Phytochemical and Antidiabetic Study of Methanolic Extract of *Portulaca oleracea* L.

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ABSTRACT: *Portulaca oleracea* L. (*P. oleracea*), a globally distributed edible herb traditionally used in ethnomedicine, has gained scientific attention due to its broad pharmacological spectrum. This study aimed to isolate and characterize bioactive compounds from the methanolic extract of *P. oleracea* and evaluate its antidiabetic potential in alloxan-induced diabetic mice. The whole plant was subjected to cold maceration, followed by chromatographic fractionation and ^{1}H NMR-based structural elucidation. Two pure compounds were isolated and tentatively identified as 5,6-Bis(nitrooxy)-3,4-dihydro-1,2,4-triazine and 3-[4-Hydroxy-3-(3,4,5-trihydroxy-6-hydroxymethyl-tetrahydropyran-2-yloxy)-phenyl]-3-methoxy-N-[2-(3-methoxybenzyl)butyl]acrylamide. *In vivo* antidiabetic activity was assessed in albino mice via oral administration of the extract (500 mg/kg body weight) over 14 days. Fasting blood glucose levels were significantly reduced (mean reduction: 59.0%) in treated diabetic mice compared to baseline. ELISA-based biochemical assays demonstrated that serum creatinine and uric acid levels remained within normal physiological ranges, suggesting no renal toxicity and a potential nephroprotective effect. These findings indicate that *P. oleracea* contains potent bioactive constituents with antidiabetic efficacy and renal safety, supporting its therapeutic potential as a natural agent for diabetes management.

Key words: *Portulaca oleracea* L., Antidiabetic,p, ^{1}H NMR, bioactive, nephroprotective.

INTRODUCTION

Portulaca oleracea L. (*P. oleracea)*, commonly known as purslane, is an annual succulent herb belonging to the family Portulacaceae. The plant is widely distributed across tropical and temperate regions of the world. [1,2] Traditionally, *P. oleracea* has been employed in various ethnomedicinal systems as a febrifuge, antiseptic, vermifuge, and anti-inflammatory agent.[1-3] The World Health Organization (WHO) has recognized it as one of the most commonly used medicinal plants, referring to it as a "Global Panacea" due to its broad spectrum of therapeutic properties.

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Numerous pharmacological studies have demonstrated the bioactivities of *P. oleracea*, including antioxidant,[4,5] anticancer,[6] antitumor,[7] antimicrobial,[8] anti-obesity,[9] and hepatoprotective[10] effects. These biological activities are primarily attributed to its rich phytochemical profile, comprising omega-3 fatty acids, polyphenols, flavonoids, terpenoids, alkaloids, ferulic amide, and various other secondary metabolites.[5,7,11–14]

Despite extensive documentation of *P. oleracea*'s medicinal benefits, few studies have addressed the antidiabetic effects of *P. oleracea*.[15,16] Diabetes mellitus (DM), a chronic metabolic disorder characterized by persistent hyperglycemia, presents in three primary forms: type I, type II, and gestational diabetes. Among them, type II diabetes is most prevalent, resulting from insulin resistance, whereas type I diabetes is an autoimmune condition leading to

the destruction of pancreatic β-cells.[17] According to the International Diabetes Federation, the global prevalence of diabetes among adults aged 20–79 years was 8.8% in 2017, with nearly half of all cases undiagnosed. The number of diabetic individuals worldwide is projected to reach 439 million by 2030 and potentially reach 592 million by 2035.[18,19]

Given the lack of a definitive cure for diabetes, identifying plant-based alternatives for diabetes prevention and management remains a research priority. Several botanicals, including *P. oleracea* (purslane),[15,16] *Momordica charantia* (bitter melon),[20] *Gymnema sylvestre* (gurmar),[21] *Trigonella foenum-graecum* (fenugreek),[22] *Syzygium cumini* (jamun),[23] *Cinnamomum zeylanicum* (cinnamon),[24] and *Curcuma longa* (turmeric)[25] have demonstrated notable antidiabetic mechanisms. *P. oleracea* holds great promise as a dual-purpose pharmacological and nutritional agent. Therefore, the present study was designed to isolate and characterize the bioactive constituents from the methanolic extract of *P. oleracea* and to evaluate its antidiabetic effects in alloxan-induced diabetic mice. The findings aim to provide insights into the therapeutic potential of *P. oleracea* and support its future development as a natural antidiabetic agent.

MATERIALS AND METHODS

Sample collection and preparation. Fresh *P. oleracea* plants were collected from Kamrangichar, Dhaka. The entire plant—including leaves, stems, and roots—was used for phytochemical analysis. A total of 10.5 kg of plant material was thoroughly washed with tap water to remove dust and debris. The whole plants were chopped into small segments and sun-dried for 15 days, yielding 2.3 kg of dried sample. All solvents used in the study, including methanol, hexane, acetonitrile, DMSO, $CDCl_3$, and distilled water, were of analytical grade.

Extraction of Crude Phytochemicals. The dried plant material (2.3 kg) was subjected to cold maceration using a 1:1 (v/v) mixture of methanol and distilled water at room temperature for 72 hours with intermittent shaking. The mixture was filtered, and the process was repeated to maximize extraction efficiency. The combined filtrates were concentrated under reduced pressure using a rotary evaporator at 55°C, yielding 48.48 g of crude methanolic extract. This extract was stored at 4°C for further phytochemical investigations.

Column Chromatography and Chromatogram Development. Column chromatography was performed using a glass column (16 in × 1.5 in) packed with 25.4 g of silica gel (60-120 mesh). A 5.0 g portion of the crude methanolic extract of entire plant was subjected to separation using a gradient elution method,[9] where solvent polarity was gradually increased to enhance compound resolution. The solvent system consisted of combinations of hexane, ethyl acetate, methanol, and water. Elution was carried out at a constant flow rate of 15-20 drops per minute. Collected fractions were monitored by thin-layer chromatography (TLC) to identify distinct bands for further purification and analysis.

Fractionation of methanolic extract via open column chromatography. The extracted sample was fractionated using a series of solvents in varying proportions, including hexane, ethyl acetate, methanol, and water. The resulting eluates were collected in test tubes and subsequently analyzed by thin-layer chromatography (TLC). Various solvent systems were initially tested to achieve optimal separation, and the methanol:toluene (6:4, v/v) mixture was found to provide the best resolution.

Spots on the TLC plates were visualized under UV light and by spraying with sulfuric acid, followed by gentle heating (charring) to develop visible spots. Based on the TLC profiles, three distinct fractions containing relatively pure compounds were identified and labeled as Fraction 1, Fraction 2, and Fraction 3. Each fraction was concentrated under reduced pressure using a rotary evaporator. The concentrated samples were then transferred into labeled vials and subsequently subjected to ^{1}H NMR spectroscopy for structural analysis.

Evaluation of antidiabetic activity in alloxan-induced diabetic mice. *Animal procurement and housing:* Female albino mice (26-30 g) were

obtained from the Institute of Food and Nutrition Science, University of Dhaka. Animals were housed under standard laboratory conditions with a 12 hrs light/dark cycle at a controlled temperature of 21-23°C. All procedures involving animals were conducted following ethical guidelines.

Induction of Type II Diabetes. Type II diabetes was induced via a single intraperitoneal injection of alloxan monohydrate (98%) at a dose of 180 mg/kg body weight, dissolved in 2.2 ml of distilled water. Following the injection, the mice were provided with 10% glucose solution overnight to counteract initial hypoglycemic shock. Blood glucose levels were measured 24 hrs post-injection using an Accu-Chek Active glucometer to confirm successful induction of hyperglycemia.

Preparation of Plant Extract Dosage. A dosage of 500 mg/kg body weight was prepared by dissolving 1.0 g of the methanolic extract in 7.7 ml of distilled water. The solution was administered orally once daily for 14 consecutive days. No mortality, behavioral abnormalities, or noticeable signs of toxicity were observed during the treatment period.

Experimental Design. Mice were randomly divided into two groups (n=7), as follows:

- **Group I (Control):** Non-diabetic mice (n=3), not injected with alloxan, received normal food and water to serve as baseline controls.
- **Group II (Diabetic + Treatment):** Diabetic mice (n=4), treated daily with 500 mg/kg of *P. oleracea* extract (whole plant) via oral gavage.

Monitoring and Sample Collection. Fasting blood glucose levels were measured on day 0 (before treatment), and subsequently on days 2, 5, 8, 11 and 14. Blood samples were collected from the tail vein after an overnight fast. Glucose levels were quantified using glucose oxidase/peroxidase reactive strips.

At the end of the treatment period, all animals were fasted for 10 hours and euthanized under approved humane conditions. Approximately 3 ml of blood was collected from the inferior vena cava for biochemical analysis. Major organs, including the liver and kidneys, were harvested for histopathological comparison between treated and control groups.

RESULTS AND DISCUSSION

^{1}H NMR Spectra of Isolated Compounds. ^{1}H NMR spectra were analyzed to propose the structures of the isolated compounds. Among the three fractions, fraction 1 and fraction 3 yielded relatively clean spectra with minimal impurities. In contrast, the spectrum of Fraction 2 was not interpretable due to a high level of impurities.

^{1}H NMR Spectrum of Fraction 1 Compound

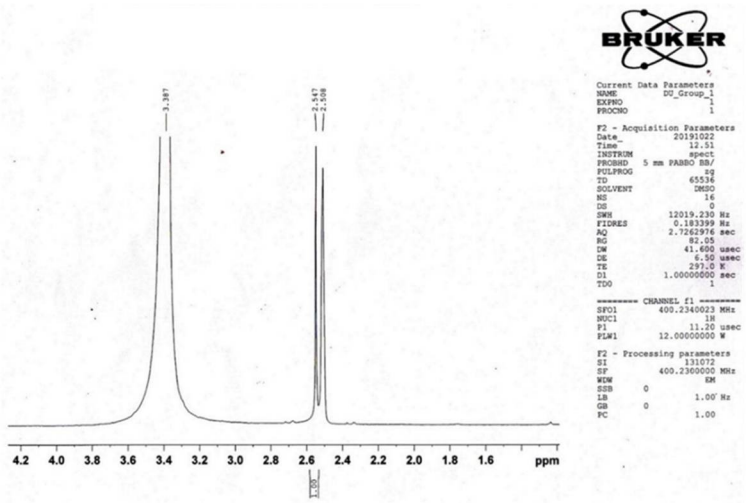


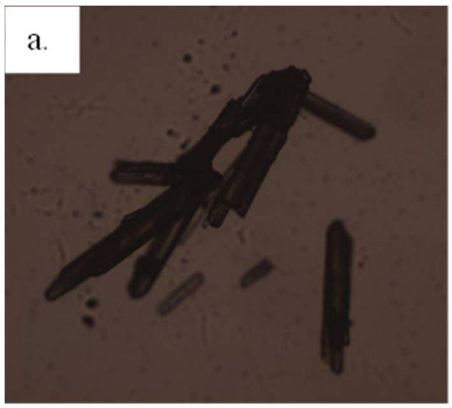
Figure 1. ^{1}H NMR spectrum of Fraction 1 compound.

The ¹H NMR spectrum (400 MHz, DMSO) of the Fraction 1 compound shows two doublets at δ 2.508 ppm and δ 2.547 ppm, corresponding to protons labeled H-3′ and H-3″, respectively. The small difference in chemical shift (Δδ = 0.039 ppm) and the splitting pattern suggest that these protons occupy axial and equatorial positions on adjacent carbon atoms within a cyclic structure. Such closely spaced doublets are characteristic of two hydrogen on adjacent carbon which will form J-J coupling.[26]

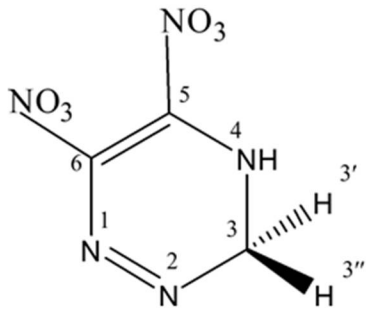
The observed coupling constant (J = 16 Hz) further supports this, as such a value is typical for trans-diaxial or axial–equatorial relationships in six-membered rings. The slight deshielding of both protons is likely due to the presence of electron-withdrawing nitrogen atoms on either side of the carbon framework, consistent with a substituted triazine ring. The –NH– proton signal is not visible, likely due to overlapping with the solvent (DMSO) signal.

Table 1. ¹H NMR spectral data for fraction 1 compound.

Protons	Nature of the peak	Chemical shift (δ_H) value for protons of compound (ppm)
H-3′	Doublet	2.508
H-3″	Doublet	2.547



b.



5,6-Bis(nitrooxy)-3,4-dihydro-1,2,4-triazine

Figure 2. (a) Crystalline structure obtained from Fraction 1 sample (b) Tentative structure of Fraction 1 compound.

¹H NMR spectrum of fraction 3 compound. The ¹H NMR spectrum (400 MHz, methanol) of the compound isolated from Fraction 3 shows a multiplet at δ 7.35-7.36 ppm corresponding to protons H-1 to H-6 and H-1′ to H-6′. This chemical shift range is characteristic of aromatic protons on a benzene ring, consistent with reported values (typically 6.5–8.0 ppm, centered around 7.3 ppm). Additional multiplets are observed for H-7, H-9, and H-10, all of which correspond to aliphatic protons, showing variation in chemical shifts depending on the nature of the neighboring functional groups. H-11 appears as a triplet at δ 1.00 ppm, typical for a methyl ($-CH_3$) group in an aliphatic environment (expected range: 0.7-1.3 ppm).

A sharp singlet at δ 2.01 ppm is assigned to the –NH– proton (H-12), while H-1″ shows a singlet at δ 5.74 ppm, consistent with a glycosidic proton in a sugar moiety. The remaining glucose ring protons (H-2″ to H-6″) give a multiplet centered around δ 3.53 ppm, which falls within the typical range for sugar protons (3.60–3.90 ppm). The proton signals at δ 3.33 ppm (H-10′) and δ 3.67 ppm (H-13) are attributed to aliphatic and aromatic ether functionalities, respectively. The chemical shift of H-8 appears higher than the expected value for a typical aliphatic proton, likely due to anisotropic deshielding effects between adjacent –C=O and –NH– groups.

It is important to note that chemical shift values are influenced by the electronic environment; electron-withdrawing or electron-donating groups on neighboring atoms can cause significant variations. Therefore, while the spectral data provide strong indications of structural features, the proposed structure based on this NMR analysis remains tentative.

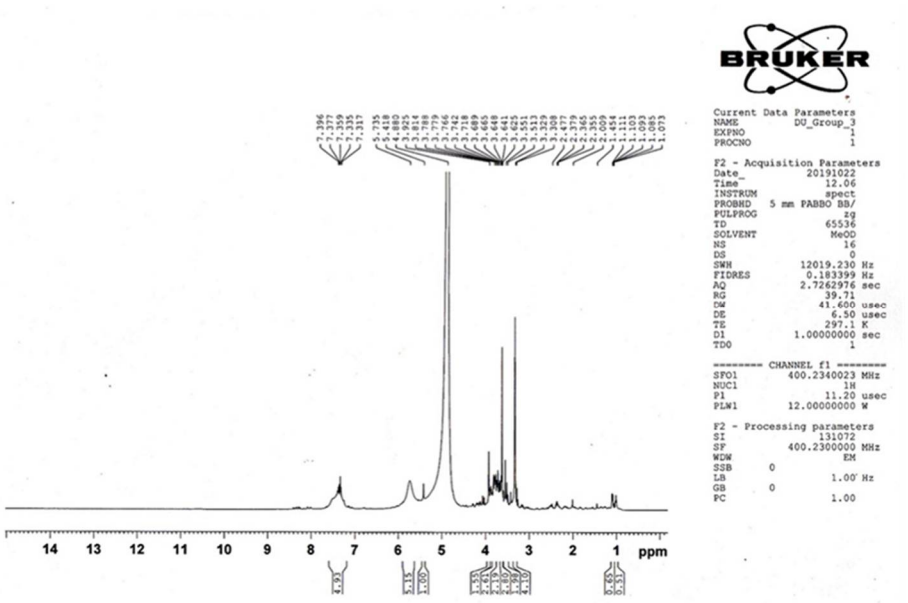


Figure 3. ^{1}H NMR spectrum of Fraction 3 compound.

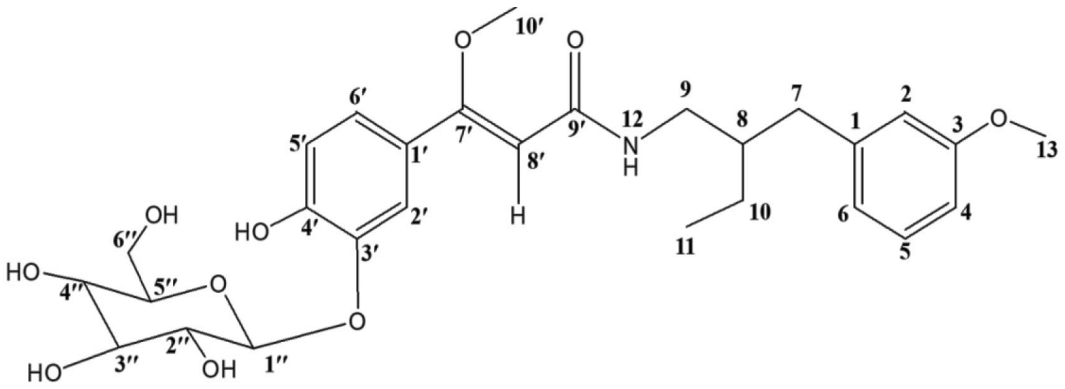
Table 2. ^{1}H NMR spectral data for fraction 3 compound.

Protons	Nature of the peak	Chemical shift (δ_H) value for protons of the compound (ppm)
H-(1-6)	Multiplet	7.35
H-7	Multiplet	2.38
H-8	Multiplet	2.36
H-9	Multiplet	2.47
H-10	Multiplet	1.10
H-11	Triplet	1.00
H-12	Singlet	2.01
H-1′	Singlet	5.74
H-(2″-6″)	Multiplet	3.53
H-(1′-6′)	Multiplet	7.36
H-8′	Singlet	5.41
H-10′	Singlet	3.33
H-13	Singlet	3.67

Analysis of anti-diabetic properties. *Blood Glucose Level Data Analysis***:** Fasting blood glucose levels were monitored to evaluate the anti-diabetic potential of the methanolic extract of *P. oleracea* in alloxan-induced diabetic mice. Control mice (Group I) maintained stable fasting glucose levels throughout the study, with mean values of 6.5 ± 1.2 mmol/L, confirming normoglycemia and physiological stability during the experimental period. In contrast, alloxan-induced diabetic mice (Group II) exhibited a marked increase in fasting glucose levels 24 hours post-induction (mean ± SD: 20.3 ± 8.4 mmol/L), confirming successful diabetes induction. Treatment with *P. oleracea* extract resulted in a progressive and significant reduction in fasting glucose over 14 days.

These results show a significant decrease in blood glucose levels after administration of *P. oleracea* extract, indicating its anti-diabetic potential. The similarity between the hypoglycemic effects observed from Day 5 to Day 14 suggests that *P. oleracea* extract reached its maximal glucose-lowering efficacy by Day 5, after which the effect was maintained. Such a sustained effect indicates that the extract may help normalize glucose metabolism rather than induce excessive hypoglycemia, which is desirable for long-term management of diabetes.

ELISA Test for creatinine. evaluation of serum biomarkers: creatinine and uric acid: To evaluate the renal safety and possible nephroprotective effects of *P. oleracea* in diabetic conditions, serum creatinine and uric acid levels were assessed in alloxan-induced diabetic mice (Group II) using ELISA-based colorimetric assays. The results were compared against standard reference values generated from known concentrations.



3-[4-Hydroxy-3-(3,4,5-trihydroxy-6-hydroxymethyl-tetrahydropyran-2-yloxy)-phenyl]-3-methoxy-N-[2-(3-methoxybenzyl)butyl]acrylamide

Figure 4. Tentative structure of fraction 3 compound.

Table 3. Effect of *P. oleracea* extract on diabetic mice (Group-II).

Mouse ID	Baseline (mmol/l)	Post-alloxan (mmol/l)	Day 2	Day 5	Day 8	Day 11	Day 14
1	5.4	25.1	11.4 (−54.6%)	6.7 (−73.3%)	8.6 (−65.7%)	7.4 (−70.5%)	5.3 (−78.9%)
2	6.1	30.0	12.9 (−57.0%)	10.6 (−64.7%)	14.8 (−50.7%)	9.5 (−68.3%)	8.2 (−72.7%)
3	5.2	11.7	8.7 (−25.6%)	7.1 (−39.3%)	6.9 (−41.0%)	8.7 (−25.6%)	6.2 (−47.0%)
4	5.6	14.3	9.2 (−35.7%)	7.5 (−47.6%)	7.2 (−49.7%)	5.3 (−62.9%)	8.4 (−41.3%)
Mean ± SD	5.6 ± 0.4	20.3 ± 8.4	10.5 ± 1.9 (−43.2%)	8.0± 1.7 (−56.7%)	9.4 ± 3.5 (−51.3%)	7.7 ± 1.6 (−56.4%)	7.0 ± 1.3 (−59.0%)

Control mice showed stable blood glucose levels throughout the study.

Creatinine, a marker of glomerular filtration rate, was measured at 4.71 µmol/L in the control group (Group I). In Group II mice, serum creatinine levels ranged from 3.83 to 4.90 µmol/L, with a mean value of 4.74 µmol/L, indicating preserved renal function despite hyperglycemic stress.

Uric acid, a biomarker commonly elevated in metabolic disorders and renal dysfunction, remained within physiologically acceptable limits. The control group (Group I) showed a average value of 79.71 µmol/L, while diabetic mice exhibited serum uric acid levels ranging from 61.14 to 82.30 µmol/L, with a mean value of 72.97 µmol/L. The summarized data are presented in Table 4**:**

Table 4. Summary of serum creatinine and uric acid levels in diabetic mice (Group II) compared to control (Group I).

Parameter	Group I average (µmol/l)	Group II mean ± SD (µmol/l)
Creatinine	4.71 µmol/l	4.74 ± 0.46
Uric acid	79.71 µmol/l	72.97 ± 8.70

These results suggest that *P. oleracea* administration did not adversely affect renal biomarkers in diabetic mice. On the contrary, the observed values support a potential nephroprotective role**,** as creatinine and uric acid levels remained well within safe limits, indicating preserved renal filtration and metabolic function. The extract appears to counteract renal stress typically associated with alloxan-induced diabetes.

CONCLUSION

The methanolic extract of *P. oleracea* showed significant antidiabetic activity in alloxan-induced diabetic mice, with marked reduction in blood glucose levels. ELISA tests confirmed that creatinine and uric acid levels remained within normal ranges, indicating no renal or hepatic toxicity. Chromatographic isolation followed by ^{1}H NMR analysis led to the tentative identification of two bioactive compounds: 5,6-Bis(nitrooxy)-3,4-dihydro-

1,2,4-triazine and a methoxy-substituted acrylamide derivative. These findings support the antidiabetic potential of *P. oleracea* and highlight the need for further pharmacological studies.

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