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***In vitro* α - amylase and α - glucosidase inhibitory activities of ethanolic extract of *Lactuca runcinata* DC.**

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ABSTRACT

The present study was intended to investigate the *in vitro* α - amylase and α - glucosidase inhibitory activities of ethanolic extract from whole plant of *Lactuca runcinata* (DC.). Postprandial hyperglycemia is a prime characteristic of diabetes mellitus and has been a focus in the therapy for diabetes. Pancreatic α -amylase and α -glucosidase inhibitors offer an effective technique to lower levels of postprandial hyperglycemia by means of control of starch breakdown. Both the therapeutic methodologies which include diminishing hyperglycemia goes for at inhibiting the enzyme α -amylase and α - glucosidase. In this study range, herbal remedies are considered convenient for management of Type 2 diabetes with postprandial hyperglycemia because their traditional adequacy and acceptability, low expenses, lesser side effects. The ethanolic extract got was subjected to *in vitro* alpha amylase and alpha glycosidase inhibitory assay utilizing starch azure as a substrate and porcine pancreatic amylase as the enzyme . The enzyme solutions were premixed with extract at distinctive concentrations (20,40,60,80 and 100 mg/ml). Substrate solutions and colorimetric reagents were added to the reaction. The release of glucose was measured by spectrophotometric method. Acarbose was utilized as the positive control. The extract (20- 100 mg/ ml) totally inhibit α - amylase and α - glucosidase activities. The extract produced higher reduction of α -glucosidase activity than α -amylase. Inhibition at various concentrations were significantly different ($p < 0.05$). The results demonstrated a significant (more than 80%) reduction in α - amylase and additionally 90% reduction in α -glucosidase activity. This finding gives the utilization of ethanolic extract of whole plant of *Lactuca runcinata* effective in inhibiting α -amylase and α -glucosidase thereby proving to be potential hostile to hyperglycemic agents.

Key words: α - amylase; α -glucosidase; *In vitro* antidiabetic; *Lactuca runcinata*.

INTRODUCTION

Diabetes mellitus (DM) is an interminable metabolic issue described by both postprandial and fasting hyperglycemia with unsettling influences in carbohydrates, fat and protein metabolism. Hyperglycemia in diabetes comes about either from a flat out inadequacy in insulin secretion (type I) or insulin activity (type II) or both. The occurrence of diabetes has expanded worldwide as of late. The assessed number of individuals with diabetes was 30 million in 1985, 150 million in 2000 and after that 246 million in 2007, as per the International Diabetes Federation. It 474 anticipates that this number will hit 380 million by 2025^[1].

Treatment of diabetes include: improvement of the activity of insulin at the objective tissues, with the utilization of sensitizers (biguanides, thiozolidinediones); incitement of endogenous insulin discharge with the utilization of sulfonylureas (glibenclamide, glimepiride), and decrease of the interest for insulin utilizing particular enzyme inhibitors (acarbose, miglitol)^[2]. Nonetheless, there is a trouble of undesirable reactions like looseness of the bowels, nausea, dyspepsia, myocardial infarction, peripheral edema and tipsiness with the utilization of these medications. Plants have been a commendable wellspring of medications that have been gotten specifically or in a roundabout way from them. It is accounted for that around 800 plants may have hostile to diabetic potential^[3]. Hypoglycemic activity of therapeutic plants is because of their capacity to restore the capacity of pancreatic tissues by bringing

about an increment in insulin yield, hindering the intestinal ingestion of glucose or encouraging metabolites in insulin subordinate processes^[4,5].

As of now medications of diabetes, notwithstanding insulin supplement incorporates numerous oral hypoglycemic agents alongside among fitting diet and exercise. One remedial methodology which may turn out to be useful for treatment of diabetes is to diminish the post-prandial hyperglycemia. This can be accomplished by hindering the ingestion of glucose through the hindrance of the carbohydrate hydrolyzing enzymes in the digestive tract. The α -glucosidase compounds, for example, an amylase are in charge of the breakdown of oligo and/or disaccharide to monosaccharides. Inhibitors of these compounds delay carbohydrate assimilation and drag out general carbohydrate processing time bringing on a checked reduction in the rate of glucose retention in this manner blunting the post prandial plasma glucose rise^[6]. Cases of such inhibitors which discover application in the clinical practice for administration of diabetes are acarbose, miglitol and voglibose^[7]. In any case, these medications are known not connected with different gastrointestinal symptoms, for example, abdominal pain, flatulence and diarrhea in the patients^[8,9]. Hence, it is the need of time to recognize and investigate the amylase inhibitors from natural sources having less side effects. The Indian traditional system of prescription rehearsed for over a great many years have reports of various against diabetic plants with no known symptoms. Numerous plants and their products have been generally recommended and utilized for diabetic treatment all around the globe with less known unthinking premise of their functioning. Subsequently, these characteristic products should be assessed scientifically in order to check for their against diabetic properties. *Lactuca runcinata* DC.; Synonyms, *L. heyneana* DC.. Family: *Compositae*; *Asteraceae*. This occurs many parts of India, as a common weed. It is considered as valuable medicinal herb in traditional systems of medicine in India. Action diuretic, slightly aperient. It is used as a diuretic in calculous affections, also for chronic obstruction of liver and bowels^[10]. A smaller var., found in western Uttar Pradesh, Rajasthan, Saurashtra and the Deccan Peninsula, is equated with *L. remotiflora* DC. However, the plant is reported to possess the activities like antibacterial activity^[11], phytoconstituents^[12], *in vitro* antioxidant activity^[13], and *in vitro* cytotoxic activity^[14]. Literature survey revealed that there is a no earlier scientific reports regarding *in vitro* studies on α - amylase and α -glucosidase inhibitory activities of this plant. Therefore, objective of the present investigation was to study the effect of ethanolic extract of whole plant of *Lactuca runcinata* (DC.) on α - amylase and α -glucosidase inhibitory activities by *in vitro* methods.

MATERIALS AND METHODS

Plant materials

The fresh whole plants of *Lactuca runcinata* (DC.) were collected from the natural habitats of Kayathar, Thoothukkudi district, Tamil Nadu, India. Taxonomic identification was made by Botanical Survey of Medical Plants Unit Siddha, Government of India, Palayamkottai. The soil particles remove from sample were washed thoroughly in running tap water and adhered debris and finally washed with sterile distilled water. The fine powder of whole plants were shade dried and ground . The powdered materials were stored in air tight polythene bags until use.

Preparation of extract

The hot continuous percolation method in Soxhlet apparatus used for the above powdered materials were successively extracted with ethanol (40-60°C) for 24 hours. Then the extract was concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

In vitro α -amylase inhibition activity^[16]

The α -amylase (0.5 mg/mL) was premixed with extract at various concentrations (20-100 μ g/ml) and starch as a substrate was added as a 0.5% starch solution to start the reaction. The reaction was carried out at 37°C for 5 min and terminated by addition of 2 mL of DNS (3,5-dinitrosalicylic acid) reagent. The reaction mixture was heated for 15 min at 100°C and diluted with 10 mL of distilled water in an ice bath. α - amylase activity was determined by measuring spectrum at 540 nm. The % α -amylase inhibitory activity is calculated by the following formula

$$\% \text{ Inhibition} = (\text{Control OD} - \text{Sample OD} / \text{Control OD}) \times 100$$

The IC₅₀ value was defined as the concentration of the sample extract to inhibit 50% of α -amylase activity under assay condition.

In vitro α -glucosidase inhibition activity

The enzyme α -glucosidase inhibitory activity was determined by premixing α -glucosidase (0.07 Units) with 20-100 μ g/mL of extract. Then 3mM p-nitrophenyl glucopyranoside was added as a substrate. This reaction mixture was incubated at 37°C for 30 min and the reaction was terminated by addition of 2 mL of sodium carbonate. The α -

glucosidase activity was determined by measuring the p-nitrophenyl release from p- nitrophenyl glucopyranoside at 400 nm. The % α -glucosidase inhibitory activity is calculated by the following formula

$$\% \text{ Inhibition} = (\text{Control OD} - \text{Sample OD} / \text{Control OD}) \times 100$$

The IC_{50} value was defined as the concentration of the sample extract to inhibit 50% of α -glucosidase activity under assay condition.

Statistical analysis

Tests were carried out in triplicates. The mean values were calculated from the triplicate values. Values were expressed as the Mean $\pm$ SD (n=3) and differences between groups were considered to be statistically significant if $p < 0.05$. The IC_{50} values were determined from plots of percentage inhibition versus log inhibitor concentration and were calculated by non linear regression analysis from the mean inhibitory values.

RESULTS

According to the outcomes got from the respective study it is being shown that there was a dose-dependent increase in percentage inhibitory activity against alpha amylase enzyme. The rate inhibition value correspondingly increases with increase in the dose concentration as indicated in the particular tables and graphs. The ethanolic extract of whole plant showed marginally more inhibitory effect, which could be expected the more polyphenols constituents being found in the ethanolic extract.

Table 1- IC_{50} value for acarbose in alpha amylase inhibitory assay of *in vitro* antidiabetic activity

S.No	Concentration of sample ($\mu\text{g/mL}$)	% of inhibition	IC_{50} ($\mu\text{g/mL}$)
1	20	35.12	32.55
2	40	56.22	
3	60	74.23	
4	80	87.22	
5	100	93.97	

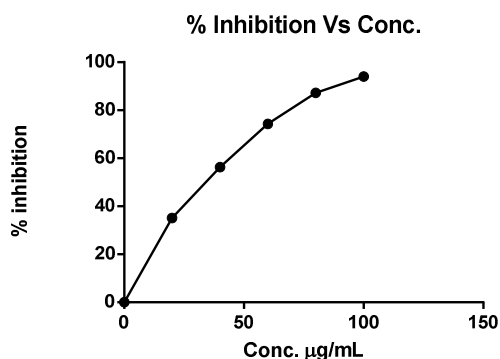


Fig 1: IC_{50} value for acarbose in alpha amylase inhibitory assay of *in vitro* antidiabetic activity

Alpha amylase inhibitory activity for the ethanolic extract of *Lactuca runcinata* presented in Table 2. The inhibition of α - amylase potential shown maximum activity of 82.23% at 100 $\mu\text{g/ml}$ and Standard (acarbose) was found to be 93.97% at 100 $\mu\text{g/ml}$. (Fig:1) The IC_{50} for the ethanolic extract of *Lactuca runcinata* and standard (acarbose) were found to be 32.55 $\mu\text{g/ml}$ and 61.75 $\mu\text{g/ml}$ respectively, the extract showed a better antidiabetic activity (Fig : 2).

Table 2- IC_{50} value for *L. runcinata* ethanolic whole plant extract in alpha amylase inhibitory assay of *in vitro* antidiabetic activity

S.No	Concentration of sample ($\mu\text{g/mL}$)	% of inhibition	IC_{50} ($\mu\text{g/mL}$)
1	20	17.79	61.75
2	40	33.33	
3	60	48.90	
4	80	60.22	
5	100	82.23	

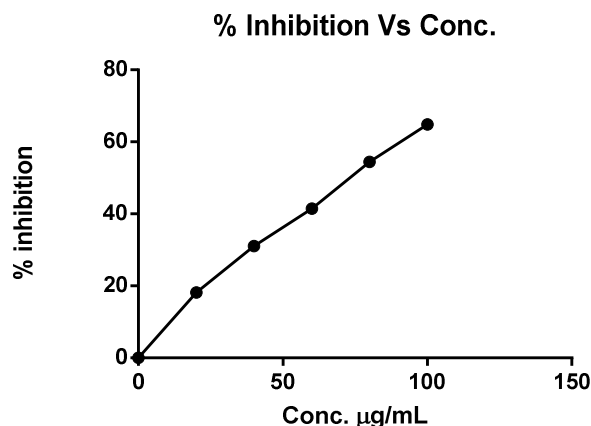


Fig 2: IC₅₀ value for *L. runcinata* ethanolic whole plant extract in alpha amylase inhibitory assay of *in vitro* antidiabetic activity

Alpha glucosidase inhibitory activity for the ethanolic extract of *Lactuca runcinata* presented in Table 3. The inhibition of α -glucosidase potential shown maximum activity of 90.70% at 100 µg/ml. The IC₅₀ for the ethanolic extract of *Lactuca runcinata* was found to be 41.35 µg/ml, the extract showed a good antidiabetic activity (Fig :3).

Table 3- IC₅₀ value for *L. runcinata* ethanolic whole plant extract in alpha glucosidase inhibitory assay of *in vitro* antidiabetic activity

S.No	Concentration of sample (µg/mL)	% of inhibition	IC ₅₀ (µg/mL)
1	20	34.35	41.35
2	40	49.65	
3	60	58.62	
4	80	73.44	
5	100	90.70	

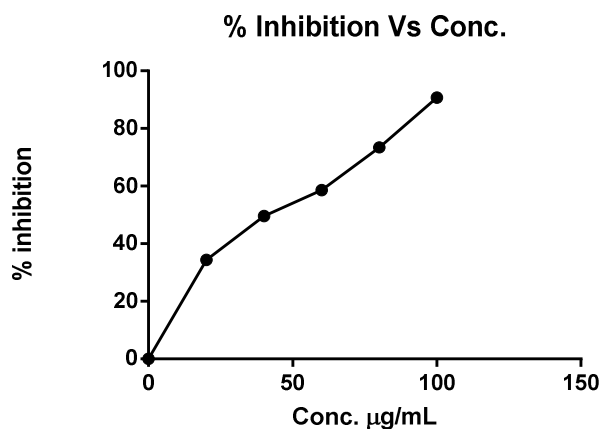


Fig 3: IC₅₀ value for *L. runcinata* ethanolic whole plant extract in alpha glucosidase inhibitory assay of *in vitro* antidiabetic activity

DISCUSSION

Diabetes mellitus is a metabolic issue and the key variable for its control is insulin. Absence of insulin in the body of an organism affects carbohydrate, fat and protein metabolism^[17]. The control over diabetes mellitus without adverse effects is a challenge to therapeutic group. Synthetic inhibitor causes adverse effect such as abdominal pain, diarrhea and soft faeces in the colon. The inhibition of alpha-amylase and alpha-glucosidase would defer the degradation of carbohydrate, which causes a diminishing in the assimilation of glucose; therefore the rise of postprandial blood glucose level decreases^[18]. Amylase inhibitors are otherwise called as starch blockers because they prevent dietary starch from being consumed by the body and in this manner lower postprandial glucose levels^[19,20]. Slowing the processing and breakdown of starch may have useful impacts on insulin resistance and glycemic index control in individuals with diabetes^[21-23]. In our examination we found that ethanolic extract moderately inhibited α -amylase. From phytochemical screening we can see that presence of saponin, steroid and terpenoid which may be responsible for this restorative activity. Natural polyphenols have been accounted to inhibit the activity of carbohydrate hydrolyzing enzymes like α -amylase, α -glucosidase^[22]. Terpenoids also a promising source for biologically active natural compounds which have potential for investigate and development of new substances with pharmacologic

activity. α -amylase inhibitory activity was connected only for oleanane, ursane and lupane type terpenoids^[24]. In a past study saponins have likewise been observed to be a probable α -amylase inhibitor^[25,26]. The mechanism by which this ethanolic extract applied this effect may be because of its action on carbohydrate binding regions of α -amylase enzymes that catalyze hydrolysis of the inside α -1, 4- glucosidic linkages in starch and other related polysaccharides have likewise been focused for the concealment of postprandial hyperglycemia. Hence, this study support the case that natural inhibitors from dietary plants have α -amylase inhibitory activity and could be utilized as successful therapy for the management of postprandial hyperglycemia with minimal adverse effects.

The inhibitors of alpha-glucosidase hinder the processing of carbohydrates and moderate down the assimilation. Acarbose and miglitol are known to be the competitive inhibitor of α -glucosidases and lessens absorption of starch and disaccharides^[27]. Henceforth the therapeutic approaches for lessening postprandial (PP) blood glucose levels in patient with diabetes mellitus is to avert absorption of carbohydrate after food intake. Postprandial blood glucose level in diabetic patients gets expanded because of the inhibition of two of these two enzymes (α -amylase and α -glucosidases)^[28]. The α - amylase inhibitors likewise go about as an against supplements and discourages the processing and assimilation of carbohydrates. Acarbose just like a complex oligosaccharide delays the absorption of carbohydrates and inhibits the action of pancreatic amylase in breakdown of starch. In the present study, investigate has been done to assess the inhibiting capability of alpha-glucosidase and alpha-amylase. The present finding reveals that *Lactuca runcinata* efficiently inhibits both alpha-amylase and alpha-glucosidase. The ethanolic extract indicated higher inhibition potential, furthermore it was observed that plant reacted more towards alpha amylase than that of alpha glucosidase.

In conclusion the result of the present study revealed that ethanolic extract of *Lactuca runcinata* DC. exhibit the potential antidiabetic activity focusing on the inhibitory effects on α -amylase and α -glucosidase. In our *in vitro* model, the outcomes give scientific support to the utilization of *Lactuca runcinata* DC. whole plant extract for the treatment of diabetes, notwithstanding, further investigation is required to validate its utilization previous to clinical implementation as therapeutic agent.

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