Research article
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Assessment of the Antidiabetic Properties of Essential Oil from *Cannabis sativa*

Saima Naz*, Ali Raza Kashif, Abdul Tawab, Muhammad Zaid Rasool, Abdul Rauf, Sabir Hussain, Umar Khan
*Department of Chemistry, University of Education Lahore, Faisalabad campus 37300 Pakistan
Corresponding author: Email: saima.naz@ue.edu.pk

Abstract
This study used the hydrodistillation method to extract essential oil from *Cannabis sativa* (*C.sativa*) leaves, while its chemical composition was examined through gas chromatography-mass spectrometry. Experiments were conducted on alloxan-induced hyperglycemic rabbits (albino), with metformin as the reference medication for comparative analysis to evaluate its potential in managing diabetes. Chemical analysis showed that the principal components of *C.sativa* essential oil were *caryophyllene* (46.63%), *d-limonene* (7.123%) and *cis-β-farnescene* (9.115%), *humulene* (13.153%) and *trans-α-bergamotene* (6.592%). The minor components (<5%) include *α-pinene* (3.720%), *β-myrcene* (1.910%), *cineole* (2.340%), *valencene* (1.614%), *β-bisabolene* (1.897%) and unidentified components (4.201-4.452%) were respectively. Intraperitoneal administration of *C. sativa* oil (0.7, 0.9 and 1.2µl/kg b.wt.) to hyperglycemia for 14 days resulted in a noteworthy decrease in both hepatic and fasting blood glucose levels. Meanwhile, there was a marked increase in the hepatic concentration of glycogen. The oil administration exhibited a reduced yet favourable anti-hyperglycemic potential compared to the reference antidiabetic drug. The study's findings indicate that the essential oil derived from *C. sativa* leaves cultivated in Pakistan is characterized as a Beta Caryophyllene chemotype. Remarkably, the oil demonstrated significant efficacy in lowering glucose levels and showed promise in mitigating hyperglycemia-induced dyslipidemic complications in rabbits induced with alloxan.

Keywords: *C. sativa; Essential oil; GC-Ms; antidiabetic, rabbits, Glucose levels*

Highlights:

- Hydrodistillation and gas chromatography-mass spectrometry are utilized for extracting essential oil from *C. sativa*
- The predominant components of *C. sativa* were identified as *caryophyllene, d-limonene, cis-β-farnescene, humulene*, and *trans-α-bergamotene*.
- Experiments were conducted on alloxan-induced hyperglycemic rabbits, with metformin used as the reference medication for comparative analysis to assess the essential oil's potential in managing diabetes.
- The antidiabetic properties of *C.sativa* oil were evaluated through intraperitoneal administration at different doses (0.7, 0.9, and 1.2µl/kg b.wt.) over 14 days.
- Noteworthy reduction in both hepatic glucose levels and fasting blood glucose following the administration of *C. sativa* oil, suggesting potential anti-hyperglycemic effects.

1. Introduction

Diabetes mellitus is a persistent metabolic disorder marked by compromised insulin function or insufficient insulin production, which poses a substantial global health burden. As the prevalence of diabetes continues to rise, there is an escalating need for innovative therapeutic approaches to complement existing treatments (Campbell & Newgard, 2021; Sugandh et al., 2023; Shankar, 2023). In recent years, natural products derived from various plant sources have gathered attention for their potential antidiabetic properties. One such candidate is the essential oil extracted from *C. sativa*, commonly known as marijuana or hemp, has been traditionally employed for its medicinal properties. Beyond its well-documented psychotropic effects, this plant exhibits a rich chemical profile, with the essential oil representing a complex mixture of bioactive compounds (Chaachouay et al., 2023; Mittal et al., 2023; Rizzo et al., 2023). Preliminary studies suggest that certain constituents within the essential oil may possess antidiabetic attributes, making it a compelling subject for further investigation (Malabadi et al., 2023; Malabadi et al., 2023).

This research systematically evaluates the antidiabetic potential of the essential oil derived from *C. sativa*. By employing rigorous experimental methodologies and comprehensive analyses, we seek to elucidate the impact of essential oil on key markers of diabetes, including glucose metabolism and insulin sensitivity. Through this assessment, we aspire to contribute valuable insights into the therapeutic potential of *C. sativa* essential oil as a novel and natural intervention for managing diabetes. The findings from this study may deepen our understanding of the plant's medicinal properties and pave the way for the development of alternative and effective antidiabetic agents.

2. Experimental materials and procedures

2.1. Instrument and chemicals

Major equipment used was a supercritical fluid extractor, spectrophotometer and centrifuge machine. All chemicals like Dimethyl sulfoxide (DMSO) employed in this study, meeting analytical-grade standards, were obtained from Merck.

2.2. Plant Material

In September-October 2022, fresh *C.sativa* plants were collected from District Chaniot Punjab, Pakistan. The plant specimens underwent further identification and authentication by a respected Taxonomist at the Department of Botany, University of Agriculture Faisalabad, Pakistan. The freshly collected leaf sample was promptly processed to extract essential oil (EO).

2.3. Isolation of Cannabis sativa essential oil

The extraction of essential oils from the leaves employed a batch process utilizing supercritical fluid at temperatures of 50°C and pressures of 90 bar. Each batch of supercritical fluid extraction utilized approximately 15kg of fresh *C.sativa* plant sample. Throughout the extraction process, continuous control of gas supply, pressure and temperature was maintained. The resulting oil yield was expressed as a percentage (% W/W). Post-extraction, The EOs were kept in vials made of black glass at temperatures between 0°C and 4°C until subjected to analysis.

2.4. Analysis of essential oil.

A Perkin Elmer Clarus 600 gas chromatograph–mass spectrometer (GC–MS) system featuring an FID detector was used to analyze the essential oil. Helium gas was employed as the carrier, maintaining a consistent flow rate of ±1 ml/min. The mass transfer line and injector temperatures were set at 250°C and 300°C, respectively. The oven temperature was programmed to ramp up from 40°C (held for 2 minutes) to 270°C at a rate of 2°C/min, followed by an isothermal hold for 10 minutes, and ultimately increased to 300°C at a rate of 10°C/min. Diluted samples (1/100, v/v, in dichloromethane) of 1µl were injected in split mode with a split ratio of 120:1. The percentages of essential oil constituents were determined based on peak areas. Identification of the chemical constituents of *C.sativa* essential oil was achieved by analyzing GC retention time and calculating retention indices based on n-alkanes (C6–C24). Individual compounds were identified by comparing their mass spectra using NIST 2005 v.2.0 and Wiley Access Pak v.7 (2003) of GC–MS systems (Sahoo et al., 2022; Yan et al.,2023)

2.5. Preparation of Alloxan solution and standard drug.

Alloxan monohydrate was dissolved in regular saline to form the alloxan solution. Glimepiride 1mg and Gum tragacanth were obtained from Shifa Pharmacy, Susan Road, Faisalabad. 2% suspension of Gum traganth was prepared by dissolving it in 100 ml water. Glimepiride was dissolved into this 2% gum suspension and made up to 10 ml volume.

2.6. Administration of Essential oil samples and standard drug

2.6.1. Essential oil doses of C. sativa

The extracted essential oil from *C. sativa*, obtained through supercritical fluid extraction at 50°C and 90 Bar pressure, was utilized. The quantity/volume of essential oil assigned to each albino rabbit was determined based on weight. The calculated volumes (0.7, 0.9, and 1.2 µl/kg) were meticulously triturated in 2 ml of a 2% gum tragacanth suspension, and the final volume was adjusted to approximately 5 ml by adding gum tragacanth suspension. This resulting suspension was then orally administered to each albino rabbit using a gastric tube connected to a 10 ml graduated syringe. The gastric tube was carefully introduced into the stomach via the esophagus, and the plunger was slowly pressed to administer the prescribed dose. Glimepiride was also administered by suspending it in 5 ml of a 2% gum tragacanth solution.

2.6.2. Induction of diabetes in Swiss albino rabbits

All groups, except group I, were induced into a diabetic state by intravenously injecting 150 mg/kg body weight of alloxan. Following the alloxan injection, all surviving rabbits' blood glucose (BG) levels were assessed using a BG testing kit. Adult albino rabbits exhibiting BG levels in the range of approximately 250-300 mg/dl were identified as diabetic and subsequently included in subsequent experimental studies (Baghel et al.,2023)

2.6.3. Grouping of animals

Eighteen albino rabbits in good health were selected and distributed randomly into four groups, as outlined in Table 1. Group IV was further divided into three subgroups (n=3) to facilitate the administration of different doses of *C. sativa* essential oil. Each rabbit had an average body weight within the range of 1.5-2kg. The rabbits underwent a one-week acclimatization period before the commencement of the experiment. Throughout the study, the animals were provided with regular seasonal fodder, and water was made available to adult albino rabbits at all times (Baghel et al.,2023).

Table 1: Diet and administration timetable for adult albino rabbits throughout the 0-14 day experimental period.

Gp #	Treatments	No of animals	Subgroups	Feeding and Drug Administration schedule
I	**Untreated control of the routine feed**		3	Routine normal feed 0 to 14 days
II	**Untreated control on alloxan at 150 mg/kg body weight**		3	Routine feed+ alloxan intravenously
III	**Treated control on the synthetic antidiabetic drug: Glimepiride at 800µg/kg body weight orally**		3	Routine feed + alloxan+ Glimepiride in 2% gum tragacanth as vehicle 4 to 14 days
IV	***C. sativa* E. oil SCFE at 50 C, 90bar**	**I**	3	Routine feed +alloxan++0.7µl/kg E. oil, 4to 14 days
		II	3	Routine feed +alloxan++0.9µl/kg E. oil, 4 to 15 days
		III	3	Routine feed +alloxan++1.2µl/kg E. oil, 4 to 15 days

2.7. Hypoglycemic Investigations

2.7.1. Blood Sample Collection

Blood samples were obtained from the jugular vein of each animal on days 0, 5, 10, and 15. In addition to the designated sampling days, samples were aseptically gathered at 0, 2, 4, 8, 16, and 24 hours on each scheduled sampling day. After clotting, serum was isolated via centrifugation and stored at 4ºC in a refrigerator.

2.7.2. Blood Glucose Measurement

The glucose level in the blood samples was assessed using the Kit method (glucose GOD-PAP, UK). The application of the glucose oxidase method ensured precise and reliable results. Currently, the glucose kit method is the most straightforward and extensively employed technique in this context.

2.8 Statistical Analysis

The results underwent evaluation through a Two-Way Analysis of Variance. The statistical distinctions between groups were examined using Duncan's Multiple Range Test at a 5% level of significance (Dash et al., 2023)

3. Results and discussion

3.1. Essential Oil Yield and Composition

Table 2 presents the yield of *C. sativa* essential oil obtained through supercritical fluid extraction. An optimal yield of 0.031 ± 0.02% was achieved at temperatures and pressures of 50°C and 90 Bar, respectively. The highest quality oil can be extracted at low temperatures (Yousefi et al., 2019). Current findings regarding the yield and explanation of supercritical fluid extraction (SCFE) closely align with the outcomes reported by (Rajput et al., 2023) for the extraction of essential oil from clove buds.

Table 2: Extraction yield (%) of *C. sativa* essential oil by SCFE

Sr No.	Temperature and pressure	Weight (Kg)	Distilled water Vol. (litr)	Time of Extraction (hr)	Oil (g)	Yield %
1	50 ºC, 90 Bar	12	Nil	3hr	3.80	0.031 ± 0.02^{a}
2		12	Nil	3hr	3.76	0.029 ± 0.01^{b}
3		12	Nil	3hr	3.74	0.025 ± 0.03^{c}

Values are presented as the mean ± standard deviation of three samples. Superscript letters within the same column indicate significant differences at $P \leq 0.05$ among the extractions of *C. sativa* EO

3.2 GC MS analysis of SCFE *C. sativa* essential oil

The chemical compositional data of the EOs from *C. sativa* essential oil obtained by SCFE are reported in Table (3) and showed in Fig.(1). Fifty compounds were identified in the EOs of *C. sativa* extracted at 50ºC, 90 bar extraction temperature and pressure respectively. At an extraction temperature of 50ºC and 80 Bar, the primary constituents of *C. sativa* essential oil included *caryophyllene* (46.63%), *humulene* (13.153%), *d-limonene* (7.123%), *cis-β-farenscene* (9.115%), and *trans-α-bergamotene* (6.592%). Additionally, the essential oils contained various minor components, with *α-pinene* (3.720%), *β-myrcene* (1.910%), *cineole* (2.340%), *valencene* (1.614%), *β-bisabolene* (1.897%), and unidentified components (4.201-4.452%) among them. Other components included many unidentified components (1.802–0.01%) and many trace components (<1%), such as *p-cymol*, *allo-aromadendrene*, *gamma-murolene (δ-murolene)*, *α-cubebene* etc. The overall identified constituents were categorized as monoterpene hydrocarbons (12 components) at 24.91%, sesquiterpene hydrocarbons (14 components) at 31.50%, oxy-monoterpenes (3 components) at 6.32%, oxy sesquiterpenes (1 component) at 2.27%, others (2 components) at 4.44%, and unidentified (UI) compounds (12 components) at 30.45% in the *C. sativa* essential oil extracted at 50°C, 90 bar.

The hydrocarbons identified in *C. sativa* encompass *n*-alkanes spanning from C9 to C39, including 2-methyl, 3-methyl, and certain dimethyl alkanes. In essential oils obtained through extraction and steam distillation, the primary alkane detected was the n-C29 alkane nonacosane, constituting 56.8% and 11.7%, respectively. Other notable alkanes included hentriacontane, heptacosane, pentacosane, 2, 6-dimethyltetradecane and hexacosane. The significant variation in essential oil compositions could be attributed to the extraction method employed and the extraction temperature (Pieracci et al., 2021).

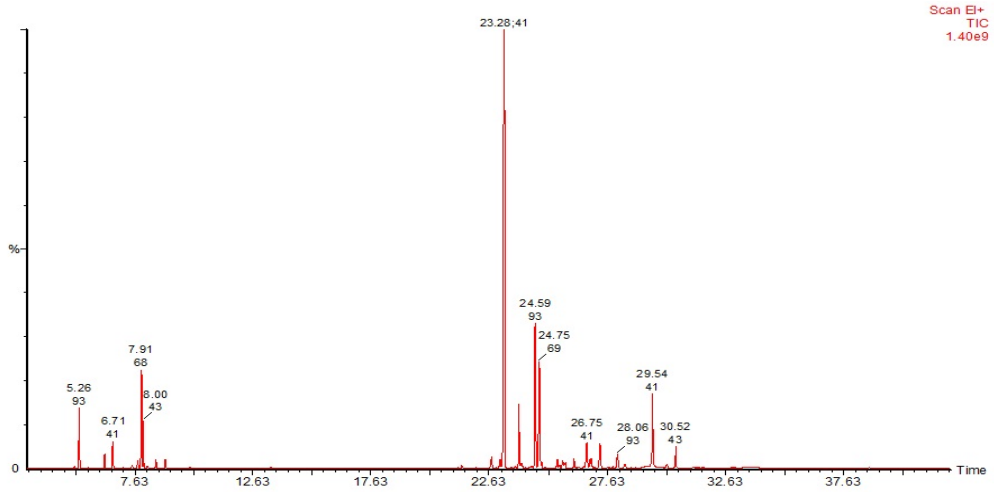

Figure 1: GC MS chromatogram of *C. sativa* SCFE EO at 50°C, 90 Bar.

A diverse array of volatile compounds, spanning various chemical classes such as alcohols, ketones, hydrocarbons, and esters, has been identified in *C. sativa* samples. The volatile fraction's chemical composition in *Cannabis*-derived products has been thoroughly documented, primarily clarified through the analysis of their essential oils (Eržen et al., 2021; Rock et al., 2021). Aromatic compounds were extracted from *C. sativa* using supercritical CO_2 extraction coupled with online fractionation. The predominant constituents in the essential oil included *terpinolene* (6.33%), *caryophyllene* (31.24%), *myrcene* (11.83%), *α-pinene* (10.08%), *α-humulene* (9.75%), *caryophyllene oxide* (6.17%) and *β-pinene* (3.65%). The composition of the essential oil exhibited notable quantitative variations when compared to essential oils extracted from different fiber hemp inflorescences, as previously reported by (Mazzara et al., 2022). However, the presence of these primary constituents was verified. The essential oil demonstrated a higher percentage of sesquiterpenes (54.23%) and associated oxygenated compounds (12.64%) compared to hydrocarbon monoterpenes (35.31%) and oxygenated monoterpenes (1.24%).

Table 3: chemical composition of *C. sativa* essential oil obtained by SCFE at different temperature and pressure conditions.

Peak #	Compound	RT	RI	50°C, 90 Bar
1	A-Thujene	5.085	925.23	0.068±0.05 [b]
2	A-pinene	5.265	939.53	3.720±0.05 [a]
3	Camphene	5.64	950.57	0.080±0.07 [b]
4	B-phellandrene	6.26	966.23	0.043±0.02 [a]
5	B-pinene	6.37	972.15	1.081±0.06 [a]
6	B-myrcene	6.71	915.62	1.910±0.01 [b]
7	(+)-4-carene	7.516	978.11	0.178±0.12 [b]
8	P-Cymol	7.762	1020.56	0.415±0.05 [a]
9	D-limonene	7.906	1037.28	7.123±0.05 [a]
10	Cineole	7.996	1040.34	2.340±0.06 [a]
11	Trans-β-ocimene	8.172	1044.09	0.186±0.11 [a]
12	B-ocimene	8.521	1054.56	0.714±0.12 [a]
13	Gamma-terpinene	8.906	1059.82	0.611±0.04 [b]
14	(+)-4-carene	9.962	1062.11	0.118±0.07 [b]
15	Nonanal	10.51	1063.23	0.023±0.02
16	Terpinen-4-ol	13.38	1178.21	0.047±0.06 [a]
17	Ylangene	21.28	1181.21	0.064±0.09 [b]
18	Copaene	21.47	1185.63	0.130±0.02 [c]
19	U.i	22.72	1193.22	0.043±0.10 [c]

20	U.i	23.08	1195.61	0.643±0.06 [a]
21	U.i	23.09	1197.54	0.927±0.06 [b]
22	Caryophyllene	23.27	1431.53	46.63±0.03 [a]
23	U.i	23.56	1440.32	0.060±0.12 [c]
24	(E)-β-famesene	23.72	1455.34	0.113±0.01
25	Trans-α bergamotene	23.89	1459.33	6.592±0.08 [a]
26	U.i	24.17	1460.22	0.046±0.02 [a]
27	U.i	24.19	1462.91	-
28	Aromandendrene	24.41	1463.22	0.189±0.04 [b]
29	Humulene	24.58	1464.11	13.153±0.09 [a]
30	Cis-β-farnesene	24.74	1465.34	9.115±0.05 [a]
31	Alloaromadendrene	24.85	1467.53	0.373±0.08 [a]
32	U.i	24.99	1473.44	0.060±0.01 [b]
33	Gamma-muurolene	25.49	1487.73	0.396±0.04 [c]
34	B-curcumene	25.60	1491.32	0.188±0.05 [a]
35	U.i	25.61	1493.56	-
37	U.i	25.86	1503.44	-
38	Valencene	26.22	1510.55	1.614±0.07 [a]
40	U.i	26.43	1514.66	-
41	A-Bulnesene	26.63	1515.69	0.149±0.03 [c]
42	B-bisabolene	26.74	1520.32	1.897±0.04 [a]
43	U.i	26.94	1523.31	1.171±0.03 [b]
44	U.i	27.32	1526.33	0.018±0.01 [c]
45	U.i	27.33	1527.85	-
46	Naphthalene,1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-	27.63	1533.99	1.902±0.01 [a]
47	1hcycloprop[e]azulene,1a,2,3,4,4a,5,6,7boctahydro1,1,4,7tetramethyl-, [1a (1a.α.,4.α.	27.85	1535.63	0.057±0.02 [c]
48	U.i	28.05	1539.07	0.120±0.04 [c]
49	Caryophyllene oxide	28.37	1543.21	0.892±0.12 [b]
50	U.i	28.62	1544.54	0.186±0.10 [a]
51	U.i	29.54	1546.22	0.025±0.01 [b]
52	U.i	30.13	1547.13	0.029±0.04
53	U.i	30.51	1548.90	4.352±0.04 [a]
54	U.i	31.24	1550.07	0.153±0.10 [c]
55	U.i	33.55	1553.34	0.990±0.05 [a]
56	U.i	31.24	1554.66	0.240±0.01 [b]
57	U.i	33.54	1557.72	0.667±0.03 [a]

1. Values represent the mean ± standard deviation of three samples of each *C. sativa* EO, individually analyzed in triplicate. Different superscript letters in the same row indicate significant differences ($p < 0.05$).
2. Compounds are presented in the order of elution from the ULTRA Dimethyl polysiloxane-packed capillary column..
3. Retention indices are relative to n-alkanes (C6–C24) on the MS Clarus 600 column.
4. RT signifies identification based on retention time; RI signifies identification based on retention index; MS signifies identification based on the comparing mass spectra. t = traces < 0.05.

3.3. Determination of Antidiabetic activity

Diabetes mellitus is a metabolic disorder marked by hyperglycemia, glucosuria, and negative nitrogen balance. It primarily results from insufficient insulin secretion in the pancreatic beta cells and desensitization of insulin receptors. The prevalence of this condition has escalated to epidemic levels in the current century (Hichri et al., 2019). Currently, various medications like biguanides and sulfonylureas exist to alleviate hyperglycemia in diabetes mellitus. However, these drugs come with associated side effects. Hence, it is imperative to explore new compound classes to address these issues (Derici et al., 2021). *C. sativa* has been employed for various medicinal purposes, including the treatment of conditions like diabetes and as an early remedy for snakebites. Given its historical use in indigenous diabetes treatments, this study explores the potential of *C. sativa* essential oil and its isolated component fractions to improve insulin sensitivity (Kim et al., 2023; Sabir et al., 2020).

3.4. Hypoglycemic Investigation

The blood glucose levels were assessed in both standard and experimental rabbits, revealing a promising antidiabetic effect of *C.sativa* essential oil, as presented in Tables (4 & 5) and Fig. (2-4). The outcomes in Table (4) demonstrate a significant reduction in fasting blood glucose levels ($p < 0.05$) over the 24-hour study period after administering a dose of 0.7 ml/kg glucose to the rabbits. In the case of chronic administration of alloxan, highly significant variations ($p < 0.001$) were

observed between the investigational and diabetic control rabbits, leading to a substantial reduction in fasting blood glucose levels. Comparative analysis with the results of the standard antidiabetic drug exhibited significant variations ($p < 0.05$) and ($p < 0.001$). The doses of essential oil and isolated fractions 0.7ml/kg, 0.9ml/kg, and 1.2 ml/kg significantly lowered blood glucose levels in all days from 0 to 14 days and showed maximum reduction with 1.2 ml/kg, was found at 14 days. 1.2ml/kg dose showed maximum reduction of glucose 243.4 mg/100ml followed by 0.9ml/kg dose, 256.5mg/100ml> 0.7ml/kg, 258.75 mg/100ml respectively as shown in Table (4).

However, there is no previous report or data available about the antidiabetic potential of *C. sativa* essential oil (Akhtar et al., 2020). studied the in vivo impact of *C. sativa* extract on blood coagulation, fat metabolism, and glucose metabolism was investigated in both standard and streptozocin-induced diabetic rats, yielding promising results. In the liver tissue of both diabetic and normal rats, there was a general decrease in glycogen content, with *C. sativa* extract-treated rats exhibiting a significantly lower amount ($P<0.001$) compared to the untreated control rats.

In this study, alloxan was selected to induce a diabetic state in mice. Alloxan is a specific toxin known for its capacity to destroy pancreatic β-cells, leading to a primary deficiency of insulin without impacting other types of islets (Caixeta et al., 2020: Aju et al., 2019). Put differently; alloxan induces hyperglycemia by significantly diminishing insulin release through the destruction of the β-cells in the islets of Langerhans (Longkumer et al., 2021). Given the increasing inclination toward incorporating natural remedies alongside conventional treatments, plants traditionally employed may offer a valuable reservoir of potential new hypoglycemic compounds (Madariaga et al., 2023). Many authors have previously documented the potential antidiabetic properties of essential oils derived from medicinal plants (Nazir et al., 2021; Usman et al., 2020). Several plants have been identified to exhibit hypoglycemic effects. The proposed mechanisms underlying these effects involve the augmentation of insulin secretion from the β-cells of the islets of Langerhans or the liberation of bound insulin. In simpler terms, the hypoglycemic effects of plant essential oils may be ascribed to their insulin-mimicking actions (Rocamora et al., 2020). It could be inferred that *C.sativa* similarly exerts its hypoglycemic activity. Therefore, the potential mechanism of action for the essential oil and isolated fractions could be linked to evocative impact akin to hypoglycemic sulfonylureas, which involves the facilitation of channels responsible for insulin secretion, membrane depolarization, and the initiation of Ca^{2+} influx. This signifies a pivotal initial stage in insulin secretion (Mukai et al., 2022; Campbell et al., 2021). The essential oil might additionally function by enhancing glucose utilization in peripheral tissues, particularly considering that alloxan treatment results in the permanent destruction of β-cells (Wariyapperuma et al., 2020: Patle et al., 2021).

Table 4. Mean ±SEM levels of blood glucose expressed in mg/100ml at various time intervals at 0 day

Treatments	Doses	0hr	2hr	4hr	8hr	16hr	24hr	Total
Control		93.65	95.54	96.2	90.63	93.59	91.39	93.5
Alloxan at 150 mg/kg b.w		254.13	257.17	256.63	257.71	259	256.55	256.87 [c]
Glimepiride at 800µg/kg b .w		235.7	238.6	241.42	244.59	248.68	251.09	243.35 [b]
E. oil (SCFE, 50°C)	**0.7 ml/Kg**	242.09	247.94	250.08	255.17	259.35	263.75	253.06
E.oil (SCFE, 50°C)	**0.9 ml/Kg**	237.84	245.69	247.83	252.92	257.1	261.5	250.48 [c]
E.oil (SCFE, 50°C)	**1.2ml/Kg**	234.34	235.84	237.19	239.4	242.9	248.4	239.68 [a]

Means sharing similar letters in a row or in a column is statistically non-significant ($P>0.05$). Small letters represent comparison among interaction means and capital letters are used for the overall mean

Table 5: Mean ±SEM levels of blood glucose expressed in mg/100ml from day 0 to 14

Doses	Treated groups	0 day (mg/100mL)	5 days (mg/100mL)	10 days (mg/100mL)	14 days (mg/100mL)
	Control	93.5	93.39	91.39	91.39
	Alloxan at 150 mg/kg b.w	256.87	296.87	282.87	276.87
	Glimepiride at 800µg/kg b .w	243.35	269.09	256.09	251.09
0.7 ml/Kg	**E.oil (SCFE, 50°C)**	253.06**	272.75***	268.75***	258.75**
0.9 ml/Kg	**E.oil (SCFE, 50°C)**	250.48**	270.5***	266.5***	256.5**
1.2ml/Kg	**E.oil (SCFE, 50°C)**	239.68***	257.4**	253.4**	243.4***

Values are given as mean ± SEM, (n = 3); ** $p<0.05$ *** $p<0.001$, Duncan test as compared to diabetic control (Group-2).

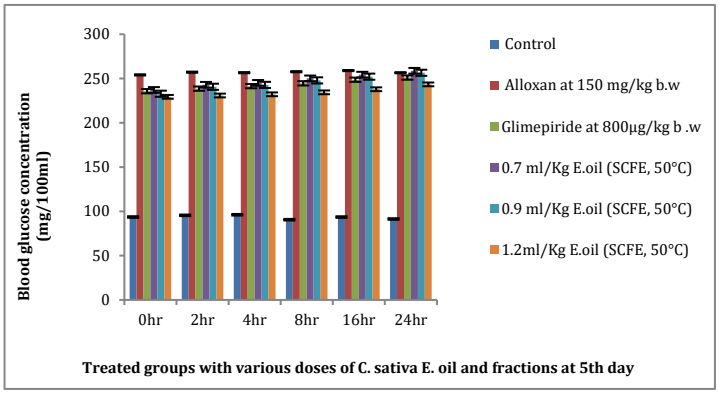

Figure 2: Blood glucose level of treated groups at 5th day

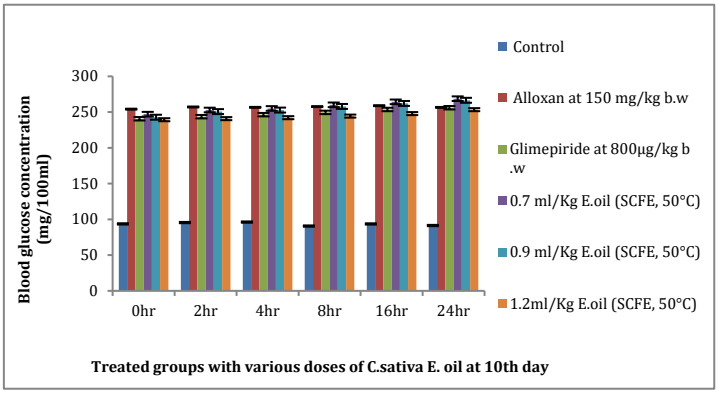

Figure 3: Blood glucose level of treated groups at 10th day

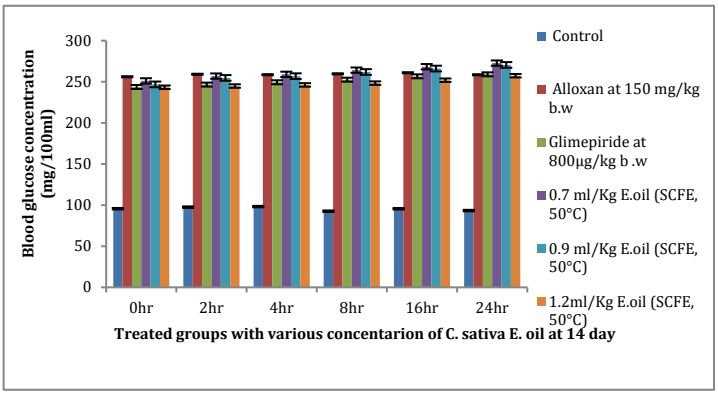

Figure 4: Blood glucose level of treated groups at 5th day

4. Conclusion

In conclusion, the investigation into the antidiabetic properties of the essential oil derived from *C. sativa* leaves has revealed promising outcomes. Through meticulous chemical analysis, the composition of the essential oil was elucidated, highlighting significant constituents such as *caryophyllene*, *d-limonene*, *cis-β-farnescene*, *humulene*, and *trans-α-bergamotene*, along with minor components including *α-pinene, β-myrcene, cineole, valencene, β-bisabolene*, and unidentified compounds. The experiment, conducted on alloxan-induced hyperglycemic rabbits over 14 days, demonstrated a significant decrease in both hepatic glucose levels and fasting blood glucose following the intraperitoneal administration of *C. sativa* oil. Additionally, a notable increase in hepatic glycogen concentration was observed. While the anti-hyperglycemic potential of the oil was slightly reduced compared to the reference antidiabetic drug metformin, it nevertheless exhibited a favourable impact. The study classifies the essential oil as a *Beta Caryophyllene* chemotype, sourced explicitly from *Cannabis sativa* leaves cultivated in Pakistan. Markedly, the oil demonstrated substantial efficacy in lowering glucose levels and showed promise in addressing hyperglycemia-induced dyslipidemia complications. These findings underscore the potential of *C. sativa* essential oil as a candidate for further exploration in the quest for effective strategies in the management of diabetes. Continued research is warranted to elucidate the underlying mechanisms and to ascertain their viability for clinical applications.

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References

Aju, B. Y., Rajalakshmi, R., & Mini, S. (2019). Protective role of Moringa oleifera leaf extract on cardiac antioxidant status and lipid peroxidation in streptozotocin induced diabetic rabbits. *Heliyon*, *5*(12).

Akhtar, M. T., Qadir, R., Bukhari, I., Ashraf, R. A., Malik, Z., Zahoor, S., ... & Saadia, M. (2020). Antidiabetic potential of Nigella sativa L seed oil in alloxaninduced diabetic rabbits. *Tropical Journal of Pharmaceutical Research*, *19*(2), 283-289.

Baghel, K., Azam, Z., Srivastava, R., Gupta, N., & Kango, N. (2023). Withaferin-A attenuates diabetes mellitus induced male reproductive dysfunction mediated by ERα in brain and testes of Swiss albino mice. *Scientific Reports*, *13*(1), 17625.

Chaachouay, N., Azeroual, A., Bencharki, B., Douira, A., & Zidane, L. (2023). Cannabis sativa L.: A Review on Traditional Uses, Botany, Phytochemistry, and Pharmacological Aspects. *Traditional and Integrative Medicine*, 97-116.

Campbell, J. E., & Newgard, C. B. (2021). Mechanisms controlling pancreatic islet cell function in insulin secretion. *Nature reviews Molecular cell biology*, *22*(2), 142-158.

Caixeta, D. C., Aguiar, E. M., Cardoso-Sousa, L., Coelho, L. M., Oliveira, S. W., Espindola, F. S., ... & Sabino-Silva, R. (2020). Salivary molecular spectroscopy: A sustainable, rapid and non-invasive monitoring tool for diabetes mellitus during insulin treatment. *PLoS One*, *15*(3), e0223461.

Dash, S. L., Gupta, P., Dubey, A., Sahu, V. K., & Mishra, A. (2023). An Experimental Models (In-Vivo and In-Vitro) Used for the Study of Antidiabetic agents. *Journal of Advanced Zoology*, *44*(4), 86-95.

Derici, G. E., Özdaş, S., Canatar, İ., & Koç, M. (2021). Antidiabetic activities of Bolanthus spergulifolius (Caryophyllaceae) extracts on insulin-resistant 3T3-L1 adipocytes. *PloS one*, *16*(6), e0252707.

Eržen, M., Košir, I. J., Ocvirk, M., Kreft, S., & Cerenak, A. (2021). Metabolomic Analysis of Cannabinoid and Essential Oil Profiles in Different Hemp (Cannabis sativa L.) Phenotypes. Plants 2021, 10, 966.

El Bakali, I., Boutahar, A., Kadiri, M., & Merzouki, A. (2022). A comparative phytochemical profiling of essential oils isolated from three hemp (Cannabis sativa L.) cultivars grown in central-northern Morocco. *Biocatalysis and Agricultural Biotechnology*, *42*, 102327. (not used in text)

Guzmán-Flores, J. M., Pérez-Vázquez, V., Martínez-Esquivias, F., Isiordia-Espinoza, M. A., & Viveros-Paredes, J. M. (2023). Molecular Docking Integrated with Network Pharmacology Explores the Therapeutic Mechanism of Cannabis sativa against Type 2 Diabetes. *Current Issues in Molecular Biology*, *45*(9), 7228-7241. (not used in text)

Hichri, F., Omri Hichri, A., Maha, M., Saad Mana Hossan, A., Flamini, G., & Ben Jannet, H. (2019). Chemical composition, antibacterial, antioxidant and in vitro antidiabetic activities of essential oils from Eruca vesicaria. *Chemistry & Biodiversity*, *16*(8), e1900183.

Kim, Y., Kim, W., Kim, S. H., Sim, K. S., Kim, K. H., Cho, K. H., ... & Kim, J. H. (2023). Protective Effects of Hemp (Cannabis sativa) Root Extracts against Insulin-Deficient Diabetes Mellitus In Mice. *Molecules*, *28*(9), 3814.

Longkumer, S., Jamir, A., Kechu, M., Ezung, S., & Pankaj, P. P. (2021). Alloxan monohydrate induced diabetes: A comprehensive review. *Sciences*, *1*(1), 1-9.

Patle, D., Vyas, M., & Khatik, G. L. (2021). A review on natural products and herbs used in the management of diabetes. *Current diabetes reviews*, *17*(2), 186-197.

Mazzara, E., Carletti, R., Petrelli, R., Mustafa, A. M., Caprioli, G., Fiorini, D., ... & Cespi, M. (2022). Green extraction of hemp (Cannabis sativa L.) using microwave method for recovery of three valuable fractions (essential oil, phenolic compounds and cannabinoids): A central composite design optimization study. *Journal of the Science of Food and Agriculture*, *102*(14), 6220-6235.

Malabadi, R. B., Kolkar, K. P., & Chalannavar, R. K. (2023). CANNABIS SATIVA: Industrial hemp (fiber type)-An Ayurvedic Traditional Herbal Medicine. *International Journal of Innovation Scientific Research and Review*, *5*(2), 4040-4046.

Malabadi, R. B., Kolkar, K. P., & Chalannavar, R. K. (2023). Medical Cannabis sativa (Marijuana or Drug type); The story of discovery of Δ9-Tetrahydrocannabinol (THC). *International Journal of Innovation Scientific Research and Review*, *5*(3), 4134-4143.

Mittal, V., Panghal, A., & Gupta, R. (2023). Hemp (Cannabis sativa L.) agronomic practices, engineering properties, bioactive compounds and utilization in food processing industry. *Harvesting Food from Weeds*, 143-181.

Madariaga-Mazón A., Naveja J.J. Medina-Franco, J. L. Noriega-Colima K. O. and Martinez-Mayorga K. (2021), DiaNat-DB: a molecular database of antidiabetic compounds from medicinal plants *RSC Advances* 11(9), 5172-5178..

Mukai, E., Fujimoto, S., & Inagaki, N. (2022). Role of reactive oxygen species in glucose metabolism disorder in diabetic pancreatic β-cells. *Biomolecules*, *12*(9), 1228.

Nazir, N., Zahoor, M., Uddin, F., & Nisar, M. (2021). Chemical composition, in vitro antioxidant, anticholinesterase, and antidiabetic potential of essential oil of Elaeagnus umbellata Thunb. *BMC Complementary medicine and therapies*, *21*(1), 1-13.

Palmieri, S., Maggio, F., Pellegrini, M., Ricci, A., Serio, A., Paparella, A., & Lo Sterzo, C. (2021). Effect of the distillation time on the chemical composition, antioxidant potential and antimicrobial activity of essential oils from different Cannabis sativa L. cultivars. *Molecules*, *26*(16), 4770. (not used in text)

Pieracci, Y., Ascrizzi, R., Terreni, V., Pistelli, L., Flamini, G., Bassolino, L., ... & Paris, R. (2021). Essential oil of Cannabis sativa L: Comparison of yield and chemical composition of 11 hemp genotypes. *Molecules*, *26*(13), 4080.

Rajput, S., Kaur, S., Panesar, P. S., & Thakur, A. (2023). Supercritical fluid extraction of essential oils from Citrus reticulata peels: Optimization and characterization studies. *Biomass Conversion and Biorefinery*, *13*(16), 14605-14614.

Rizzo, G., Storz, M. A., & Calapai, G. (2023). The Role of Hemp (Cannabis sativa L.) as a Functional Food in Vegetarian Nutrition. *Foods*, *12*(18), 3505.

Richter, G., Hazzah, T., Hartsel, J. A., Eades, J., Hickory, B., & Makriyannis, A. (2021). Cannabis sativa: An overview. *Nutraceuticals*, 603-624. (not used in text)

Rocamora, C. R., Ramasamy, K., Lim, S. M., Majeed, A. B. A., & Agatonovic-Kustrin, S. (2020). HPTLC based approach for bioassay-guided evaluation of antidiabetic and neuroprotective effects of eight essential oils of the Lamiaceae family plants. *Journal of Pharmaceutical and Biomedical Analysis*, *178*, 112909.

Rock, E. M., & Parker, L. A. (2021). Constituents of Cannabis sativa. *Cannabinoids and Neuropsychiatric Disorders*, 1-13.

Sahoo, C., Champati, B. B., Dash, B., Jena, S., Ray, A., Panda, P. C., ... & Sahoo, A. (2022). Volatile profiling of magnolia champaca accessions by gas chromatography mass spectrometry coupled with chemometrics. *Molecules*, *27*(21), 7302.

Salehi, B., & Ata, A. (2019). V Anil Kumar N, Sharopov F, Ramírez-Alarcón K, Ruiz-Ortega A, et al. *Antidiabetic potential of medicinal plants and their active components. Biomolecules*, *9*(10), 551.

Sugandh, F. N. U., Chandio, M., Raveena, F. N. U., Kumar, L., Karishma, F. N. U., Khuwaja, S., ... & Sugandh, F. (2023). Advances in the management of diabetes mellitus: a focus on personalized medicine. *Cureus*, *15*(8).

Shankar, A. (2023). A narrative review of pathogenesis of gestational diabetes melletus. *Student's Journal of Health Research Africa*, *4*(12), 12-12.3.

Usman, L. A., Oguntoye, O. S., & Ismaeel, R. O. (2020). Effect of seasonal variation on chemical composition, antidiabetic and antioxidant potentials of leaf essential oil of Eucalyptus globulus L. *Journal of Essential Oil Bearing Plants*, *23*(6), 1314-1323

Wariyapperuma, W. N. M., Kannangara, S., Wijayasinghe, Y. S., Subramanium, S., & Jayawardena, B. (2020). In vitro antidiabetic effects and phytochemical profiling of novel varieties of Cinnamomum zeylanicum (L.) extracts. *PeerJ*, *8*, e10070.

Yan, C., Zeng, J., Li, H., Pan, X., Liu, J., & Wei, Y. (2023). Research on the chemical composition of Mentha haplocalyx volatile oils from different geographical origins by comprehensive two-dimensional gas chromatography/time-of-flight mass spectrometry in combination with principal component analysis and the enrichment of bioactive compounds by particle-assisted solvent sublation. *Microchemical Journal*, *188*, 108477.

Yousefi, M., Rahimi-Nasrabadi, M., Pourmortazavi, S. M., Wysokowski, M., Jesionowski, T., Ehrlich, H., & Mirsadeghi, S. (2019). Supercritical fluid extraction of essential oils. *TrAC Trends in Analytical Chemistry*, *118*, 182-193.

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