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Original Research Article

Investigation of the Antidiabetic Activity and GC-MS Analysis of Extracts of *Lilium polyphyllum*

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ABSTRACT

Diabetes is a disease characterized by high blood glucose (hyperglycaemia) due to insufficient insulin production or impaired tissue response to insulin. The hormone Insulin, produced in the pancreas, helps in the regulation of blood glucose. The present study is aimed at finding an alternative approach to blood glucose regulation by the use of *Lilium polyphyllum* Stem extract as potential inhibitor of the enzymes; *α*-amylase and *α*-glycosidase, which are responsible for the breakdown of oligosaccharides into glucose molecules. The α-amylase and α-glycosidase inhibitory activities of the extracts of *Lilium polyphyllum in vitro* using standard procedures. The ethanol extract of the plant was subjected to gas chromatographic-mass spectrometric (GC-MS) analysis. The results showed inhibition of α-amylase by 54.95% (water extract), 53.01% (ethanol extract) and 47.87% (dichloromethane extract). The inhibition of *α*-glucosidase was found to be 48.48%, 48.48% and 43.85% for water, ethanol and dichloromethane extracts, respectively. The possible compounds that could be responsible for the enzyme inhibition were identified by GC-MS analysis as; Methyl 2-furoate, 5-hydroxymethyl furfural, Piperine, Palmitic acid, and Methyl Palmitate. The results revealed that the extracts of *Lilium polyphyllum* could be a possible source of natural antidiabetic medicine.

Keywords: Antidiabetic, *Lilium polyphyllum*, Alpha amylase, Alpha glycosidase.

Introduction

Diabetes is a metabolic disease associated with elevated blood glucose levels, leading to major complications such as diabetic neuropathy, nephropathy, retinopathy and cardiovascular diseases.[1] More than 100 million of the world's population are diabetic, and the disease reportedly now kills more people than AIDS.[2] Report over the past two decades revealed that diabetic patients are easily prone to obesity, stress, decrease in physical activities, and appetite loss.[3] People with Type 1 Diabetes do not produce insulin while the Type 2 Diabetics do not respond to insulin and often do not produce enough insulin. In conventional therapy, Type I diabetes is treated with exogenous insulin and Type 2 with oral hypoglycemic agents (sulphonylureas, biguanides, etc.)[4] In 2007, it was reported by[5] that diabetes caused about 3.5 million deaths globally. Diabetes initially considered as 'a disease of the rich now spread among all population[6] in India and the whole world.[7] Commercially a large number of oral hypoglycaemic drugs belonging to different classes such as biguanides, sulfonylureas, meglitinides and thiazolidinediones are available to control and treat type 2 diabetes. However, none of these drugs are known to completely cure the disease. On the other hand, long term use of these drugs exhibits several side effects and complications which ultimately lead to cardiovascular problems, liver Disease, kidney disease and weight gain. Like many other drugs, anti-diabetic drugs are also known to interfere and interact with other non-anti-diabetic drugs, especially when used for a long time. To combat the side effects of these drugs, complementary treatments may be found as a preventive measure and more promising in the management of the disease. Reports available from a large number of studies stated that complementary therapies may include physical exercises, dietary supplements and Nutraceuticals. Although, different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes, there is an increasing need for the use of natural products with antidiabetic activity.[8]

Herbal medicine has been part of human existence and its as old as life itself.[9] The use of herbs is an integral part of traditional medicine of different cultures around the world. Herbs are used for the prevention, treatment and mitigation of variety of diseases both in man and animals.[10] Nearly 80% of the human population of developing countries depend directly on traditional medicines, involving the use of plants, for their primary healthcare needs.

Lilium polyphyllum commonly known as Kshirkakoli or white lilly (family Liliaceae) is extensively used in many indigenous preparations from time immemorial. Kshirkakoli have been found to contain Sugar, Alkaloids, Flavonoids, and essential steroids.[11] Medicinally, the bulb of this specie is used as diuretic, antipyretic and as energy tonic.[12, 13] Few species of the plant have good economic importance, as they serve as medicines for treatment of diseases and are also taken as food.[14, 15] In the traditional system of medicine, the species have been reported to restore health and act as an antioxidant.[16,17] The present study, therefore, seek to investigate the enzyme; α-amylase and *α*-glycosidase inhibitory activity with a view to assessing the potential antidiabetic activity of the plant.

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Materials and Methods

Chemicals

All the chemicals used were of analytical reagent (AR) grade and were purchased from Sigma Merck. De-ionized water was used in the whole study.

Plant collection

Lilium polyphyllum Stem was collected from Kishtwar region of Jammu and Kashmir in July 2019, and was authenticated at FRI Dehradun with a reference number of 1575. The plant sample was air dried and grounded.

Extraction

Lilium polyphyllum Stem powder (75 g) was segregated from foreign matter, dried and weighed accurately after which extraction was carried out in a Soxhlet Apparatus. Various solvents with different polarity index were used in order of their increasing polarity (Dichloromethane (DCM), Ethanol and Water).

The powdered plant sample was first extracted with DCM. After complete extraction with DCM, the extract solution of DCM solvent was collected and subjected to filtration and the filtrate was evaporated to remove the volatile solvent to one-fourth of its volume over a water bath at a suitable temperature. The filtrate was completely dried into a powder form in an oven at 50- 60^{o}C.

The solid material left after complete extraction with DCM was then extracted with Ethanol in the same manner as mentioned above.

After extraction with ethanol, the plant material was collected from the thimble and was subjected to water extraction by decoction technique, in which the plant material was dissolved in 500 mL of water. The solution was heated over a water bath to remove all the water from the extract and then additional 500 mL of water was added to the extract, and the extract solution was finally evaporated to remove nearly 250 mL of water. The solution was filtered and the filtrate was evaporated to one-fourth of its volume, and then evaporated to dryness in an oven at 30-50^{o}C. The percentage yield of all the extracts was determined.

In vitro antidiabetic studies

The antidiabetic activity was evaluated by the inhibition of two main enzymes (α-amylase and α-glucosidase) which are responsible for the breakdown of higher polysaccharides into smaller molecular weight monosaccharides, like glucose, galactose, etc. The inhibition of these two enzymes limits the concentration of glucose in the blood stream, which in turn leads to antidiabetic effect.[18]

Inhibition of alpha-amylase

A total of 500 µL (100-1000 µg/mL) of test samples and standard drug (Acarbose) were added to 500 µL of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5 mg/mL) and were incubated at 25°C for 10 min. Thereafter, 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 incubated at 25°C for 10 min. The reaction was stopped with 1.0 mL of 3, 5-dinitro salicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min, and cooled to room temperature. The reaction mixture was diluted by the addition of 10 mL distilled water and absorbance was measured at 540 nm. Control represent 100% enzyme activity and were conducted in a similar way by replacing extract with the vehicle.[19, 20]

Inhibition of alpha-glycosidase

The alpha-glycosidase inhibitory activity was determined by incubating a 1 mL solution of starch substrate (2% w/v maltose or sucrose) with 0.2 M Tris buffer (pH 8.0) and various concentrations of the 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 540 nm.[21]

Calculation of 50% Inhibitory Concentration (IC_{50})

The concentration of the plant extracts required to inhibit 50% of the enzyme activity (IC_{50}) was calculated by using the percentage inhibitory activities at five different concentrations of the extract. Percentage inhibition (I %) was calculated as follows;

$$I\ \% = (Ac-As)/Ac\ X\ 100$$

Where Ac is the absorbance of the control and *As* is the absorbance of the sample.[22]

GC-MS Analysis

The GC-MS analysis of the ethanol extract of the plant was carried out using REX Column and acetone as a standard.

The Gas Chromatography-Mass spectrometry (GC-MS) analysis of ethanol extract was carried out by the method described by Hema *et al*.[23] The GC-MS analysis of the extract was performed using a GC-MS (Model; QP 2015 series, Shimadzu, Tokyo, Japan) equipped with a VF-5ms fused silica capillary column of 60 m length, 0.25 mm diameter and 0.25 mm film thickness. The injection mode used was split with flow control mode while Pressure (173.3 kPa), linear velocity (28.9cm/sec), Purge flow (3.0 mL/min) and split ratio (10.0). For GC-MS detection (GC-2010), an electron ionization system with ionization energy of 70eV was used. Helium gas (99.99%) was used as a carrier gas at a constant flow rate- total flow (16.3 mL/min.) and column flow (1.21 mL/min.), injector and mass transfer line temperature were set at 200 and 240°C, respectively. Total running time of GC-MS is 28 min. The software adopted to handle mass spectra and chromatograms was a Turbomass.

Identification of components/Interpretation of mass spectrum of GC-MS was done using the database of National Institute of Standard and Technology NIST-08 LIB.16 and WILEY-8 LIB.17 library sources were used for matching the identified components from the plant material having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library.

Statistical analysis

For the enzyme inhibitory activity, data were analysed using student-t test. All the experiments were performed in triplicate and the results are expressed in mean ± standard deviation. Significance of all the values obtained was tested by one way ANOVA at $P < 0.05$.

Results and Discussion

In this study, Acarbose was used as a standard drug for the inhibition of *α*-amylase and *α*-glucosidase, and it had been found that highest inhibition of the concerned enzymes was shown by water extract followed by ethanol and least results were observed for the DCM extracts. The results are shown in the respective figures below.

From Figures 1-8, it was be observed that the inhibition of α-amylase and *α*-glycosidase were dose-dependent. The percentage enzyme inhibition increases as the concentration of the plant extracts increased.

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

The ethanol extract of the concerned plant when analysed by GC-MS technique, the following compounds; Methyl 2-furoate, 5-hydroxymethyl furfural, Piperine, Palmitic acid, and Methyl Palmitate were identified (Table 1).

Diabetes mellitus is a metabolic disorder and the key factor responsible for it is insulin. The decline of insulin in the body has a direct effect on carbohydrate, fat and protein metabolism.[24] Presently. The use of allopathic medicines in the control diabetes mellitus is

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associated with numerous side effects, hence the reason scientists are looking towards herbal medicines as they are believed to be less associated with side effects. For this reason, the present study is based on the inhibition of alpha-amylase and alpha-glycosidase with the use of *Lilium polyphyllum* extract with the aim to delay the breakdown of carbohydrate, thereby causing a decline in the concentration of blood glucose. As a result, the increase in postprandial blood glucose level is reduced.[25]

The inhibitors of alpha-amylase and alpha-glucosidase decrease the digestion of carbohydrates, and reduce their absorption. Two standard drugs (Acarbose and Miglitol) are well-known inhibitors of α-glucosidases that reduces the absorption of starch and disaccharides.[26] Many researches are ongoing to investigate the role of herbal remedies in reducing 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 be reduced by the inhibition of *α*-amylase and *α*-glucosidase.[27] The inhibitors of *α*-amylase act as anti-nutrients, by obstructing the digestion and absorption of carbohydrates, among which Acarbose is a well-known inhibitor because of its complex oligosaccharide nature, it delays the digestion of carbohydrates, and so suppresses the action of pancreatic amylase in the breakdown of starch.

The present study therefore evaluated the alpha-glucosidase and alpha-amylase inhibitory activities of various extracts of *Lilium polyphyllum*. The finding reveals that *Lilium polyphyllum* efficiently inhibited both alpha-amylase and alpha-glucosidase. From the study, it had been found that the inhibitory potential of the various extracts increases correspondingly with the polar nature of the solvents.

The ethanol extract of *Lilium polyphyllum* was subjected to GC-MS analysis and the compounds Methyl 2-furoate[28], 5-hydroxymethyl furfural[29], Piperine[30] Palmitic acid[31] Methyl Palmitate[32] (Figure 9) which have been identified may contribute to the antidiabetic properties of the plant.

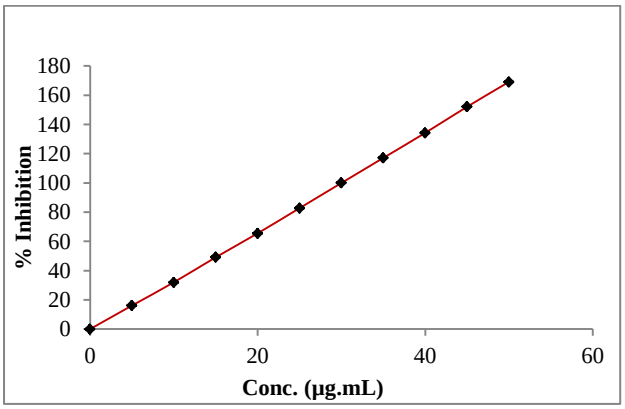

Figure 1: Inhibition of alpha-amylase by Standard Drug (Acarbose)

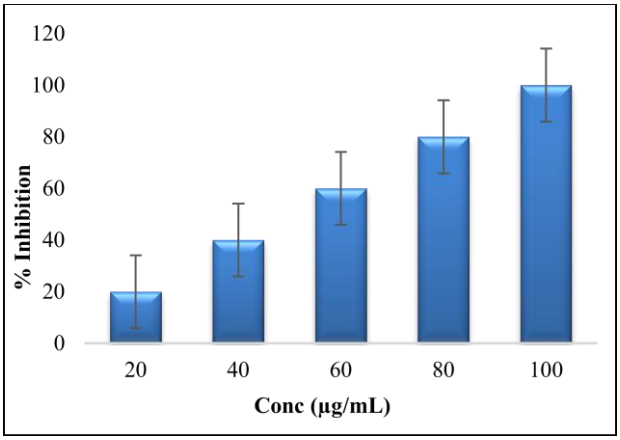

Figure 2: Inhibition of alpha-amylase by Water Extract

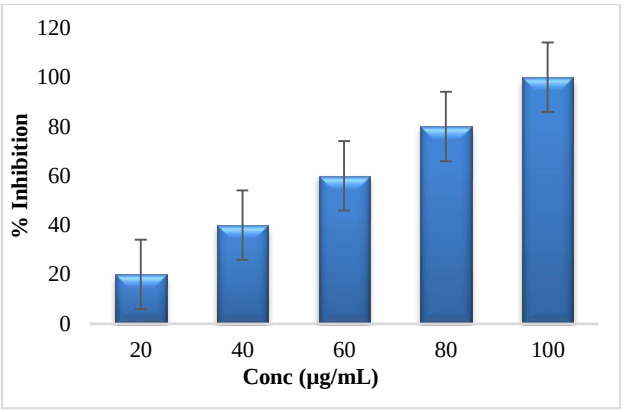

Figure 3: Inhibition of alpha-amylase by Ethanol Extract

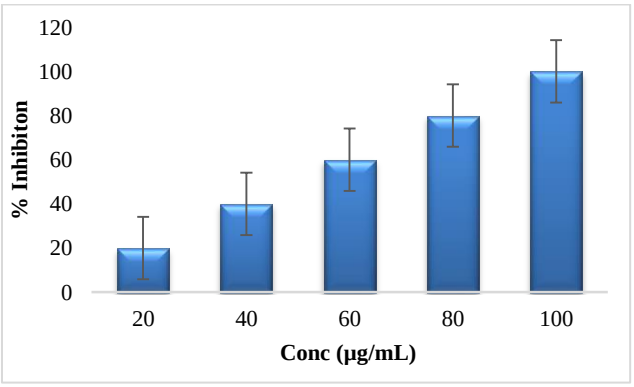

Figure 4: Inhibition of alpha-amylase by DCM Extract

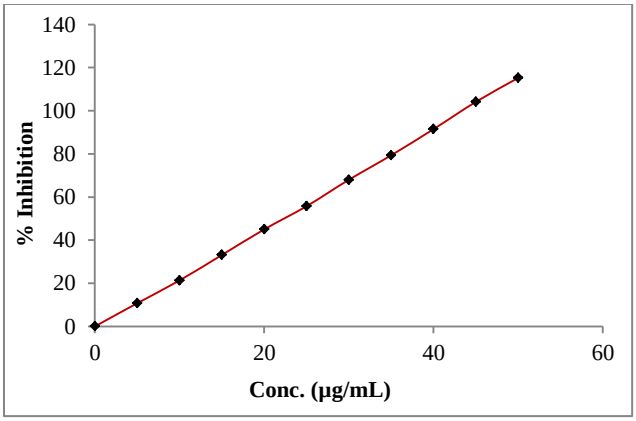

Figure 5: Inhibition of alpha-glycosidase by Acarbose

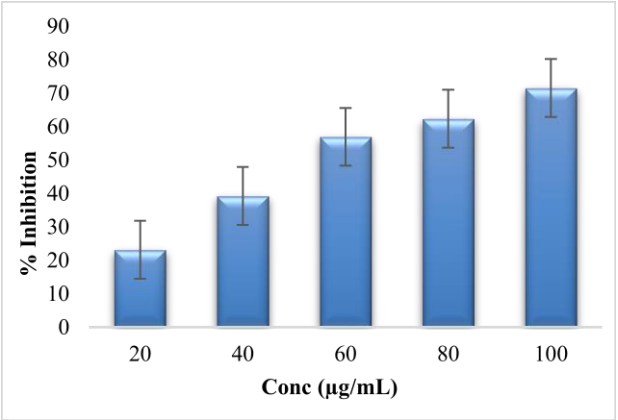

Figure 6: Inhibition of alpha-glycosidase by water Extract

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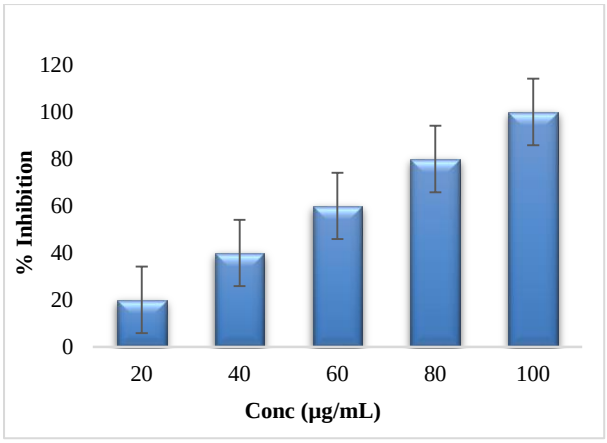

Figure 7: Inhibition of alpha-glycosidase by Ethanol Extract

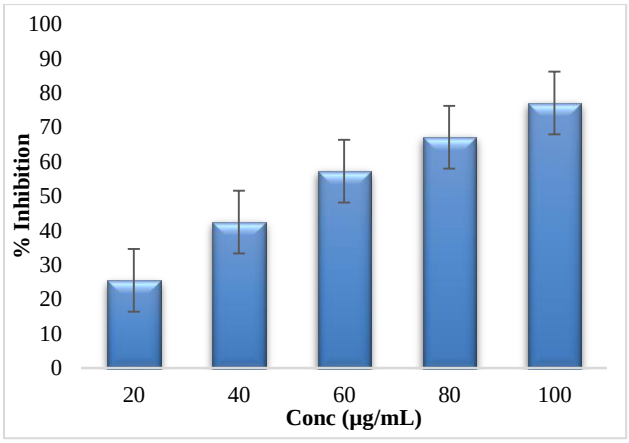

Figure 8: Inhibition of alpha-glycosidase by DCM Extract

Table 1: Compounds identified from the GC-MS Analysis of the ethanol extract of Lilium

S/N	R.T.	Compound Name	Molecular weight	Molecular formula	Activity
1.	11.303	Methyl 2-furoate	126.11	$C_6H_6O_3$	Anti-inflammatory, Antifungal
2.	10.797	5-hydroxymethyl furfural	126.03	$C_7H_5O_3$	Anti-inflammatory, Antimicrobial, Anticancer, treatment for asthma
3.	22.917	Piperine	285.34	$C_{17}H_{19}NO_3$	Rheumatoid, arthritis, Antibacterial, antifungal
4.	28.113	Palmitic acid	256.42	$C_{16}H_{32}O_2$	rheumatoid, arthritis, Antidiabetic, antifungal
5.	26.833	Methyl palmitate	270.45	$C_{17}H_{24}O_2$	Antimicrobial, Antifungal

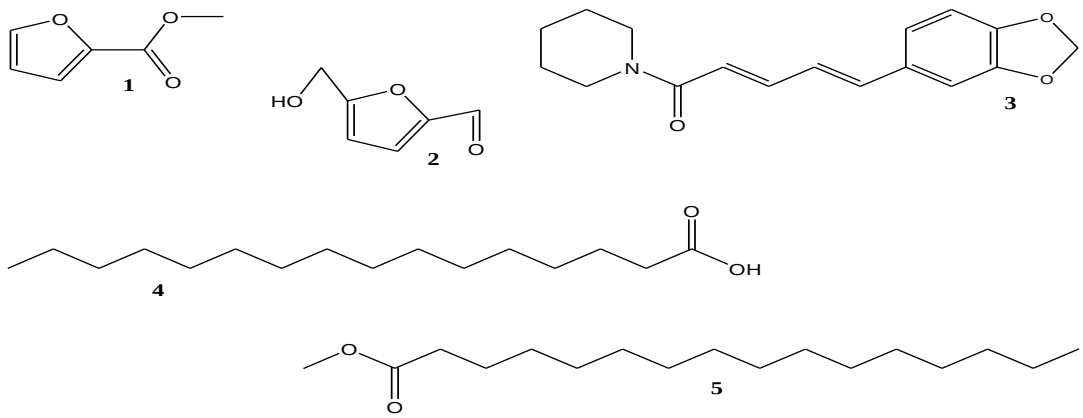

Figure 9: Chemical Structures of compounds identified from GC-MS analysis of Lilium polyphyllum extract

Conclusion

The present study showed that *Lilium polyphyllum* extracts (DCM, Ethanol and Water) have good α-amylase and *α*-glycosidase inhibitory potential. The amylase and glycosidase inhibitory potential of natural products have been found to act as a good choice for diabetic patients as they are believed to have little or no side effect. Therefore, *Lilium polyphyllum* may be a good source of herbal supplement in the control of diabetes so far it is consumed in moderate doses.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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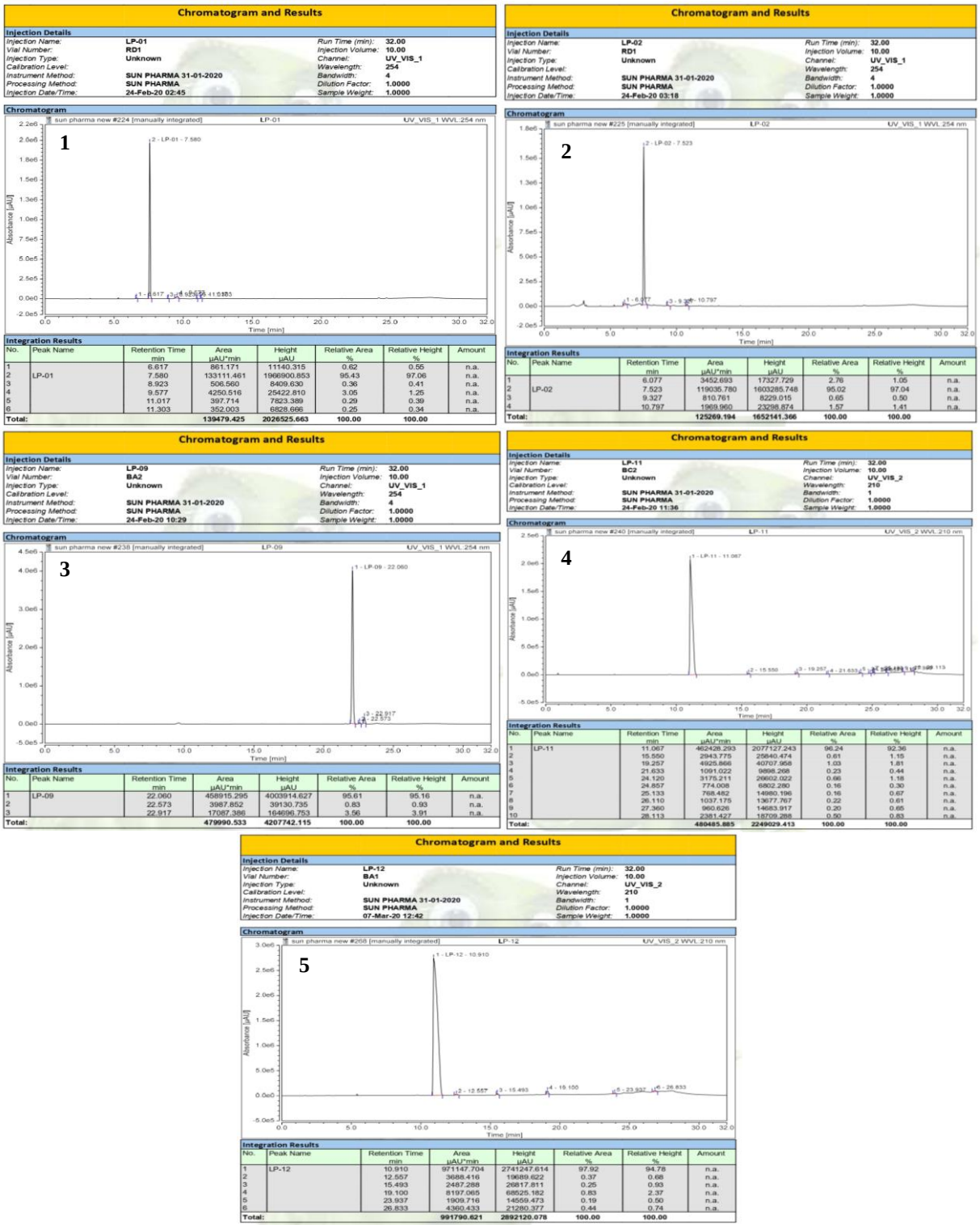

Figure 10: GC Chromatograms of compounds 1 – 5

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