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Phytochemical Profiling by HR-LCMS and *In Vitro* Assessment of Antioxidant and Antidiabetic Activities of *Cordia obliqua* Leaves

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Abstract

Background: *Cordia obliqua* Willd., valued in Indian ethnomedicine for the treatment of metabolic disorders and inflammation, lacks scientific validation of its phytochemicals and bioactivity. This study identifies key metabolites and evaluates their therapeutic potential, substantiating traditional uses and advancing novel plant-based treatments. **Aim:** To profile the phytochemical constituents of the hydroalcoholic extract of *C. obliqua* (HECO) leaves using HR-LCMS and evaluate its *in vitro* antioxidant and antidiabetic activities. **Methods:** Fresh leaves of *C. obliqua* were extracted using hydroalcoholic solvent, and phytochemical profiling was performed through High-Resolution Liquid Chromatography–Mass Spectrometry (HR-LCMS). *In vitro* antioxidant activity was assessed using DPPH, hydrogen peroxide scavenging, ferric ion reducing power, and hydroxyl radical scavenging assays. The antidiabetic potential was evaluated using α-amylase and α-glucosidase inhibition assays, with ascorbic acid and acarbose serving as reference standards. IC_{50} values were calculated to compare extract potency with standard drug controls. **Results:** HR-LCMS analysis revealed a diverse phytochemical spectrum, including flavonoids, phenolic acids, terpenoids, alkaloids, and glycosides, previously associated with metabolic protection, antioxidant effects, and enzyme modulation. The HECO demonstrated dose-dependent antioxidant activity across all assays, exhibiting values comparable to those of ascorbic acid. HECO also significantly inhibited carbohydrate-hydrolysing enzymes, with IC_{50} values of 92.75 µg/mL (α-amylase) and 179.5 µg/mL (α-glucosidase), indicating its potential to reduce postprandial glucose spikes. **Conclusion:** The findings validate the ethnomedicinal use of *C. obliqua* for oxidative stress and diabetes management. The synergistic presence of multiple phytoconstituents confers strong antioxidant capacity and enzymatic inhibition, supporting its promise as a cost-effective phytotherapeutic agent. Additional *in vivo* studies and toxicity profiling are recommended to establish safety, mechanism of action, and translational potential for phytopharmaceutical development.

Major Findings: HR-LCMS revealed multiple bioactive metabolites in HECO. The extract exhibited strong antioxidant activity comparable to that of ascorbic acid. Significant α-amylase and α-glucosidase inhibition supports its antidiabetic potential.

Keywords: *Cordia obliqua,* DPPH, HR-LCMS, *In vitro* Antioxidant, *In vitro* Anti Diabetic, Alpha-glucosidase

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1. Introduction

Herbal medicines are gaining prominence in pharmaceutical research due to their substantial therapeutic benefits, safer profile, and widespread patient acceptance. As concerns about the side effecincrease; thetic drugs increase, there is a growing global interest in plant-based treatments. These herbal

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remedies, derived from various plant components and phytochemicals, provide advantages like enhanced bioavailability, lower toxicity, and affordability, making them particularly attractive for treating chronic conditions. Moreover, the pharmaceutical sector is increasingly turning to natural products, with many new drugs being developed from or modelled after plant-based compounds[1-3].

Cordia obliqua Willd., also known as *Clammy Cherry*, belongs to the family *Boraginaceae* and is a medium-sized deciduous tree native to tropical and subtropical Asia, particularly India[4]. It grows commonly along roadsides, in forests, and wastelands, and is extensively used in tribal medicine. The *Cordia* genus encompasses numerous species—such as *C. alliodora*, *C. fragrantissima*, *C. trichotoma*, *C. americana*, *C. corymbosa*, *C. curassavica*, *C. linnaei*, *C. polycephala*, *C. leucocephala*, *C. globosa*, and *C. globifera*—that serve as rich sources of bioactive quinones and meroterpenoids[5].

Phytochemical studies confirm the presence of hydroquinones, meroterpenoid benzoquinones, naphthoquinones, and terpenoid quinones across several species—including antifungal and larvicidal cordiaquinones from *C. curassavica* and *C. linnaei*, and cytotoxic cordiaquinones from *C. polycephala*[6]. These compounds often have broad-spectrum activities: antioxidant, antimicrobial, anti-inflammatory, anticancer, antimalarial, and more[7].

Specifically, *Cordia obliqua* has been traditionally applied as an anthelmintic, purgative, diuretic, expectorant, antipyretic, hepatoprotective, hypotensive, respiratory stimulant, analgesic, antimicrobial, anti-inflammatory, and antioxidant[4]. Its edible fruits are used in pickles, and the mucilage (gum) is employed as a tablet binder or paper adhesive[4]. Methanolic extracts of leaves and seeds have shown potent antimicrobial activity against oral pathogens and fungi[8].

Cordia obliqua is a promising candidate for developing herbal therapeutics, particularly for diabetes. It combines traditional use, cost-effectiveness, and a robust phytochemical basis for further *in vitro* and *in vivo* validation.

Diabetes mellitus, a chronic metabolic disorder marked by persistent hyperglycemia, is strongly linked to oxidative stress. The excessive generation of Reactive Oxygen Species (ROS) leads to significant cellular damage, contributing to diabetes-related complications such as nephropathy, neuropathy, and cardiovascular diseases[9,10]. Antioxidants play a crucial role in neutralising ROS, thereby reducing oxidative stress and mitigating disease progression. Natural antioxidants, particularly from medicinal plants, are gaining attention for their potential in managing diabetes and its associated complications[11].

Cordia obliqua, traditionally used in Indian folk medicine, is rich in bioactive compounds such as hydroquinones and meroterpenoids, which exhibit diverse biological activities including antioxidant and antidiabetic effects[12,13]. This study aims to bridge the knowledge gap by profiling the phytochemicals present in the Hydroalcoholic Extract of *Cordia obliqua* (HECO) leaves using High-Resolution Liquid Chromatography–Mass Spectrometry (HR-LCMS). Furthermore, it evaluates the extract's *in vitro* antioxidant and antidiabetic activities, highlighting its potential as a natural therapeutic agent for diabetes and oxidative stress management.

2. Materials and Methods

2.1 Collection and Authentication of Medicinal Plant

In September and October 2023, the *Cordia obliqua* plant was collected from the Gokhulpur-Panjer region of Rohtas, Bihar, India (GPS Coordinates: N 25°01'59.7" E 84°00'36.7"). The specimens were verified by the Botanical Survey of India at the Central National Herbarium in Howrah, with voucher specimen number NSHM/SS-02. The leaves were carefully removed, washed thoroughly with water, and then dried in the shade. Once dried, they were pressed and stored in an airtight container for later use.

2.2 Extraction

The subsequent extraction was performed with a Soxhlet apparatus using organic solvents, including petroleum ether, chloroform, and 70% ethanol, over 24-26 hours. The heating mantle temperature was set between 40-60°C. After extraction, the sample's extract was filtered, concentrated and stored in a vacuum desiccator for further use[14].

2.3 HR-LCMS Study

High-Resolution Liquid Chromatography Mass Spectrometry (HR-LCMS) was performed to analyse

the chemical composition of the hydroalcoholic *C. obliqua* (HECO) extract. The instrument employed was the HRLCMS-QTOF from Agilent Technologies, USA. Data acquisition was conducted using Agilent Mass Hunter software, with data processed through Agilent Mass Hunter Qualitative Analysis B.06. The column used was ZORBAX Eclipse Plus - C18, 150 x 2.1 mm, 5 microns (Agilent). Solvent A consisted of 0.1% formic acid in Milli-Q water, while Solvent B was Acetonitrile.

2.4 *In Vitro* Antioxidant Activity

2.4.1 DPPH (2,2-Diphenyl-1-picrylhydrazyl) Assay

Various concentrations of the extracts were made by dissolving them in methanol (ranging from 10 to 100 µg/ml). A 200 µM solution of DPPH in methanol was prepared, and 1.5 ml of this solution was combined with 1.5 ml of the methanol extract solutions at varying concentrations, followed by a 30-minute incubation at room temperature in the dark. After the incubation period, the absorbance of each solution was recorded at 517 nm using methanol as the blank[15]. The same was done for standard Ascorbic Acid (AA) at the same concentration.

2.4.2 Hydrogen Peroxide Scavenging Assay

A phosphate buffer (pH 7.4) was used to prepare a 20 mM solution of hydrogen peroxide. Extracts at varying concentrations (10 µg/ml to 100 µg/ml) were made in double-distilled water and subsequently added to the hydrogen peroxide solution. The absorbance of the hydrogen peroxide at 230 nm was measured 10 minutes later in comparison to a blank solution that consisted of the phosphate buffer without hydrogen peroxide[16]. The same was done for standard AA at the same concentration.

2.4.3 Reducing Power Assay

An extract (0.75 mL) of varying concentrations was combined with 0.75 mL of phosphate buffer (0.2 M, pH 6.6) and 0.75 mL of potassium hexacyanoferrate (K3Fe(CN)6) (1%, w/v) and then incubated at 50°C in a water bath for 20 minutes. The reaction was halted by introducing 0.75 mL of a trichloroacetic acid (TCA) solution (10%), and the mixture was centrifuged at 800 g for 10 minutes. 1.5 mL of the resultant supernatant was combined with 1.5 mL of distilled water and 0.1 mL of ferric chloride (FeCl3) solution (0.1%, w/v) for 10 minutes. The absorbance of the reaction mixture was measured at 700 nm. A higher absorbance value of the reaction mixture reflected a greater reducing power[17]. The same was done for standard AA at the same concentration.

2.4.4 Hydroxyl Radical Scavenging Activity

The Fe3+ -ascorbate-EDTA-H_2O_2 system produced hydroxyl radicals. The assay involved measuring the degradation product of 2-deoxy-D-ribose, which developed a pink chromogen when heated with TBA at a low pH. The reaction mixture included 0.8 ml of Phosphate-Buffered Saline (PBS) (50 mmol L^{-1}, pH 7.4), extract/standard at varying concentrations (100, 200, 300, 400 µg/ml), EDTA (1.04 mmol L^{-1}), FeCl3 (1 mmol L^{-1}), and 2-deoxy-D-ribose (28 mmol L^{-1}). The mixture was incubated in a water bath at 37°C for one hour. Ascorbic acid and H_2O_2 (10 mmol L^{-1}) were then added to initiate the reaction. The reaction mixture was subsequently kept at 37°C for an additional hour. Cold thiobarbituric acid (TBA) was incorporated into the incubated mixture, followed by the addition of HCl (25%). The mixture was heated at 100°C for 15 minutes and then allowed to cool. The absorbance of the resulting solution was measured at 532 nm using a spectrophotometer[18]. The same was done for standard AA at the same concentration.

2.5 *In Vitro* Antidiabetic Activity

2.5.1 Alpha-amylase Inhibition Assay

Solutions were prepared in DMSO ranging from concentrations of 10 µg/mL to 250 µg/mL. The DNS solution consisted of 20 mL of 96 mM 3,5-dinitrosalicylic acid, 12 g of sodium potassium tartrate in 8 mL of 2 M NaOH, and 12 mL of deionised water, which acted as the colouring agent. The experiments were divided into three groups: test, blank, and control. A mixture containing 1 mL of the test solution and enzyme solution was incubated at 25°C for 30 minutes. Following this, 1 mL of starch solution was introduced and incubated for an additional 3 minutes. Subsequently, 1 mL of DNS was added, and the tube was placed in a water bath at 85°C for 15 minutes. After cooling, the mixture was diluted with 9 mL of distilled water, mixed thoroughly, and the absorbance was recorded at 540 nm. For the blank group, DNS was added before the starch. In the control group, DMSO replaced the plant extract.

Acarbose served as a positive control. The α-amylase inhibitory activity was calculated according to the equation given below:

$$\text{Inhibition \%} = (A_{Control} - A_{Sample}) / A_{control} * 100$$

Where $A_{control}$ is the absorbance of the control (without drug extracts); A_{Sample} is the absorbance in the presence of drug extracts[19-21].

2.5.2 *Alpha-glucosidase Inhibition Assay*

The reaction mixture for α-glucosidase contained 2.9 mM of p-nitrophenyl-α-glucopyranoside (pNPG), a range of drug extract concentrations (from 10 µg/mL to 250 µg/mL), and 1.0 U/mL of α-glucosidase in a sodium phosphate buffer at pH 6.9. Control tubes included only DMSO along with the enzyme and substrate, while in the positive control, the sample extract was substituted with acarbose. Mixtures lacking the enzyme, sample extract, and acarbose acted as blanks. The reaction mixtures were incubated for 5 minutes at 25 °C and then boiled for 2 minutes. The absorbance of the resulting p-nitrophenol (pNP) was measured at 405 nm using a spectrophotometer, which was directly correlated to enzyme activity. The IC50 values were derived from percentage inhibition graphs plotted against the logarithm of the inhibitor concentration and were determined using non-linear regression analysis based on the average inhibitory values. Acarbose served as the reference drug for the alpha-glucosidase inhibition assay. All tests were conducted in triplicate[22,23].

3. Results

3.1 HR-LCMS Report Analysis

The HR-LCMS method was utilised to distinguish and identify the phytoconstituents based on their retention time, differences in the database (library), experimental m/z values, MS/MS fragments, classes of metabolites, and proposed compounds. Mass spectrometry data were acquired in both negative and positive ionisation modes. By examining the mass of the molecular ion peak along with its fragments, considering neutral mass loss and established fragmentation patterns, as well as comparing it with existing literature, the phytochemical analysis of HECO indicated the presence of various known and newly identified compounds (Figures 1 and 2). Table 1 lists the identified compounds along with their mass (m/z), retention time, and bioactivity, in terms of abundance.

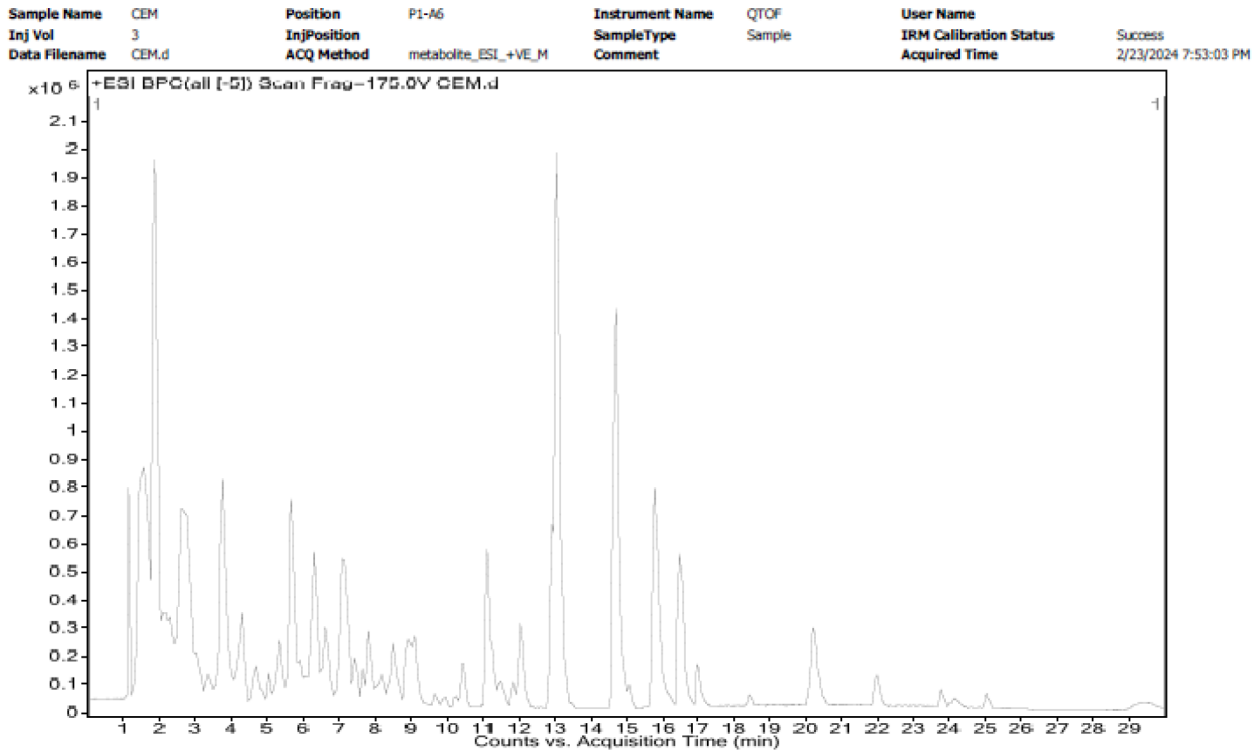


Figure 1. HR-LCMS chromatogram of HECO (+ve). HECO: Hydroalcoholic extract of *Cordia obliqua*.

3.2 *In Vitro* Antioxidant Activity

The hydroalcoholic extract of *Cordia obliqua* leaves (HECO) was assessed for its antioxidant potential *in vitro* using several established assays, with its effectiveness being compared to that of ascorbic acid as a reference. In the DPPH free radical scavenging assay, HECO showed a concentration-dependent increase in inhibitory activity (Figure 3), demonstrating 40% inhibition at 25 µg/mL and reaching up to 90% at 125 µg/mL, which was similar to ascorbic acid (96% inhibition at the same concentration). Likewise, in the hydrogen peroxide scavenging assay, HECO showed moderate activity (Figure 4), with inhibition values between 18% at 25 µg/mL and 68% at 125 µg/mL. Although these values were lower than those of ascorbic acid (32% to 79% across the same concentrations), the extract still exhibited significant potential for scavenging peroxide. In the ferric ion reducing power assay, HECO demonstrated a strong reducing capacity (Figure 5), recording 48% inhibition at 25 µg/mL and increasing to 86% at 125 µg/mL. Notably, these results were comparable to those of ascorbic acid, which showed inhibition ranging from 45% to 94%. Additionally, in the hydroxyl radical scavenging assay, HECO displayed progressive activity (Figure 6), with inhibition rising from 17% at 25 µg/mL to 63% at 125 µg/mL, while ascorbic acid recorded slightly higher inhibition (27% to 72% across the tested concentrations). In summary, these results indicate that HECO exhibits robust antioxidant activity through various mechanisms, highlighting its potential in alleviating oxidative stress.

3.3 *In Vitro* Antidiabetic Activity

The HECO was tested for its potential to inhibit diabetes *in vitro* through α-amylase and α-glucosidase inhibition assays. Acarbose, a widely recognised synthetic drug for diabetes treatment, served as the standard reference in both tests.

3.3.1 *Alpha-amylase Inhibition Assay*

The HECO was assessed for its ability to inhibit the α-amylase enzyme, with acarbose serving as a standard reference drug (Figure 7). HECO exhibited a concentration-dependent inhibitory effect, beginning with a 20% inhibition at 10 µg/mL and increasing steadily to 73% inhibition at 250 µg/mL. In contrast,

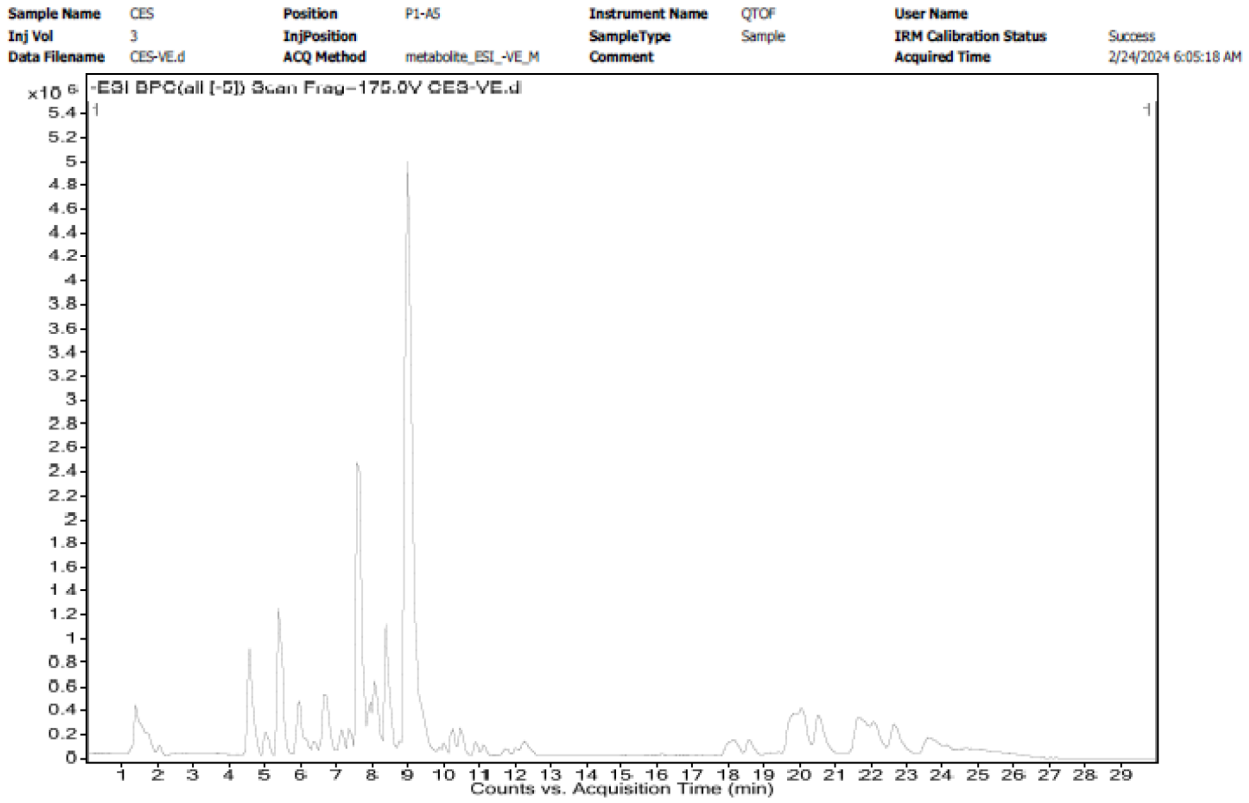


Figure 2. HR-LCMS chromatogram of HECO (-ve). HECO: Hydroalcoholic extract of *Cordia obliqua*.

Table 1. List of dominant compounds identified in HECO by HR-LCMS-QTOF

Sl No.	Compound Name	Molecular Formula	Class of Compounds/ Biological Activity	Retention time (Min.)	Mass	m/z	Abundance
1	(-)-Ormosanine	$C_{20}H_{35}N_3$	Quinolizidine+I3:I41 alkaloid; reported antimicrobial and cytotoxic activities.	12.917	317.2804	318.288	20706
2	p-Menthane-2,6,8,9-tetrol	$C_{10}H_{20}O_4$	Monoterpenoid alcohol exhibits antioxidant and anti-inflammatory effects.	7.194	204.1363	227.126	57654
3	2-Amino-4-hydroxy-3-methylpentanoic acid	$C_6H_{13}NO_3$	Isoleucine derivative; amino acid; contributes to metabolic regulation.	1.987	147.0901	170.079	81595
4	2-(Methylthiomethyl)-3-phenyl-2-propenal	$C_{11}H_{12}OS$	Aromatic aldehyde; flavour compound; possible antimicrobial properties.	1.396	192.0613	191.054	159638
5	2-Ethyl-5-methylpyridine	$C_8H_{11}N$	Methylpyridine derivative; flavour compound; has antioxidant and flavour-enhancing activities.	6.291	121.0902	144.08	202514
6	3β,6β-Dihydroxynortropane	$C_7H_{13}NO_2$	Nortropane alkaloid; shows anticholinergic and neuroactive effects.	1.808	143.0935	144.101	547633
7	3-Nonanon-1-yl acetate	$C_{11}H_{20}O_3$	Fatty alcohol ester; flavour compound; exhibits antimicrobial activity.	8.235	200.1412	223.13	85003
8	4-Feruloyl-1,5-quinolactone	$C_{17}H_{18}O_8$	Phenolic lactone possesses antioxidant and anti-inflammatory properties.	4.617	350.0991	395.097	239995
9	4-Oxocy clohexanecarboxylate	$C_7H_{10}O_3$	Cyclohexanecarboxylic acid derivative; may exhibit anti-inflammatory activity.	2.406	142.0637	165.053	156924
10	7-Epi-12-hydroxyjasmonic acid glucoside	$C_{18}H_{28}O_9$	Jasmonate derivative; plant hormone conjugate; involved in plant defence and stress responses.	6.343	388.1705	411.159	296359
11	9,10-Dihydroxy-12,13-epoxyoctadecanoate	$C_{18}H_{32}O_5$	Epoxy fatty acid; plant lipid metabolite; reported anti-inflammatory effects.	11.709	330.2396	329.232	133483
12	Alginic acid	$C_{12}H_{20}O_{12}P_2$	Polysaccharide; seaweed-derived; used as an antioxidant and immunomodulatory agent.	8.973	418.0397	417.034	34669
13	Anthranilic acid	$C_7H_7NO_2$	Aromatic amine; precursor to alkaloids; known anti-inflammatory and analgesic effects.	1.484	137.0466	138.054	439472
14	Astragalin 7-rhamnoside	$C_{27}H_{30}O_{15}$	Flavonoid glycoside exhibits antioxidant and anti-inflammatory activities.	8.244	594.1576	593.151	37854
15	Benzyl O-[arabinofuranosyl-(1->6)-glucoside]	$C_{17}H_{24}O_{10}$	Glycoside; reported antimicrobial and hepatoprotective activities.	6.087	402.1516	447.15	57893

Table 1. Continued...

Sl No.	Compound Name	Molecular Formula	Class of Compounds/ Biological Activity	Retention time (Min.)	Mass	m/z	Abundance
16	Benzylsuccinate	$C_{10}H_{12}O_4$	Aromatic dicarboxylic acid derivative; microbial metabolite; implicated in metabolic regulation.	10.834	208.0718	207.065	206035
17	Caffeic acid	$C_9H_8O_4$	Phenolic acid; antioxidant; shows strong free radical scavenging activity.	6.56	180.0405	179.033	371010
18	Chlorophyllide	$C_{55}H_{72}MgN_4O_5$	Chlorophyll derivative; photosynthetic pigment; potential antioxidant and wound healing effects.	8.338	614.2423	673.25	18887
19	Chrysosplenol D	$C_{17}H_{14}O_7$	Flavonoid displays anti-inflammatory and anticancer activities.	9.236	360.0839	359.077	374131
20	Citrusin B	$C_{20}H_{24}O_{10}$	Flavonoid; citrus-derived; possesses antioxidant and anti-inflammatory properties.	7.336	568.2129	567.206	16984
21	Corchorifatty acid F	$C_{18}H_{30}O_2$	Fatty acid derivative; may show cytoprotective effects.	11.088	328.2238	327.217	149429
22	Crosatoside B	$C_{27}H_{30}O_{15}$	Flavonoid glycoside; antioxidant and anti-inflammatory potential.	8.402	446.1782	445.171	29902
23	Dambonitol	$C_6H_{14}O_6$	Sugar alcohol has diuretic and hepatoprotective activities.	1.806	208.0927	231.082	1E+06
24	Divaricoside	$C_{30}H_{46}O_{10}$	Cardiac glycoside; used in heart failure treatment.	9.103	534.3177	533.311	18970
25	Esculetin	$C_9H_6O_4$	Coumarin derivative; strong antioxidant and anti-inflammatory effects.	6.438	178.0242	177.017	19464
26	Guavin B	$C_{15}H_{20}O_6$	Flavonoid: antioxidant and anticancer activities.	9.134	694.1282	739.126	
27	Isomelitric acid A	$C_{16}H_{12}O_7$	Phenolic acid shows antioxidant and antimicrobial activities.	7.515	538.1101	537.103	4509
28	Kaempferol 3-rhamnoside 7-xyloside	$C_{27}H_{30}O_{15}$	Flavonoid glycoside exhibits antioxidant and antidiabetic effects.	6.918	564.1472	563.14	47761
29	L-2-Amino-3-methylenehexanoic acid	$C_7H_{13}NO_2$	Non-proteinogenic amino acid; involved in metabolic pathways.	3.727	143.0954	166.085	164168
30	Lotaustralin	$C_{14}H_{19}NO_6$	Cyanogenic glycoside; plant defence compound; potential anticancer and antimicrobial effects.	1.569	261.1185	262.126	38679
31	Macrocarpal B	$C_{30}H_{44}O_5$	Terpenoids show antimicrobial and anti-inflammatory activities.	22.617	472.2818	471.275	28172

Table 1. Continued...

Sl No.	Compound Name	Molecular Formula	Class of Compounds/ Biological Activity	Retention time (Min.)	Mass	m/z	Abundance
32	Medicanine	$C_{16}H_{13}NO_4$	Isoflavonoid: antioxidant and anti-inflammatory activities.	2.705	159.0895	182.079	127892
33	Menthyl lactate	$C_{13}H_{24}O_2$	Monoterpene ester; flavour compound; cooling, soothing, and antimicrobial effects.	1.185	228.1691	251.158	452326
34	Methyl N-methylanthranilate	$C_9H_{11}NO_2$	Aromatic ester; flavour compound; exhibits insect-repellent and antimicrobial properties.	5.296	191623	188.069	191623
35	Myricetin 7-rhamnoside	$C_{21}H_{20}O_{12}$	Flavonoid glycoside; antioxidant and anti-inflammatory effects.	7.886	464.0949	463.087	40713
36	Nigakilactone B	$C_{20}H_{24}O_6$	Quassinoid displays antimalarial and antitumor activities.	15.722	392.2189	415.208	406613
37	Physagulin D	$C_{28}H_{36}O_{10}$	Withanolide has anti-inflammatory and immunomodulatory effects.	9.56	620.355	679.369	49104
38	Quercetin	$C_{15}H_{10}O_7$	Flavonoid; antioxidant; exhibits strong anti-inflammatory, antidiabetic, and anticancer activities.	7.689	302.0398	303.047	47433
39	Quercetin 3-rhamnoside-7-glucoside	$C_{27}H_{30}O_{16}$	Flavonoid glycoside: potent antioxidant and anti-inflammatory.	7.539	610.1537	609.147	255336
40	Rutin	$C_{27}H_{30}O_{16}$	Flavonoid glycoside; antioxidant; strengthens blood vessels and reduces oxidative stress.	7.805	610.1523	609.145	40538
41	Sagerinic acid	$C_{36}H_{32}O_{16}$	Lignan: plant-derived antioxidant and hepatoprotective properties.	8.961	720.1693	719.162	181882
42	Salvianolic acid L	$C_{36}H_{30}O_{16}$	Phenolic acid; antioxidant; cardioprotective and hepatoprotective activities.	8.582	718.1519	717.145	86783
43	Silibinin	$C_{25}H_{22}O_{10}$	Flavonolignan; hepatoprotective agent that also exhibits antioxidant and anticancer effects.	8.799	482.12	527.118	61516
44	Sudachiin A	$C_{24}H_{26}O_{13}$	Flavonoid; citrus-derived; anti-inflammatory and antioxidant activities.	7.956	522.1362	521.129	31075
45	Theaflavin-3-gallate	$C_{36}H_{28}O_{16}$	Polyphenol; tea-derived antioxidant; known for antidiabetic and anticancer effects.	8.377	714.1303	713.114	
46	Tricin 7-diglucuronoside	$C_{29}H_{28}O_{17}$	Flavonoid glycoside; antioxidant and anti-inflammatory.	8.97	682.1296	741.144	106533

Table 1. Continued...

Sl No.	Compound Name	Molecular Formula	Class of Compounds/ Biological Activity	Retention time (Min.)	Mass	m/z	Abundance
47	Vicinin 2	$C_{27}H_{30}O_{15}$	Flavonoid glycoside; antioxidant and hepatoprotective.	12.405	626.1413	671.14	104242
48	N^{1},N^{10}-Dicoumaroylspermidine	$C_{25}H_{31}N_3O_4$	Phenolamide: antimicrobial and anti-inflammatory effects.	7.749	437.2266	438.234	43959
49	Phytosphingosine	$C_{18}H_{39}NO_3$	Sphingoid base; lipid component in plants and animals; antimicrobial and skin barrier protective.	13.222	317.2902	318.297	846222
50	Retronecine	$C_8H_{13}NO_2$	Pyrrolizidine alkaloid; hepatotoxic but may have insect deterrent activity.	1.551	155.0934	156.101	389912
51	Saxitoxin	$C_{10}H_{17}N_7O_4$	Neurotoxin: blocks sodium channels.	2.222	299.1339	300.141	54592
52	Sphinganine	$C_{18}H_{39}NO_2$	Sphingoid base exhibits skin protective and antimicrobial effects.	14.662	301.2955	302.303	611801
53	Threonate	$C_4H_7O_5^-$	Metabolite; derivative of threonine; involved in mineral metabolism and antioxidative functions.	3.402	136.0374	137.045	333684

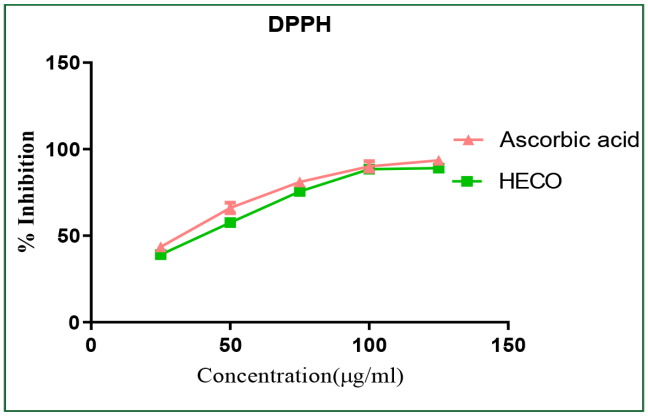


Figure 3. The data represent the percentage inhibition of 2,2-diphenyl-1-picrylhydrazyl by HECO against ascorbic acid (standard). Each point represents the values obtained from experiments, performed in triplicate (mean±standard error of the mean). HECO: Hydroalcoholic extract of *Cordia obliqua*.

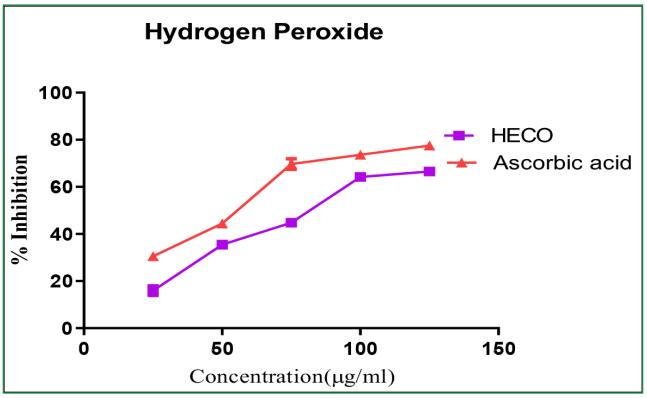


Figure 4. The data represent the percentage inhibition of Hydrogen peroxide by HECO against ascorbic acid (standard). Each point represents the values obtained from experiments, performed in triplicate (mean±standard error of the mean). HECO: Hydroalcoholic extract of *Cordia obliqua*.

acarbose displayed slightly greater inhibition at all concentrations, ranging from 23% at 10 µg/mL to 87% at 250 µg/mL.

The IC_{50} value for HECO regarding α-amylase inhibition was determined to be 92.75 µg/mL, suggesting its potential effectiveness in inhibiting carbohydrate-hydrolysing enzymes and thereby reducing postprandial hyperglycemia.

3.3.2 Alpha-glucosidase Inhibition Assay

HECO demonstrated significant inhibitory effects on the α-glucosidase enzyme. At the lowest concentration

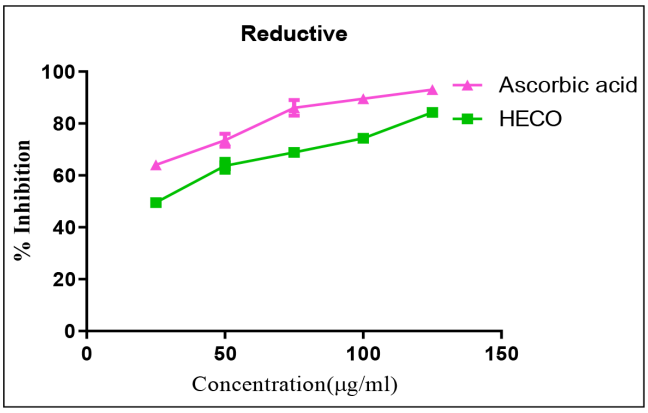


Figure 5. The data represent the percentage inhibition of Ferric ion reduction by HECO against ascorbic acid (standard). Each point represents the values obtained from experiments, performed in triplicate (mean±standard error of the mean). HECO: Hydroalcoholic extract of *Cordia obliqua*.

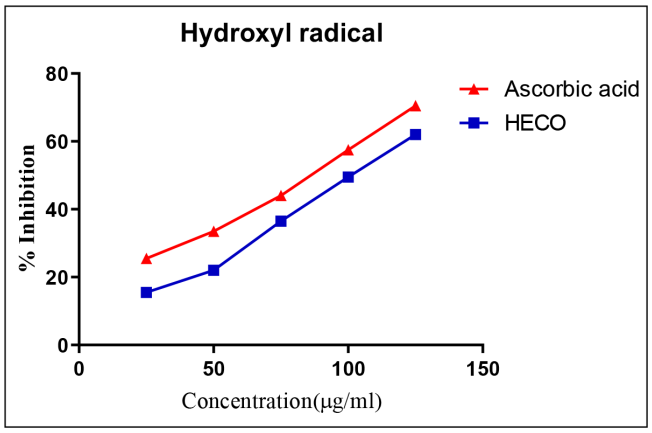


Figure 6. The data represent the percentage inhibition of Hydroxyl radical reduction by HECO against ascorbic acid (standard). Each point represents the values obtained from experiments, performed in triplicate (mean±standard error of the mean). HECO: Hydroalcoholic extract of *Cordia obliqua*.

studied (10 µg/mL), HECO exhibited 15% inhibition, which progressively increased to 71% at 250 µg/mL (Figure 8). Acarbose, utilised as a reference standard, displayed greater inhibitory effects, ranging from 29.6% at 10 µg/mL to 77% at 250 µg/mL. The IC_{50} value for HECO regarding α-glucosidase inhibition was determined to be 179.5 µg/mL. These findings indicate that HECO has considerable α-glucosidase inhibitory potential, which complements its α-amylase inhibitory activity, thereby supporting its potential role in controlling postprandial blood glucose levels.

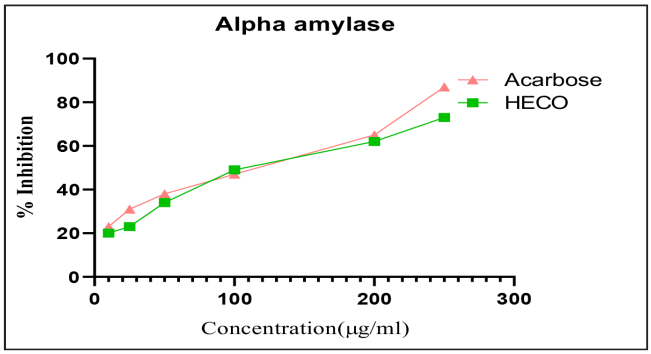


Figure 7. Inhibition of alpha-amylase by HECO: The data represent the percentage inhibition of *α*-amylase by HECO against acarbose (standard). Each point represents the values obtained from experiments, performed in triplicate (mean±standard error of the mean).

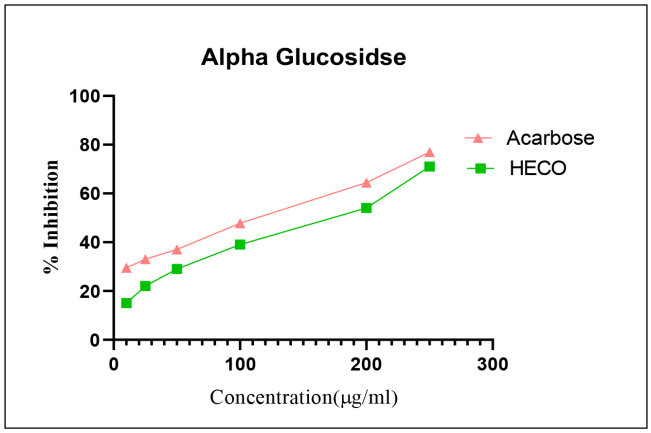


Figure 8. Inhibition of alpha-glycosidase by HECO: The data represent the percentage inhibition of α-glucosidase by HECO against acarbose (standard). Each point represents the values obtained from experiments, performed in triplicate (mean± standard error of the mean).

4. Discussion

The detailed phytochemical analysis of *C. obliqua* leaves using HR-LCMS uncovered a variety of bioactive compounds, shedding light on its potential therapeutic applications. The identification of several classes of phytochemicals—such as flavonoids, phenolic acids, alkaloids, terpenoids, glycosides, lignans, and fatty acid derivatives—highlights the extensive pharmacological capabilities of this plant. Notably, compounds like quercetin, rutin, caffeic acid, and kaempferol derivatives are recognised for their strong antioxidant and anti-inflammatory properties, which correspond with the

observed *in vitro* antioxidant findings in the present study[11-13].

The flavonoids identified, such as quercetin, myricetin 7-rhamnoside, astragalin 7-rhamnoside, and vicinin 2, are recognised for their strong capabilities in scavenging free radicals and chelating metals, which significantly aid the plant in reducing oxidative stress[24,25]. Furthermore, phenolic acids, including caffeic acid, salvianolic acid L, and isomelitric acid A, have well-documented functions in preventing lipid peroxidation and protecting cellular integrity from reactive oxygen species[26]. The presence of lignans, such as sagerinic acid, further enhances the antioxidative defence mechanisms, highlighting the potential of *C. obliqua* in mitigating oxidative damage associated with chronic metabolic conditions, including diabetes[27].

Additionally, the presence of glycosides like rutin, quercetin 3-rhamnoside-7-glucoside, and benzyl O-[arabinofuranosyl-(1→6)-glucoside] indicates potential health advantages, including vasoprotective and hepatoprotective effects, as these substances are recognised for their ability to stabilise vascular structures and enhance liver function[28,29]. Terpenoid compounds such as macrocarpal B and physagulin D, along with alkaloids like (-)-ormosanine and retronecine, are linked to antimicrobial, anti-inflammatory, and neuroactive effects, thus expanding the therapeutic uses of the extract beyond just antioxidant and antidiabetic properties[30,31].

The identification of compounds such as the cyanogenic glycoside lotaustralin and the withanolide physagulin D showcases the plant's natural defence systems against herbivores and pathogens, while also presenting potential pharmacological benefits, including immunomodulation and anticancer properties[32,33]. The presence of phytosphingosine and sphinganine, both types of sphingoid bases, further reinforces protective and antimicrobial functions for the skin, indicating potential use in dermatological products[34].

The diverse range of these phytochemicals can synergistically act to produce multiple effects, which is vital for addressing complex issues like diabetes mellitus. The noteworthy α-amylase and α-glucosidase inhibitory activities found in this study demonstrate its ability to influence carbohydrate digestion and glucose uptake, thus managing postprandial hyperglycemia[35,36]. Coupled with its strong antioxidant characteristics, these attributes advocate for the traditional use of *C. obliqua* and scientifically substantiate its role as a natural antidiabetic and nephroprotective agent[12,13].

In summary, the chemical variety and numerous bioactivities of the compounds found imply that *C. obliqua* has great potential for creating multifunctional herbal formulations. The interaction of antioxidants, enzyme inhibitors, anti-inflammatory compounds, and protective secondary metabolites present in the extract lays a solid groundwork for further *in vivo* investigations and eventual clinical applications. This wealth of phytochemicals not only supports the ethnomedicinal significance of *C. obliqua* but also paves the way for its integration into contemporary phytopharmaceutical products aimed at managing diabetes and its related complications with limited side effects[37,38].

5. Conclusion

The current research provides significant scientific endorsement for the traditional application of *C. obliqua* leaves as a medicinal plant with possible antidiabetic and antioxidant properties. The comprehensive HR-LCMS-based phytochemical analysis unveiled a wide range of bioactive compounds, including flavonoids, phenolic acids, alkaloids, terpenoids, glycosides, lignans, and fatty acid derivatives. Notable compounds such as quercetin, rutin, caffeic acid, astragalin derivatives, and sagerinic acid are recognised for their potent antioxidant, anti-inflammatory, and metabolic regulatory effects, supporting the medicinal claims associated with the plant.

In vitro antioxidant tests revealed that the HECO exhibits strong free radical scavenging and reducing capabilities, comparable to ascorbic acid, affirming its effectiveness in alleviating oxidative stress, a major factor in diabetic complications. Furthermore, the *in vitro* antidiabetic tests demonstrated that HECO shows significant inhibitory activities against α-amylase and α-glucosidase, emphasising its potential to lower postprandial hyperglycemia by influencing carbohydrate digestion and glucose absorption pathways.

In summary, the diverse phytochemical profile and bioactivities of *C. obliqua* highlight its potential as a natural therapeutic agent for diabetes and related

conditions caused by oxidative stress. These results not only validate its traditional use among indigenous populations but also pave the way for its development into standardised phytopharmaceutical products that may have fewer side effects compared to conventional synthetic medications.

Additional *in vivo* research and clinical assessments are needed to fully confirm its pharmacological effectiveness and safety, ultimately aiding its incorporation into evidence-based herbal remedies and functional food products.

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Author Contribution

Arvind Kumar was responsible for conceptualising the study, executing the experiments, acquiring data, interpreting the results, and preparing the manuscript, including its graphical and visual components. Mrinmoy Basak provided academic supervision, contributed to critical manuscript review, and offered guidance in structuring and refining the written work. All authors have read and agreed to the published version of the manuscript.