic potential of a drug can be accessed through several in vitro assays, providing clues for its in vivo antidiabetic potential. Beside the antioxidant assays [5], several other indicator assays include (i) potential of glucose uptake across cell membrane such as that of yeast cells [6], adipose cells [7], or muscle cells [8]; (ii) capability of glucose adsorption [6]; (iii) inhibition of alpha amylase and alpha glucosidase enzymes [9].

2. Methodology

Plant material:

The leaves of Pulsatilla nuttalliana were collected from Chandragiri forest area, Tirupati and was authenticated by Dr. K. Madhava Chetty, Asst. Professor, Department of Botany, S V University, Tirupati.

Preparation of coarse powder and Extraction technique

The leaves were shade dried at room temperature for 10 days. Then these were milled into powder by mechanical grinder. This powder was sequentially extracted to their increasing polarity with Petroleum ether, Ethyl acetate, Ethanol respectively. About 500gm of powdered leaves was uniformly packed into a thimble in a soxhlet apparatus and extracted with 1000ml Petroleum ether, Ethyl acetate and Ethanol, respectively. Constant heat was provided by Mantox heater for recycling of the solvent. The process of extraction continues for 1-2 hours for each solvent. The excess solvent was evaporated and the dried extracts were kept in refrigerator at 4ºC for their future use in phytochemical analysis and pharmacological screenings.

In-vitro Antidiabetic Activity of Pulsatilla nuttalliana Leaves Extracts

Alpha-Amylase Inhibition Assay

Chemicals or Reagents

Potato starch, trichloroacetic acid, Folin-Ciocalteau reagents were purchased from SD Fine Pvt. Ltd., Mumbai, 3,5-dinitrosalicylic acid, Tris buffer, linoleic acid, ammonium molybdate, were purchased from Hi-Media Pvt. Ltd., Mumbai, α- amylase, α-glucosidase enzymes, xanthine oxidase, quercetin, hypoxanthine, pyrocatechol were purchased from SRL Pvt. Ltd., Mumbai. Glucose assay kit from Agappe diagnostic Pvt. Ltd., Kerala, Acarbose was obtained from Bicon Pvt. Ltd., Chennai, ferrozine, (2'2-azobis (2-amidino propane) dihydrochloride), butylated hydroxy toluene from Loba Cheme. All other chemicals used in the study were obtained commercially and were of analytical grade.

Instrument used

UV-visible Spectrometer (Systronic double beam- UV-2201).

Preparation of extract

Leaves extraction used in invitro studies were prepared by using suitable solvents (Carboxy methyl cellulose).

Experimental procedure for α-amylase inhibition assay

The alpha-amylase assay was performed according to the method described by Odeyemi [10]. Briefly, 15 µl of the plant extract at different concentrations (50 µg/ml – 200 µg/ml) (diluted in a phosphate buffer) was added to 5 µl of enzyme porcine pancreatic solution into 96-well plate. After 10 min of incubation at 37°C, the reaction was initiated by adding 20 µl of starch solution and further incubated for 30 min at 37°C. The reaction was then stopped by adding 10 µl 1M of HCl to each well followed by 75 µl of iodine reagent. A blank containing phosphate buffer (pH 6.9) instead of the extract and a positive control (acarbose, 64µg/ml) were prepared. No enzyme control

and no starch control were included for each test sample. The absorbance was measured at 580 nm and the percentage inhibitory activity was calculated by using the following equation:

Inhibition (*%*) = [Ac−(As−Asb)/Ac]×100

Where,

Ac=Absorbance of the negative control (uninhibited reaction), As=Absorbance of the sample (inhibited reaction), and Asb=Absorbance of the sample blank (enzyme omitted).

3. Results and Discussion

Table No. 1: Results of ethanolic extract of *Pulsatilla nuttalliana*

Class of compounds	Tests performed	Results
Carbohydrates	*Molisch's test*	-
	Fehling's test	-
Phenols	*Phosphomolybdic acid test*	+++
Flavonoids	*Shinoda test*	++
	Lead acetate test	++
Tannins	*Braemer's test*	_
Alkaloids	*Wagner's*	+
	Mayer's	+
	Draggendrof's test	+
Glycosides	*Legal's test*	+
	Brontranger's test	+
Saponins	*Foam test*	+
Sterols	*Salkowski's test*	_
Amino acids	*Ninhydrin test*	_
Terpenoids	*Lieberman Burchardt test*	+

+ In traces, ++ Present in moderate amount, +++ More amounts is present, -Absence

Table No: 2, α-Amylase Inhibition of Petroleum Ether extract of *Pulsatilla nuttalliana* leaves

Concentration(µg/ml)	IC_{50} %
0	0
25	29
50	33
75	42
100	53
125	56

Table No.3: α-Amylase Inhibition of Ethyl acetate Extract of *Pulsatilla nuttalliana* leaves

Concentration(µg/ml)	IC_{50} %
0	0
25	33
50	42
75	49
100	57
125	61

Table No.4: α-Amylase Inhibition of Ethanolic Extract of Pulsatilla nuttalliana leaves

Concentration(µg/ml)	IC_{50} %
0	0
25	30
50	35

75	51
100	55
125	62

Table No: 5, α-Amylase Inhibition of Acarbose (Positive control)

Concentration(µg/ml)	IC_{50} %
0	0
25	25
50	45
75	53
100	56
125	65

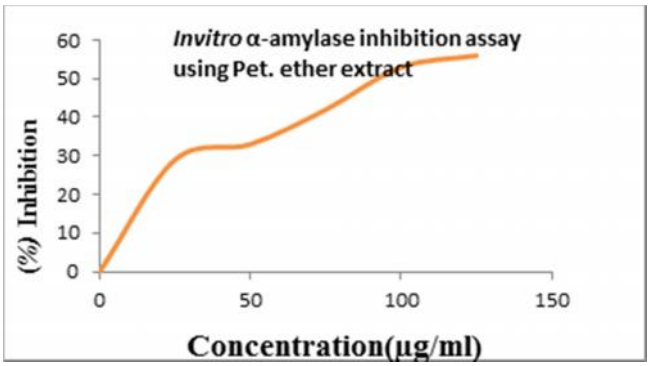

Fig No. 1: α amylase inhibition of petroleum Ether extract of *Pulsatillanuttalliana*

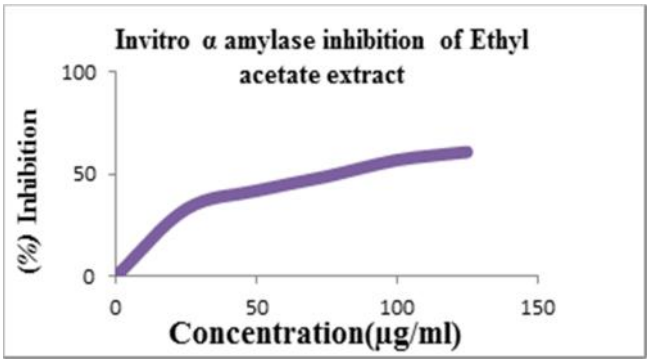

Fig No. 2: α amylase inhibition of Ethyl acetate extract of *Pulsatilla nuttalliana*

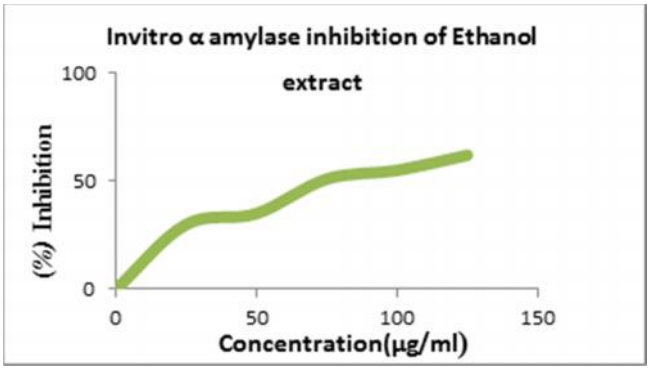

Figure No.3: α amylase inhibition of Ethanolic Extract of *Pulsatilla nuttalliana*

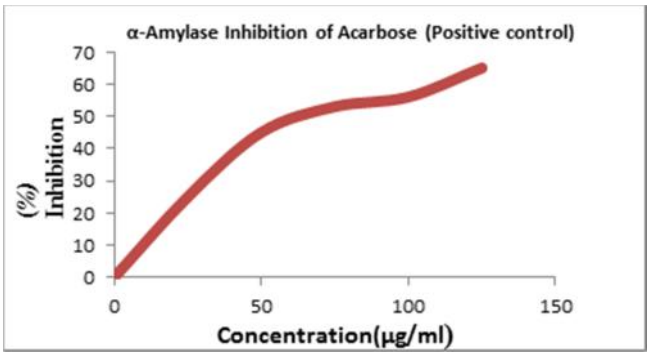

Figure No: 7 α-Amylase Inhibition of Acarbose

Discussion:

The α-amylase, enzyme that plays a role in digestion of starch and glycogen are considered a strategy for the treatment of disorders in carbohydrate uptake, such as diabetes. Pancreatic α-amylase is a key enzyme in the digestive system and catalyses the initial step in hydrolysis of starch to a mixture of smaller. Sequential extraction was done according to increasing polarity order (Petrolium ether, Ethyl acetate and Ethanol). Each extracts were tested for α-amylase inhibition to get the extraction with minimum IC50 value. As per the above mechanism all the extract have concentration dependent affinity towards the inhibition of α-amylase. So the ethanolic extract of Pulsatilla nuttalliana showed antidiabetic activity. This work will be useful for further diabetes mellitus and its related diseases research works to develop new entity for the treatment of diabetes mellitus.

4. Conclusion

The leaves extraction has been performed by sequential extraction method The leaves *of Pulsatilla nuttalliana using* the solvent with increasing polarity order (petroleum ether, ethyl acetate and ethanol) and the active extract was tested by *in-vitro* anti diabetic screening method. The *in-vitro* antidiabetic study has been performed based on the α-amylase inhibition assay. Each extracts were tested for α-amylase inhibition and the extracts with minimum IC50 have been undergone phytochemical screening. The preliminary phytochemical tests was performed to identify

the active phytochemicals present in the ethanolic extract of *Pulsatilla nuttalliana* and showed the presence of Phenols, Flavanoids, Alkaloids, Glycosides, Saponins and Terpenoids. The present study suggested that the isolation of active constituents from ethanolic extract of *Pulsatilla nuttalliana* leaf and characterize the compounds by using preliminary phytochemical studies.

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