Exploring the Antioxidant, Antidiabetic, and Anti-Inflammatory Properties of Cold Macerated *Rumex nepalensis* Leaf Extract: A Study on Its Role in Hyperglycaemia Control, Free Radical Scavenging, and the Development of a Nanogel for Therapeutic Use

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Abstract
The present study aimed to evaluate the phytochemical composition, antioxidant, antidiabetic, and anti-inflammatory properties of methanolic leaf extract of *Rumex nepalensis*, codenamed RNME-L, along with the formulation and characterization of a nanogel incorporating solid lipid nanoparticles (SLNs). Preliminary phytochemical screening confirmed the presence of alkaloids, flavonoids, phenols, phytosterols, tannins, anthraquinones, and saponins, indicating potential pharmacological activities. The antioxidant evaluation using DPPH, ABTS, and reducing power assays demonstrated dose-dependent radical scavenging effects, with RNME-L exhibiting significant activity, though slightly lower than standard antioxidants like BHT and quercetin. The IC_{50} values confirmed its strong free radical neutralization potential. Antidiabetic analysis through α-amylase, α-glucosidase inhibition, and glucose uptake assays revealed RNME-L as a potent inhibitor of carbohydrate-digesting enzymes while enhancing cellular glucose absorption, suggesting its role in glycaemic control. The anti-inflammatory assays showed a concentration-dependent inhibition of COX-1 and COX-2, highlighting its potential in inflammation modulation. Additionally, a nanogel was successfully formulated using SLNs and optimized for stability, viscosity, and spreadability. In vitro drug release studies confirmed the sustained release profile of selected formulations. These findings suggest that RNME-L holds promise as a natural antioxidant, antidiabetic, and anti-inflammatory agent, with the nanogel formulation offering a novel approach for enhanced therapeutic efficacy in topical applications.

Keywords: *Rumex nepalensis*, Antioxidant activity, Anti-inflammatory activity, Solid Lipid Nanoparticles, Antidiabetic properties, Nanogel

1. INTRODUCTION

Oxidative stress is a critical biological phenomenon resulting from an imbalance between the production of reactive oxygen species (ROS) and the body's ability to neutralize them using antioxidants. ROS, including superoxide anions, hydroxyl radicals, and hydrogen peroxide, are generated during normal cellular metabolism and play essential roles in cell signalling and homeostasis [1, 2]. However, excessive ROS accumulation can lead to cellular damage, lipid peroxidation, protein oxidation, and DNA fragmentation, ultimately contributing to various pathological conditions such as neurodegenerative disorders, cardiovascular diseases, and metabolic syndromes. Antioxidants, both enzymatic (superoxide dismutase, catalase, and glutathione peroxidase) and non-enzymatic (vitamins C and E, flavonoids, and polyphenols), help mitigate oxidative damage by neutralizing free radicals. In this context, identifying natural sources of antioxidants has become a crucial area of research, with medicinal plants being a promising reservoir of bioactive compounds capable of scavenging ROS and preventing oxidative stress-induced damage [3-5].

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycaemia resulting from impaired insulin secretion, insulin resistance, or both. Uncontrolled diabetes leads to severe complications, including cardiovascular diseases, nephropathy, neuropathy, and retinopathy. Oxidative stress plays a significant role in the pathogenesis and progression of diabetes and its associated complications [6-8]. Hyperglycaemia induces excessive ROS production through multiple mechanisms, including mitochondrial dysfunction, glucose autoxidation, and activation of advanced glycation end products (AGEs). These oxidative pathways contribute to β-cell dysfunction, insulin resistance, and endothelial damage, exacerbating diabetic complications. Given the

pivotal role of oxidative stress in diabetes, therapeutic strategies focusing on antioxidants and enzyme inhibitors have gained increasing attention for managing hyperglycemia and preventing long-term complications [9-12].

Natural products, particularly medicinal plants, have long been explored for their pharmacological benefits in managing diabetes and oxidative stress-related disorders. Several plant-derived bioactive compounds, including polyphenols, flavonoids, alkaloids, and saponins, have demonstrated potent antioxidant and antidiabetic properties. Many medicinal plants exhibit α-amylase and α-glucosidase inhibitory activity, delaying carbohydrate digestion and glucose absorption, thereby controlling postprandial hyperglycemia [6, 13]. Additionally, plant-based compounds can enhance glucose uptake, improve insulin sensitivity, and reduce oxidative stress, making them valuable alternatives to synthetic antidiabetic drugs, which often have undesirable side effects. Given the growing demand for safer and more effective natural therapies, investigating the bioactive potential of medicinal plants is essential for developing novel antioxidant and antidiabetic agents [6, 8, 10, 11].

Rumex nepalensis, commonly known as Himalayan dock, belongs to the Polygonaceae family and is widely distributed in the Himalayan regions [14]. Traditionally, it has been used in folk medicine for treating skin disorders, gastrointestinal ailments, and inflammatory conditions. Phytochemical studies have identified the presence of flavonoids, phenolic compounds, tannins, alkaloids, and saponins in *R. nepalensis,* which contribute to its antioxidant, anti-inflammatory, and antimicrobial activities [14, 15]. Given its rich phytochemical composition, *R. nepalensis* holds promise as a natural source of bioactive compounds for managing oxidative stress and diabetes. However, despite its traditional use, scientific validation of its antidiabetic and antioxidant properties remains limited, necessitating further research [15, 16].

The present study aims to evaluate the antioxidant and antidiabetic potential of *Rumex nepalensis* leaf extract (RNME-L) using in vitro assays. The antioxidant activity will be assessed through DPPH, ABTS, and reducing power assays, while the antidiabetic potential will be determined by α-amylase and α-glucosidase inhibition assays and glucose uptake studies. The study will also include preliminary phytochemical screening to identify the bioactive compounds responsible for these activities. By investigating the pharmacological properties of *R. nepalensis*, this research seeks to establish its therapeutic relevance as a natural antioxidant and antidiabetic agent.

2. EXPERIMENTAL

Chemicals, Drugs and Reagents

DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), potassium persulfate, ferric chloride ($FeCl_3$), potassium ferricyanide, trichloroacetic acid, alpha-amylase, alpha-glucosidase, p-Nitrophenyl glucopyranoside (pNPG), sodium carbonate, iodine, and phosphate buffer were all procured from Loba Chemical Company, Sigma Aldrich or Himedia Laboratories, India. Zolen Pharma, located in Karnal, India, provided a complimentary sample of phospholipon 80. Stearic acid and pluronic F68 were procured from Loba Chemical Company, located in Mumbai, India. All additional reagents and chemicals were of analytical quality and were exclusively purchased from verified suppliers.

Identification and Extraction

The leaves of the plant species *Rumex nepalensis* were gathered from Herbal Trenz in Kullu, Himachal Pradesh, and Dr. S. Kumar of the College of Life Sciences' Department of Botany made the identification. After these plants were identified, the cold maceration process was used to extract their beneficial components. The cold maceration method was employed for the extraction of *Rumex nepalensis* leaves to preserve bioactive compounds. Fresh leaves were washed, air-dried in shade for 7–10 days, and ground into a coarse powder. A known quantity of 100 g of the powdered material was soaked in 500 mL of 80% methanol in an airtight glass container. The mixture was left at room temperature (25–28°C) for 72 hours with occasional stirring to enhance the extraction process. The extract was then filtered through muslin cloth and Whatman No. 1 filter paper to remove plant residues. The filtrate was concentrated using a rotary evaporator at 40°C under reduced pressure. The semi-solid crude extract (RNME-L) was stored at 4°C for further analysis.

Preliminary phytochemical screening

In order to determine the active ingredients causing therapeutic benefits, preliminary phytochemical screening is an essential stage in the study of medicinal plants. To find several types of phytochemicals, including alkaloids, flavonoids, tannins, saponins, terpenoids, steroids, and phenolic compounds, this screening entails a number of qualitative tests. As previously mentioned, standard procedures were followed to carry out initial phytochemical screening in a lab setting.

Antioxidant activity

DPPH Radical Scavenging Assay

The antioxidant potential of the samples was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. A fresh stock solution of DPPH was prepared by dissolving 0.1 mM of DPPH in methanol and kept in a dark environment at room temperature. The test samples were prepared in methanol at different concentrations. For the assay, 1.0 mL of the DPPH solution was mixed with 1.0 mL of the prepared sample solutions. The reaction mixtures were incubated at room temperature in the dark for 30 minutes to ensure adequate

interaction between the DPPH radicals and the antioxidants in the sample. Following the incubation, the absorbance was recorded at 517 nm using a UV-visible spectrophotometer, with methanol used as a blank. A control solution was also prepared by mixing 1.0 mL of DPPH solution with 1.0 mL of methanol to serve as a reference. Ascorbic acid was used as the standard antioxidant for comparison [17, 18]. The ability of the test sample to neutralize DPPH radicals was determined using the following formula:

DPPH Scavenging Activity (%) = (Acontrol – Asample / Acontrol) × 100

where Acontrol represents the absorbance of the control solution, and Asample is the absorbance of the test sample. The IC_{50} value, which indicates the concentration of the sample required to inhibit 50% of the DPPH radicals, was calculated from the dose-response curve. All experiments were conducted in triplicate, and the results were expressed as mean ± standard deviation to ensure accuracy and reliability

ABTS Radical Cation Decolorization Assay

The antioxidant capacity of the test samples was evaluated using the ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation decolorization assay. The $ABTS^{\bullet+}$ solution was generated by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate. This mixture was incubated in the dark at room temperature for 12–16 hours to allow the formation of stable $ABTS^{\bullet+}$ radicals. Before use, the $ABTS^{\bullet+}$ solution was diluted with methanol or phosphate-buffered saline (pH 7.4) to obtain an absorbance of approximately 0.700 ± 0.02 at 734 nm. For the assay, 1.0 mL of the diluted $ABTS^{\bullet+}$ solution was mixed with 1.0 mL of the test sample at different concentrations. The reaction mixture was incubated at room temperature in the dark for 10 minutes, allowing the antioxidant compounds in the sample to interact with the $ABTS^{\bullet+}$ radicals. After incubation, the absorbance was measured at 734 nm using a UV-visible spectrophotometer. A control solution containing $ABTS^{\bullet+}$ without the test sample was prepared to establish the baseline absorbance, while Trolox was used as the standard antioxidant for comparison [19, 20]. The percentage of $ABTS^{\bullet+}$ scavenging activity was calculated using the following formula:

ABTS Scavenging Activity (%) = (Acontrol−Asample /Acontrol) × 100

where Acontrolrepresents the absorbance of the control solution, and Asample is the absorbance of the test sample. The IC_{50} value, which represents the concentration of the sample required to scavenge 50% of $ABTS^{\bullet+}$ radicals, was determined using a dose-response curve. All measurements were performed in triplicate, and the results were reported as mean ± standard deviation.

Reducing Power Assay

The reducing power of the test samples was determined based on their ability to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), which was measured using the potassium ferricyanide reduction method. For the assay, different concentrations of the test samples were prepared in methanol or phosphate buffer (pH 6.6). A reaction mixture was prepared by adding 1.0 mL of the sample solution to 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide solution. The mixture was incubated at 50°C for 20 minutes to allow the reduction reaction to take place. After incubation, 2.5 mL of 10% trichloroacetic acid was added to terminate the reaction. The mixture was then centrifuged at 3000 rpm for 10 minutes to obtain a clear supernatant. A volume of 2.5 mL of the supernatant was transferred to a fresh test tube and mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride solution. The reaction mixture was allowed to stand for 10 minutes, and the absorbance was recorded at 700 nm using a UV-visible spectrophotometer. A higher absorbance indicated a stronger reducing power. Ascorbic acid was used as the standard antioxidant, and a blank sample containing all reagents except the test compound was prepared as a reference. The reducing power of the samples was expressed as the absorbance at 700 nm, and all measurements were performed in triplicate to ensure accuracy and reproducibility. The results were reported as mean ± standard deviation [21, 22].

Antidiabetic activity

Alpha-amylase inhibition assay

The α-amylase inhibitory activity of the test samples was evaluated using a colorimetric method based on the hydrolysis of starch by α-amylase. A stock solution of porcine pancreatic α-amylase (1.0 U/mL) was prepared in phosphate buffer (0.02 M, pH 6.9) and stored at 4°C until use. A starch solution (1% w/v) was also prepared by dissolving soluble starch in phosphate buffer and heating it to 60°C with continuous stirring. For the assay, different concentrations of the test samples were prepared in phosphate buffer. A reaction mixture containing 500 µL of the sample solution and 500 µL of α-amylase solution was preincubated at 37°C for 10 minutes. Following incubation, 500 µL of the starch solution was added to initiate the enzymatic reaction. The mixture was further incubated at 37°C for 10 minutes to allow enzymatic hydrolysis. After incubation, 1.0 mL of dinitrosalicylic acid (DNS) reagent was added to each tube to terminate the reaction. The tubes were then heated in a boiling water bath for 5 minutes to develop a color, followed by cooling to room temperature. The absorbance of the resulting solution was measured at 540 nm using a UV-visible spectrophotometer. A control sample containing α-amylase and starch solution without the test sample was prepared to represent 100% enzyme activity [23, 24]. Acarbose was used as a standard inhibitor for comparison. The percentage of α-amylase inhibition was calculated using the following formula:

Inhibition (%) = (Acontrol −Asample / Acontrol) × 100
where Acontrol is the absorbance of the control, and Asample is the absorbance of the test sample. The IC_{50} value, which represents the concentration of the sample required to inhibit 50% of α-amylase activity, was determined from a dose-response curve. All experiments were conducted in triplicate, and the results were reported as mean ± standard deviation.

Alpha-glucosidase inhibition assay

The α-glucosidase inhibitory activity of the test samples was determined using a colorimetric method based on the hydrolysis of p-nitrophenyl-α-D-glucopyranoside (pNPG). A stock solution of α-glucosidase (1.0 U/mL) was prepared in phosphate buffer (0.1 M, pH 6.8) and kept at 4°C until use. The substrate solution was prepared by dissolving pNPG (5 mM) in the same phosphate buffer. For the assay, different concentrations of the test samples were prepared in phosphate buffer. A reaction mixture containing 50 µL of the test sample and 50 µL of α-glucosidase solution was preincubated at 37°C for 10 minutes. After preincubation, 50 µL of the pNPG substrate solution was added to initiate the enzymatic reaction. The mixture was further incubated at 37°C for 20 minutes to allow the reaction to proceed. Following incubation, 100 µL of 0.2 M sodium carbonate (Na_2CO_3) solution was added to stop the reaction. The absorbance of the resulting mixture was measured at 405 nm using a UV-visible spectrophotometer. A control sample containing α-glucosidase and the substrate without the test sample was prepared to represent 100% enzyme activity. Acarbose was used as the standard inhibitor for comparison [23, 25]. The percentage of α-glucosidase inhibition was calculated using the following equation:
Inhibition (%) = (Acontrol – Asample / Acontrol) × 100
where Acontrol is the absorbance of the control, and Asample is the absorbance of the test sample. The IC_{50} value, which represents the concentration of the sample required to inhibit 50% of α-glucosidase activity, was determined from a dose-response curve. All experiments were performed in triplicate, and the results were reported as mean ± standard deviation.

Glucose uptake assay

The glucose uptake assay was conducted to evaluate the effect of the test samples on glucose absorption in cultured cells. The assay was performed using L6 myotubes, depending on the experimental design. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin in a CO_2 incubator at 37°C with 5% CO_2 until they reached confluence. Differentiation into adipocytes or myotubes was induced using standard differentiation media for 7–10 days. Before the assay, the cells were serum-starved in DMEM containing 0.5% FBS for 12 hours to synchronize glucose metabolism. The test samples were prepared at different concentrations in serum-free DMEM. The cells were then treated with the test samples in the presence or absence of insulin (100 nM) for 24 hours. Following incubation, the medium was aspirated, and the cells were washed with phosphate-buffered saline (PBS, pH 7.4). For glucose uptake measurement, 2-deoxy-D-glucose (2-DG, 0.1 mM) or fluorescently labelled 2-NBDG (100 µM) was added to the cells and incubated for 30 minutes at 37°C. The reaction was terminated by washing the cells with ice-cold PBS. The intracellular glucose uptake was quantified by measuring the absorbance at 540 nm (for 2-DG) or fluorescence intensity at excitation 485 nm/emission 535 nm (for 2-NBDG) using a microplate reader. A control without the test sample was used to determine the baseline glucose uptake, while metformin (1 mM) served as the positive control [26, 27]. The percentage increase in glucose uptake was calculated using the following equation:
Glucose Uptake (%) = (Asample – Acontrol / Acontrol) × 100
where Asample is the absorbance or fluorescence of treated cells and Acontrol is the absorbance or fluorescence of untreated cells. All experiments were performed in triplicate, and results were reported as mean ± standard deviation. Statistical analysis was conducted using ANOVA, with significance set at $p < 0.05$.

Anti-inflammatory activity

Cycloxygenase–1 (COX–1) and cycloxygenase–2 (COX–2) assays

The COX-1 assay was conducted using the previously reported methodology [28, 29]. 190 µL of 0.1 M Tris-HCL, 18 µL of L-adrenaline–D-hydrogentartrate, and 10 µL of hematine were mixed with sample solution (10 µL). After adding COX-1 (0.2 U) and letting it sit for five minutes, five microlitres of arachidonic acid were added. To end the incubation at 37 °C, 10% formic acid (10 µL) was added after 20 minutes. PGE 2 concentration was then determined using PGE 2 enzyme-immunoassay (R and D systems). The COX-2 assay was carried out using the previously described methodology [28, 29]. Hematine (10 µL), 190 µL of 0.1 M Tris-HCL buffer, 18 µL of L-adrenaline-D-hydrogentartrate, and 10 µL of disodium edetate (Na2-EDTA) were combined with the sample solution (10 µL). After adding 0.2 U of COX-2 to the mixture, it was pre-incubated for five minutes. 5 µL of arachidonic acid was added to this once more. By adding 10 µL of 10% formic acid, the incubation at 37 °C was stopped after 20 minutes. Ultimately, PGE2-enzyme-immunoassay (R and D systems) was employed to quantify PGE 2 levels.

Formulation of Nanogel using solid lipid nanoparticles (SLN)

The solid lipid nanoparticle (SLN) formulations containing *Rumex nepalensis* leaf extract (RNME-L) were prepared using hot homogenization followed by ultrasonication, and the resulting dispersion was incorporated into a Carbopol-based gel to enhance topical delivery. The lipid phase was prepared by melting stearic acid at 75°C, followed by the addition of Phospholipon 80 and RNME-L at specified concentrations. The aqueous phase, containing Pluronic F68, was heated separately to the same temperature and added dropwise to the molten lipid phase under continuous stirring at 10,000 rpm for 10 minutes using a high-speed homogenizer. The resultant coarse emulsion was subjected to ultrasonication (40% amplitude) for 10 minutes to reduce particle size and enhance stability. The SLN dispersion was allowed to cool to room temperature, solidifying the lipid nanoparticles [30]. The SLN dispersion was incorporated into Carbopol 940-based gel to enhance viscosity and facilitate skin application. Carbopol 940 (1% w/w) was dispersed in deionized water under continuous stirring and allowed to swell for 24 hours at room temperature. The SLN dispersion was gradually added to the hydrated Carbopol gel under mild stirring to ensure uniform distribution. The pH of the gel was adjusted to 5.5–6.5 using triethanolamine to achieve skin compatibility. The formulations (NGF-1 to NGF-7) were developed with varying concentrations of RNME-L (0.5%–3.5% w/w) and optimized for lipid composition, surfactant concentration, and gel consistency. The final formulations were stored in sterile containers at 4°C until further characterization and evaluation [31].

Table 1. Composition of the Formulations (SLN and Gel)

Formulation Code	RNME-L (%w/w)	Stearic Acid (%w/w)	Pluronic F68 (%w/w)	Phospholipon 80 (%w/w)	Carbopol 940 (%w/w)	Water (%w/w)
NGF-1	0.5	2.0	1.0	0.5	1.0	95.0
NGF-2	1.0	2.5	1.5	1.0	1.0	93.0
NGF-3	1.5	3.0	2.0	1.5	1.0	91.0
NGF-4	2.0	3.5	2.5	2.0	1.0	89.0
NGF-5	2.5	4.0	3.0	2.5	1.0	87.0
NGF-6	3.0	4.5	3.5	3.0	1.0	85.0
NGF-7	3.5	5.0	4.0	3.5	1.0	83.0

Characterization of solid lipid nanoparticles (SLN) and Nanogel

The prepared solid lipid nanoparticles (SLNs) were characterized to ensure their stability, drug encapsulation, and release profile. Particle size and zeta potential were measured using Dynamic Light Scattering (DLS) to evaluate uniformity and stability, with zeta potential indicating surface charge and aggregation behaviour. Drug loading and entrapment efficiency were assessed by analyzing the amount of drug incorporated within the lipid matrix. The SLN sediment obtained after centrifugation was diluted with acetonitrile, and drug content was determined at 362 nm using a UV-Visible spectrophotometer. Similarly, entrapment efficiency was determined. In vitro drug release studies were conducted to analyze the release kinetics of the active ingredient. The formulation with the most favourable characteristics was selected for further development, including surface morphology analysis and in vivo efficacy studies. These assessments confirmed the stability, encapsulation efficiency, and controlled release behaviour of the optimized SLN formulation, supporting its potential for effective drug delivery applications [32].

Statistical analysis

The analysis was conducted using GraphPad Prism software (Version 8), which is particularly useful when comparing multiple experimental groups to a control group. An ANOVA was performed, followed by Dunnett's test as a post hoc. The findings of these studies were presented as mean ± SD. A p value of less than 0.05 was considered to be the threshold for significance.

3. RESULTS AND DISCUSSION

Preliminary phytochemical screening

The preliminary phytochemical screening of RNME-L revealed the presence of various bioactive compounds, indicating its potential pharmacological significance. The presence of alkaloids (+) suggests possible neuroprotective, antimicrobial, and antidiabetic properties. The test sample exhibited a higher concentration of flavonoids (++) and phenols (++), both of which are well-known for their strong antioxidant, anti-inflammatory, and free radical scavenging activities. The detection of phytosterols (+) implies a possible role in cholesterol-lowering effects and metabolic regulation, contributing to anti-hyperlipidaemic potential. The Borntrager test (+) indicates the presence of anthraquinones, which are often linked to laxative, antimicrobial, and anticancer properties. Additionally, the presence of tannins (+) suggests astringent and antimicrobial properties, which may contribute to anti-inflammatory and wound healing effects. The presence of saponins (+) further supports antidiabetic, anticancer, and immunomodulatory potential. Overall, these findings suggest that RNME-L is rich

in bioactive compounds, particularly flavonoids and phenols. The presence of diverse phytochemicals indicates its potential therapeutic applications in managing oxidative stress, metabolic disorders, and microbial infections.

Table 2. Results of Preliminary phytochemical screening

Phytochemical constituent	RNME-L
Alkaloids	+
Flavonoids	++
Phytosterols	+
Borntrager test	+
Phenols	++
Tannins	+
Saponins	+

+: Presence of moderate active constituents**, ++:** Presence of maximum active constituents

Antioxidant activity

DPPH Radical Scavenging Assay

The results of the DPPH radical scavenging assay demonstrate a concentration-dependent increase in antioxidant activity for both BHT (Butylated Hydroxytoluene) and RNME-L. At the lowest concentration of 50 µg/mL, RNME-L exhibited a higher scavenging activity (16.78± 1.28%) compared to BHT (9.56 ± 0.89%), suggesting that RNME-L may have a stronger free radical neutralization ability at lower concentrations. However, at 100 µg/mL, BHT (43.68 ± 0.99%) showed greater scavenging activity than RNME-L (35.76 ± 0.99%), indicating that the synthetic antioxidant becomes more effective at moderate concentrations. As the concentration increased to 150 µg/mL, the activity of both samples became nearly comparable, with BHT (70.45 ± 1.03%) slightly surpassing RNME-L (68.90 ± 1.09%). At the highest concentrations of 200 and 250 µg/mL, BHT maintained a marginally superior scavenging capacity (88.67 ± 0.97% and 92.94 ± 1.08%) compared to RNME-L (87.78 ± 1.35% and 88.08 ± 1.56%), although both exhibited nearly similar efficacy. These findings highlight the dose-dependent antioxidant potential of RNME-L, which demonstrated substantial DPPH scavenging activity across all tested concentrations. While BHT, a synthetic antioxidant, showed higher activity at higher concentrations, RNME-L displayed strong efficacy at lower concentrations, suggesting the presence of potent bioactive compounds contributing to its radical scavenging properties. The results imply that RNME-L could be a promising natural antioxidant with a performance comparable to that of BHT at higher concentrations. Further studies, including IC_{50} determination, will be necessary to confirm its effectiveness and explore its potential applications in pharmaceutical and nutraceutical formulations.

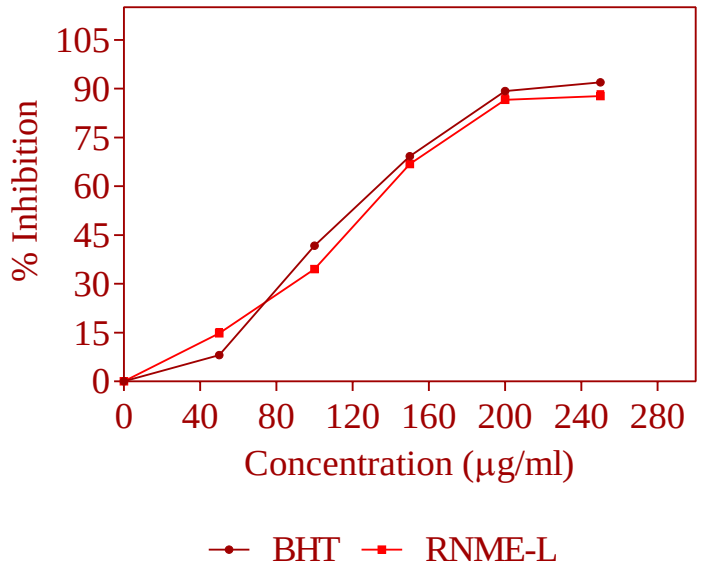

Figure 1. DPPH Radical Cation Decolorization Assay of RNME-L

ABTS Radical Cation Decolorization Assay

The ABTS radical scavenging assay results reveal a concentration-dependent increase in antioxidant activity for both Quercetin (a standard natural antioxidant) and RNME-L. At 50 µg/mL, quercetin exhibited a scavenging activity of 45.13 ± 1.75%, which was higher than RNME-L (35.73 ± 1.039%), indicating that quercetin was more effective at lower concentrations. As the concentration increased to 100 µg/mL, the scavenging ability of both compounds significantly improved, with quercetin at 79.14 ± 1.65% and RNME-L at 64.9 ± 1.09%, still maintaining a notable difference in activity. At 150 µg/mL, quercetin continued to show superior radical scavenging (88.69 ± 1.89%) compared to RNME-L (76.68 ± 1.03%). However, at 200 µg/mL, the activity of both samples nearly converged, with quercetin at 95.79 ± 1.09% and RNME-L at 95.67 ± 2.01%, indicating that RNME-L exhibits similar antioxidant efficacy at higher concentrations. At the highest concentration of 250 µg/mL, quercetin reached 99.12 ± 1.76%, while RNME-L showed 95.17 ± 1.9%, further supporting its potent antioxidant capacity. These findings suggest that RNME-L demonstrates substantial ABTS radical scavenging activity, approaching that of quercetin at higher concentrations. While quercetin exhibited superior antioxidant potential across all concentrations, the difference became negligible at 200 µg/mL and beyond, highlighting RNME-L as a promising natural antioxidant candidate. The results indicate that RNME-L may contain bioactive compounds capable of neutralizing free radicals efficiently, supporting its potential application in pharmaceutical and nutraceutical formulations as an alternative natural antioxidant. Further studies, including IC_{50} determination and mechanistic evaluations, will be beneficial to better understand its effectiveness and practical applications.

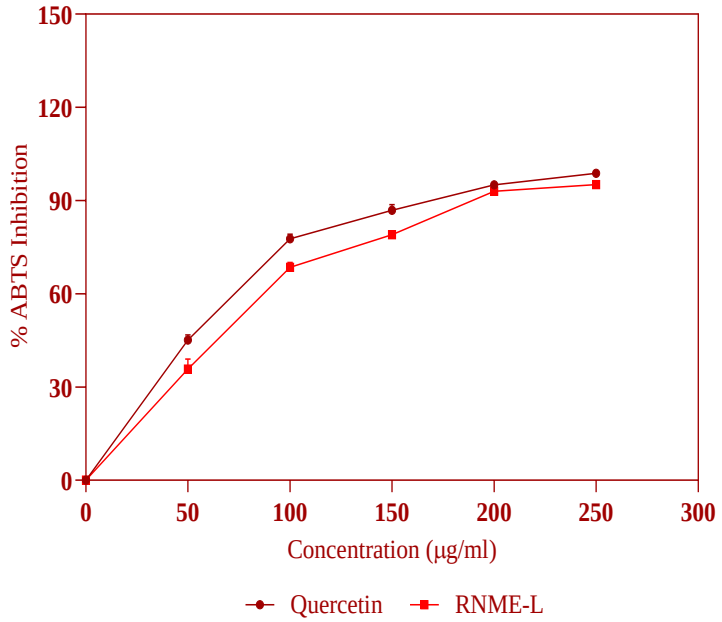

Figure 2. ABTS Radical Cation Decolorization Assay of RNME-L

Reducing Power Assay

The results of the Reducing Power Assay indicate a concentration-dependent increase in the reducing ability of RNME-L and Butylated Hydroxyanisole (BHA, a synthetic antioxidant). At 50 µg/mL, RNME-L exhibited an absorbance of 0.277 ± 0.0047, which was significantly lower than BHA (0.689 ± 0.0046), suggesting that BHA possesses a stronger electron-donating capacity at lower concentrations. As the concentration increased to 100 µg/mL, the reducing power of both samples improved, with RNME-L showing an absorbance of 0.379 ± 0.0046 and BHA at 0.779 ± 0.0077. At 150 µg/mL, the reducing power of RNME-L increased to 0.478 ± 0.0029, while BHA continued to demonstrate a superior effect at 0.878 ± 0.0084. This trend persisted at 200 µg/mL, where RNME-L reached 0.586 ± 0.0069, while BHA exhibited 0.946 ± 0.0095. At the highest concentration of 250 µg/mL, RNME-L recorded an absorbance of 0.677 ± 0.0047, whereas BHA showed a much higher absorbance of 1.399 ± 0.017, indicating its stronger reducing potential. These findings suggest that while RNME-L possesses notable reducing power, it remains lower than BHA at all tested concentrations. The reducing ability of an antioxidant correlates with its capacity to donate electrons and neutralize free radicals, implying that RNME-L has significant but moderate reducing power compared to the synthetic antioxidant BHA. However, the steady increase in RNME-L's activity with concentration suggests that it contains bioactive compounds contributing to its antioxidant potential. Further studies, such as IC_{50} determination and correlation with total phenolic content, could provide deeper insights into its effectiveness as a natural antioxidant alternative in pharmaceutical and nutraceutical applications.

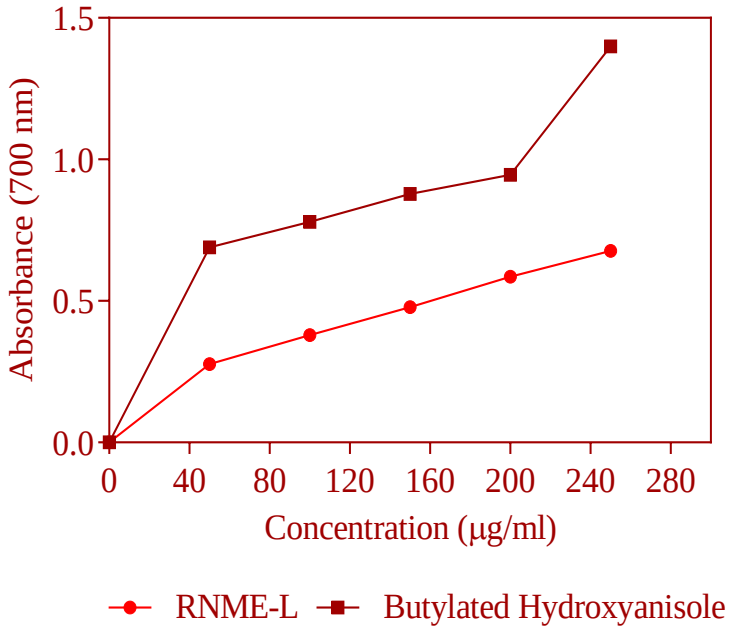

Figure 3. Reducing power assay of RNME-L

The IC_{50} values

The IC_{50} values of RNME-L for the DPPH and ABTS radical scavenging assays indicate its antioxidant potential in comparison to BHT (Butylated Hydroxytoluene, a synthetic antioxidant). The IC_{50} value represents the concentration of a substance required to inhibit 50% of free radicals, with lower values indicating higher antioxidant activity. For the DPPH assay, BHT exhibited a lower IC_{50} (97.98 µg/mL) compared to RNME-L (109.69 µg/mL), suggesting that BHT was slightly more effective in scavenging DPPH radicals. Similarly, in the ABTS assay, BHT (104.84 µg/mL) again displayed a stronger antioxidant effect than RNME-L (122.69 µg/mL). Although RNME-L exhibited slightly higher IC_{50} values, it still demonstrated notable antioxidant activity, suggesting the presence of bioactive compounds contributing to radical scavenging. The results indicate that RNME-L is a promising natural antioxidant, although slightly less potent than the synthetic standard BHT. Further investigations, such as total phenolic and flavonoid content analysis, could help understand the molecular components responsible for its antioxidant potential.

Table 3. The estimated IC50 values of RNME-L for the antioxidant models

Assay Type	Substance	IC50 Value
DPPH	BHT	97.98 g/ml
	RNME-L	109.69 g/ml
ABTS	BHT	104.84 µg/ml
	RNME-L	122.69 µg/ml

Antidiabetic activity

Alpha-Amylase Inhibition Assay

The α-amylase inhibition assay results indicate a concentration-dependent increase in the inhibitory effect of RNME-L against the α-amylase enzyme, which is essential for carbohydrate digestion. At 0 mg/mL (control), no inhibition was observed (0 ± 0%), confirming that the enzyme activity remained unaffected in the absence of RNME-L. At 0.1 mg/mL, RNME-L exhibited 10.86 ± 0.99% inhibition, demonstrating a mild effect at low concentrations. As the concentration increased to 0.5 mg/mL, the inhibition improved significantly to 25.44 ± 0.99%, suggesting a stronger interaction between RNME-L and α-amylase. A substantial inhibitory effect was observed at 1 mg/mL (45.37 ± 1.02%), indicating that nearly half of the enzyme's activity was inhibited. Higher concentrations, such as 2.5 mg/mL and 5 mg/mL, resulted in 71.48 ± 1.01% and 86.76 ± 1.09% inhibition, respectively, highlighting the potent α-amylase inhibitory effect of RNME-L at elevated doses. The steep increase in inhibition from 1 mg/mL onwards suggests the presence of bioactive compounds in RNME-L capable of interfering with enzymatic activity. These results indicate that RNME-L has strong potential as a natural α-amylase inhibitor, which may help reduce postprandial hyperglycaemia by slowing carbohydrate digestion. Further analysis, including IC_{50} determination and comparison with standard inhibitors like acarbose, will provide deeper insights into its potential for antidiabetic applications.

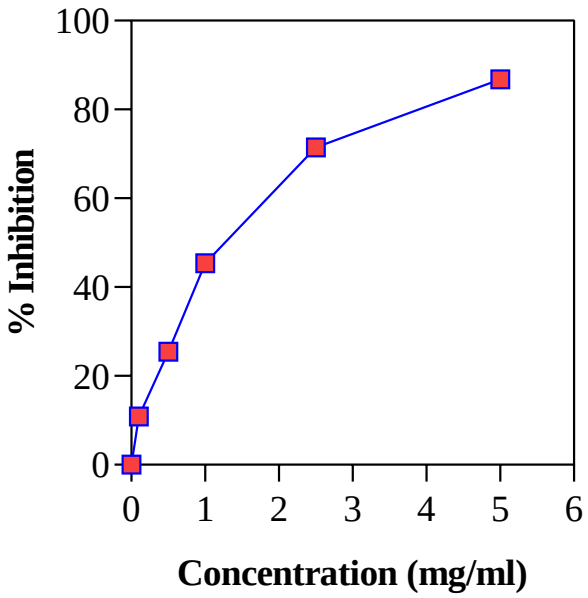

Figure 4. Alpha-Amylase Inhibition Assay of RNME-L

Alpha-Glucosidase Inhibition Assay

The α-glucosidase inhibition assay results indicate a dose-dependent increase in the inhibitory activity of RNME-L against the α-glucosidase enzyme, which plays a crucial role in carbohydrate digestion and glucose absorption. At 0 mg/mL (control), no inhibition was observed (0%), confirming that the enzyme was fully active in the absence of RNME-L. At 0.1 mg/mL, RNME-L exhibited 17.67 ± 0.99% inhibition, indicating mild enzyme suppression at a low concentration. As the concentration increased to 0.5 mg/mL, the inhibition improved significantly to 38.66 ± 0.98%, showing a notable effect on enzyme activity. A substantial inhibition of 59.97 ± 0.98% was recorded at 1 mg/mL, suggesting that RNME-L effectively interferes with α-glucosidase activity at moderate doses. Higher concentrations resulted in 78.56 ± 1.03% inhibition at 2.5 mg/mL and 94.76 ± 1.09% at 5 mg/mL, indicating a strong inhibitory effect at elevated doses. The steep increase in inhibition from 1 mg/mL onwards suggests that RNME-L contains bioactive compounds capable of significantly reducing enzymatic activity. These findings highlight RNME-L as a potent natural α-glucosidase inhibitor, suggesting its potential for managing postprandial hyperglycemia by delaying carbohydrate breakdown and glucose absorption. Further evaluation, including IC_{50} determination and comparison with standard inhibitors such as acarbose, will provide deeper insights into its antidiabetic potential.

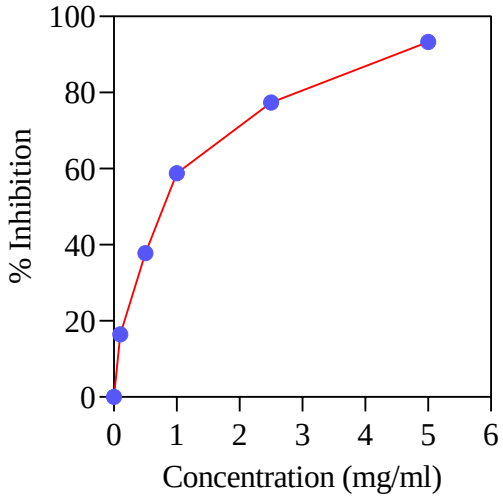

Figure 5. Alpha-Glucosidase Inhibition Assay of RNME-L

Glucose Uptake Assay

The glucose uptake assay results reveal a dose-dependent enhancement in glucose uptake by RNME-L, suggesting its potential to improve cellular glucose absorption. At 0 mg/mL (control), no increase in glucose uptake was observed (0%), indicating baseline glucose transport in the absence of RNME-L. At 0.1 mg/mL, RNME-L exhibited a moderate glucose uptake increase (9.46 ± 0.99%), suggesting an initial stimulation of glucose transport. As the concentration increased to 0.5 mg/mL, the uptake improved significantly to 25.97 ± 0.99%, indicating enhanced glucose absorption by the cells. A substantial increase (44.56 ± 0.99%) was observed at 1 mg/mL, implying that RNME-L effectively promotes glucose transport at moderate doses. At higher concentrations, glucose uptake further increased, reaching 68.76 ± 1.01% at 2.5 mg/mL and 88.98 ± 1.09% at 5 mg/mL, indicating a strong glucose uptake effect at elevated doses. The consistent increase in uptake with concentration suggests that RNME-L contains bioactive compounds capable of facilitating glucose transport, possibly through insulin-mimetic or insulin-sensitizing mechanisms. These findings highlight RNME-L as a potential agent for improving glucose utilization, which could be beneficial for diabetes management by enhancing glucose uptake in peripheral tissues, thereby reducing blood glucose levels. Further studies, including mechanistic evaluations on GLUT4 translocation and insulin pathway modulation, will provide deeper insights into its antidiabetic potential.

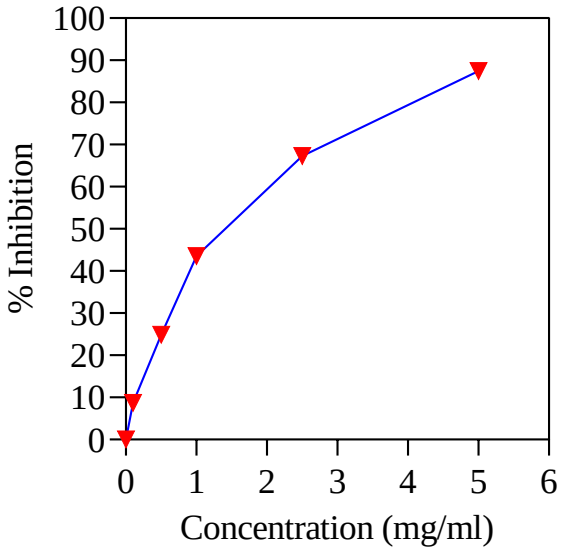

Figure 6. Glucose Uptake Assay of RNME-L

The IC_{50} values of RNME-L for the antidiabetic models

The IC_{50} values of RNME-L for the antidiabetic models indicate its efficacy in glucose uptake enhancement and enzyme inhibition. The IC_{50} value represents the concentration at which 50% of the desired biological activity is achieved, with a lower IC_{50} indicating higher potency. In the α-glucosidase inhibition assay, RNME-L showed the lowest IC_{50} (0.86 mg/mL) with a Hill slope of 1.01, suggesting strong inhibitory potential against α-glucosidase, which is crucial for slowing carbohydrate digestion and glucose absorption. This low IC_{50} value indicates that RNME-L can effectively inhibit α-glucosidase at relatively low concentrations, making it a promising postprandial hyperglycemia regulator. For the α-amylase inhibition assay, the IC_{50} was slightly higher (1.12 mg/mL) with a Hill slope of 1.07, suggesting moderate inhibition of α-amylase, which is responsible for starch breakdown. Although RNME-L exhibited weaker inhibition against α-amylase compared to α-glucosidase, it still demonstrated significant activity, indicating its role in slowing down carbohydrate digestion and reducing blood glucose spikes. In the glucose uptake assay, RNME-L exhibited an IC_{50} of 1.17 mg/mL with a Hill slope of 1.06, showing its ability to enhance glucose uptake in cells at relatively low concentrations. While its glucose uptake-promoting activity was slightly weaker compared to its enzyme inhibition potential, the positive effect on glucose absorption suggests insulin-sensitizing or insulin-mimetic properties, making it beneficial for diabetes management. Overall, RNME-L demonstrated potent antidiabetic activity, with α-glucosidase inhibition being its strongest effect (IC_{50} = 0.86 mg/mL), followed by α-amylase inhibition (IC_{50} = 1.12 mg/mL) and glucose uptake enhancement (IC_{50} = 1.17 mg/mL). These findings suggest that RNME-L could serve as a natural therapeutic agent for diabetes management, particularly by slowing carbohydrate digestion and enhancing glucose utilization.

Further studies, such as in vivo validation and mechanistic evaluations, will provide deeper insights into its potential applications.

Table 4. The estimated IC50 values of RNME-L for the antidiabetic models

Assay Type	Hill Slope	IC50 (mg/mL)
Glucose Uptake Assay	1.06	1.17
Alpha-Glucosidase Inhibition Assay	1.01	0.86
Alpha-Amylase Inhibition Assay	1.07	1.12

Anti-inflammatory activity

Cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) assays

The COX-1 and COX-2 inhibition assays demonstrate a concentration-dependent increase in the inhibitory activity of RNME-L against cyclooxygenase enzymes, which are key mediators of inflammatory pathways. At 50 µg/mL, RNME-L exhibited minimal inhibition of COX-1 (4.856 ± 0.035%) and COX-2 (6.930 ± 0.131%), indicating a weak initial effect. However, as the concentration increased to 100 µg/mL, the inhibition significantly improved to 9.652 ± 0.168% for COX-1 and 14.381 ± 0.134% for COX-2, suggesting a stronger interaction with the enzymes. At 150 µg/mL, RNME-L showed 26.342 ± 0.067% COX-1 inhibition and 31.785 ± 0.169% COX-2 inhibition, further confirming its anti-inflammatory potential. A more pronounced effect was observed at 200 µg/mL, where COX-1 inhibition reached 49.528 ± 0.076%, and COX-2 inhibition was 48.879 ± 0.187%, indicating nearly equal efficacy against both isoforms. At the highest tested concentration (250 µg/mL), RNME-L exhibited strong inhibition of COX-1 (98.873 ± 0.076%) and COX-2 (90.548 ± 0.168%), demonstrating its significant ability to suppress inflammatory pathways. The IC_{50} values for COX-1 (168.98 µg/mL) and COX-2 (176.64 µg/mL) suggest that RNME-L has a slightly stronger affinity for COX-1 than COX-2, although both are effectively inhibited at higher concentrations. This indicates that RNME-L may have potential as a natural anti-inflammatory agent, capable of modulating both constitutive (COX-1) and inducible (COX-2) enzyme pathways. While COX-2 selective inhibition is desirable to reduce gastrointestinal side effects, the balanced inhibition observed in RNME-L suggests it could be effective in managing inflammatory conditions with a broad mechanism of action. Further studies, including mechanistic evaluations and in vivo anti-inflammatory models, will be needed to establish its therapeutic relevance in inflammation-related disorders.

Table 5. Percentage inhibition of the Cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) by the RNME-L.

Concentration (µg/mL)	% Inhibition	
	Cyclooxygenase-1 (COX-1)	Cyclooxygenase-2 (COX-2)
50	4.856±0.035	6.930±0.131
100	9.652±0.168	14.381±0.134
150	26.342±0.067	31.785±0.169
200	49.528±0.076	48.879±0.187
250	98.873±0.076	90.548±0.168
IC_{50}	168.98 µg/mL	176.64 µg/mL

Fabrication of Nanogel using solid lipid nanoparticles (SLN)

The nanogel was formulated by incorporating solid lipid nanoparticles (SLNs) into a Carbopol 940-based gel matrix to enhance stability and topical delivery. The SLNs were prepared using hot homogenization and ultrasonication, ensuring uniform nanoparticle dispersion. The gel base was formed by dispersing Carbopol 940 (1% w/w) in deionized water, followed by neutralization with triethanolamine to adjust the pH to 5.5–6.5. The optimized SLN dispersion was gradually added to the gel under continuous stirring to achieve homogeneity. The final nanogel exhibited a smooth texture, good spreadability, and suitable viscosity, making it ideal for topical drug delivery applications.

Characterization of solid lipid nanoparticles (SLN)

The particle size, polydispersity index (PDI), zeta potential, drug loading, and entrapment efficiency of the NGF formulations exhibited significant variations, influencing their stability and drug encapsulation capacity. NGF-1 had the largest particle size (935.43 nm) with a moderate PDI (0.243) and high entrapment efficiency (66.89%), indicating a relatively stable system but with larger aggregates. NGF-2 showed the smallest particle size (321.32 nm), the lowest PDI (0.212), and the highest entrapment efficiency (71.91%), suggesting a well-optimized

formulation with uniform dispersion and excellent drug retention. NGF-3, NGF-4, and NGF-5 exhibited intermediate particle sizes (561.12 nm, 418.65 nm, and 321.43 nm, respectively) but had relatively higher PDI values, indicating greater heterogeneity. NGF-6 and NGF-7, despite their smaller sizes (309.11 nm and 405.23 nm, respectively), had higher PDI values (1.00 and 0.312) and the lowest entrapment efficiency (57.72% and 53.56%), suggesting lower stability and reduced drug retention. The zeta potential values ranged from -17.8 to -22.8 mV, with NGF-2 and NGF-6 (-22.7 and -22.8 mV, respectively) showing the highest stability due to greater electrostatic repulsion. Drug loading values were relatively low across all formulations, with NGF-1 showing the highest (0.591%) and NGF-5 the lowest (0.454%). Overall, NGF-2 emerged as the most stable and efficient formulation due to its small particle size, low PDI, high zeta potential, and superior entrapment efficiency, making it the most promising candidate for further development.

Table 6. Particle size (nm), PDI, Zeta potential (mV), % Drug loading and % Entrapment efficiency

Formulation code	Particle size (nm)	PDI	Zeta potential (mV)	% Drug loading (Mean + S.D) *	% Entrapment efficiency (Mean + SD) *
NGF-1	935.43	0.243	-20.5	0.591+ 0.076	66.89 + 1.11
NGF-2	321.32	0.212	-22.7	0.555 + 0.033	71.91 + 2.01
NGF-3	561.12	0.317	-17.8	0.476 + 0.056	62.60 + 2.11
NGF-4	418.65	0.732	-18.8	0.522 + 0.074	67.58 + 2.12
NGF-5	321.43	1.00	-18.7	0.454 + 0.024	65.55+ 1.15
NGF-6	309.11	1.00	-22.8	0.454 + 0.086	57.72+ 1.18
NGF-7	405.23	0.312	-21.5	0.524 + 0.042	53.56 + 1.41

In vitro drug release study

The in vitro drug release study of formulations NGF1–NGF7 over 24 hours revealed significant variations in release profiles, highlighting differences in sustained drug delivery potential. NF2 exhibited the slowest release (47.23 ± 1.04%), suggesting strong drug entrapment within the lipid matrix, making it ideal for prolonged drug delivery. In contrast, NF7 demonstrated the highest release (89.12 ± 1.05%), indicating a faster release profile, possibly due to lower entrapment efficiency. NGF4, NGF5, and NGF6 showed intermediate release patterns (77.54–78.99%), balancing between sustained and rapid release. NGF1 (49.76 ± 1.22%) and NGF3 (42.88 ± 1.16%) exhibited slower release kinetics, which may be beneficial for prolonged drug availability. Overall, NGF2 displayed the most controlled release, while NGF4–NGF6 offered a balance between sustained and faster release. These findings suggest that the choice of formulation should be guided by the desired therapeutic outcome, whether for prolonged or immediate drug action.

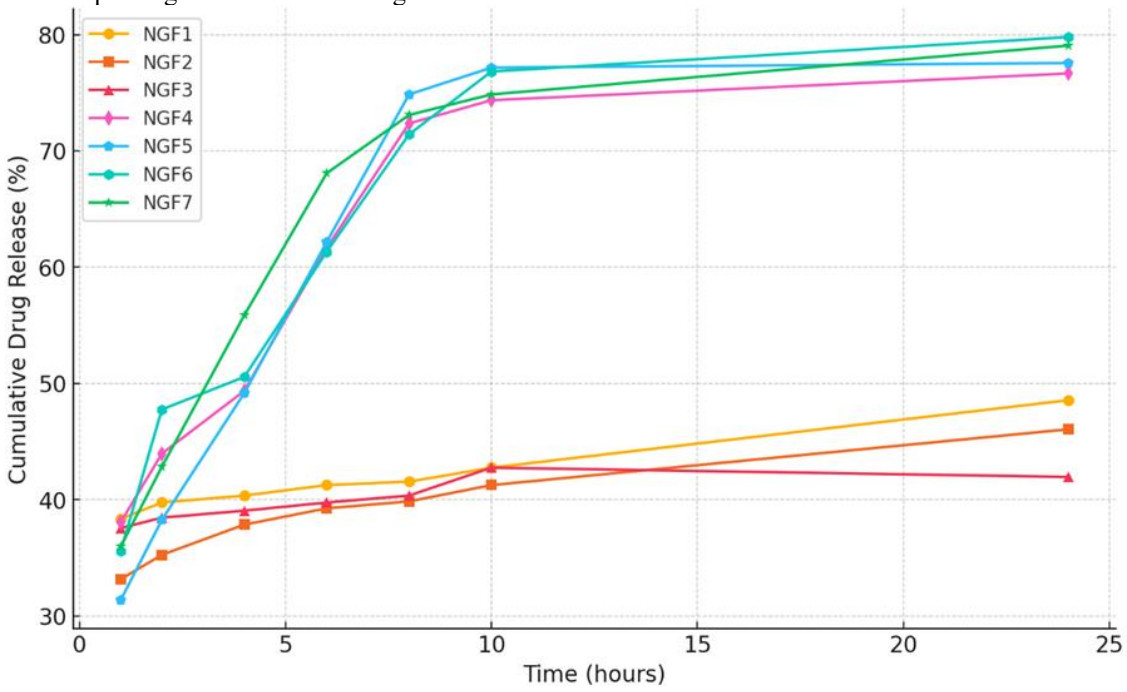

Figure 7. The in vitro drug release study of formulations NGF1–NGF7

Characterization of Nanogel

The gel formulation exhibited a clear, thick, and uniform consistency, ensuring excellent homogeneity and smooth texture. Its translucent appearance and optimal spreadability (38.42 g·cm/sec) allowed for easy application and even distribution on the skin. The viscosity of the gel was assessed using a Brookfield viscometer, confirming its suitability for topical use. Thixotropic behavior was observed, meaning the gel's viscosity decreased under shear stress (during application) and returned to its original state upon rest. This property enhances ease of application, prolonged skin contact, and improved user experience, making the gel highly effective for topical delivery. These findings suggest that the formulation is well-suited for skin application, providing good spreadability and stable rheological properties

4. CONCLUSIONS

The findings of this study highlight the pharmacological potential of methanolic leaf extract of *Rumex nepalensis* (RNME-L), demonstrating its strong antioxidant, antidiabetic, and anti-inflammatory properties. The phytochemical screening confirmed the presence of bioactive compounds, particularly flavonoids and phenols, which contribute to its observed biological activities. The antioxidant assays revealed that RNME-L exhibited dose-dependent radical scavenging potential, comparable to synthetic antioxidants at higher concentrations. The antidiabetic assays showed its ability to inhibit α-amylase and α-glucosidase while enhancing glucose uptake, suggesting its potential for managing postprandial hyperglycaemia. Additionally, its significant COX-1 and COX-2 inhibitory effects indicate its role in inflammation modulation. The successful fabrication of a nanogel using SLNs demonstrated its stability, suitable rheological properties, and sustained drug release, making it a promising candidate for topical drug delivery. The overall results suggest that RNME-L, in both its crude extract and nanogel formulation, could serve as a valuable natural therapeutic for oxidative stress-related diseases, diabetes, and inflammation. Further in vivo studies and mechanistic evaluations will be necessary to confirm its efficacy and establish its potential applications in pharmaceutical and nutraceutical formulations.

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