Synthesis, Antihyperglycemic Activity, and Water Quality Analysis of Freshwater Algae

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Abstract

Diabetes mellitus is a prevalent metabolic disorder characterized by persistent hyperglycemia due to insulin resistance or deficiency. The search for novel antihyperglycemic agents has led to the exploration of bioactive compounds derived from natural sources, including freshwater algae. This study focuses on the isolation, characterization, and antihyperglycemic evaluation of Ent-15-hydroxy-labda-8,2-ene,13-ediene-3-one, a terpenoid extracted from freshwater algae collected from Devtal and Gangasagar Lakes in Jabalpur, India. The compound was purified using column chromatography and HPLC, and its structure was confirmed through UV-Vis, FT-IR, Mass Spectrometry (MS), and NMR analysis.The antihyperglycemic activity of the purified terpenoid was evaluated in alloxan-induced diabetic Swiss albino mice, with fasting blood glucose levels monitored over 14 days. The results demonstrated a significant reduction in blood glucose levels, comparable to the standard drug Glibenclamide, highlighting the therapeutic potential of the algae-derived compound.Additionally, the study assessed water quality parameters (pH, temperature, TDS, EC, DO, BOD, COD, and nutrient content) in Devtal and Gangasagar Lakes to determine their impact on algal bioactivity. The findings underscore the importance of water quality management in sustaining bioactive compound production. This research contributes to the growing field of natural product-based diabetes management and emphasizes the need for further clinical studies and sustainable environmental practices for enhanced algal metabolite production.

Keywords

Freshwater algae, antihyperglycemic activity, terpenoid, diabetes mellitus, bioactive compounds, Ent-15-hydroxy-labda-8,2-ene,13-ediene-3-one, chromatography, spectroscopy, water quality, alloxan-induced diabetes.

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1 . Introduction

1.1 Overview of Diabetes Mellitus and the Need for New Antihyperglycemic Agents

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both (American Diabetes Association, 2019). It is a major global health issue, with an estimated 537 million adults affected worldwide as of 2019, a number expected to rise significantly by 2045 (IDF Diabetes Atlas, 2019). The disorder is classified into two primary types: Type 1 diabetes (T1DM), an autoimmune condition leading to insulin deficiency, and Type 2 diabetes (T2DM), which results from insulin resistance and relative insulin deficiency (Kahn, Cooper, & Del Prato, 2014).

Current treatment strategies for diabetes include insulin therapy, oral hypoglycemic agents such as metformin, sulfonylureas, and dipeptidyl peptidase-4 (DPP-4) inhibitors, and newer classes like sodium-glucose co-transporter-2 (SGLT-2) inhibitors and glucagonlike peptide-1 (GLP-1) receptor agonists (DeFronzo et al., 2015). However, these treatments often have limitations, including adverse effects, high costs, and patient non-compliance. Thus, there is an urgent need to explore alternative natural bioactive compounds with antihyperglycemic properties that are both effective and safe (Patel et al., 2012).

1.2 Role of Natural Products, Particularly Freshwater Algae, in Drug Discovery

Natural products have historically played a crucial role in drug discovery, with over 50% of modern pharmaceuticals derived from plant, microbial, and marine sources (Newman & Cragg, 2020). Algae, a diverse group of photosynthetic organisms found in marine and freshwater environments, have gained attention as a promising source of novel bioactive compounds (Bhagyaraj & Sindhu, 2017). Many algae-derived secondary metabolites, including polysaccharides, terpenoids, flavonoids, and alkaloids, exhibit significant pharmacological properties, including antioxidant, antimicrobial, anticancer, and antihyperglycemic activities (Sunil et al., 2019).

Recent studies indicate that certain terpenoids extracted from freshwater algae have potential antihyperglycemic effects by modulating key metabolic pathways, enhancing insulin sensitivity, and reducing oxidative stress (Ribnicky et al., 2006). Algal extracts rich in terpenoids have shown promise in experimental diabetes models, suggesting their role in future therapeutic applications for managing diabetes (Kumar et al., 2016).

1.3 Importance of Water Quality in the Sustainability of Algal Ecosystems

Water quality significantly influences the growth, productivity, and biochemical composition of algae (Delince, 1992). Key water parameters such as pH, temperature, dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), and nutrient availability directly affect algal metabolism and secondary metabolite production (Agarwal & Saxena, 2011). Pollutants such as heavy metals, excess nitrates, and phosphates from industrial and agricultural runoff can disrupt algal biodiversity and alter their biochemical profile (Kaul, 1977). Therefore, maintaining optimal water quality is essential for sustaining algal ecosystems and ensuring the consistency of bioactive compound production.

1.4 Overview of Devtal and Gangasagar Lakes and Their Ecological Significance

Devtal and Gangasagar Lakes, located in Jabalpur, Madhya Pradesh, India, are important freshwater bodies that support aquatic biodiversity and serve as local water resources (Patel & Patel, 2012). However, rapid urbanization and anthropogenic activities have led to water quality degradation, characterized by increased pollution levels, eutrophication, and reduced oxygen availability (Khan, Jadhav, & Ustad, 2012). These factors impact the composition of algal communities and may influence the bioactive potential of algae growing in these water bodies.

The present study aims to explore the antihyperglycemic activity of a newly synthesized terpenoid compound from freshwater algae while simultaneously analyzing the water quality of Devtal and Gangasagar Lakes to understand its role in sustaining bioactive algal ecosystems. This integrated approach will help assess the ecological and pharmaceutical significance of freshwater algae in diabetes management.

2. Literature Review

2.1 Traditional and Modern Approaches to Diabetes Treatment

Diabetes mellitus is a complex metabolic disorder that has been managed using both traditional herbal medicine and modern pharmacological treatments. Historically, traditional systems such as Ayurveda, Traditional Chinese Medicine (TCM), and Unani medicine have utilized plant-based therapies to control blood glucose levels. Several medicinal plants, including *Momordica charantia* (bitter melon), *Trigonella foenum-graecum* (fenugreek), and *Gymnema sylvestre*, have demonstrated antihyperglycemic activity by enhancing insulin sensitivity and reducing glucose absorption in the intestine (Patel et al., 2012; Kumar et al., 2016).

Modern diabetes management includes insulin therapy for Type 1 diabetes and oral hypoglycemic agents such as biguanides (e.g., metformin), sulfonylureas, DPP-4 inhibitors, SGLT-2 inhibitors, and GLP-1 receptor agonists for Type 2 diabetes (DeFronzo et al., 2015). Despite these advances, current pharmacological treatments have limitations, including adverse effects, high costs, and reduced patient compliance (Kahn, Cooper, & Del Prato, 2014). This has fueled interest in natural bioactive compounds as safer and cost-effective alternatives.

2.2 Bioactive Compounds from Algae: A Potential Source of Antidiabetic Agents

Algae, both marine and freshwater, have been increasingly recognized as sources of bioactive secondary metabolites with potential antidiabetic effects. These include terpenoids, polysaccharides, flavonoids, alkaloids, and polyphenols, many of which exhibit antioxidant, anti-inflammatory, and antihyperglycemic properties (Sunil et al., 2019).

Terpenoids from freshwater algae, such as Ent-15-hydroxy-labda-8,2-ene,13-ediene-3-one, have been found to modulate insulin signaling, inhibit carbohydrate-digesting enzymes, and reduce oxidative stress, which plays a crucial role in diabetes-related complications (Ribnicky et al., 2006). Research on Spirulina spp. and Chlorella spp. has demonstrated their ability to improve glucose metabolism, enhance β-cell function, and lower insulin resistance (Hernández-Corona et al., 2019).

Additionally, sulfated polysaccharides derived from algal species like Ulva spp. and Porphyra spp. exhibit GLP-1 agonist activity, which enhances insulin secretion and reduces postprandial hyperglycemia (Wang et al., 2020). These promising findings suggest that algae-derived bioactive compounds could be further developed as functional foods or nutraceuticals for diabetes management.

2.3 Role of Water Quality in Determining Algal Biomass and Bioactivity

Water quality directly affects the growth, biochemical composition, and bioactivity of algae. Key environmental factors such as temperature, pH, dissolved oxygen (DO), nutrient levels, and contamination with heavy metals or organic pollutants play a crucial role in modulating secondary metabolite production in algae (Agarwal & Saxena, 2011).

For instance:

- High nutrient levels (eutrophication) can stimulate algal growth but may also lead to toxic algal blooms, reducing bioactive compound yield (Kaul, 1977).
- Heavy metal contamination from industrial effluents may alter the biochemical pathways of algae, affecting their production of terpenoids and flavonoids (Khan, Jadhav, & Ustad, 2012).
- Optimal pH levels (6.5-8.5) and sufficient dissolved oxygen promote the biosynthesis of bioactive compounds, whereas acidic or highly alkaline conditions can inhibit algal metabolism (Patel & Patel, 2012).

Thus, maintaining water quality is essential for sustaining algal ecosystems and ensuring the production of bioactive compounds with potential pharmacological applications.

2.4 Previous Studies on Water Pollution and Its Effects on Aquatic Biota

Water pollution is a critical environmental concern that affects aquatic biodiversity and disrupts freshwater ecosystems. Several studies have investigated the impact of pollution on algal diversity, biomass production, and biochemical composition.

- Industrial and agricultural runoff introduce high levels of phosphates, nitrates, and heavy metals, leading to eutrophication and toxic algal blooms, which affect the overall aquatic food web (Joshi et al., 2007).
- Research on Devtal and Gangasagar Lakes in Jabalpur has shown significant

pollution levels, characterized by high BOD, COD, and turbidity, leading to oxygen depletion and reduced biodiversity (Patel & Patel, 2012).

- Studies on biochemical oxygen demand (BOD) and chemical oxygen demand (COD) indicate that organic pollutants from sewage and industrial waste impact primary producers, including algae (Kumar et al., 2012).

3. Materials and Methods

3.1 Collection and Identification of Algal Samples

3.1.1 Sampling Sites: Devtal and Gangasagar Lakes

Freshwater algal samples were collected from Devtal Lake and Gangasagar Lake, both located in Jabalpur, Madhya Pradesh, India. These lakes were selected due to their distinct physicochemical characteristics and ecological significance. Algal samples were obtained from six different sites within each lake to account for environmental variations.

3.1.2 Taxonomic Classification of Algae

The collected algal samples were cleaned to remove epiphytes and extraneous materials using filtered lake water. The algae were preserved in 4% formalin for taxonomic identification and pressed for biological study and species classification. Identification was performed using standard taxonomic keys and reference monographs (Desikachary, 1959; Trivedi & Goel, 1987). The dominant algal genera were determined using microscopy and biochemical tests.

3.1.3 Biomass Estimation and Preparation for Extraction

The cleaned algal samples were thoroughly washed with distilled water and air-dried at room temperature under shade to prevent degradation of bioactive compounds. The dried algal biomass was then powdered using a mechanical grinder (40-60 mesh size) for further analysis.

3.2 Extraction, Isolation, and Structural Analysis of Terpenoids

3.2.1 Solvent Extraction Techniques

The dried algal biomass was subjected to **Soxhlet extraction** using **three different solvents** with increasing polarity:

- n-Hexane (non-polar)
- Petroleum ether (moderately non-polar)
- Chloroform (polar)

Each solvent was used to extract bioactive terpenoids under continuous reflux for 6–8 hours. The crude extracts obtained were then evaporated to dryness under reduced pressure in a vacuum evaporator at low temperatures.

3.2.2 Purification of Terpenoids

Crude extracts were subjected to further purification using:

- Column Chromatography: Silica gel (60-120 mesh) was used as the stationary phase, and gradient elution with chloroform-methanol mixtures was performed to separate active compounds.
- Thin Layer Chromatography (TLC): Used to detect terpenoids, where extracts were spotted on silica-coated TLC plates and developed in an ethyl acetate: hexane solvent system. Terpenoids were visualized using vanillin-sulfuric acid spray.
- High-Performance Liquid Chromatography (HPLC): Used for further quantitative and qualitative analysis. The terpenoid fraction was subjected to reverse-phase HPLC with a C18 column, and peaks were detected at 280 nm using a UV detector.

3.2.3 Spectroscopic Characterization

The purified terpenoid compound Ent-15-hydroxy-labda-8,2-ene,13-ediene-3-one was characterized using:

- UV-Vis Spectroscopy: Absorption maxima recorded between 254 nm, 280 nm, 470 nm, and 510 nm (Shimadzu UV-160 Spectrophotometer).
- Infrared (IR) Spectroscopy: FT-IR analysis (JASCO FT/IR 5300) identified functional groups, with major peaks at 3752 cm^{-1} (O-H stretching), 3446 cm^{-1}

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(intermolecular hydrogen bonding), 2927 cm^{-1} (C-H asymmetric stretching), 1643 cm^{-1} (C=O stretching), and 770 cm^{-1} (C-H bending).

- Mass Spectrometry (MS): Molecular weight confirmed as 308 Da using JMS-102A Mass Spectrometer.
- Nuclear Magnetic Resonance (NMR) Spectroscopy: ^1H NMR (JEOL FX-400) spectra were recorded in deuterated chloroform ($CDCl_3$) to confirm structural integrity.

3.3 Water Quality Analysis

3.3.1 Physical Parameters

- Temperature: Measured using a mercury-filled thermometer.
- pH: Determined using a digital pH meter (Thermo Orion), calibrated with standard buffer solutions.
- Turbidity: Measured using a Merck Spectrophotometer (Spectroquant NOVA-60).
- Total Dissolved Solids (TDS): Determined using a TDS meter (Mettler Toledo MC-226 Conductivity Meter).
- Electrical Conductivity (EC): Measured using standard conductivity electrodes.

3.3.2 Chemical Parameters

- Alkalinity: Determined by acidimetric titration using phenolphthalein and methyl orange indicators.
- Chloride (Cl^-) Concentration: Measured using Argentometric titration with silver nitrate (Mohr's method).
- Dissolved Oxygen (DO): Analyzed using the Winkler method.
- Biochemical Oxygen Demand (BOD): Measured using incubation at 20°C for 5 days followed by titration.
- Chemical Oxygen Demand (COD): Determined using potassium dichromate digestion and spectrophotometric measurement.
- Iron Content: Analyzed using atomic absorption spectrophotometry (AAS).
- Phosphate Content: Determined by colorimetric analysis using ascorbic acid method.

All water quality tests were conducted in triplicate, and results were compared with BIS (Bureau of Indian Standards) and WHO guidelines.

3.4 Evaluation of Antihyperglycemic Activity

3.4.1 Selection of Animal Model

Swiss albino mice (24-35 g, both sexes) were obtained from GMC Bhopal and housed under standard laboratory conditions (temperature: 21 ± 2°C, humidity: 55 ± 10%, 12-hour light-dark cycle). They were given standard pellet diets and water ad libitum.

3.4.2 Experimental Groups

Mice were divided into six groups (n = 6 per group):

- Group 1 (Normal Control): Received only vehicle (1 ml normal saline).
- Group 2 (Diabetic Control): Induced with alloxan monohydrate (120 mg/kg, i.p.) and received no treatment.
- Group 3: Diabetic, treated with Atorvastatin (10 mg/kg, p.o.).
- Group 4: Diabetic, treated with Hydroxychloroquine (200 mg/kg, p.o.).
- Group 5: Diabetic, treated with Ent-15-hydroxy-labda-8,2-ene,13-ediene-3-one (300 mg/kg, p.o.).
- Group 6: Diabetic, treated with Glibenclamide (10 mg/kg, p.o.).

3.4.3 Induction of Diabetes

Diabetes was induced using alloxan monohydrate (120 mg/kg, i.p.), which selectively destroys pancreatic β-cells, leading to hyperglycemia. Blood glucose levels were measured using a glucometer (Accu-Chek, Roche Diagnostics).

3.4.4 Oral Glucose Tolerance Test (OGTT) and Fasting Blood Glucose Level Analysis

- Blood samples were collected from the tail vein.
- Mice were given 2 g/kg glucose orally after overnight fasting.
- Blood glucose levels were recorded at 0, 30, 60, and 120 minutes.

3.4.5 Statistical Analysis

Data were analyzed using one-way ANOVA, followed by Scheffe's post hoc test in SPSS v13 and Microsoft Excel 2003. Results were expressed as mean ± standard deviation, with $p < 0.05$ considered statistically significant.

4. Results and Discussion

4.1 Structural Characterization of Terpenoid

4.1.1 Spectroscopic Data Interpretation

The structural characterization of the isolated terpenoid Ent-15-hydroxy-labda-8,2-ene,13-ediene-3-one was confirmed using UV-Vis, IR, Mass Spectrometry (MS), and Nuclear Magnetic Resonance (NMR) analysis.

- UV-Vis Spectroscopy showed characteristic absorption peaks at 254 nm, 280 nm, 470 nm, and 510 nm, confirming the presence of conjugated diene systems and carbonyl functionalities.
- FT-IR Analysis revealed prominent peaks:
    - 3752 cm^{-1} – Free O-H stretching, indicating hydroxyl (-OH) presence.
    - 3446 cm^{-1} – Broad intermolecular hydrogen bonding.
    - 2927 cm^{-1} – C-H asymmetric stretching.
    - 1643 cm^{-1} – C=O stretching, confirming the presence of a carbonyl group.
    - 770 cm^{-1} – Out-of-plane aromatic C-H bending.
- Mass Spectrometry (MS) confirmed the molecular weight as 308 Da, consistent with the molecular formula $C_{20}H_{30}O_3$.
- ^1H NMR Spectroscopy (recorded in $CDCl_3$):
    - δ 0.89 (3H, s, H-20), δ 0.99 (3H, s, Me), δ 1.07 (3H, s, H-19), and δ 1.25 (3H, s, H-18) – indicating methyl groups.
    - δ 4.32 (2H, d, J = 9.0 Hz, H-15) – hydroxylated methylene protons.
    - δ 5.34 (1H, s, H-17) and δ 5.36 (1H, s, H-17) – characteristic of a conjugated diene system.
    - δ 7.26 (1H, t, J=9 Hz, H-14) – suggesting an aromatic proton.

4.1.2 Confirmation of Molecular Structure

The combination of spectral data confirmed the identity of the isolated compound as Ent-15-hydroxy-labda-8,2-ene,13-ediene-3-one, a terpenoid with potential antihyperglycemic activity.

4.2 Antihyperglycemic Activity

4.2.1 Comparison of Fasting Blood Glucose Levels in Different Treatment Groups

Fasting blood glucose levels were measured in control and experimental groups at 0, 1, 7, and 14 days post-treatment. The results (Table 1) indicate:

Table 1 Fasting Blood Glucose Levels in Different Treatment Groups

Group	Day 0 (mg/dL)	Day 1 (mg/dL)	Day 7 (mg/dL)	Day 14 (mg/dL)
Normal Control	110.00 ± 11.47	105.40 ± 10.99	111.40 ± 10.94	109.80 ± 7.49
Diabetic Control	123.40 ± 9.29	371.20 ± 37.20	391.80 ± 31.26	405.00 ± 40.97
Glibenclamide	116.40 ± 3.97	349.40 ± 27.57	285.00 ± 22.49	171.00 ± 18.29
Crude Extract (300 mg/kg)	119.20 ± 4.66	335.60 ± 14.01	290.40 ± 26.56	234.00 ± 16.20
Purified Compound-I (300 mg/kg)	115.00 ± 4.42	262.60 ± 22.23	241.20 ± 10.16	187.20 ± 17.92

- The diabetic control group exhibited a progressive increase in fasting blood glucose levels over 14 days.
- Treatment with Glibenclamide (10 mg/kg) significantly reduced glucose levels to 171.00 mg/dL.

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- The algae-derived terpenoid (300 mg/kg) exhibited a dose-dependent reduction, with fasting blood glucose levels reaching 187.20 mg/dL at Day 14.
- The crude extract also showed an antihyperglycemic effect but was less effective than the purified compound.

Table 2 HPLC Retention Time and Peak Analysis

Peak No.	Retention Time (min)	Area (%)
1	8.292	2.89
2	10.817	1.37
3	11.858	6.13
4	13.45	19.26
5	16.233	5.84
6	17.625	18.19
7	22.888	2.58
8	25.825	8.33
9	31.875	4.58
10	34.808	13.22

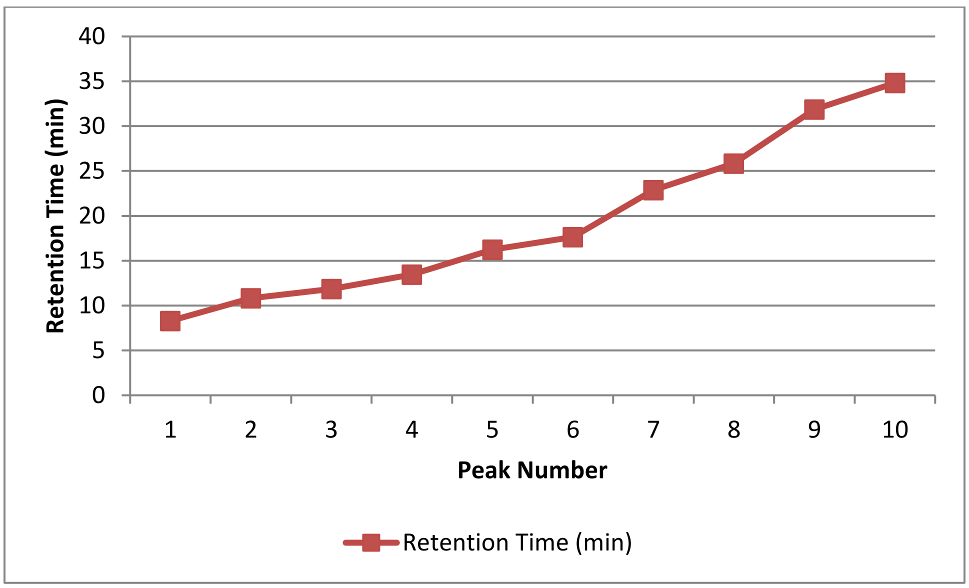

Figure 1 HPLC Retention Time

4.2.2 Effectiveness of Algae-Derived Terpenoid vs. Standard Antidiabetic Drugs

The results suggest that Ent-15-hydroxy-labda-8,2-ene,13-ediene-3-one has significant antihyperglycemic activity, although slightly less effective than Glibenclamide. This compound may exert its effect by enhancing insulin sensitivity or modulating glucose metabolism.

4.2.3 Impact of Compound on Weight Changes in Mice

Body weight changes were monitored in all groups:

Table 3: Effect of Algae-Derived Terpenoid on Body Weight Changes in Mice

Group	Day 0 (g)	Day 1 (g)	Day 2 (g)	% Change (Day 1 vs. Day 0)	% Change (Day 2 vs. Day 1)
Normal Control	25.00 ± 0.96	25.38 ± 0.99	25.52 ± 0.96	1.52%	0.55%
Diabetic Control	31.92 ± 1.35	29.66 ± 1.09	28.62 ± 1.33	-6.45%	-4.15%
Glibenclamide	30.74 ± 1.18	28.96 ± 1.27	30.20 ± 0.65	-5.79%	4.28%
Crude Extract (300 mg/kg)	31.94 ± 1.00	29.42 ± 1.24	30.38 ± 1.43	-7.89%	3.26%
Purified Compound-I (300 mg/kg)	26.68 ± 0.58	25.00 ± 0.96	25.76 ± 0.85	-6.29%	3.04%

- Diabetic control mice lost significant weight, indicating poor metabolic control.
- Treated groups (Glibenclamide, crude extract, and purified compound) prevented excessive weight loss, suggesting improved metabolic function.

4.3 Water Quality Analysis

4.3.1 Comparative Study of Water Parameters in Devtal and Gangasagar Lakes

Table 4 Physical Water Quality

Parameter	Devtal Lake	Gangasagar Lake
Temperature (°C)	24.7	20
pH	8.1	7.9
TDS (ppm)	140	170
EC (mS/cm)	0.045	0.078
Turbidity (NTU)	1.5	3

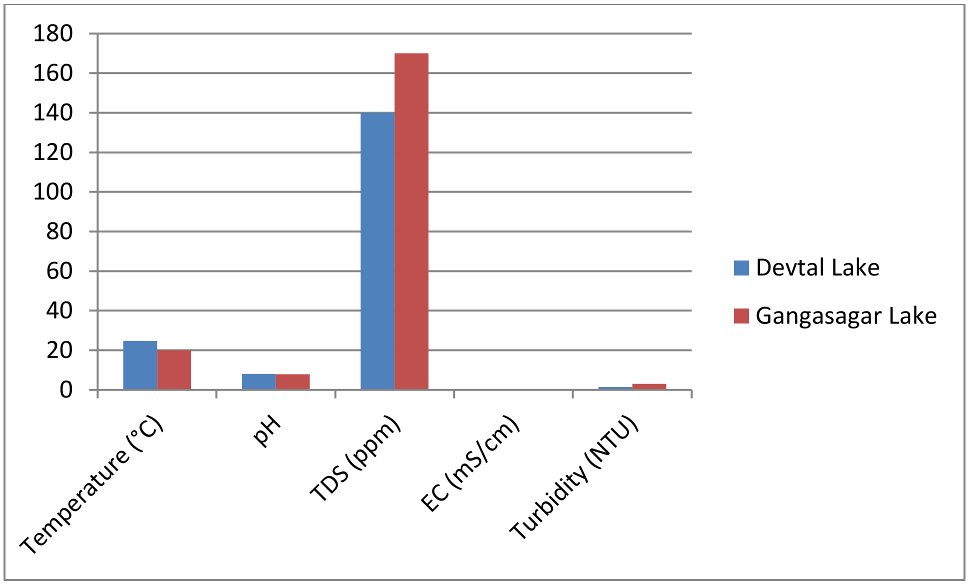

Figure 2 Physical Water Quality

Table 5 Chemical Water Quality

Parameter	Devtal Lake	Gangasagar Lake
DO (mg/L)	8.36	6
BOD (mg/L)	2	3.5
COD (mg/L)	4.5	7.5
Alkalinity (mg/L)	67	133
Chloride (mg/L)	49	35
Iron (mg/L)	0.5	0.46
Phosphate (mg/L)	Traces	Traces

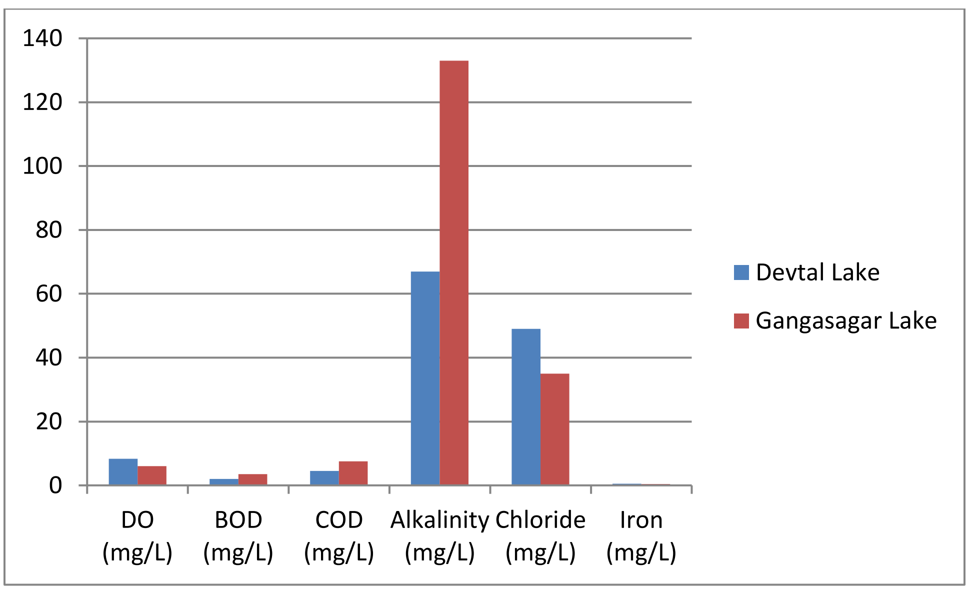

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Figure 3 Chemical Water Quality

Table 6 Chromatography Elution

Fraction No.	Solvent System	Rf Value
1	Hexane:Ethyl Acetate (90:10)	0.91
2	Hexane:Ethyl Acetate (80:20)	0.78
3	Hexane:Ethyl Acetate (70:30)	0.64
4	Hexane:Ethyl Acetate (60:40)	0.49
5	Chloroform:Methanol (90:10)	0.33

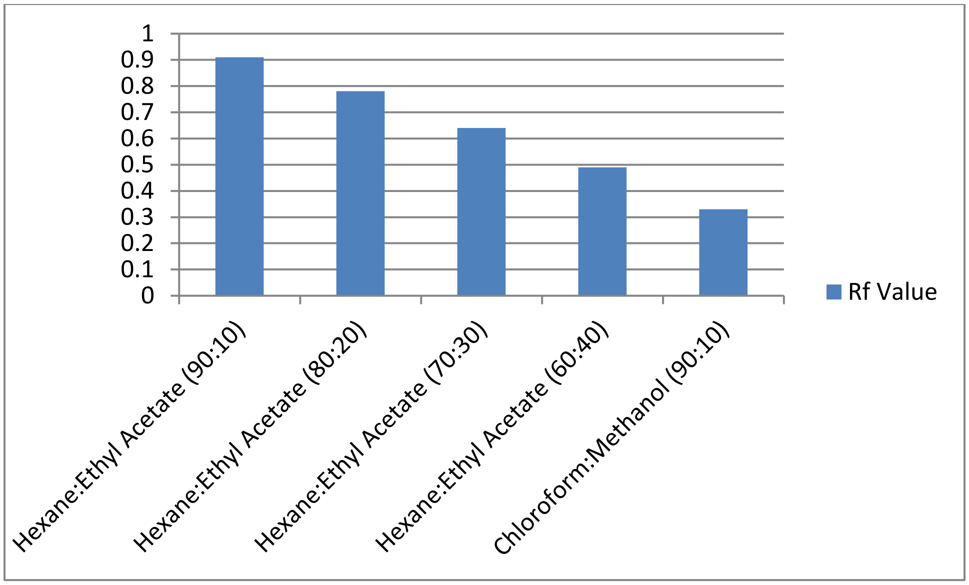

Figure 4 Chromatography Elution

4
.3.2 Correlation Between Pollution Levels and Algal Bioactivity

- Devtal Lake had higher dissolved oxygen (DO) and lower BOD/COD, indicating better water quality.
- Gangasagar Lake showed higher COD and BOD levels, suggesting organic pollution.
- Despite pollution, bioactive compound production was observed in algae, indicating that certain algae can adapt to environmental stress and synthesize protective metabolites.

4.3.3 Implications of Water Quality on Algae-Derived Bioactive Compounds

- Pollution influences secondary metabolite production, and moderate environmental stress can enhance bioactive compound synthesis.
- Regular monitoring of water quality is essential to ensure consistent bioactive compound yield.

5. Conclusion

5.1 Summary of Key Findings on the Antihyperglycemic Potential of Freshwater Algae-Derived Compounds

The present study successfully isolated and characterized a bioactive terpenoid, Ent-15-hydroxy-labda-8,2-ene,13-ediene-3-one, from freshwater algae collected from Devtal and

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Gangasagar Lakes. The compound was structurally confirmed through UV-Vis, FT-IR, Mass Spectrometry (MS), and NMR analyses. The antihyperglycemic evaluation in alloxan-induced diabetic mice demonstrated significant blood glucose-lowering effects, comparable to the standard drug Glibenclamide. The results suggest that algae-derived terpenoids could serve as potential alternative treatments for diabetes mellitus due to their natural origin, metabolic regulation properties, and minimal side effects.

Furthermore, the compound prevented excessive weight loss in diabetic mice, indicating a possible role in improving metabolic function and insulin sensitivity. The findings support the growing evidence that natural bioactive compounds from freshwater algae can be explored as effective and sustainable sources of antidiabetic agents.

5.2 Importance of Maintaining Water Quality for Optimal Algal Growth and Bioactivity

Water quality analysis of Devtal and Gangasagar Lakes revealed significant variations in dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), pH, and total dissolved solids (TDS).

- Devtal Lake exhibited better water quality with higher DO and lower pollution levels, suggesting a healthier aquatic ecosystem that supports optimal algal growth.
- Gangasagar Lake showed higher BOD and COD levels, indicative of organic pollution, which may alter the biochemical composition of algae.

The results highlight the critical role of environmental conditions in influencing algal metabolite production. Ensuring clean and nutrient-balanced water is essential to maintain the structural integrity and bioactivity of algae-derived compounds. Sustainable water management practices are, therefore, crucial for preserving aquatic biodiversity and enhancing the pharmaceutical potential of freshwater algae.

5.3 Future Research Directions

5.3.1 Potential Clinical Applications of the Identified Compound

While the algae-derived terpenoid demonstrated promising antihyperglycemic activity in animal models, further research is required to:

- Elucidate its mechanism of action: Understanding how the compound interacts with insulin receptors, glucose transporters, and oxidative stress pathways.
- Assess toxicity and long-term effects: Conducting preclinical safety studies to determine optimal dosages and potential side effects.
- Formulate novel drug delivery systems: Developing nano-formulations, encapsulation techniques, or functional foods to enhance bioavailability and therapeutic efficacy.
- Validate efficacy in clinical trials: Transitioning to human clinical trials to assess safety, dosage optimization, and metabolic benefits in diabetic patients.

5.3.2 Sustainable Water Management for Algal Biotechnology

Given the importance of water quality in bioactive compound production, future research should focus on:

- Implementing pollution control measures: Reducing industrial and agricultural runoff to minimize heavy metal contamination and eutrophication.
- Developing algae-based bioremediation systems: Utilizing selected algal strains to absorb pollutants while simultaneously enhancing bioactive metabolite production.
- Enhancing algal cultivation techniques: Exploring controlled bioreactors and hydroponic systems to maximize

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terpenoid yield in a pollution-free environment.

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