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Screening of *invitro* antidiabetic activity and Thin layer chromatography of *Saussurea hypoleuca* Spreng. root

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Diabetes is a common metabolic disorder which is characterized by hyperglycemia due to the absolute or relative deficiency of insulin. Recent decades have experienced a sharp increase in the incidence and prevalence of Diabetes mellitus (DM). One antidiabetic approach is to reduce the gastrointestinal glucose absorption and production through the carbohydrate digesting enzymes such as α-amylase and α-glucosidase. Inhibition of these enzymes can significantly decrease the post prandial increase of blood glucose after a mix carbohydrate diet that can be an important strategy for blood glucose management. The current study was designed to screen the root methanolic extract of *Saussurea hypoleuca* against α-amylase and α-glucosidase. The study also focuses on Thin layer chromatography (TLC) of various fractions of plant root methanolic crude extracts for the isolation of phytoconstitutents.

Keywords: Diabetes mellitus, Thin layer chromatography, *Saussurea hypoleuca,* insulin, phytoconstitutents.

INTRODUCTION

Diabetes mellitus (DM) has become one of the most perplexing health problems of 21st century with increasing prevalence all over the world. It is a chronic disorder categorized by increased plasma glucose level resulting from inadequate amount of insulin or insulin resistance or both along with the disturbance of carbohydrate, fat and protein metabolism result as hyperglycemia (Keerthana et al. 2013). Many diverse therapeutical strategies are available for the treatment of DM. One therapeutical approach is to decrease the post prandial hypoglycemia to delay the digestion. Alpha glucosidase is responsible for the breakdown of polysaccharide to monosaccharides which are absorbed from the mucosal border of small intestine (Rehman et al. 2022). Inhibitory action of this enzyme will decrease the blood glucose level (Alexander, 1992). Another effective method is the inhibition of alpha amylase activity which cause the conversion of starch into more simple sugar. Alpha amylase inhibitor's delay glucose absorption rate and maintained the serum glucose level in hyperglycemic individuals (Dineshkumar et al. 2010).

An enormous quantity of anti-diabetic agents is accessible in market for the treatment of diabetes but none of these effectively control complications and side effects associated with it. Hence people are more sentient about health and imminent to the natural products (Akbar et al. 2022; Ajaib et al. 2022).

Saussurea hypoleuca is a medicinal plant belongs to family Asteraceae. Mostly Asteraceae family plants are herbaceous and gained popuity in alternative and traditional medicinal system. *Saussurea* root is ethnomedicinally as liver tonic (Shakya and Shukla 2011). It also has potential for different biological activities due the presence of large number of phytochemicals (Arshad and Ishtiaq, 2019). However, no studies have been done to assess the antidiabetic effects of this plant root. Therefore, this article directs the evaluation of antidiabetic activity by employing in vitro assay method.

MATERIALS AND METHODS

Plant collection and preparation of extracts

Saussurea roots have been collected from Quetta, Baluchistan, Pakistan. Specimen was identified by Dr. Zaheer -ul -din taxonomist department of Botany, GC

University, Lahore under the voucher number of GC. Herb. Bot.3453. Plant roots were pulverized after drying under shade. Root methanolic extracts (RME) was made by cold maceration using rotary evaporator under reduced pressure. Fractionation of the methanolic extract was performed with different solvents using polarity order. Each fraction was dried and preserved for biological activities (Arshad and Ishtiaq, 2019).

Enzyme inhibitory activity by Alpha Amylase

A total of 200 µl of test samples (RME) and standard were added (100-1000µl/ml) to 200 µl of 0.2Mm phosphate buffer (6.9pH) containing alpha amylase (0.5mg/ml) solution and were incubated at 37℃ for 15minutes. 200µl of 1% starch solution in 0.02M sodium phosphate was added in each test tube. The mixture was again incubated at 37℃for 15minutes. The reaction was stopped with 1ml of DNSA color reagent. The test tubes were then incubated in boiling water for 5minutes and then cooled. The reaction was then diluted with 10ml distilled water and absorbance was measured at 540nm. Control samples were prepared without any test sample (Gómez-Villegas et al. 2021).Assay was repeated in triplicates and absorbance was recorded. Following equation was used to determine the percentage of inhibition.

$$\%inhibition = Abs(control) - \frac{Abs(sample)}{Abs}(Control) * 100$$

1C_{50} of RME and fractions was calculated.

Enzyme inhibitory activity by Alpha glucosidase

Enzyme inhibitory activity was determined by incubating 1ml solution of starch substrate (2%W/V maltose /sucrose) with 0.2M Tris buffer (8.0 pH) and various concentrations of plant RME (100-1000µl/ml) at 37℃ for 15minutes. Reaction was initiated by adding 1ml solution of alpha glucosidase. Reaction mixture was again incubated at 37℃ for 15minutes. The reaction was stopped by boiling the reaction mixture in water bath for 5minutes. The liberated glucose was measured by glucose oxidase peroxidase methods (Ichimura et al. 2021).

Thin layer Chromatography

Thin layer chromatography of RME and all organic fractions were performed on aluminum coated TLC plates by using M.P system (A) n-hexane: E.A (1:4) as reported by (Wagner 2004). RME and all fractions were dissolved in their respective solvents and applied on TLC plates by capillary tubes. These plates were subjected into chromatographic jar to developed having 20 mL M.P with saturated conditions. After drying plates were observed under UV lamp to locate the spots. R_f values of each spots were calculated and photographed.

Statistical Analysis

All results were expressed as mean ± SD. Microsoft Excel 2016 was used for the calculation of all observed study data as well as for standard and regression curve analysis.

RESULTS

Evaluation of *in vitro* alpha amylase inhibitory activity

The findings of the assay are presented in table 1. There is a dose dependent increase in percentage inhibitory activity against alpha amylase. RME at 100µg/ml concentrations was shown percentage inhibition 24.09% and at 1000µg/ml the inhibitory activity was 63.41%. IC50 value of RME was 616.46 for alpha amylase as shown in fig.2 while 50% inhibition of standard drug Acarbose was observed 522.36 for alpha amylase enzyme (figure1).

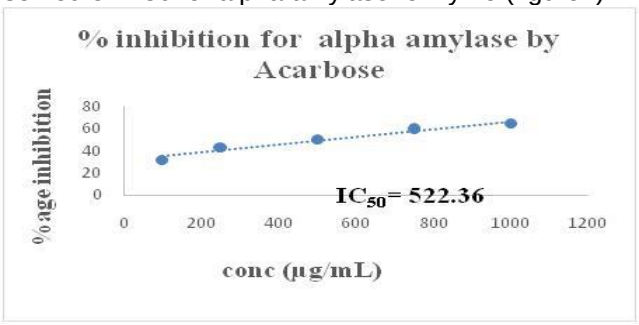


Figure 1: Percentage inhibition of Alpha-amylase enzyme by Acarbose

Evaluation of *in vitro* alpha glucosidase inhibitory activity

Table.2 demonstrates the inhibitory activity of RME for alpha gulcosidase enzyme. RME of *Saussurea* has displayed significant percentage inhibition at dose dependent manner. The percentage inhibition was varied from lowest concentrations 12.87% for 100µg/ml to highest concentrations 50.95% for 1000µg/ml. IC50 value of standard drug acarbose was found to be 442.38 (figure 3) where as IC50 value of RME was 992.89 for alpha glucosidase enzyme as shown in figure 4.

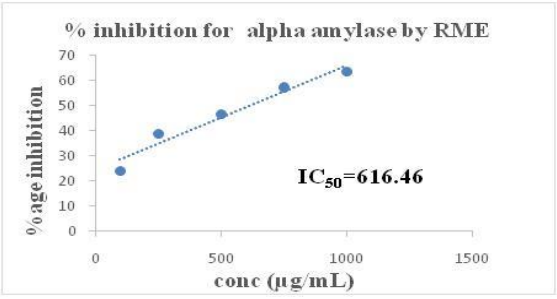


Figure 2: Percentage inhibition of Alpha-amylase enzyme by RME of *Saussurea hypoleuca*

Table 1: *In-vitro* antidiabetic activity of RME using alpha amylase method vs standard drug Acarbose

Concentration (µg/mL)	Mean ± SD (Acarb.)	%inhibition by Acarb.	IC_{50} of Acarb. (µg/mL)	Mean ± SD (RME)	%inhibition by RME	IC_{50} of RME (µg/mL)
100	0.144 ± 0.002	32.05%		0.109 ± 0.008	24.09%	
250	0.173 ± 0.001	43.17%		0.106 ± 0.001	38.72%	
500	0.197 ± 0.002	50.25%	522.16	0.105 ± 0.00	46.70%	616.46
750	0.245 ± 0.002	59.87%		0.105 ± 0.001	57.14%	
1000	0.275 ± 0.003	64.25%		0.100 ± 0.006	63.41%	

All values are carried out in triplicate manner and expressed as the mean ±SD. The IC_{50} value is defined as the concentration of inhibitor to inhibit 50% of its activity under the assay.

Table 2: *In-vitro* antidiabetic activity of RME using alpha glycosidase method vs standard drug Acarbose

Concentration (µg/mL)	Mean ± SD (Acarb.)	%inhibition by Acarb.	IC_{50} of Acarb. (µg/mL)	Mean ± SD (RME)	%inhibition by RME	IC_{50} of RME (µg/mL)
100	0.132± 0.001	32.57%		0.115± 0.115	12.87%	
250	0.147±0.005	39.45%		0.121± 0.006	17.68%	
500	0.1986 ± 0.0056	55.05%	442.38	0.137± 0.004	30.80%	992.89
750	0.2643 ± 0.011	66.28%		0.163± 0.004	38.25%	
1000	0.3653 ± 0.014	75.61%		0.179± 0.004	50.95%	

All values are carried out in triplicate manner and expressed as the mean ±SD. The IC_{50} value is defined as the concentration of inhibitor to inhibit 50% of its activity under the assay.

Table 3: TLC of RME and Fractions of *S. hypoleuca* in M.P (n-hex: E.A 1:4) System (A)

RME and Fractions	M.P	NO. of spots	Retardation Factor	Observation in 254nm &366nm
CF	n-hex: E.A (1:4)	3	0.48,0.73,0.93	1-blu, 2-blu,-3-blu
E.AF	n-hex: E.A (1:4)	7	0.13,0.18,0.42,0.66, 0.82,0.90,0.93	1-l-blu, 2-d-blu,3-f-blu,4-l-blu,5-l-blu,6-l-org,7-blu
n-HF	n-hex: E.A (1:4)	2	0.15,0.71	1-d-blu,2-l-blu
n-BF	n-hex: E.A (1:4)	3	0.2,0.66,0.92	1-d-blu,2-d-blu,3-l-blu
RME	n-hex: E.A (1:4)	4	0.2,0.66,0.90,0.93	1-l-blu, 2-d-blu,3-l-blu,4l-blu
AF	n-hex: E.A (1:4)	1	0.66	1-l-blu
CF	n-hex: E.A (1:4)	3	0.15,0.73,0.93	1-l-bro, 2- d-bro,-3-d-bro
E.AF	n-hex: E.A (1:4)	5	0.42,0.66, 0.82,0.87,0.92	1-l-bro, 2-l-bro,3-d-bro,4-l-bro,5-l-bro
n-HF	n-hex: E.A (1:4)	1	0.15	1-l-bro
n-BF	n-hex: E.A (1:4)	4	0.13,0.25,0.66,0.71	1-l-bro,2-l-bro,3-d-bro,4-d-bro
RME	n-hex: E.A (1:4)	2	0.74,0.94	1-l-bro,2-l-bro
AF	n-hex: E.A (1:4)	-	-	-

l- light, blu- blue, d-dark, f-florescent, bro-brown,CF-Chloroform fraction, E.AF ethyle acetate fraction, n-HF- n-hexane fraction, n-BF- n-butanol fraction, RME- root methanolic extract, AF- aqueous fraction. M.P- mobile phase.

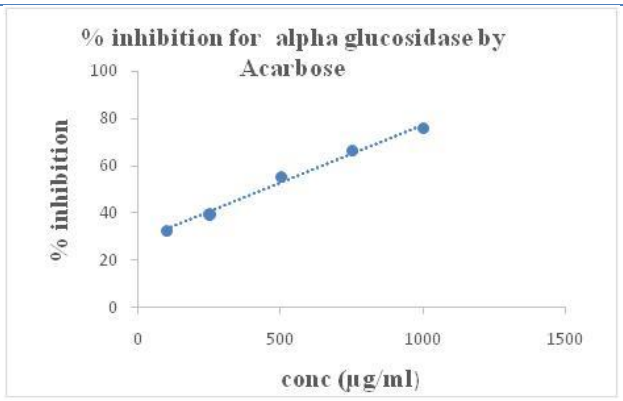


Figure 3: Percentage inhibition of Alpha-glucosidase enzyme by Acarbose

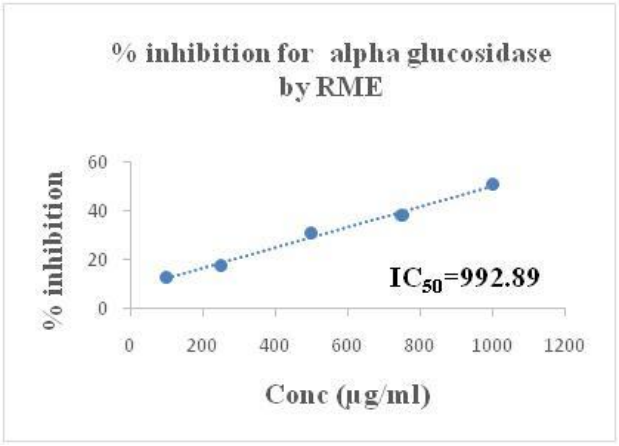
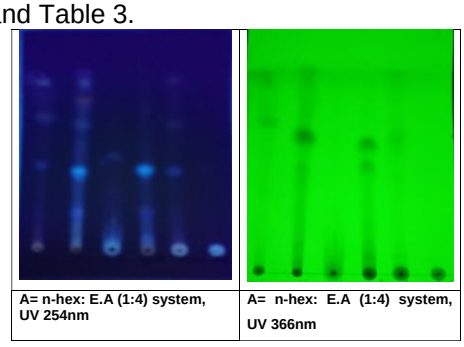


Figure 4: Percentage inhibition of Alpha-glucosidase enzyme by RME of *Saussurea hypoleuca*

TLC Profiling

Thin layer chromatography of RME and fractions were carried out in solvent systems (A) and photographed. R_f values are calculated at 254 nm and 366 nm presented in Figure 5 and Table 3.



A= n-hex: E.A (1:4) system, UV 254nm	A= n-hex: E.A (1:4) system, UV 366nm

Figure 5: TLC of RME and Fractions in Solvent System (A)

DISCUSSION

Diabetes is one of the major cause of prenature death in all over the world. It is associated with cardiovascular complications. An enormous quantity of anti-diabetic agents is accessible in market for the treatment of diabetes but none of these effectively control complications and side effects associated with it. Hence, there is immense need to locate the excellent therapeutical agents from natural origin for the discovery of novel oral hypoglycemic agent which have lesser side effects and are safe, economic and effective. Literature shows various plant extract reduced blood glucose level by increasing insulin secretion from pancreatic beta cells or by increasing peripheral insulin sensitivity (Rehman et al. 2022). Alpha amylase and alpha glucosidase breaks the starch into monosaccharides and glucose. For controlling this metabolic disease, the inhibitors of both enzymes could interrupted or lessen the synthesis of glucose which are targets for developing antidiabetic drugs (Narkhede et al. 2011). These enzymes are present in epithelium of small intestine. Consequently on inhibiting these enzyme cause decrease the digestion rate of carbohydrate and delays the total absorption rate of glucose and declines the postprandial peak of blood glucose in diabetic individuals (Kumar and Kumara et al. 2013).The verdicts of current research, RME revealed inhibition against alpha amylase and alpha glucosidase enzyme which shows that RME of *Saussurea* is a potent inhibitor of alpha amylase and alpha glucosidase. More over IC_{50} values of RME was very close to standard drug Acarbose which proposes that plant can be useful as an effective therapy for postprandial hyperglycemia with minimal side effects. The research work also supports the data that natural inhibitors from plants have strong inhibition towards the activity of enzymes as compared to Acarbose.

TLC is another significant technique for the detection of phytoconstituents present in crude plant extracts which is very imperative with respect to their pharmacological effects. It is rapid, simple, and sensitive method with low cost to study the herbal products to assure their quality. RME and various fractions of *Saussurea* were spotted on TLC cards by capillary tube with different M.P n-hexane: E.A (1:4) in TLC jars to give maximum separation. It was analyzed under UV lamp and Rf values of each spots were calculated as shown in (table 3 &figure 5).

CONCLUSION

The current research work revealed that RME of *Saussurea hypoleuca* produce alpha amylase and alpha glucosidase inhibitory activity. It is prophesied that RME overturn the glycemic response in diabetic illnesses. The antidiabetic activity of RME can also be attributed to the intestinal alpha amylase and alpha glucosidase inhibitory activity. Further *in vivo* ant diabetic study was required to endorse the extract mechanism of RME of *Saussurea hypoleuca*.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

NA performed the experimental work, SA rechecked the manuscript. SA wrote the manuscript and provided the study design. SR and UR helped in performance of experimental work, FZ, ED and SK helped in compilation of data. TA, SI and SM helped in performing and compiling TLC results.

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