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IMPACT OF JASMINUM OFFICINALE FRACTIONS AS ENZYME INHIBITORS

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ABSTRACT

The present study deals with the assessment of inhibitory activity of two important enzymes, involved in carbohydrate metabolism viz. α-Amylase and α-glucosidase, of different fractions of Jasminum officinale leaves obtained by successive extraction with various solvents of increasing polarity viz. hexane, chloroform, ethanol, methanol and water. Since, these enzymes are involved in breakdown of carbohydrates into glucose and then it's gastrointestinal absorption, therefore, activity inhibition of these enzymes has been used in this study as a tool for evaluating the anti-hyperglycemic potential of J. officinale leaves by lowering the postprandial blood glucose level. It is evident from the results that the aqueous fraction of J. officinale leaves is a potent activity inhibitor for both the enzymes, α-amylase as well as α-glucosidase. Inhibitory activity of all the fractions was found to be of the following order, aqueous > methanol > ethanol> chloroform > hexane, indicating thereby that the aqueous fraction had appreciable α-amylase inhibitory activity of 70.47±1.63% with IC_{50} value at 4.22±0.18 mg ml^{-1} in addition to significant α-glucosidase inhibitory activity of 81.24±1.13% with IC_{50} at 1.21 ±0.03mg ml^{-1}. Hence, the aqueous fraction of J. officinale leaves could be developed as α-amylase and α-glucosidase inhibitors for treating diabetes and its complications.

KEY WORDS

α-Amylase; α-Glucosidase; inhibitory activity; J. officinale etc.

INTRODUCTION

Diabetes is a collective disorder of carbohydrate, protein and fat metabolism, characterized by the elevated blood sugar [1]. In India, the number of people with diabetes is projected to rise from 19 to 57 million, between 1995 and 2025. Studies have revealed that in addition to genetic factors causing Type 1 diabetes, life style disorders is also the major cause behind development of Type 2 diabetes and hence urbanization of rural India has doubled this rate [2]. In present scenario prevention of Type 2 diabetes is better than its cure [3]. Currently available drugs for diabetes such as sulfonylureas, biguanides, metformin and insulin have serious undesired effects [4], therefore, herbal drugs are coming up as an alternative of these synthetic medicines for the treatment of diabetes being cost effective and high efficacy associated with great margin of safety [5].

The dietary carbohydrates are generally hydrolyzed to monosaccharides, by the enzymes α-amylase and α-glucosidases, which can be absorbed easily from intestinal lumen and transported into blood stream to provide energy to the system [6]. Impedance of carbohydrate digestion by inhibition of activity of key carbohydrate hydrolyzing enzymes i.e. α-amylase and α-glucosidase would lead to blood glucose level reduction and hence could be a therapeutic strategy for the treatment of diabetes. α-amylase and α-glucosidase inhibitors diminish the level of blood glucose by slowing

down the rate with which these enzymes can convert the complex polysaccharide such as starch into simple monomers and hence, such inhibitors play a significant role in management of diabetes.

The natural products present in medicinal plants help in retarding the absorption of glucose by inhibiting the carbohydrate hydrolyzing enzymes, such as pancreatic amylase. The inhibition of this enzyme delay carbohydrate digestion and prolong complete carbohydrate digestion time, resulting in the reduction in glucose absorption rate and subsequently reducing the postprandial plasma glucose level in blood.

The plant *Jasminum officinale* Linn., family Oleaceae locally known as 'Chameli', cultivated throughout India, was selected for the present study as ethnopharmacological reports have revealed that all parts of the *Jasminum officinale* possess significant medicinal value. The roots of *Jasminum officinale* are used in the treatment of ringworm, flowers are reported as antiseptic, anti-spasmodic, leaves possess wound-healing property and also demonstrated detectable antibacterial activity as well [7].

Significant antioxidant efficacy [8] of *J. officinale* leaves, already reported in our previous study encouraged us to explore these fractions further for their antihyperglycemic potential. Hence, the present study deals with a systematic and scientific exploration of *J. officinale* leave fractions to assess their role as *α*-amylase and *α*-glucosidase inhibitors in order to develop it as a novel antidiabetic agent for treating diabetes and its complications.

MATERIALS AND METHODS

Plant materials

Fresh leaves of *J. officinale* L. (500 g) were collected from the local area of Allahabad, India and authenticated by Prof. D. K. Chauhan, Taxonomist, Department of Botany, University of Allahabad, India. The leaves of the *J. officinale* were washed well with distilled water, shade dried at room temperature, coarsely powdered (500 g), and then extracted with each solvent in increasing order polarity from hexane, chloroform, ethanol, methanol and finally with distilled water successively, using Soxhlet apparatus for 8 hrs at 24° C±5. The collected fractions were concentrated using a rotatory vacuum evaporator at 40° C for drying of sample. Dried powder of different fractions of *J. officinale* leaves were stored in bottles, labeled as *viz.* hexane (**J1**), chloroform (**J2**), ethanol (**J3**), methanol (**J4**) and aqueous (**J5**) fractions, and kept in refrigerator for further experimental analysis.

Chemicals and Reagents

α –Glucosidase from *Saccharomyces cerevisiae*, *α*-amylase from *porcine pancreas*, p-nitrophenyl *α* -D-glucopyranoside, sodium potassium tartarate (SPT) were purchased from Sigma-Aldrich. Acarbose, 3,5-Dinitrosalicylic acid (DNS), starch, NaOH pellets, Na_2HPO_4, NaH_2PO_4, NaCl, Na_2CO_3 from Himedia. Other solvents were of analytical grade.

Experimental design

Carbohydrate hydrolyzing enzyme inhibitory activity assays were performed at different concentrations of five fractions of *J. officinale* leaves viz. hexane (**J1**), chloroform (**J2**), ethanol (**J3**), methanol (**J4**) and aqueous (**J5**) fractions for *α*-amylase and *α*-glucosidase. The results were compared with acarbose, the reference. The IC_{50} values of fractions and acarbose were calculated. A lower IC_{50} values indicates higher inhibitory activity.

α-Amylase Inhibitory assay

The inhibitory action of the *J. officinale* fractions of on α-amylase was carried out using a modified procedure of McCue and Shetty [9]. Briefly, 250 µL extract of varied concentration ranging from 1.0 to 10 mg ml^{-1} was placed in a tube and 250 µL of pancreatic α-amylase solution (0.5 mg ml^{-1}) in 0.02 M sodium phosphate buffer (pH 6.9) was added. The mixture was incubated at 25°C for 10 min, after which 250 µL of starch solution (1 %) in 0.02 M sodium phosphate buffer (pH 6.9) was added. This reaction mixture was again incubated at 25°C for 10 min. The reaction was finally quenched by 500 µL of a reagent, 96 mM 3,5-dinitrosalicylic acid (DNS), and further incubated in boiling water for 5 min and then cooled to room temperature. Reaction further proceed by incubating the reaction mixture at 100° C in boiling water bath for 5 minutes, immediately after that it was kept in the ice bath for 3 minutes followed by the dilution with 5 ml distilled water and the absorbance was measured at 540 nm in a spectrophotometer. Absorbance of control was also measured.The assay was performed in triplicate. The results were expressed as percent inhibition of *α*-amylase inhibitory activity using the following formula:

$$\% \text{ Inhibition} = \left[\frac{Ac - As}{Ac}\right] \times 100$$

Where, *Ac* is absorbance of control and *As* is absorbance of sample.

α-Glucosidase Inhibitory assay

The inhibitory action of the *J. officinale* fractions of on α-glucosidase was determined according to method described by Kim et al. [10] with slight modifications. Firstly, 50 µL of extract of varied concentration ranging from 1.0 to 10 mg ml^{-1} was preincubated with 100 µL 0.02 M sodium phosphate buffer (pH 6.9) containing α-glucosidase (1.0 mg/ml) was added at 25 °C for 10 minutes, after which 50 µL of 3.0mM substrate solution of pNPG in 20 mM phosphate buffer (pH 6.9) was added to start reaction. The reaction mixture was incubated at 37̊ C for 20 minutes. After then 2 ml of (0.1 M) Na_2CO_3 added to terminate the reaction. The α-glucosidase inhibitory activity was determined by measuring the absorbance of the yellow colored reaction mixture at 405 nm. Absorbance of control was also measured. The assay was performed in triplicate. The results were expressed as percent inhibition of α-glucosidase inhibitory activity using the following formula:

% Inhibition = $\left[\frac{Ac - As}{Ac}\right] \times 100$

Where, *Ac* is absorbance of control and *As* is absorbance of sample.

Statistical Analysis

The entire group of data was statistically evaluated using one-way ANOVA, followed by a post hoc Scheffe's test using the SPSS computer software, version 7.5. The values were considered significant when $P<0.05$. Experiments were done in triplicate and the mean value was reported as mean ± S.D.

RESULTS AND DISCUSSION

α-Amylase Inhibitory Activity

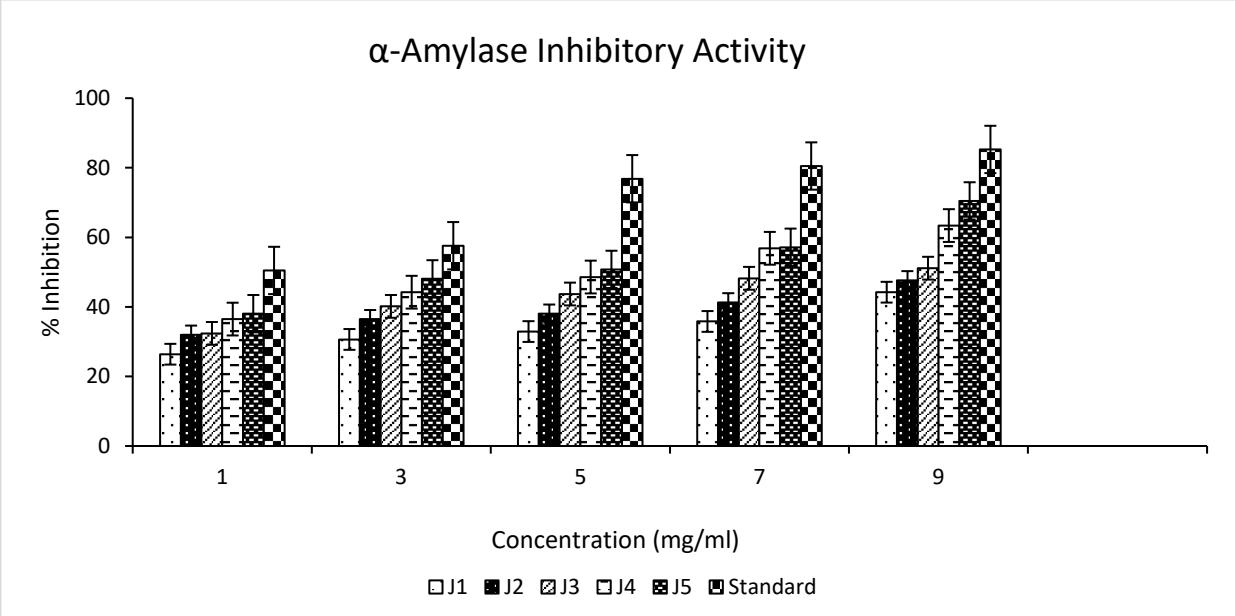

Fig. 1: Comparative percent inhibition of α-Amylase Inhibitory activity in fractions J1, J2, J3, J4, J5 and, Acarbose (reference).

Fig. 1: reveals the α-amylase inhibitory activity of different fractions viz. J1, J2, J3, J4, J5 and Acarbose (reference) in terms of percent inhibition. The highest percent inhibition obtained in case of the fractions J1, J2, J3, J4, J5 and Acarbose were found to be 44.22±1.32, 47.67±1.28, 51.12±1.26, 63.38±1.05, 70.47±1.63 and 85.26±1.13%, respectively at highest evaluated concentration 9mg/ml. Moreover, the IC_{50} values of the fractions viz. J1, J2, J3, J4, J5 and reference obtained were 12.84, 11.02, 8.02, 5.03, 4.22 and 0.65 mg/mL, respectively. Results revealed that fraction J5 has the highest α-amylase inhibitory activity of 70.47±1.63% with IC_{50} value at 4.22 ±0.18 mg ml^{-1} among all the five

evaluated fractions, whereas, fraction J1 had the lowest inhibitory activity at the same concentration of 9mg/ml.

α-Glucosidase Inhibitory Activity

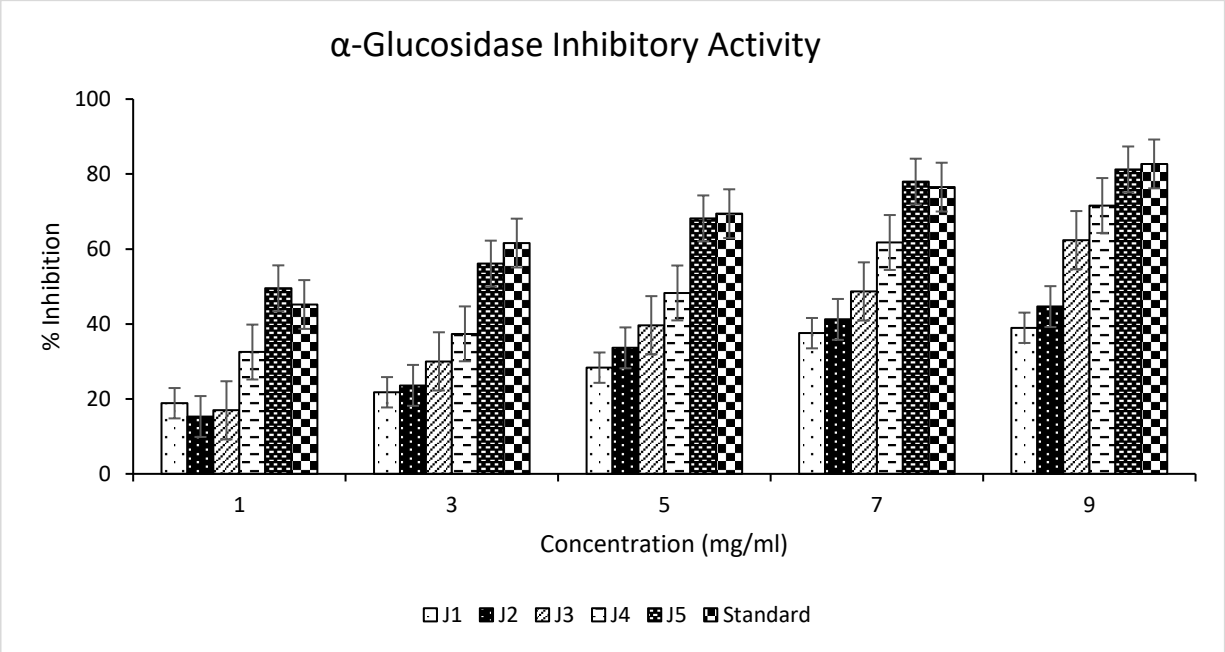

Fig. 2: Comparative percent inhibition of α-Glucosidase Inhibitory activity in fractions J1, J2, J3, J4, J5 and, Acarbose (reference).

Fig. 2, shows that α-glusosidase inhibitory activity of fractions viz. J1, J2, J3, J4, J5 and, Acarbose (reference) in terms of percent inhibition. The highest percent inhibition obtained in case of the fractions J1, J2, J3, J4, J5 and Acarbose were found to be 38.97±0.41, 44.63±0.84, 62.36±1.27, 71.6±1.10, 81.24±1.13 and 82.7±0.83%, respectively at highest evaluated concentration 9mg/ml. Moreover, the IC_{50} values of the fractions viz. J1, J2, J3, J4, J5 and reference obtained were found to be 10.37, 8.69, 6.69, 4.81, 1.21 and 1.16 mg/mL, respectively. Results revealed that fraction J5 had the highest α-glucosidase inhibitory activity of 81.24±1.13% with IC_{50} at 1.21 ±0.03mg ml^{-1} among all the five evaluated fractions at same concentration of 9mg/ml. Thus, α-glusosidase inhibitory activity of J5 was the most significant ad comparable with the standard Acarbose as well, whereas fraction J1 had the lowest inhibitory activity against α-glusosidase.

Table 1: Comparative IC_{50} values of J1, J2, J3, J4, J5 and reference, Acarbose for α –Amylase and α –Glucosidase inhibitory assay.

	IC_{50} values in mg/mL					
	J1	**J2**	**J3**	**J4**	**J5**	**Acarbose**
α -Amylase	12.84±0.73	11.02±0.77	8.02±0.46	5.03±0.15	4.22 ±0.18	0.65 ±0.03
α -Glucosidase	10.37 ±0.62	8.69±0.46	6.69 ±0.45	4.81 ±0.18	1.21 ±0.03	1.16±0.15

Each value is presented as mean ± S.D. (n=3)

Table 1. Shows the comparative IC_{50} values of *J. officinale* leave fractions and Acarbose, the reference, for α –Amylase and α –Glucosidase inhibitory assays. The results clearly reveal that the fraction J5 is the most effective fraction in both the inhibitory assays having the lowest IC_{50} value and its efficacy is also comparable with the standard, Acarbose.

On the whole, across both evaluated anti-diabetic *in vitro* enzymatic assays, the aqueous fraction was found to be the most effective fraction and this efficacy could be correlated well with the presence of phytochemicals like polyphenols or their synergistic effects [8]. From the present study, thus it may be concluded, enzyme inhibiting potency of *J. officinale* leaves fractions is in

the order of aqueous >methanol >ethanol> chloroform >hexane.

Management of the blood glucose level is an essential approach in the control of diabetes complications. Current therapeutic strategy for the control of postprandial hyperglycemia is the inhibition of two members of glycoside hydrolases viz. *α*-amylase and *α*-glucosidase, resulting in delay of carbohydrate digestion to absorbable monosaccharides [11]. Before being absorbed into the intestine and entering blood circulation, starch and other complex polysaccharides are hydrolyzed by *α*-amylase to oligosaccharides, which are further hydrolyzed to simpler glucose by intestinal *α*-glucosidase. Therefore, inhibitors of *α*-amylase and *α*-glucosidase will eventually reduce the flow of glucose from dietary carbohydrates into the bloodstream, waning the postprandial hyperglycemia.

Moreover, the leading glucosidase inhibitors, acarbose and miglitol, are often reported to produce diarrhoea and other intestinal troubles, in addition to bloating, flatulence, cramping and abdominal pain [12]. Interestingly, plant-based agents were reported to be more acceptable source of glucosidase inhibitors due to low cost and relatively better safety levels, including lower incidence of serious gastrointestinal side effects [13]. Thus, the search for nontoxic alternative agents with potent enzyme inhibitory properties which could decrease postprandial hyperglycemia also, is the need of the hour.

CONCLUSION

Since, the results of the present study provide ample evidence of significant enzyme inhibitory activity of *J. officinale* leaves due to the presence of specific phytoconstituents, therefore, it could be developed as a potent anti-hyperglycemic agent and hence extension of this study is warranted as a forwarding step in order to develop it as an anti-diabetic phyto-remedy.

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