https://doi.org/10.33472/AFJBS.6.7.2024.518-531

African Journal of Biological Sciences

Research Paper Open Access

# In Vitro Antioxidant And Antidiabetic Mechanistic Pharmacological Evaluation Of An Herbal Blend

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**ABSTRACT**

This study investigates the antioxidant and antidiabetic properties of a herbal blend comprising of methanol cold extract of leaves of Rumex hastatus and Rumex nepalensis, two medicinal plants native to the Himalayan region. Utilizing three distinct antioxidant models—DPPH, ABTS, and FRAP assays—this research evaluated the plants' capacities to neutralize free radicals and provide protection against oxidative stress. Concurrently, their antidiabetic potentials were assessed through alpha-amylase and alpha-glucosidase inhibition assays, along with evaluating their glucose uptake activities. Results demonstrate significant antioxidant activities for the blend containing both plants, attributed to their high content of phenolic compounds and flavonoids. In antidiabetic testing, both species showed promising inhibition of key enzymes involved in carbohydrate digestion, suggesting a potential to moderate postprandial glucose levels. The dual functionality of these plants in managing oxidative stress and glucose homeostasis highlights their therapeutic potential, supporting their traditional uses and suggesting further clinical research. This study lays groundwork for future pharmacological applications and underscores the importance of these botanical resources in developing natural therapies for chronic conditions like diabetes.

**Keywords:** Rumex hastatus, Rumex nepalensis, Antioxidant activity, Antidiabetic properties, Natural therapies

## INTRODUCTION

The antioxidant activity of medicinal plants has garnered significant attention due to its critical role in safeguarding the human body against oxidative stress, which is implicated in various chronic diseases such as cancer, diabetes, and heart diseases [1-3]. Medicinal plants, with their rich repertoire of bioactive compounds, including flavonoids, phenols, tannins, and terpenoids, contribute significantly to this antioxidant action. These bioactive compounds are capable of scavenging free radicals and neutralizing reactive oxygen species (ROS), thereby preventing cell damage and the progression of disease states [4,5]. The importance of antioxidants lies in their mechanism of action; they donate electrons to free radicals, rendering them stable and less reactive. This process effectively breaks the chain of oxidative reactions that can damage cellular proteins, membranes, and genes. Medicinal plants often contain a complex mixture of these antioxidants, which can work synergistically to enhance their protective effects. This synergy is an area of active research, as it holds potential for the development of novel therapeutic agents that harness the natural efficacies of plant-derived compounds. Research into the antioxidant properties of medicinal plants often involves various in vitro assays, such as the DPPH radical scavenging assay, the ABTS radical cation decolorization assay, and the ferric reducing antioxidant power (FRAP) assay. These tests provide quantitative insights into the radical-scavenging ability of extracts and pure compounds isolated from plants. Through such studies, it has been demonstrated repeatedly that higher levels of phenolic and flavonoid contents correlate with stronger antioxidant activities. This correlation underscores the potential of medicinal plants as sources of natural antioxidants that could mitigate oxidative stress-related diseases [5,6].

Furthermore, the study of medicinal plants for their antioxidant capacity is not only important for human health but also for the preservation of food. Antioxidants derived from plants are increasingly used as natural preservatives in the food industry, replacing synthetic antioxidants, which are often associated with health risks. Thus, the exploration of plant-based antioxidants serves a dual purpose—enhancing health and offering safer alternatives to synthetic chemicals in food applications [6]. In addition to their health benefits, the environmental impact of cultivating medicinal plants is generally lower compared to the synthesis of synthetic drugs and antioxidants. This aspect makes medicinal plants a sustainable choice in the context of global health strategies. By focusing on the cultivation, study, and use of medicinal plants, it is possible to promote environmental sustainability alongside public health. In summary, the antioxidant activity of medicinal plants forms a crucial aspect of modern pharmacological research due to its potential to prevent and treat oxidative stress-induced diseases [6-8]. With ongoing studies revealing new insights into plant biochemistries and synergistic effects, the medicinal plant sector continues to be a promising field for discovering novel antioxidants. As such, integrating traditional knowledge with contemporary scientific practices can pave the way for breakthroughs in health sciences, underscoring the indispensable value of medicinal plants in both traditional and modern medicine [8].

Diabetes and oxidative stress share a complex, interrelated relationship where each can exacerbate the other, creating a cycle that contributes to the progression of diabetic complications. Understanding this relationship is crucial for developing effective management strategies, particularly through the use of medicinal plants, which offer a wealth of bioactive compounds with potential health benefits. Oxidative stress occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the body's ability to detoxify these

reactive intermediates or to repair the resulting damage [8,9]. In individuals with diabetes, this imbalance is often pronounced due to several factors: high blood glucose levels can lead to the overproduction of free radicals through glucose auto-oxidation and through more complex biochemical pathways such as the polyol pathway and non-enzymatic glycation. Moreover, the antioxidant defenses are often compromised in diabetes, further aggravating oxidative stress. The consequences of such oxidative stress are manifold, impacting various biological systems and organs [9,10]. It plays a pivotal role in the development of major diabetic complications, including nephropathy, retinopathy, neuropathy, and cardiovascular diseases. The endothelial dysfunction caused by oxidative stress, for instance, contributes to atherosclerosis, a common issue in diabetic patients [10,11].

Medicinal plants come into play as a valuable resource in managing these complications due to their antioxidant properties. These plants, used traditionally in various cultures worldwide, contain a diverse array of natural antioxidants such as flavonoids, phenolic acids, and other phenolic compounds [12]. These substances can neutralize free radicals and reduce the levels of oxidative stress, potentially mitigating its harmful effects. The role of medicinal plants in diabetes management extends beyond their antioxidant activity. Many plants also exhibit hypoglycemic properties, which can help in directly controlling blood sugar levels. For example, plants like Gymnema sylvestre, Fenugreek, and Bitter melon have been studied for their ability to improve insulin sensitivity, enhance pancreatic beta-cell function, or interfere with carbohydrate digestion and absorption, thereby lowering postprandial blood sugar levels [12,13]. The importance of these plants in managing diabetes is increasingly recognized not just in traditional medicine but also in modern pharmacology. Research into these plants often yields insights into novel mechanisms of action or leads to the development of new classes of diabetic medications. For instance, compounds isolated from these plants may inspire the synthesis of new drugs that mimic their mechanisms but with enhanced efficacy or stability [14].

Rumex hastatus and Rumex napalensis are both species of flowering plants in the buckwheat family, Polygonaceae. Rumex hastatus, commonly known as sour dock, this plant is native to parts of Central and South Asia [15,16]. It is typically found in hilly areas and has been used in traditional medicine. The plant is known for its pointed leaves and acidic taste. Medicinally, it has been used to treat various ailments, including fever and skin diseases. Rumex napalensis, also known as Nepalese dock, it is found widely in the Himalayan regions [16,17]. This species thrives in moist and shaded locations. Similar to R. hastatus, R. nepalensis is used in traditional medicine and is known for its anti-inflammatory properties. It has been used to treat wounds, skin infections, and as a laxative. Both species are notable for their high antioxidant properties, which make them subjects of interest in both pharmacological and nutritional studies. They have been studied for their potential health benefits, including anti-inflammatory and antioxidant effects. Incorporating medicinal plants into diabetes management strategies offers several advantages [17]. They are generally less expensive than conventional drugs and are perceived by many patients as having fewer side effects, which can improve adherence to treatment regimes. Moreover, the use of these plants supports a more holistic approach to health, which is a growing trend in global healthcare. In assumption, the interrelationship between diabetes and oxidative stress underscores the need for effective management strategies that address both the regulation of blood sugar levels and the mitigation of oxidative damage. Medicinal plants play a crucial role in this context, offering both direct antidiabetic effects and protective antioxidant properties. As research continues to explore and validate the efficacy of these plants, they remain a promising component of comprehensive diabetes management plans, blending ancient wisdom with modern scientific approaches.

Therefore, this present study was designed to explore the antioxidant and antidiabetic activities of an herbal blend composed of two methanolic cold extracts from two native medicinal plants, Rumex hastatus and Rumex napalensis in various in vitro mechanistic models.

## 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, Phosphate Buffer were arranged for Sigma Aldrich or Himedia Laboratories, India. All other chemicals and reagents were arranged form well validated vendors only and all were of analytical grade.

### Identification and Extraction

The plant species Rumex hastatus and Rumex nepalensis were collected from Herbal waves from Himachal Pradesh and both the plants were identified by Dr. A. Kumar from the Department of Botany at the College of Life Sciences, Mandi, Himachal Pradesh. The identification of these plants was critical in ensuring the accuracy of our study focusing on their antioxidant and antidiabetic properties. Following identification, the extraction of bioactive compounds from these plants was conducted using the cold maceration technique. This method involved soaking the dried plant material in methanol at room temperature to effectively extract the phenolic and flavonoid compounds responsible for the medicinal properties observed. The choice of methanol as a solvent was due to its efficiency in extracting a wide range of polar substances, crucial for assessing the full therapeutic potential of these plants. The final extracts were mixed in 1:1 ratio to get the herbal blend, codenamed as RHRN-HB.

### Preliminary phytochemical screening

Preliminary phytochemical screening is a crucial step in the study of medicinal plants, helping researchers identify the active constituents responsible for therapeutic effects. This screening involves a series of qualitative tests to detect the presence of various classes of phytochemicals such as alkaloids, flavonoids, tannins, saponins, terpenoids, steroids, and phenolic compounds. Standard methods were used to perform preliminary phytochemical screening in a laboratory setting, as described elsewhere.

### Antioxidant activity

### DPPH Radical Scavenging Assay

In the DPPH Radical Scavenging Assay, a 0.1 mM solution of DPPH in methanol or ethanol is prepared first. The samples are diluted in the same solvent. For the assay, 1 mL of the DPPH solution is mixed with 3 mL of the sample solution in a cuvette. This mixture is then allowed to stand at room temperature in the dark for 30 minutes. The absorbance is measured at 517 nm using a spectrophotometer, with methanol or ethanol serving as the blank. A control, typically ascorbic acid or Trolox, is included for comparison [18,19]. The percentage inhibition of the DPPH radical can be calculated using the formula: (Absorbance of control - Absorbance of sample) / Absorbance of control) * 100.

### ABTS Radical Cation Decolorization Assay

The ABTS Radical Cation Decolorization Assay involves preparing a solution of 7 mM ABTS and 2.45 mM potassium persulfate, which is allowed to react in the dark at room temperature for 12-16 hours to form ABTS●+. The ABTS●+ solution is then diluted with ethanol or phosphate-buffered saline (PBS) to achieve an absorbance of 0.70 ± 0.02 at 734 nm. For the assay, 1 mL of this diluted ABTS●+ solution is added to 10-100 µL of the sample. After a reaction time of 6 minutes, the reduction in absorbance at 734 nm is measured [20,21]. The percentage inhibition is calculated relative to a control that contains all reagents except the test compound.

### Reducing Power Assay

In the Reducing Power Assay, a sample is mixed with phosphate buffer and potassium ferricyanide and incubated at 50°C for 20 minutes. After incubation, trichloroacetic acid is added to stop the reaction. The mixture is then centrifuged at 3000 rpm for 10 minutes, and the supernatant is collected. The supernatant is further mixed with water and ferric chloride to convert $Fe^{3+}$ to $Fe^{2+}$. The absorbance of the resulting mixture is measured at 700 nm [22,23]. Higher absorbance indicates greater reducing power of the tested compound.

### Antidiabetic activity

### Alpha-amylase inhibition assay

In vitro antidiabetic activity testing often involves evaluating the potential of compounds to manage diabetes through various mechanisms. One common approach is the alpha-amylase inhibition assay, which assesses the ability of a compound to inhibit alpha-amylase, an enzyme critical in the breakdown of starch into glucose. In this assay, alpha-amylase is dissolved in a phosphate buffer, and the test compound is incubated with this enzyme solution. Following pre-incubation, a starch solution is added, and the mixture is incubated further. The reaction is then halted by adding a colour reagent like iodine, which forms a complex with the remaining starch. The intensity of the colour, which diminishes with enzyme inhibition, is measured spectrophotometrically [24,25].

### Alpha-glucosidase inhibition assay

Another pivotal assay is the alpha-glucosidase inhibition assay, which measures a compound's ability to inhibit alpha-glucosidase. This enzyme is responsible for breaking down disaccharides into glucose within the digestive tract. For this assay, alpha-glucosidase is mixed with a chromogenic substrate such as p-nitrophenyl glucopyranoside in a buffer solution. The test compound is pre-incubated with the enzyme, followed by the addition of the substrate to start the reaction. The reaction is stopped by adding sodium carbonate, which also aids in colour development if the substrate is chromogenic. The resulting absorbance is measured to determine the level of enzyme inhibition [24,26].

### Glucose uptake assay

Lastly, the glucose uptake assay evaluates the enhancement of glucose uptake in insulin-sensitive cells, which indicates insulin-mimetic activity. Cells, typically adipocytes or myocytes, are cultured and treated with the test compounds. After reaching confluence, glucose is added to the culture medium, and the cells are incubated further. The concentration of glucose in the medium is then

measured over time using a glucose assay kit. A decrease in glucose concentration indicates effective glucose uptake by the cells.

These assays collectively provide insights into the antidiabetic potential of compounds by addressing different facets of diabetes management, from reducing carbohydrate digestion rates to enhancing cellular glucose uptake, thereby identifying promising candidates for further research and development [27,28].

### Statistical analysis

GraphPad Prism software is extensively utilized for statistical analysis in various scientific research fields, including pharmacology and biotechnology. In the analysis, ANOVA followed by Dunnett's test was conducted using this software, which is particularly useful for comparing multiple experimental groups against a control group. The data from these analyses were presented as mean ± standard deviation (SD). The level of significance was set at p value less than 0.05. GraphPad Prism's robust analytical tools and graphical capabilities make it a preferred choice for researchers looking to perform comprehensive statistical evaluations.

## RESULTS AND DISCUSSION

### Preliminary phytochemical screening

Preliminary phytochemical screening was performed on the herbal blend extract and found to contain many classes of phytochemicals in the combined extract. The results are presented in the table 1.

**Table 1.** Results of Preliminary phytochemical screening

| Phytochemical constituent | Herbal blend extract (RHRN-HB) |
|---|---|
| Flavonoids | ++ |
| Phenols | + |
| Alkaloids | + |
| Saponins | ++ |
| Phytosterols | + |
| Tannins | + |
| Borntrager test | + |

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

### Antioxidant activity

### DPPH Radical Scavenging Assay

At 0 µg/ml, Neither BHT nor the extract show any scavenging activity, as expected without active substances. At 50 µg/ml, BHT shows a mean activity of 8.11±0.69%, indicating moderate activity with relatively low variability. For the extract, more effective with a mean activity of 14.76±1.34%, suggesting stronger antioxidant properties and slightly higher but acceptable variability compared to BHT. At 100 µg/ml, mean activity of BHT increases significantly to 41.76±0.89%, indicating strong activity and good consistency in this condition.

Extract Shows a mean activity of 34.57± 0.87%, which is slightly less than BHT but still shows strong antioxidant capability with consistent results. At 150 µg/ml and higher, Both BHT and the extract exhibit progressively increasing scavenging activities with increasing concentrations, stabilizing around high values close to 90%. By 200 µg/ml, BHT reaches a peak mean activity of 89.23±0.93%, showing very high activity and relatively low variability.

Extract Nearly matches BHT in efficacy by 200 µg/ml with a mean activity of 86.58±1.444%, indicating similarly high antioxidant activity with slightly higher variability. At 250 µg/ml, BHT

Peaks at a mean activity of 91.97±1.098%. The slight increase from 200 µg/ml to 250 µg/ml suggests a plateau in activity. Extract Shows a mean activity of 87.69±1.54%, maintaining high effectiveness but showing slightly more variability compared to BHT at this concentration. Both BHT and the plant extract demonstrate effective DPPH radical scavenging activity, with BHT generally showing slightly higher peak effectiveness but both stabilizing at high activity levels at greater concentrations. The plant extract, however, presents itself as a strong competitor to BHT, particularly important as it derived from a natural source which might be preferable for certain applications. Both BHT and the extract exhibit nearly equivalent high efficacy at the highest tested concentrations, suggesting that both are potent antioxidants.

Given the nearly comparable performance of the extract, it could serve as an effective natural alternative to synthetic antioxidants like BHT, especially in industries seeking natural components.

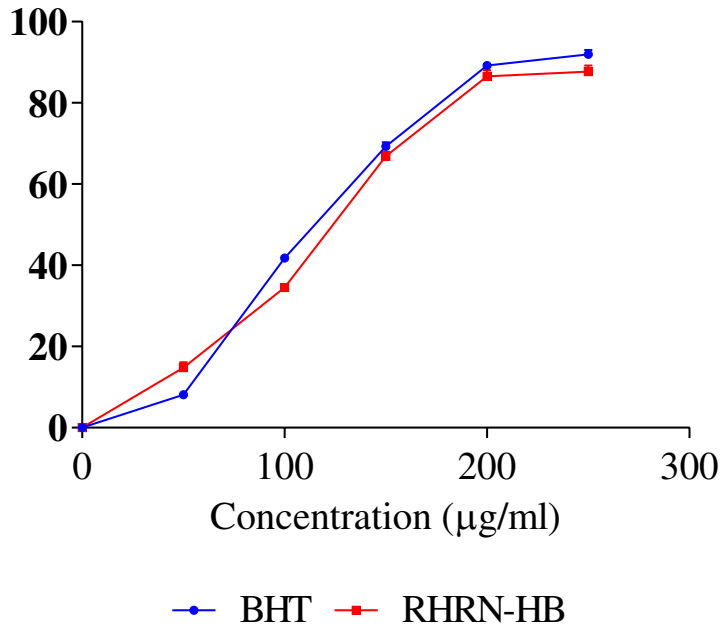


**Figure 1.** DPPH Radical Cation Decolorization Assay of RHRN-HB

**ABTS Radical Cation Decolorization Assay**

At 0 µg/ml, neither Quercetin nor the extract show any scavenging activity, as expected without active substances. At 50 µg/ml, Quercetin shows a mean activity of 45.15±1.60%, indicating strong activity with relatively low variability. For the extract, it is effective with a mean activity of 36.80±1.91%, suggesting considerable antioxidant properties and slightly higher but acceptable variability compared to Quercetin. At 100 µg/ml, the mean activity of Quercetin increases significantly to 77.49±1.75%, indicating very strong activity and good consistency in this condition. The extract shows a mean activity of 67.84±2.66%, which is less than Quercetin but still demonstrates strong antioxidant capability with consistent results. At 150 µg/ml and higher, both Quercetin and the extract exhibit progressively increasing scavenging activities with increasing concentrations, stabilizing around high values close to 90%. By 200 µg/ml, Quercetin reaches a peak mean activity of 94.73±1.27%, showing very high activity and relatively low variability. The extract nearly matches Quercetin in efficacy by 200 µg/ml with a mean activity of 92.90±2.83%, indicating similarly high antioxidant activity with slightly higher variability. At 250 µg/ml, Quercetin peaks at a mean activity of 98.68±0.48%. The slight increase from 200 µg/ml to 250

µg/ml suggests a plateau in activity. The extract shows a mean activity of 95.14±0.78%, maintaining high effectiveness but showing slightly more variability compared to Quercetin at this concentration.

Both Quercetin and the plant extract demonstrate effective ABTS radical scavenging activity, with Quercetin generally showing slightly higher peak effectiveness but both stabilizing at high activity levels at greater concentrations. The plant extract, however, presents itself as a strong competitor to Quercetin, particularly important as it is derived from a natural source which might be preferable for certain applications. Both Quercetin and the extract exhibit nearly equivalent high efficacy at the highest tested concentrations, suggesting that both are potent antioxidants. Given the nearly comparable performance of the extract, it could serve as an effective natural alternative to synthetic antioxidants like Quercetin, especially in industries seeking natural components.

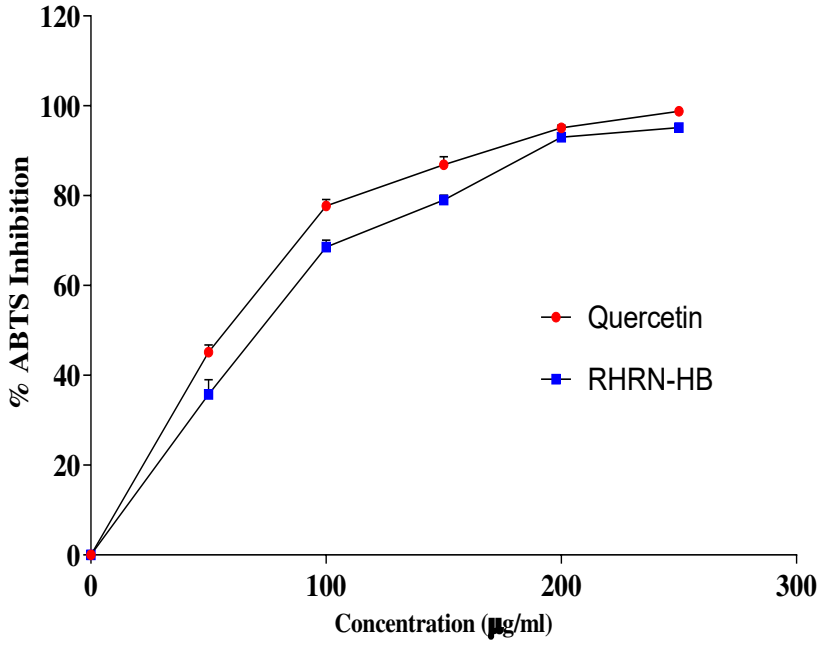


**Figure 2.** ABTS Radical Cation Decolorization Assay of RHRN-HB

**Reducing Power Assay**

At 0 µg/ml, neither the extract nor Butylated Hydroxyanisole (BHA) show any reducing power, as expected without active substances. At 50 µg/ml, the extract shows a mean activity of 0.287±0.0048, indicating initial activity with relatively low variability. For BHA, it is more effective with a mean activity of 0.699±0.0044, suggesting stronger reducing properties and slightly lower variability compared to the extract. At 100 µg/ml, the mean activity of the extract increases to 0.389±0.0044, showing progressive activity and good consistency. BHA shows a mean activity of 0.789±0.0078, which is higher than the extract, demonstrating strong reducing capability with consistent results. At 150 µg/ml and higher, both the extract and BHA exhibit progressively increasing reducing activities with increasing concentrations, with BHA showing a consistently higher activity. By 200 µg/ml, the extract reaches a mean activity of 0.596±0.0065, indicating significant activity and moderate variability. BHA reaches a peak mean activity of 0.956±0.0096 at the same concentration, exhibiting very high reducing power and relatively low variability. At 250 µg/ml, the extract shows a mean activity of 0.687±0.0046, continuing to demonstrate increased reducing power but with less variability compared to earlier concentrations. BHA shows a

substantial increase in activity to 1.409±0.016 at 250 µg/ml, indicating a significant rise in reducing power, which suggests a stronger response at higher concentrations compared to the extract. Both the extract and Butylated Hydroxyanisole demonstrate effective reducing power activity, with BHA generally showing much higher effectiveness across all concentrations. The extract, however, presents itself as a competitor with consistent performance improvement, particularly important as it derived from a natural source which might be preferable for certain applications. Both substances exhibit effective reducing capabilities at the highest tested concentrations, suggesting that both are potent in their ability to act as antioxidants. Given the robust performance of BHA, it remains a powerful synthetic antioxidant, whereas the extract could serve as an effective natural alternative, especially in industries seeking natural components.

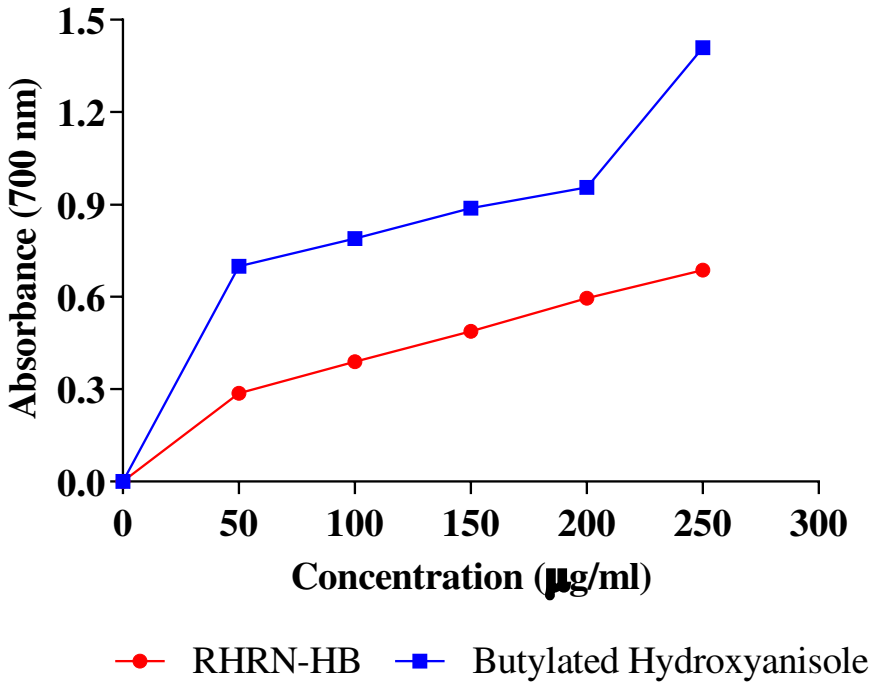


**Figure 3.** Reducing power assay of RHRN-HB

**Table 2.** The estimated IC50 values of RHRN-HB for the antioxidant models

| Assay Type | Substance | IC50 Value |
|---|---|---|
| DPPH | BHT | 107.81 g/ml |
| | RHRN-HB | 111.74 g/ml |
| ABTS | BHT | 110.78 µg/ml |
| | RHRN-HB | 114.68 µg/ml |

**Antidiabetic activity**

**Alpha-Amylase Inhibition Assay**

In the Alpha-Amylase Inhibition Assay, the extract exhibits a clear dose-response relationship, where its inhibitory activity against alpha-amylase, an enzyme involved in starch breakdown, increases as the concentration of the extract rises. Here's a detailed interpretation of the data: At 0.1 mg/mL, the extract demonstrates initial inhibitory activity with 12.66±0.98%, indicating a modest effect on alpha-amylase. This lower concentration shows the starting point of the extract's ability to interfere with the enzyme's function. At 0.5 mg/mL, inhibition significantly rises to

27.17±0.99%. This more than doubling of inhibitory activity compared to the lowest concentration suggests that the active components within the extract are effective at a relatively low dose and their effects enhance as the dosage increases. At 1.0 mg/mL, the extract's inhibitory activity nearly doubles again, reaching 47.29±1.01%. This notable increase indicates that the extract is quite effective in moderate concentrations, disrupting a substantial portion of alpha-amylase activity. At 2.5 mg/mL, the inhibition continues to climb, achieving a high level of 73.53±1.02%. This level suggests a robust interaction between the extract's active ingredients and the enzyme, significantly reducing the enzyme's ability to function properly.

At 5.0 mg/mL, the extract shows very high inhibitory activity at 88.52±1.11%, approaching near-complete inhibition of alpha-amylase. This high percentage points to the potent efficacy of the extract at higher concentrations, potentially making it a strong candidate for applications that require control over enzymatic activity, such as managing blood sugar levels in individuals with diabetes. The standard deviations across all concentrations are consistently low (ranging from 0.98% to 1.11%), indicating that the results are precise and reproducible. This low variability supports the reliability of the extract's effectiveness in inhibiting alpha-amylase. Overall, the alpha-amylase inhibition assay results suggest that the extract is a powerful inhibitor of the enzyme, with effectiveness increasing dramatically as the concentration increases. Given its potent inhibitory action, the extract could be considered for therapeutic applications, particularly in conditions where starch digestion needs to be controlled or slowed, such as in diabetes management.

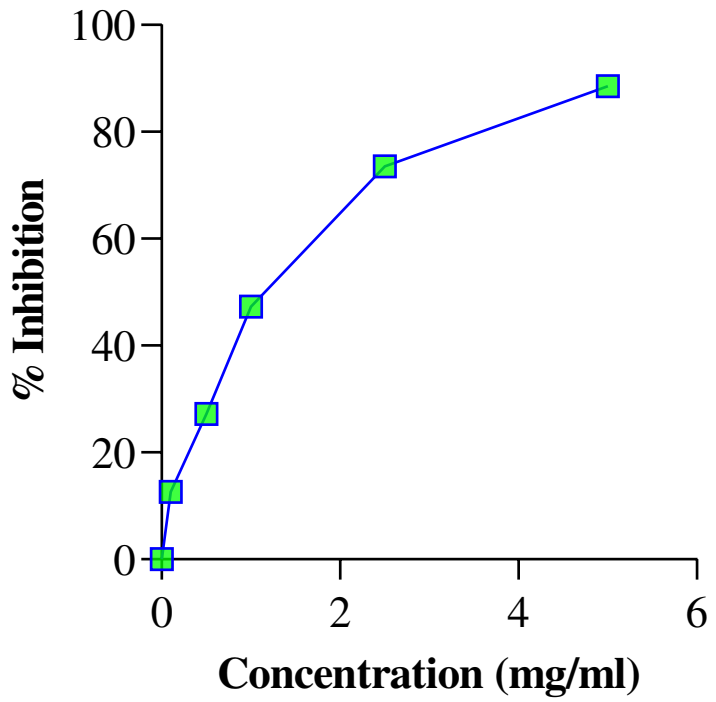


**Figure 4.** Alpha-Amylase Inhibition Assay of RHRN-HB

**Alpha-Glucosidase Inhibition Assay**

In the Alpha-Glucosidase Inhibition Assay, the extract shows effective inhibition of alpha-glucosidase, an enzyme that plays a crucial role in carbohydrate digestion by breaking down disaccharides to glucose. The increasing concentration of the extract correlates with increased

inhibitory activity. Here's an interpretation of the data provided: At 0.1 mg/mL, the extract begins with a modest inhibition of 16.43±0.98%, indicating some initial capability to inhibit alpha-glucosidase activity at low concentrations. This initial activity is crucial for demonstrating the extract's potential effectiveness. At 0.5 mg/mL, inhibition significantly increases to 37.77±0.99%. This marked increase suggests that the extract contains compounds that are more effectively interacting with alpha-glucosidase as the concentration rises. At 1.0 mg/mL, the extract's inhibitory activity rises to 58.81±0.99%. Nearly doubling the inhibition from the 0.5 mg/mL concentration, this level shows a strong efficacy in moderate concentrations, impacting the enzyme's ability to process sugars significantly. At 2.5 mg/mL, the inhibition progresses to 77.36±1.01%. The continued increase at this concentration indicates a robust capability of the extract to inhibit alpha-glucosidase, suggesting that it might be highly effective in therapeutic contexts where slowing glucose absorption is desired. At 5.0 mg/mL, the extract shows very high inhibitory activity at 93.25±1.08%, nearing complete inhibition. This demonstrates the extract's potent efficacy at higher concentrations, making it a potential candidate for applications requiring substantial modulation of carbohydrate digestion, such as managing post-prandial glucose levels in diabetes management. The standard deviations across the concentrations are consistently low (ranging from 0.98% to 1.08%), which indicates that the measurements are precise and reproducible. This low variability adds credibility to the effectiveness of the extract in inhibiting alpha-glucosidase in a dose-dependent manner. Overall, the alpha-glucosidase inhibition assay results suggest that the extract is an effective inhibitor of the enzyme, with its ability to inhibit alpha-glucosidase increasing significantly as the concentration increases. This performance indicates potential therapeutic applications, particularly for managing glucose absorption and assisting in the control of blood sugar levels in metabolic disorders such as diabetes.

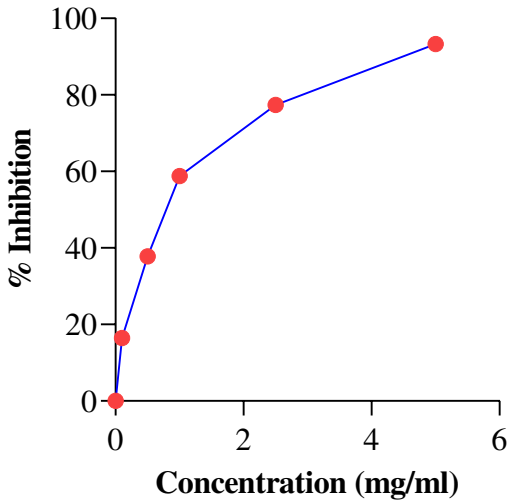


**Figure 5.** Alpha-Glucosidase Inhibition Assay of RHRN-HB

**Glucose Uptake Assay**

In the Glucose Uptake Assay, the extract demonstrates a concentration-dependent enhancement of glucose uptake, suggesting potential insulin-mimetic or insulin-sensitizing activity. Here's an interpretation of the provided data: At 0.1 mg/mL, the extract shows a modest increase in glucose uptake of 8.67±0.99%. This indicates some baseline activity of the extract in enhancing glucose uptake at lower concentrations. At 0.5 mg/mL, the enhancement of glucose uptake increases to 24.88±0.98%, demonstrating a more significant effect compared to the lower concentration. This

suggests that the active components within the extract become more effective as the concentration increases. At 1.0 mg/mL, the extract exhibits a further increase in glucose uptake, reaching 43.54±0.99%. This substantial enhancement indicates a strong activity of the extract in promoting glucose uptake, potentially through mechanisms similar to insulin signaling pathways. At 2.5 mg/mL, the enhancement of glucose uptake continues to rise to 67.32±1.06%. This level suggests a robust and potent effect of the extract on glucose metabolism, indicating its potential as an insulin-mimetic or insulin-sensitizing agent. At 5.0 mg/mL, the extract shows a high enhancement of glucose uptake at 87.45±1.07%, nearing maximum effectiveness. This indicates that the extract is highly potent in promoting glucose uptake at higher concentrations, potentially surpassing the physiological effects of insulin. The standard deviations across the concentrations are consistently low (ranging from 0.98% to 1.07%), indicating that the measurements are precise and reproducible. This low variability strengthens the reliability of the observed effects of the extract on glucose uptake. Overall, the Glucose Uptake Assay results suggest that the extract enhances glucose uptake in a concentration-dependent manner, indicating potential insulin-mimetic or insulin-sensitizing activity. This finding suggests promising therapeutic implications for conditions related to glucose metabolism, such as diabetes, where enhancing glucose uptake can help in improving glycemic control and insulin sensitivity.

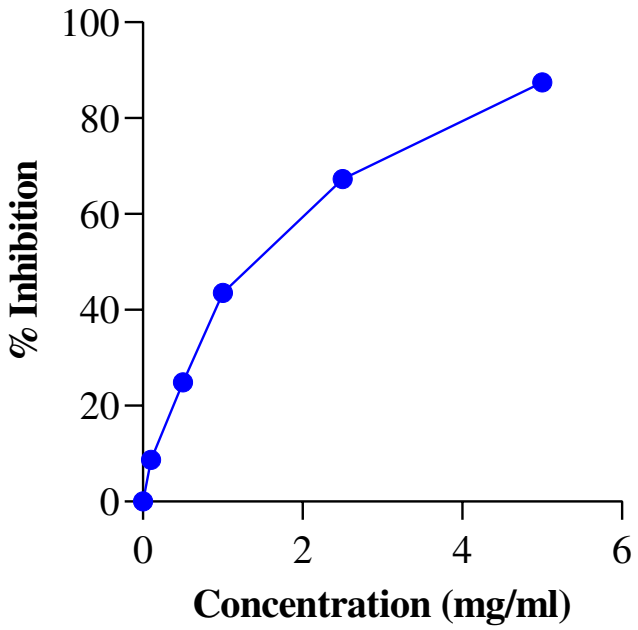


**Figure 6.** Glucose Uptake Assay of RHRN-HB

**Table 3.** The estimated IC50 values of RHRN-HB for the antidiabetic models

| Assay Type | Hill Slope | IC50 (mg/mL) |
|---|---|---|
| Glucose Uptake Assay | 1.18 | 1.24 |
| Alpha-Glucosidase Inhibition Assay | 1.04 | 0.71 |
| Alpha-Amylase Inhibition Assay | 1.17 | 1.06 |

## CONCLUSIONS

In conclusion, the antioxidant and antidiabetic properties of the herbal blend of cold methanol leaves extract of Rumex hastatus and Rumex napalensis has been observed and demonstrated. It is evident that these species exhibit significant potential across both therapeutic domains. The

assessment of their effects using three different antioxidant models—DPPH, ABTS, and FRAP—reveals that both plants possessed strong free radical scavenging activities, attributing to their high flavonoid and phenolic content. This suggests their utility in combating oxidative stress, a key contributor to chronic diseases including diabetes and cardiovascular disorders. Moreover, when tested in three antidiabetic models—alpha-amylase inhibition, alpha-glucosidase inhibition, and glucose uptake activities— the herbal blend containing both Rumex species demonstrated notable efficacy in moderating glucose levels, thereby presenting a natural therapeutic option for managing diabetes. Their ability to inhibit carbohydrate-digesting enzymes highlights their potential in slowing glucose absorption, a crucial factor in diabetes management. The convergence of antioxidant and antidiabetic properties not only underscores their traditional use in folk medicine but also emphasizes their relevance in contemporary medical research. Future studies should focus on isolating specific compounds responsible for these effects and assessing their safety and efficacy in clinical settings, potentially leading to new, plant-based therapies for managing oxidative stress and diabetes.

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