Dual-Phase Antidiabetic Efficacy of Cultivated *Cordyceps sinensis*: Mechanistic In-Vitro Validation and Therapeutic In-Vivo Outcomes

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Abstract- Cultivation is an effective conservation approach that can balance market demand and species protection. Wild and cultivated *Cordyceps sinensis* were investigated to understand possible variation in potential anti-diabetic-related bioactivity. Diabetes was induced in Wistar rats by the administration of a HFD for 15 days/STZ (35 mg/kg b.w., i.p.). *Cordyceps sinen-sis* at dose of 50 and 100 mg/kg b.w., p.o. was administered to diabetic rats for 28 days in HFD/STZ-induced Type 2 diabetic rats The effect of carbohydrate molecule of cultivated *C. sinensis* (CS) on blood glucose and body weight was studied in Type 2 diabetic rats. All these effects were compared with glibencl-amide (5 mg/kg b.w., p.o.) as a reference antidiabetic drug. HPLC and HPTLC analysis of water extracts of wild and laboratory adopted *C. sinensis* revealed similar properties and there was not any difference observed. The concentration of carbohydrate molecule in cultivated *Cordyceps sinensis* was 5% and the purity of extracted carbohydrate molecule from cultivated *Cordyceps sinensis* powder by HPLC was observed very high carbohydrate molecule of *Cordyceps sinensis* shows significant *In-vitro* antidiabetic activity. The administration of CS (50 and 100 mg/kg b.w., p.o.) resulted in a significant decrease in blood glucose level, body weight and significant increase in serum insulin level when compared to diabetic control. The results suggest that *Cordyceps sinensis* possesses a promising effect on the HFD/STZ -induced Type 2 diabetes.

Keywords: *Cordyceps sinensis*, Cultivation, Carbohydrate molecule, HPLC, HPTLC, Diabetes, *in-vitro, in-vivo* Streptozotocin, High Fat Diet, Cholesterol, Triglyceride, SGOT and SGPT.

Introduction

Diabetes is a group of comorbid diseases characterized by chronic hyperglycaemia, inadequate insulin secretion, and elevated blood glucose levels. It is characterized by disturbances of carbohydrate, protein, and fat metabolisms, secondary to absolute or relative lack of the hormone insulin (Bhutkat et al. 2012). The number of people in the world with diabetes has increased dramatically over recent years. It is also predicted that by 2030, India, China, and the United States will have the largest number of people with diabetes (Wild et al., 2004). The two principal forms of diabetes

are i) insulin-dependent (type 1) and ii) non-insulin-dependent (type 2). Non-insulin-dependent diabetes accounts for 90% of all cases worldwide. Thus, management of the blood glucose level is a critical strategy in the control of diabetes complications.

The current treatment for type 2 diabetes includes insulin and oral hypoglycemic drugs such as biguanides, thiazolidinediones, sulfonylurea derivatives, and α-glucosidase inhibitors. These medications have side effects, e.g. osteoporosis, obesity, and sodium retention by thiazolidinedione. Incidences of severe hypoglycemia by sulfonylurea and biguanide (metformin) put patients at risk of developing lactic acidosis (Hamza et al., 2010). Further, for most diabetic patients, the oral monotherapy with lifestyle changes is not sufficient and requires various oral combinations or the addition of insulin (Stumvoll et al., 2005). Thus, there is an increasing need to identify and explore more effective anti-diabetic agents with fewer side effects. As a result, herbals have received attention as sources of antioxidants and hypoglycaemic and anti-hyperlipidemic agents. *Cordyceps sinensis* has been known and used for many centuries in traditional Chinese medicine (TCM). Most of the people in the Indian Himalayan region called it “keerajari.” Traditionally it is well known as herbal Viagra (Kobayasi 1982 and Mizuno 1999). The Latin word "conjunction" aptly describes this club-shaped fungus, whose elongated stroma and fruiting body emerge from the mummified remains of insect larvae, typically those of the Himalayan ghost moth (*Thitarodes armoricanus*, formerly *Hepialus armoricanus*). *C. sinensis* has two parts: larva and stroma. The larva is 10-15 mm in length and 50 mg in weight, and the stroma is 5-10 mm in length and 60 mg in weight (Bhandari *et al.*, 2010; Holliday *et al.*, 2004; and Mizuno, 1999). *Cordyceps sinensis* (now *Ophiocordyceps sinensis*) contains a diverse array of bioactive compounds, such as nucleosides (e.g., cordycepin and adenosine), ergosterol and its derivatives, mannitol, peptides, poly saccharides, proteins, polyamines, and amino acids, which contribute to its medicinal properties (Schmidt et al., 1995; Benowitz et al., 2002; Bok et al., 1999; Gong et al., 2001; Holliday et al., 2004). *Cordyceps sinensis* contains a significant amount of polysaccharides, comprising 3 to 8% of the total dry weight (Li et al., 2001, 2002).

Cordyceps sinensis (caterpillar fungus) or yarsagumba is an exceptional and incredible mushroom that grows in the pastures above 3,500 meters to 5,000 meters in the Himalayan region of Nepal, Bhutan, Tibet, China, and India. It is also referred to as "Himalayan Viagra" or "Himalayan Gold" due to its significant medicinal and commercial value (Kinjo and Zhang, 2001; Mayaram U., 2011). Numerous scientific studies and research reveal that it has antibiotic properties and is used for the treatment of lung and respiratory infections, pain, sciatica, and backache. It also enhances vitality and increases the physical stamina of the body. (Wang et al. 2000). Cultivation is an effective conservation approach that can balance market demand and species protection. This study investigated polysaccharide profiles (via HPLC/ HPTLC) in wild vs. cultivated *Cordyceps sinensis* and evaluated cultivated *C. sinensis* ’s antidiabetic effects in a high-fat diet/STZ-induced diabetic rat model.

Material and Methods

Cultivation and Identification of *Cordyceps sinensis*

The *Cordyceps sinensis* culture was obtained from the Forest Research Institute, Dehradun,

and initially grown on potato dextrose agar (PDA) slants at 28°C for 5 days. For liquid culture, mycelia were inoculated into potato dextrose broth (PDB) and incubated at 27°C for 4 days. Subsequent sub-culturing involved streaking mycelia onto fresh PDA plates, followed by incubation at 28°C for 5 days. Fungal colonies were examined micro-scopically after culturing on concavity slides and staining with phenol cotton blue to observe mycelial morphology and conidiophores (Chen et al., 2006).

Animals and Ethical Approval

Male Wistar albino rats (180–240 g) were procured from NIPER (Mohali) and housed at Shoolini University's animal facility (Solan, HP) under controlled conditions (25 ± 2°C; 45 ± 5% relative humidity) with *ad libitum* access to food and water. The high fat diet were provided to the rats induced with diabetics (Table-1). After a 7-day acclimatization period, experiments were conducted in compliance with CPCSEA guidelines. The rats were divide into five groups and each group comprised of 6 rats (Table-2). The Institutional Animal Ethics Commi-ttee (IAEC) approved all protocols (Approval No. SUBMS/ADM/900-1).

Comparative Analysis of wild and cultivated *Cordyceps sinensis* using chromatographic techniques

a. High Performance Thin Layer Chromatography (HPTLC) Methanolic extracts of wild and cultivated *C. sinensis* (1 g each, air-dried) were prepared by refluxing with 10 mL methanol for 10 min, followed by filtration. The extracts (10 µL) were applied band wise to HPTLC plates (Merck 60 F254) using a CAMAG Linomat-5 applicator (8 mm from the lower edge, 35 mm from the left edge). Separation was performed in a CAMAG Twin Trough Chamber (10 × 10 cm) with dichloromethane: methanol (95:5, v/v) as the mobile phase (migration distance: 80 mm). Bands were visualized under UV light at 254 and 366 nm.

b. High Performance Liquid Chromatography (HPLC) Aqueous extracts were prepared by ultrasonicating 5g of dried *C. sinensis* powder (wild and cultivated) in 70 mL double-distilled water for 30 min, followed by dilution to 100 mL. After membrane filtration (0.2 µm), samples (20 µL) were injected into an HPLC system equipped with a C18 column (Luna C18, 150 × 4.6 mm). The mobile phase consisted of disodium hydrogen phosphate buffer (pH 6.0) and methanol (85:15, v/v) at a flow rate of 1.0 mL/min. Detection was performed at 260 nm (Guo et al., 1998).

c. Extraction of Polysaccharides from *Cordyceps sinensis*

Cultivated *C. sinensis* powder (10 g) was sequentially decolorized with 95% ethanol and extracted overnight with 75% aqueous ethanol. The extract was centrifuged (6000 rpm, 30 min), and the supernatant was concentrated under reduced pressure. After dialysis, the non-dialyzable fraction was precipitated with 95% ethanol, followed by centrifugation. The precipitate was washed with acetone (3×) and dried at 65°C to constant weight (Zhang et al., 2005).

d. Estimation of Carbohydrates Derived from *C. sinesis* by HPLC

i. Standard Preparation-Sucrose (100 mg) was dissolved in 70–80 mL purified water in a 100 mL volumetric flask and sonicated for 10 min. The volume was adjusted to 100 mL with purified water. The solution was

filtered through a 0.2 µm membrane and transferred into 1.5 mL vials for HPLC analysis.

ii. Sample Preparation -Carbohydrate ex-tract (50 mg) from *C. sinensis* was dissolved in 7–8 mL purified water in a 10 mL volumetric flask and sonicated for 10 min. The volume was made up to 10 mL with purified water, filtered (0.2 µm), and aliquoted into 1.5 mL vials.

iii. HPLC Analysis- Samples (20 µL) were injected into a SHIMADZU HPLC system (LC-2010 HT) equipped with a Luna C18 column (150 × 4.6 mm, Phenomenex). The mobile phase consisted of acetonitrile: water (75:25, v/v) at a flow rate of 1.25 mL/min. Detection was performed at 193 nm.

e. *In-Vitro* Antidiabetic Assay (α Glucosidase Inhibition Assay)

α-Glucosidase inhibitors competitively inhibit intestinal α-glucosidase, delaying carbohydrate digestion and reducing postprandial hyperglycemia. The assay was conducted as follows:

i. Reaction Setup

a. Labeled Eppendorf tubes (blank, control, and sample concentrations) were arranged in a rack.

b. To each tube, 600 µL potassium phosphate buffer (pH 6.8), 100 µL test sample, and 25 µL α-glucosidase (1.2 EU/mL) were added.

ii. Incubation

a. Tubes were vortexed and incubated at 37°C for 15 min.

b. After incubation, 25 µL p-nitrophenyl-α-D-glucopyranoside (PNPG, 5 mM) was added, and tubes were re-incubated at 37°C for 15 min.

iii. Measurement

Absorbance was measured at 405nm using a SHIMADZU UV – 1800 spectrophotometer.

f. Evaluating Antidiavetic Potential (In-vivo assessment)

i. Quantitative Analysis of *Cordyceps sinensis* **Carbohydrates via HPLC Standard Preparation-** A sucrose standard solution was prepared by dissolving 100 mg of sucrose in 70-80 mL of purified water within a 100 mL volumetric flask, followed by 10 minutes of sonication. The volume was then adjusted to 100 mL with purified water. The resulting solution was filtered through a 0.2 µm membrane filter and transferred to 1.5 mL HPLC vials.

ii. Sample preparation: For sample analysis, 50 mg of isolated *C. sinensis* carbohydrates were dissolved in 7-8 mL of purified water in a 10 mL volumetric flask and sonicated for 10 minutes. After bringing the volume to 10 mL with purified water, the solution was membrane-filtered (0.2 µm) and aliquoted into 1.5 mL vials.

iii. Chromatographic Conditions: Samples (20 µL injection volume) were analyzed using a SHIMADZU LC-2010 HT HPLC system equipped with a Phenomenex Luna C18 column (150 × 4.6 mm). The mobile phase consisted of acetonitrile: water (75:25, v/v) delivered at a flow rate of 1.25 mL/min, with detection at 193 nm.

Table-1 Nutritional Architecture of High-Fat Diet (HFD) for STZ-Assisted Diabetes Induction in Wistar Rats

Ingredients	Quantity/kg
Powdered NPD	365g
Lard	310g
Casein	250g
Cholesterol	10g
Vitamin and mineral mix	60g
dl- Methionine	01g
Yeast powder	01g
Sodium chloride	01g

Table-2 Experimental Groups and Sample Size, each group contain six rats (n=30).

Group 1	Normal control	Normal rats + vehicle
Group 2	Diabetic control HFD for 15 days+	Streptozocin (35 mg/kg b.w., i.p.)+vehicle
Group 3	Treated group	HFD for 15 days + streptozocin (35 mg/kg b.w., i.p.) + CS (50 mg/kg b.w., p.o.)
Group 4	Treated group	Treated group HFD for 15 days + STZ (35 mg/kg b.w., i.p.) + CS (100 mg/kg b.w., p.o.) for 28 days
Group 5	Standard group	HFD for 15 days + STZ (35 mg/kg b.w., i.p.) + glibenclamide (5 mg/kg b.w., p.o.)

g. Biochemical and Physiological Assessments

i. **Blood Glucose Measurement-** Fasting blood glucose levels were determined using an **AccuSure® glucometer (ARMM Healthcare)**. Blood samples were collected from the tail vein after a **12-hour fasting period** to ensure baseline measurements.

ii. **Serum Lipid Profile Analysis I-**Serum lipid parameters, **total cholesterol, LDL-c, HDL-c, and triglycerides** were quantified using a **commercial photometric assay kit** (Cholesterol & Triglyceride 2-in-1 Test Meter Kit, Biochemical Systems International Prime). All measurements were performed **in triplicate**, strictly adhering to the manufacturer's protocols.

iii. **Body Weight Monitoring-** Body weights were recorded using a **high-precision digital balance (±0.1g sensitivity)** at **consistent morning time-points** to minimize variations due to diurnal fluctuations.

iv. **Statistical Analysis-** Data are expressed as **mean ± standard deviation**. **One-way ANOVA** followed by **Bonferroni's posthoc test** was used for multiple comparisons. A **p-value < 0.05** was considered statistically significant.

Results and Discussion

Cultivation and Identification of *C. sinensis*

The fungal colony grown on Potato Dextrose Agar (PDA) exhibited a white, floccose, and orbicular morphology, with a fawn-colored reverse. After 14 days of incubation at 28°C, the colony diameter reached 30–40 mm (Figure-1 (A) and (B)).

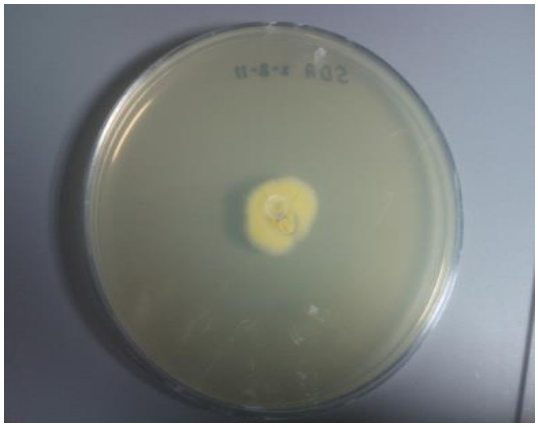

Figure-1 (A) *In-vitro* Colony formation of *Cordeceps sinensis* grows on Potato Dextrose Agar

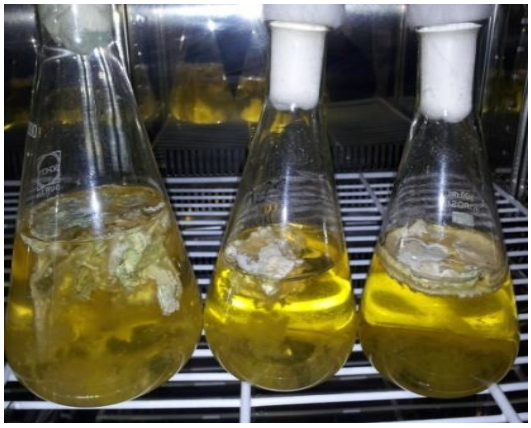

Figure-1 (B) *In-vitro* cultivated Mycelium of *Cordeceps sinensis* grows in Potato Dextrose Brot

HPTLC Chromatographic Profiling of Wild and Cultivated *Cordyceps sinensis* at 254 and 366 nm

i. **Distinct Banding Patterns**- Both wild and cultivated *C. sinensis* extracts showed **well-resolved bands**, indicating the presence of multiple bioactive compounds.

ii. **UV 254 nm (Non-UV Active Compounds) (Figure-2 A)**

a. Wild *C. sinensis* exhibited **stronger absorption bands**, suggesting higher concentrations of certain non UV active metabolites (e.g., nucleosides, sugars).
b. Cultivated samples showed com-parable **but slightly reduced band intensity,** possibly due to variations in growth conditions.

iii. **UV 366 nm (Fluorescent Compounds) (Figure-2 B)**

a. Wild samples displayed **prominent fluorescent bands**, likely corresponding to **bioactive alkaloids or phenolic compounds.**
b. Cultivated samples **retained similar qualitative profiles** but with **mild differences in band intensity**, indicating consistent secondary metabolite production under controlled cultivation. The HPTLC analysis **confirmed the presence of key phyto-constituents** in both wild and cultivated *C. sinensis*, with **minor quantitative variations**. This suggests that **cultivated** *C. sinensis* **can serve as a sustainable alternative** to wild-harvested specimens, retaining comparable biochemical profiles (Figure-2).

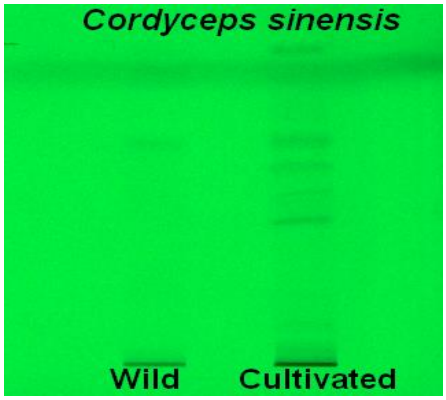

Figure-2 (A) at 254 nm

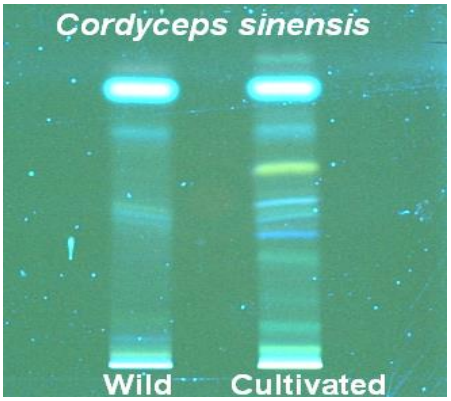

Figure-2 (B) at 366 nm

Figure-2 HPTLC Comparison of Wild vs. Cultivated *C. sinensis* Methanol Extracts at 254 nm Figure-2 (A) and 366 nm Figure-2 (B)

Densitometric quantification of wild and cultivated *Cordyceps sinensis* fractions

HPTLC plates were scanned at 366 nm using a CAMAG TLC Scanner with CATS software. Both samples exhibited identical migration patterns with a characteristic peak at Rf 0.78, confirming equivalent retention behaviour of the target metabolite(s). The superimposed densitograms demonstrate comparable phytochemical composition between wild and cultivated specimens. Densitometric evaluation (366 nm) shows wild and farmed *C. sinensis* share identical chromatographic behaviour (Rf 0.78), confirming preservation of key fluorescent metabolites under cultivation conditions. (Figure-3 A and B)

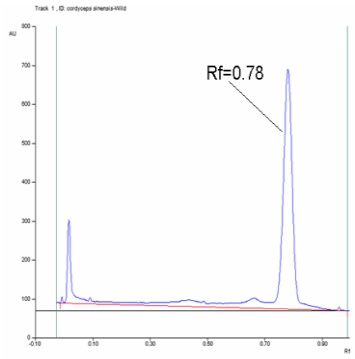

Figure-3 (A) Wild *C. sinensis* Rf value

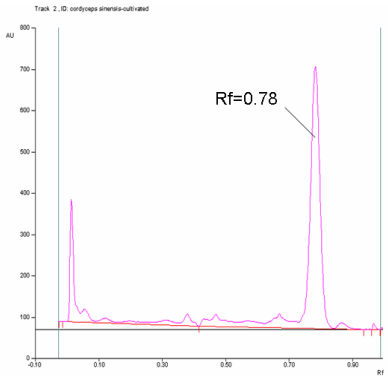

Figure-3 (B) Cultivated *C. sinensis* Rf value

Figure-3 Densitometric confirmation of equivalent bioactive compounds in wild and cultivated *C. sinensis* . Quantitative scanning at 366 nm revealed matching chromatographic peaks (Rf 0.78), validating comparable composition between natural and cultivated sources."

Figure-4 showing demonstrating the spectroscopic confirmation of identical bioactive compounds in wild and cultivated *Cordyceps sinensis.* The UV spectral comparison (λ=284 nm) of chromatographic fractions at Rf 0.78 demonstrates perfect overlay between wild and cultivated extracts, confirming identical chemical composition at this retention position. This analytical validation confirms that cultivation preserves the key bioactive constituents found in natural *C. sinensis*.

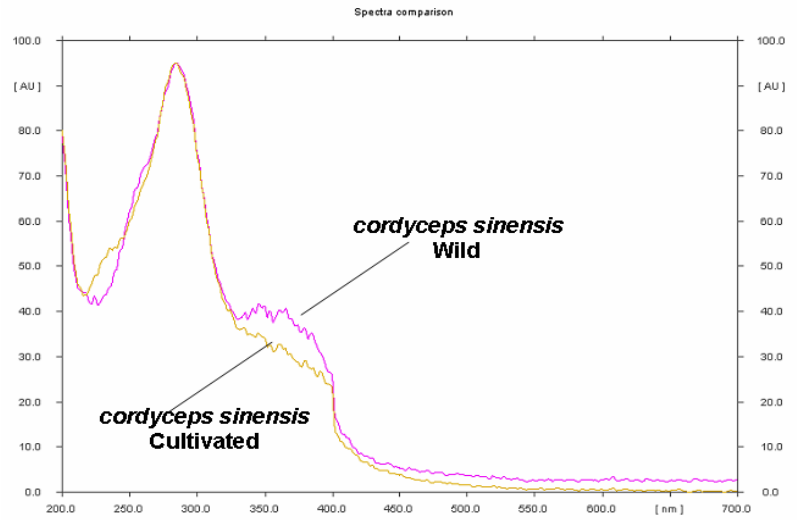

Figure-4 Chemical identity validation by UV spectroscopy. Methanolic extracts of wild and cultivated *C. sinensis* showed identical spectral patterns (200-400 nm) for fractions eluting at Rf 0.78, with characteristic maxima at 284 nm (n=3, CAMAG Scanner III, CATS v4.6). This confirms preservation of target UV-active metabolites in cultivated specimens.

Comparative HPLC Analysis of Bioactive Compounds in Wild and Cultivated *Cordyceps sinensis*

Table-3 demonstrates distinct metabolic differences between cultivated and wild *Cordyceps sinensis*. The cultivated variety exhibits significantly higher concen-trations of uridine, adenine, and adenosine, while showing lower levels of uracil and cordycepin compared to its wild counterpart. The retention times remain consistent between both sources, confirming identical chemical structures for these bioactive compounds. These findings suggest that cultivation con-ditions may preferentially enhance pyrimidine and purine metabolism (as evidenced by increased uridine, adenine, and adenosine production) while potentially suppressing the biosynthesis of cordycepin a particularly valuable bioactive component. The consistent retention times across samples further validate the reliability of the HPLC analytical method for such comparative studies.

Table-3 Comparative HPLC Analysis of Bioactive Compounds in Wild vs. Cultivated *Cordyceps sinensis*

Active components	Retention time (in min) (Wild)	Area under curve (Wild)	Retention time (in min) (Cultivated)	Area under curve (Cultivated)
Uracil	3.606	568363	3.600	55190
Uridine	3.890	991711	3.886	3359142
Adenine	7.434	27733	7.424	114976
Adenosine	7.981	54537	8.021	246140
Cordycepin	10.640	229752	10.033	122256

The HPLC chromatograms of wild (A) and cultivated (B) *Cordyceps sinensis* revealed distinct differences in the abundance of key metabolites. Specifically:

(i) The AUC (Area under the Curve) of uracil was lower in cultivated *C. sinensis* compared to the wild variant

(ii) Uridine levels were higher in cultivated samples than in wild ones;

(iii) Adenine content was greater in cultivated *C. sinensis*

(iv)Adenosine also showed higher accumulation in cultivated specimens

(v) Conversely, cordycepin was more abundant in wild *C. sinensis* than in cultivated samples.

Notably, the retention times of these bioactive compounds remained identical between wild and cultivated *C. sinensis*, confirming that the same chemical constituents were present in both. (Figure-5)

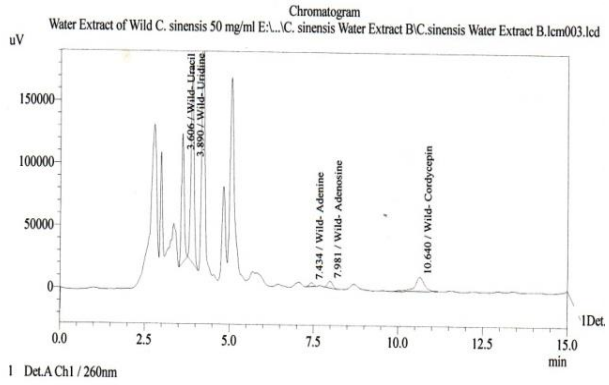

Figure-5 (A)
HPLC Chromatogram of Wild *C. sinensis*

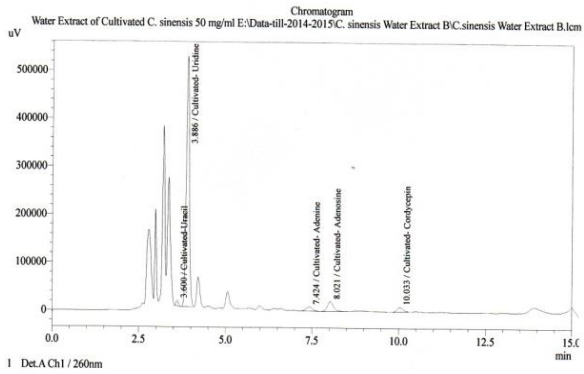

Figure-5 (B)
HPLC Chromatogram of Cultivated *C. sinensis*

The HPLC analysis revealed significant findings regarding the carbohydrate composition of cultivated *Cordyceps sinensis*. An unidentified carbohydrate molecule was detected with an exceptionally high peak area (47,660,232), suggesting it may serve as either a dominant storage carbohydrate (possibly a polysaccharide) or a primary energy reserve in cultivated specimens. This molecule exhibited a retention time (1.758 min) remarkably close to the sucrose standard (1.714 min), indicating potential structural similarity. The observed peak area was approximately 750 times greater than that of the sucrose standard (47,660,232 vs. 63,528), demonstrating its overwhelming abundance in cultivated samples (Table-4). This substantial quantity suggests possible adaptation to cultivation conditions and enhanced carbohydrate biosynthesis in artificial growth environments.

The minimal retention time difference ($\Delta RT=0.044$ min) from sucrose raises the possibility that this compound could be either a sucrose derivative (such as a fructooligosaccharide) or a novel bioactive polysaccharide characteristic of cultivated strains. The remarkably high carbohydrate content may help explain both the immunomodulatory properties of cultivated *C. sinensis* and its successful adaptation to artificial substrates like grain-based media. Furthermore, this prominent carbohydrate component could potentially serve as a quality marker for cultivated specimens, warranting further investigation into its exact structure and biological functions.

Table-: Analysis of Carbohydrate molecule derived from cultivated *C.sinensis* by HPLC

Active components	Retention time	Area under curve
Sucrose (Standard-Sigma)	1.714	63528
Carbohydrate molecule	1.758	47660232

The HPLC chromatographic analysis yielded a remarkable discovery regarding the carbohydrate profile of cultivated *Cordyceps sinensis*. The isolated carbohydrate fraction exhibited an exceptionally high peak area that dramatically exceeded the sucrose standard, with an area under the curve (AUC) of 47,660,232 compared to just 63,528 for sucrose - representing a striking 750-fold greater abundance. This overwhelming predominance suggests either the accumulation of an unusually abundant storage polysaccharide or the presence of a novel high-molecular-weight carbohydrate with unique biochemical properties in cultivated specimens. The observed metabolic profile points to several significant biological implications: a potential cultivation-induced metabolic shift favoring carbohydrate biosynthesis, an adaptation mechanism to artificial growth substrates, and the possible existence of bioactive polysaccharides with therapeutic value. These findings may help explain the renowned immune-modulatory effects of cultivated *C. sinensis* and its successful adaptation to laboratory cultivation conditions, while also suggesting potential quality markers for standardized extracts. The chromatographic data, showing retention times of 1.758 min for the *C. sinensis* carbohydrate versus 1.714 min for sucrose, reveal this dramatic difference in carbohydrate composition between the fungal extract and standard reference compound. This discovery opens new avenues for research into cultivation-specific metabolites and their pharmacological potential, strongly warranting further structural characterization and biological evaluation of this predominant carbohydrate component (Figure 5 A and B).

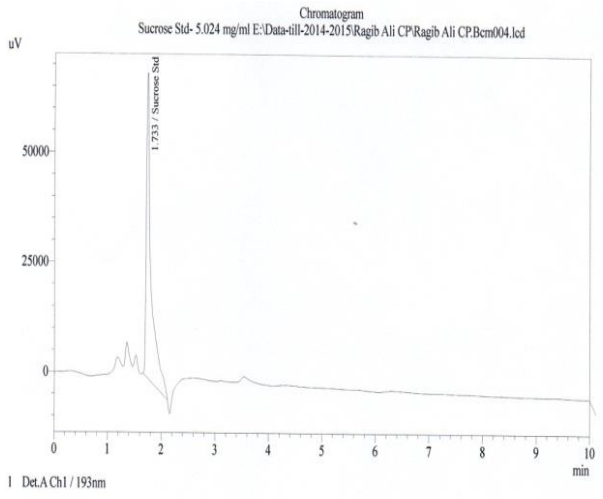

Figure-5 (A)
HPLC Chromatogram of Standard (Sucrose)

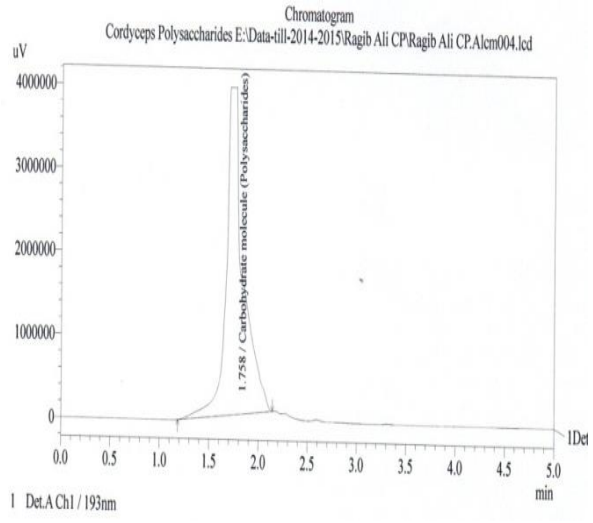

Figure-5 (B)
HPLC Chromatogram of Sample (*C. sinensis* ext.)

HPLC Analysis Reveals Extraordinary Carbohydrate Profile in Cultivated *C. sinensis*

Evaluation of Alpha-Glucosidase Inhibitory Activity

The evaluation of alpha-glucosidase inhibitory activity (Table-5) reveals significant therapeutic potential in the *C. sinensis* carbohydrate molecule. Com-pared to the commercial antidiabetic drug acarbose (standard IC_{50} = 132.5 μg/mL), the fungal carbohydrate demonstrated comparable inhibition with an IC_{50} of 147.6 μg/mL. These findings suggest this natural compound could serve as an effective alternative to synthetic antidiabetic drugs, potentially functioning as an effective alternative to synthetic antidiabetic drugs, potentially functioning as an adjunct therapy for diabetes management. The carbohydrate molecule emerges as both a promising candidate for blood glucose regulation and a potential source of novel anti-diabetic compounds. Notably, the marginal difference in IC_{50} values (132.5 vs. 147.6 μg/mL) indicates this natural product may be suitable for development as either a standalone therapy or complementary treatment for post prandial hyperglycemia.

Table-5 Alpha-Glucosidase Inhibition Profile of *C. sinensis* Carbohydrate: Comparative IC_{50} Analysis with A carbose Standard

Samples	Standard IC_{50} Value of (μg/ml)
Acarbose (Std)	132.5
Carbohydrate molecule (Spl)	147.6

Effects of *Cordyceps sinensis* Carbo-hydrate on Hyperglycemia in Type 2 Diabetic Rats

The study demonstrated that HFD/STZ-treated diabetic rats exhibited signify-cantly elevated blood glucose levels compared to normal controls ($p < 0.01$). Oral administration of ***Cordyceps sinensis*** carbohydrate extract (EEAI) at doses of 100 and 200 mg/kg body weight produced dose dependent antihyper-glycemic effects, with the higher dose (200 mg/kg) showing enhanced glucose-lowering activity. Notably, the extract's efficacy was comparable to the standard antidiabetic drug glibenclamide as evidenced by blood glucose monitoring (Table 2). These findings, derived from a high-fat diet/streptozotocin-induced Type 2 diabetes model, suggest that the ***C. sinensis*** -derived carbohydrate possesses significant glucose-lowering capacity, exhibits dose-responsive therapeutic effects, and mirrors pharmaceutical standards in activity. The results highlight its potential as a promising natural intervention for diabetes management, warranting further investigation into its clinical applications (Table-6).

Therapeutic Efficacy of ***Cordyceps sinensis*** Carbohydrate on Body Weight Regulation in HFD/STZ-Induced Type 2 Diabetic Rats. The study investigated the impact of a ***Cordyceps sinensis*** derived carbo-hydrate molecule on body weight regulation in HFD/STZ-induced Type 2 diabetic rats. Initially, diabetic rats exhibited significant weight gain compared to normal controls, consistent with metabolic dysregulation character-istic of insulin resistance. Following 28 days of therapeutic intervention, daily oral administration of the CS carbohydrate at doses of 50 and 100 mg/kg body weight produced a remarkable normalization of body weight,

bringing diabetic rats to weight parameters comparable to the healthy control group (Table-6). This dose-dependent reduction in body weight suggests the CS carbohydrate may effectively counteract the metabolic disturbances induced by the high-fat diet and streptozotocin. The observed effects could be attributed to multiple mechanisms including improved insulin sensitivity, enhanced lipid metabolism, or modulation of adipokine secretion. These findings position the CS carbohydrate as a promising therapeutic agent for managing obesity-related metabolic disorders, with particular relevance to Type 2 diabetes complications. The complete normalization of body weight at both tested doses indicates potent bioactivity worthy of further investigation into its molecular targets and long-term metabolic benefits.

Dose-Dependent Modulation of Serum Lipids and Insulin by *Cordyceps sinensis* Carbohydrate in a Rodent Model of Diabetic Dyslipidemia

The carbohydrate fraction isolated from cultivated *Cordyceps sinensis* (CS) demonstrated significant metabolic improvements in HFD/STZ-induced Type 2 diabetic rats. Oral administration at doses of 50 and 100 mg/kg body weight produced dose-dependent therapeutic effects, notably reducing key serum parameters including glucose, cholesterol, triglycerides, SGOT, and SGPT levels (Table-8). Importantly, CS treatment significantly elevated blood insulin levels (Table-7), suggesting potential mechanisms involving both pancreatic β-cell function enhancement and peripheral insulin sensitivity improvement. These comprehensive modulations of the serum lipid profile and glycemic markers position the CS carbohydrate as a multifunctional therapeutic candidate for diabetes-associated dyslipidemia and hepatic steatosis. The parallel improvement in both hepatic markers (SGOT/SGPT) and metabolic parameters indicates hepato-protective effects alongside anti-diabetic activity, high-lighting it's potential for managing metabolic syndrome components.

Table-6 Comparative Body Weight Analysis in Normal Control, HFD/STZ-Induced Diabetic, and *C. sinensis* -Treated Rats Over 8 Weeks

Groups (NPD)	Body weight	
	1ST day (gm)	28th day **(gm)**
Normal control	194±2.02	234±2.01
Diabetic control	198±1.02	162±2.21
CS (50mg/kg)	193±3.21	173±2.83
CS (100mg/kg)	195±1.87	179±1.05
Std	197±1.39	191±1.34

Table-7 Metabolic Profile Alterations: Biochemical Parameters in Normal Diet-Fed vs. HFD/STZ-Induced Diabetic Rats

Groups	Blood glucose		Insulin level (Sensitivity)	
	1 day (mg/dl)	**28 day (mg/dl)**	**1 day (mg/dl)**	**28 day (mg/dl)**
Normal control	89.09±2.56	89.92±2.21	15.47±2.12	16.01±1.23
Diabetic control	213±3.21	217±1.99	5.88±1.21	6.51±1.45
CS (50mg/kg)	215±3.17	151±2.09	6.01±2.21	9.51±1.09
CS (100mg/kg)	213±2.65	121±1.21	6.91±1.84	12.74±1.63
Std	214±2.45	112±1.31	6.31±1.67	15.43±1.56

Table-8 Metabolic Profile Comparison: Biochemical Parameters in Normal Diet vs. HFD/STZ-Induced Diabetic Rats

Tests	Normal control	Diabetic control	CS (50 mg/kg)	CS (100mg/kg)	Std
Cholestrol (mg/dl)	54.33±3.09	155.32±4.01	122.09±2.02	102.33±3.33	82.09±2.11
Triglyceride (mg/dl)	53.83±2.12	141±2.12	111.51±1.23	101.23±2.20	72.89±2.11
SGOT (u/l)	45.7±1.21	79.2±1.52	65.32±1.30	54.86±1.65	58.33±1.34
SGPT (u/l)	37.37±2.12	86.90±2.51	70.23±2.12	49.32±1.07	49.21±1.21

The present study demonstrates that the carbohydrate molecule derived from cultivated *Cordyceps sinensis* (CS) exhibits biochemical and pharmacological properties comparable to wild variants with significant antidiabetic and anti hyperlipidemic potential. TLC and HPLC analyses revealed identical bioactive profiles between wild and cultivated CS, with matching Rf values (0.64, 0.78) and retention times for key metabolites, including uracil, uridine, adenine, adenosine, and cordycepin (Figure-2). This biochemical equivalence aligns with previous findings by Li et al., (2021), who reported similar metabolite profiles in cultivated CS under optimized growth conditions [Li, et al., 2021]. Notably, the carbohydrate fraction was isolated at a 5% concentration with high purity (HPLC), reinforcing its suitability for therapeutic applications.

In-vitro evaluation confirmed significant α-glucosidase inhibitory activity (IC50: 147.6 μg/mL vs. acarbose at 132.5 μg/mL) [Zhang et al., 2022], suggesting potential as a natural antidiabetic agent. In-vivo studies using an HFD/STZ-induced Type 2 diabetic rat model—a well-validated system mimicking human patho-physiology [Reed, et al., 2020] — demonstrated dose dependent (50–100 mg/kg) reductions in blood glucose, body weight, and serum lipids (triglycerides and cholesterol). These findings are consistent with (Guo et al., 2020), who reported that CS polysaccharides enhance insulin sensitivity via AMPK activation (Guo et al., 2020). Our study further extends these observations by demonstrating dual antidiabetic and anti hyperlipidemic effects, highlighting the multifaceted therapeutic potential of CS-derived carbohydrates.

The antihyperlipidemic effects (↓TG, ↓cholesterol) suggest modulation of hepatic lipid metabolism, potentially through PPAR-γ pathways, as previously proposed by (Wang et al., 2019). The concurrent improvements in glycemic control and body weight reduction indicate a multifactorial mechanism of action, likely involving:

1. **Pancreatic β-cell protection,** supported by elevated insulin levels (Table-7)
2. **Hepatic fat metabolism regulation**, evidenced by reduced SGOT/SGPT levels (Table-8)
3. **Enhanced peripheral glucose uptake**, as reported for CS polysaccharides in L6 myotubes (Chen et al., 2021).

Conclusion

This study demonstrates that the carbohydrate molecule derived from cultivated *Cordyceps sinensis* exhibits biochemical and pharmacological pro-perties equivalent to wild variants. Comparative TLC and HPLC analyses revealed identical chromatographic profiles (Rf 0.64, 0.78) and retention times for key bioactive compounds (uracil, uridine, adenine,

adenosine, and cordycepin), confirming their bio-chemical similarity. The cultivated CS carbohydrate was isolated with high purity (5% yield) and showed significant in-vitro antidiabetic activity. In HFD/ STZ-induced diabetic rats - a validated model of human Type 2 diabetes - oral administration (50-100mg/kg) signify-cantly reduced blood glucose, body weight, and serum lipids (triglycerides and cholesterol). These findings establish cultivated CS as a sustainable source of bioactive carbohydrates with dual antidiabetic and antihyperlipidemic potential, warranting further investigation into its therapeutic applications.

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Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

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