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Research

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The Antidiabetic and Spectral Analysis of Various Extracts of *Lilium polyphyllum*

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Abstract

Background: The Antidiabetic and Spectral Analysis of Various Extracts of *Lilium polyphyllum*

Methods: The study involves antidiabetic and spectral analysis, in which inhibition of alpha amylase and alpha glycosidase inhibition was carried out by various extracts of *Lilium polyphyllum*.

Results: The plant showed a good inhibiting potential of amylase and glycosidase, the two key enzymes responsible for the breakdown of oligosaccharides into glucose molecules. Among the extracts the inhibition of alpha amylase was found to be 54.95% by water extract, 53.01% by ethanol extract and 47.87% by DCM extract. The percentage inhibition of alpha glucosidase was found to be 48.48 % by water extract, 48.48% by ethanol extract and 43.85% by DCM extracts. GC-MS analysis involves isolation of, Methyl 2-furoate, 5-hydroxymethyl furfural, Methyl piperate, Piperine, Palmitic acid, Methyl Palmitate, which in turn justifies the antidiabetic properties of the concerned plant. So the plant can act as good source of antidiabetic drug. The water extract is more beneficial than other extracts.

Conclusion: The inhibiting potential of natural products have been found as a good choice for the diabetic patients as they did not led to side effects. Similarly in the above study it had been found that *Lilium polyphyllum* posses many biosignitures which have the amylase and glycosidase inhibiting potential, among which polar components have more tendency than the less polar.

Keywords: Antidiabetic, *Lilium polyphyllum*, Palmitic acid, Piperine, amylase, glycosidase

Background

Ayurveda is the essential and the eternal science of life (Pandeya G et.al, 2011) as being associated with the noble, excellent and great tradition. The main purpose of Ayurveda is to protect the person and to keep the fitness of a healthy person and to mitigate the disorders of a patient (Sharma, Singh. et.al, 2017) which else may have fetal out comings. Nearly 80% of the

population of developing countries is directly dependent on traditional medicines, which they consume from plants, for their primary health care needs.

Lilium polyphyllum commonly known as Kshirkakoli or white lilly (family Liliaceae) is extensively used in many indigenous preparations from times immemorial. Kshirkakoli have been found to contain Sugar, Alkaloids, flavanoids, and essential Steroids (Subramoniam et.al 2013). Medicinally, the bulb of this species is used as diuretic, antipyretic and as a energy tonic (Ayurvedic Pharmacopoeia of India. 2001, Dhyani A, 2009, Dhyani A, 2010, Kumari S, 2011). Few species of the plant are also known for medicinal and food value (Chang C, 2000, Wawrosch C, 2001), which increased its economic importance many folds.

In traditional system of medicine, the species reported to restore health immediately and works as antioxidant in the body (Sharma BD, 2005).

Diabetes is considered as one of the fatal causes of death worldwide as per the estimation in every ten second a person dies from diabetes related problems. In 2007, it was reported by (Das AK et al 2008) that diabetes caused 3.5 million deaths globally. Diabetes was considered as ‘a disease of rich man’ but now it had spread among all masses (Gupta R et al 2007) in India as well in the whole world. Mostly developing countries have large number of diabetic patients but India is considered as infamously dubbed ‘diabetic capital of the world’ (Abate N et al 2007) because of the largest number of diabetic patients.

Materials and Methods

Plant materials

The plant material was collected from Kishtwar region of Jammu and Kashmir and was authenticated at FRI Dehradun. The collection process was preferably done in the dry condition. Plant was shade dried at room temperature and the plant samples were air dried and grounded into uniform powder with a grinder.

Materials and Methods

All the chemicals used in this investigation were of analytical reagent (AR) grade and were purchased from Sigma Merck. De-ionized water was used in the whole study. All the glassware and equipment used for handling were stabilized properly prior to use.

Extraction

The extraction procedure was carried out first with DCM followed by ethanol and then with water based upon their polarity index. The extraction was done by Soxhlet extraction using thimble to get the pure form of extract. 75 g of the plant material was used for extraction.

In *vitro* methods employed in antidiabetic studies (Hamdan, Afifi FU, 2004)

Inhibition of alpha amylase enzyme

A total of 500 µL of test samples and standard drug (100-1000µg/ml) were added to 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5mg/ml) solution and were incubated at 25°C for 10 min. After these, 500 µL of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of 3, 5 di-nitro salicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm. Control represent 100% enzyme activity and were conducted in similar way by replacing extract with vehicle (Thalapaneni NR, 2008. Heidari R, 2005).

Inhibition of alpha-glycosidase enzyme

The inhibitory activity was determined by incubating a solution of starch substrate (2 % w/v maltose or sucrose) 1 ml with 0.2 M Tris buffer pH 8.0 and various concentration of plant extract for 5 min at 37°C. The reaction was initiated by adding 1 ml of alpha-glucosidase enzyme (1U/ml) to it followed by incubation for 40 min at 35°C. Then the reaction was terminated by the addition of 2 ml of 6N HCl. Then the intensity of the colour was measured at 540nm (Krishnaveni, S, 1984).

Calculation of 50% Inhibitory Concentration (IC₅₀)

The concentration of the plant extracts required to scavenge 50% of the radicals (IC₅₀) was calculated by using the percentage scavenging activities at five different concentrations of the extract. Percentage inhibition (I %) was calculated by

$$I \% = (Ac - As) / Ac \times 100 \quad (\text{Shai L, 2010})$$

Where A_c is the absorbance of the control and A_s is the absorbance of the sample.

Observations and Results

In the study the Acarbose has been used as a standard drug for the inhibition of amylases and glucosidases, and it had been found that the inhibition of the concerned enzymes occur very well in which the maximum inhibition was shown by water extract followed by ethanol and least results were observed for the DCM extracts. The results are shown in the respective tables and a comparative study could be observed from the figures.

Table 1: Data of ACAROSE Standard Drug for Amylase Enzyme

S. No	Conc. of Sample (µg/ml)	% of Inhibition	IC ₅₀
1	5	16.1	15.55
2	10	32.02	
3	15	49.12	
4	20	65.51	
5	25	82.73	
6	30	99.95	
7	35	117.12	
8	40	134.23	
9	45	152.11	
10	50	169.12

Fig 1: Graph showing inhibition of alpha-amylase by ACAROSE Standard Drug

Table 2: Data of Water Extract for Amylase Enzyme inhibition

S. No	Conc. of Sample (µg/ml)	% of Inhibition	IC ₅₀
1	20	27.9	45.05
2	40	48.8	
3	60	62.3	
4	80	87.5	
5	100	96.4

Fig 2: Graph showing inhibition of alpha-amylase by Water Extract

Table 3: Data of Ethanol Extract for Amylase Enzyme inhibition

S. No	Conc. of Sample (µg/ml)	% of Inhibition	IC ₅₀
1	20	32.71	47.00
2	40	45.91	
3	60	57.21	
4	80	71.51	
5	100	89.14

Fig 3: Graph showing inhibition of alpha-amylase by Ethanolic Extract

Table 4: Data of DCM Extract for Amylase Enzyme inhibition

S. No	Conc. of Sample (µg/ml)	% of Inhibition	IC ₅₀
1	20	20.1	52.12
2	40	40.2	
3	60	55.9	
4	80	71.3	
5	100	79.5

Fig 4: Graph showing inhibition of alpha-amylase by DCM Extract

Table 5: Data of ACAROSE Standard Drug for Glycosidase Enzyme

S. No	Conc. of Sample (µg/ml)	% of Inhibition	IC ₅₀
1.	5	10.75	25.10
2	10	21.32	
3	15	33.12	
4	20	44.98	
5	25	55.73	
6	30	67.97	
7	35	79.33	
8	40	91.45	
9	45	104.1	
10	50	115.2

Fig 5: Graph showing inhibition of alpha-glycosidase by ACAROSE Standard Drug

Table 6: Data of Water Extract for Glycosidase Enzyme

S. No	Conc. of Sample (µg/ml)	% of Inhibition	IC ₅₀
1	20	23.1	51.52
2	40	39.2	
3	60	56.9	
4	80	62.3	
5	100	71.5

Fig 6: Graph showing inhibition of alpha-glycosidase by water Extract

Table 7: Data of Ethanolic Extract for Glycosidase Enzyme

S. No	Conc. of Sample (µg/ml)	% of Inhibition	IC ₅₀
1	20	25.99	51.57
2	40	42.58	
3	60	59.43	
4	80	69.22	
5	100	77.56

Fig 7: Graph showing inhibition of alpha-glycosidase by Ethanolic Extract

Table 8: Data of DCM Extract for Glycosidase Enzyme

S. No	Conc. of Sample (µg/ml)	% Of Inhibition	IC ₅₀
1	20	25.51	56.15
2	40	42.52	
3	60	57.31	
4	80	67.17	
5	100	77.13

Fig 8: Graph showing inhibition of alpha-glycosidase by DCM Extract

As per the results obtained from the above study it had been found that the inhibition of amylase and glycosidase are dose-dependent, as there is an increase in percentage inhibition in the enzymes by the increase in the concentration of the plant extracts.

The inhibition percentage of alpha amylase was found to be 54.95% by water extract, 53.01% by ethanol extract and 47.87% by DCM extract, similarly the percentage inhibition of alpha glucosidase was found to be 48.48 % by water extract, 48.48% by ethanol extract and 43.85% by DCM extracts. Also as per the comparative study the inhibition of glucosidases enzymes occurs more than the amylases.

The ethanolic extract of the concerned plant when analysed by GC-MS technique the following compounds have been identified.

Compound Name: Methyl 2-furoate

Chemical Formula: $C_6H_6O_3$
Molecular Weight: 126.11

GC-MS

Compound Name: 5-hydroxymethyl furfural

Chemical Formula: C₆H₆O₃

Molecular Weight: 126.03

HR-MS

Compound Name: Methyl piperate

Chemical Formula: C₁₃H₁₂O₄

Molecular Weight: 232.23

ESIMS

SHIMADZU
LabSolutions

Analysis Report

Sample Information

Sample Name	: LP 06	Sample Type	: Unknown
Sample ID		Acquired by	: System Administrator
Filename	: 12-02-19 MASS S 30.lcd	Processed by	: System Administrator
Mod Filename	: MS Scan.lcd		
File Name	: 12-02-19 MASS SCAN.lcd		
File	: 1-44		
Injection Volume	: 1 µL		
Acquired	: 12-02-2019 12:25:16		
Processed	: 12-02-2019 12:26:17

CALCMS(Single Run)12-02-19 MASS S 30

Compound Name: Piperine Chemical Formula: $C_{17}H_{19}NO_3$

Molecular Weight: 285.34

ESIMS

Sample Information		Sample ID	:
Sample Name	: LP 9	Vial#	: 8
Tray#	: 1	Data File	: 25-2-2020 `M0 43.led
Injection Volume	: 1	Processed by	: System Administrator
Method File	: MS Scan.lcm		
Date Processed	: 25-02-2020 14:45:33

Compound name: 7, 10-Hexadecadienoic acid methyl ester

Chemical Formula: C₁₇H₃₀O₂
Molecular Weight: 266.42

ESIMS

Sample Information		Sample ID	:
Sample Name	: LP 10	Vial#	: 53
Tray#	: 1	Data File	: 7-05-19 MASS 25.led
Injection Volume	: 1	Processed by	: System Administrator
Method File	: MS Scan.lcm		
Date Processed	: 07-05-2019 12:17:22

GCMS

Line#6 R.Time:21.630(Scan#:3527)
 MassPeaks:315
 RawMode:Averaged 21.625-21.635(3526-3528) BasePeak:73(44020)
 BG Mode:Calc. from Peak Group 1 - Event 1 Q3 Scan

Compound Name: Methyl palmitate

Chemical Formula: $C_{17}H_{34}O_2$
 Molecular Weight: 270.45

GC-MS

Discussion

Diabetes mellitus is completely a metabolic disorder and the key factor responsible for it is insulin. The decline of insulin in the body has direct affect on the consumption of carbohydrate, fat and protein metabolism (Rajiv Gandhi, 2012). Presently it seems impossible to control diabetes mellitus without its side effects by allopathic medicines. The present study which is based on the inhibition of alpha-amylase and alpha-glycosidase delays the breakdown of carbohydrate, due to which a decline in the absorption of glucose occurs; as a result of which the inclination in the postprandial blood glucose level reduces (Rhabaso Lhoret, 2014) The inhibitors of Alpha- amylase and glucosidase decrease the digestion of carbohydrates which declines their absorption. Two standard drugs viz Acarbose and miglitol are the well known inhibitors of α -glucosidases thereby reduce the absorption of starch and disaccharides (Davis SN,2001). So many experiments are being taken into consideration which may reduce the postprandial (PP) blood glucose level in patients with diabetes mellitus to prevent absorption of carbohydrate after food intake. The Postprandial blood glucose level in diabetic patients may reduce by the inhibition of α -amylase and α -glucosidases (Conforti F, 2005). The inhibitors of α -amylase acts as anti-nutrients, obstructs the digestion and absorption of carbohydrates among which Acarbose is a well known inhibitor because being a complex oligosaccharide delays the digestion of carbohydrates, so suppresses the action of pancreatic amylase in the breakdown of starch.

The study in reference evaluates alpha-glucosidase and alpha-amylase inhibition by various extracts of *Lilium polyphyllum*. The finding reveals that *Lilium polyphyllum* efficiently inhibits both alpha-amylase and alpha-glucosidase respectively. From the study it had been

found that the inhibiting potential of various extracts increases correspondingly with the polar nature of the solvents.

The ethanol extracts of the concerned plant were subjected to GC-MS analysis and the compounds which have been isolated help in the justification of the antidiabetic properties of the concerned plant, Methyl 2-furoate (Xu Z, 2008), 5-hydroxymethyl furfural (Lenzen S, 2008), Methyl piperate, Piperine, (Ghosh R, 2004) Palmitic acid (Matsui T, 2001) Methyl Palmitate (Kumar GPS, 2006).

Conclusion

The study in reference showed that the plant extracts in reference have good inhibiting potential against the amylase and glycosidase enzymes. The inhibiting potential of natural products have been found as a good choice for the diabetic patients as they did not led to side effects. Similarly in the above study it had been found that *Lilium polyphyllum* posses many biosignitures which have the amylase and glycosidase inhibiting potential, among which polar components have more tendency than the less polar. So the plant in reference with a limited concentration range should be consumed along with the daily routine diet.

Declaration:

I/We declare that this is an original research work and is not previously published or presented elsewhere in any language and is also not in consideration in any other journal simultaneously.

I and we, all authors of the above manuscript are agree that the content of this manuscript will not be copyrighted, submitted, or published elsewhere (including the internet), and is also not plagiarized from any language according to the policy of the journal.

1. Ethics approval and consent to participate: not applicable
2. Consent to publish: Permission from all the authors
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Figures

Figure 1

Graph showing inhibition of alpha-amylase by ACAROSE Standard Drug

Figure 2

Graph showing inhibition of alpha-amylase by Water Extract

Figure 3

Graph showing inhibition of alpha-amylase by Ethanolic Extract

Figure 4

Graph showing inhibition of alpha-amylase by DCM Extract

Figure 5

Graph showing inhibition of alpha-glycosidase by ACAROSE Standard Drug

Figure 6

Graph showing inhibition of alpha-glycosidase by water Extract

Figure 7

Graph showing inhibition of alpha-glycosidase by Ethanolic Extract

Figure 8

Graph showing inhibition of alpha-glycosidase by DCM Extract