*Article*

# Exploring the Therapeutic Promise of *Drynaria coronans*: Phytochemical Analysis, Antioxidant Capacity, α-Amylase Inhibition with Safety Assessment

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Received: 1 December 2024; Revised: 9 January 2025; Accepted: 7 February 2025; Published: 10 February 2025

**Abstract:** Background: Secondary metabolites derived from plants have been identified as potential natural antioxidants, exhibiting robust capabilities in neutralizing oxidative stress that can emerge under various pathological conditions. Aim: The aim of this study was to analyze the phytochemical composition of ethanolic extract of *Drynaria coronans (D. coronans)*, and its antioxidant potential along with α-amylase inhibition and oral toxicity studies. Methods: Preliminary qualitative phytochemical screening was conducted for *D. coronans* while quantitative phytochemical analysis involved the estimation of total phenolic and flavonoid contents. In vitro antioxidant activities were evaluated by using ferrous reducing-antioxidant power (FRAP), hydrogen peroxide ($H_2O_2$) scavenging activity, nitric oxide scavenging activity and DPPH free radical scavenging assays. The in vitro antidiabetic activity was evaluated by using the α-amylase inhibition assay. In vivo oral acute toxicity studies were evaluated in the rats as per the Organization for Economic Cooperation and Development guidelines. Results: The extraction yield of *Drynaria coronans* was found to be 11.94% using ethanol as the primary solvent and plant sample with solvent in a 4:1 ratio. Phytochemical analysis of the rhizome revealed the presence of secondary metabolites, including alkaloids, phenols, tannins, glycosides, and carbohydrates, which were further validated by TLC profiling. The dried extract contained 56.38 ± 0.09 mg gallic acid equivalent (GAE/g) of total phenols and 202.54 ± 0.22 mg quercetin equivalents (QE/g) of flavonoids. The extract demonstrated notable antioxidant properties, with $IC_{50}$ values of 43.59 (DPPH), 758.94 (NO), and 715.60 ($H_2O_2$), while also exhibiting strong reducing power (0.164 ± 0.011) at 700 nm. Additionally, it showed effective α-amylase inhibition with an $IC_{50}$ of 889.84 µg/mL. When tested for acute oral toxicity at a dose of 5000 mg/kg, no behavioral changes, morbidity, or mortality were observed in Swiss albino rats. Conclusion: The ethanolic extract of *Drynaria coronans* rhizomes have a positive correlation of total phenolic and flavonoid contents with the antioxidant, and inhibitory potential of the α-amylase.

**Keywords:** α-amylase; antioxidant activity; *Drynaria coronans*; phytochemical analysis; toxicity studies

## 1. Introduction

Oxidative stress arises from an excess of reactive oxygen species (ROS) and reactive nitrogen species (RNS) [1]. These terms collectively describe free radicals and other non-radical reactive oxidants. ROS include dioxygen ($O2^{\bullet-}$), hydrogen peroxide ($H_2O_2$), and hydroxide ($^{\bullet}OH$), while RNS include nitrogen dioxide ($NO_2$), nitric oxide ($NO^{\bullet}$), and peroxynitrite ($ONOO^-$) [2,3]. An accumulation of ROS and RNS leads to oxidative damage to nearly all molecules. Under normal physiological conditions, ROS and RNS groups are not harmful [4,5]. However, when the body fails to adequately eliminate them, oxidative stress promotes atherosclerotic plaque formation, increasing the risk of atherosclerosis, cancer and even type 2 diabetes mellitus [6–8].

α-Amylase, a class of hydrolytic enzymes, is synthesized by various organisms including humans, animals, fungi, bacteria, and plants. In the human body, these enzymes are produced in the salivary glands and subsequently released into the oral cavity. Additionally, the pancreas secretes α-amylase directly into the small intestine [9]. The primary function of α-amylase is to catalyze the breakdown of starch molecules. Specifically, these enzymes

target and cleave the α-(1,4)-glycosidic bonds within starch, leading to the production of several compounds such as glucose, maltodextrins, maltotetraose, maltose, and maltotriose [9].

Oxidative stress has been linked to numerous health conditions, including diabetes, cancer, neurodegenerative disorders, and cardiovascular diseases [10,11]. Additionally, diabetes-related cardiovascular issues can develop through various mechanisms, with oxidative stress being a significant factor. Consequently, managing oxidative stress and blood sugar levels is crucial for overall health. The natural world has consistently provided a rich source of beneficial compounds that contribute to human well-being [12]. Substantial evidence demonstrates that plants and other natural food sources are primary providers of antioxidants and have been shown to inhibit α-amylase and α-glucosidase activities [13].

*Drynaria coronans* (Wall. ex Mett.) J.Sm. ex T.Moore (Family: Polypodiaceae), is one of the medicinal plants of Nepal which are commonly found densely on tree trunks at higher elevations of approximately 1500–2000 m altitude [14]. The rhizome of *D. coronans* is used to treat constipation, bone fractures and backaches [15–17]. There was a paucity of research on the *D. coronans* rhizome as per the literature review since the search for bioactive plant components from this plant is a deciding factor in the search for new bioactive phytoconstituents. Investigating the *D.coronans* rhizome will help to improve the proper use of this plant in various medical conditions as an alternative treatment plan and aiding in the discovery of therapeutic agents for specific diseases. The purpose of this study was to investigate the in vitro antioxidant activity, phytochemical profile, α-amylase inhibitory activity, and acute oral toxicity of the rhizome of *D. coronans.*

## 2. Material and Methods

### *2.1. Collection and Authentication of Plant Material*

*D. coronans* rhizomes (Figure S1) were collected from Pokhara-31, Kaski district of Nepal, with the help of the local people. The plant was authenticated by the taxonomist via macroscopical and microscopical evaluation methods from the National Herbarium and Plant Laboratories, Godawari, Nepal. The parts of plant were collected from their natural habitat, and plant samples were stored in the Pharmacognosy Laboratory of the School of Health and Allied Sciences, Pokhara University, Kaski, with the Herbarium Sheet voucher number PUH-2022-40.

### *2.2. Drugs and Chemicals*

Ethanol was purchased from Central Drug House (P.) Ltd.,Delhi, India, while Times Pharmaceutical Limited, Chitwan, Nepal, provided the standard reference drug, acarbose. Ascorbic acid, gallic acid, quercetin, and DPPH were purchased from HiMedia Pvt. Ltd., Maharashtra, India. All the reagents and chemicals used in this study were of analytical grade.

### *2.3. Plant Extract Preparation and Percentage Yield*

A modified version of the cold maceration method was used for extraction. The plant sample (100 g) was macerated twice for 24 h in ethanol (800 mL). A rotary evaporator was used to evaporate and dry the extract. The extracts were stored in glass vials with silica crystals for drying in vacuum desiccators operating at 60 mbar. The extract weights were recorded daily until a steady dry weight was obtained. The percentage yield was calculated from the weight of the dried extract. The percentage of extract yield was determined using the following formula:

$$\text{Extract Yield Percentage} = [(\text{Weight of dry extract} / \text{Weight of crude drug sample}) \times 100\%]$$

### *2.4. Phytochemical Screening*

Phytochemical screening was performed to identify the secondary metabolites present in the ethanolic extract of *D. coronans* using color reactions with different reagents [18]. The ethanolic extracts were tested for carbohydrates, flavonoids, glycosides, alkaloids, tannins, saponins, proteins, and steroids.

### *2.5. TLC Profiling of the Plant Extracts*

TLC profiling of the extracts was performed for qualitative analysis [19,20]. One gram of dried extract of *D. coronans* rhizome was mixed with ethanol to prepare sample. TLC profiling was then carried out by applying the sample into three different TLC plates, which were allowed to run separately in the mobile phase of a solvent mixture (chloroform:methanol:water = 7:3:0.5). The TLC plates were then observed under UV lamps at both 365 nm and 254 nm wavelengths. The spraying reagents were 10% $FeCl_3$, 10% $H_2SO_4$, and 100 µM DPPH solution.

### *2.6. Total Phenolic and Flavonoid Compound Analysis*

Using the Folin-Ciocalteu technique, the total phenolic content of extracts was determined [21] using certain adjustments, and the results were expressed as gallic acid (GA) equivalents in milligrams (mg) per gram of dry rhizome extract (mg GAE/g).

The total flavonoid content was evaluated using the aluminum chloride method [10] with a few minor adjustments, and the total flavonoid content was expressed as milligrams of quercetin equivalents (QE) per gram of extract from dried rhizome (mg QE/g).

### *2.7. Invitro Antioxidant Activity*

#### 2.7.1. DPPH Free Radical Scavenging Assays

The scavenging activity of plant extracts towards the stable free radical DPPH was measured using a previously established method with some modifications [22,23]. A small portion (2 mL) of different concentrations of the extract and ascorbic acid solutions (1, 2.5, 5, 7.5, 15, 30, and 60 µg/mL) in ethanol was mixed with 100 µM DPPH solution (2 mL). The mixture was allowed to stand in the dark for 30 min. After 30 min, the absorbance of each test sample was measured at 517 nm by using a UV Spectrophotometer. Ascorbic acid was used as the standard reference. All findings were expressed in the $IC_{50}$ values, which represent the 50% inhibition of the free radicals with the plant extract and standard reference. The radical scavenging activity of each sample was calculated using the following formula [24]:

$$\text{DPPH free radical scavenging activity (\%)} = [(A_{\text{control 517}} - A_{\text{Sample 517}}) / A_{\text{control 517}}] \times 100$$

where, $A_{\text{control 517}}$ is the absorbance control (sample was replaced with distilled water); $A_{\text{sample 517}}$ is the plant extract sample absorbance

#### 2.7.2. Nitric Oxide Scavenging Activity

The nitric oxide radical scavenging activity of the plant extracts was determined using a previous method [25]. The plant extract (1 mL) of different concentrations (0.1 µg/mL, 1 µg/mL, 10 µg/mL, 100 µg/mL, 250 µg/mL, 500 µg/mL, 1000 µg/mL) was mixed with SNP (1 mL) solution and incubated for 2.5 h at 29 °C. During incubation, the extract scavenges nitric oxide radicals, and the Griess reagent determines the quantity of remaining nitric oxide radicals. After 2.5 h, Griess (2 mL) reagent was added to the reaction mixture and absorption was measured at 548 nm using a UV spectrophotometer. Curcumin was used as the standard reference. The NO scavenging activity was calculated using the following formula:

$$\text{NO free radical scavenging activity (\%)} = [(A_{\text{control 548}} - A_{\text{Sample 548}}) / A_{\text{control 548}}] \times 100$$

where, $A_{\text{control 548}}$ is the absorbance control $A_{\text{sample 548}}$ is the plant extract sample absorbance

#### 2.7.3. Hydrogen Peroxide Scavenging Activity

The ability of the extracts to scavenge hydrogen peroxide was performed by the method of [26] with little modification 1 mL of the extracts from (0.1 µg/mL, 1 µg/mL, 10 µg/mL, 100 µg/mL, 250 µg/mL, 500 µg/mL, 1000 µg/mL) was added to 20 mM $H_2O_2$ (2 mL) solution prepared in 0.1 M phosphate buffer saline (pH 7.4). The mixture was incubated for 10 min and the absorbance was read at 230 nm using a UV spectrophotometer against a buffer blank. Ascorbic acid was used as the standard reference. The control was similar to that of the reaction mixture but without a test sample. Similarly, the plant control was a reaction mixture of the extract and buffer only. The scavenging activity was calculated using the following equation:

$$\text{Hydrogen Peroxide scavenging activity (\%)} = [(A_{\text{control 230}} - A_{\text{Sample 230}}) / A_{\text{control 230}}] \times 100$$

where, $A_{\text{control 230}}$ is the absorbance control $A_{\text{sample 230}}$ is the plant extract sample absorbance

#### 2.7.4. Ferrous Reducing-Antioxidant Power (FRAP)

The ability of the plant extracts to reduce antioxidant power was determined [27,28] with a slight modification. The ethanolic extract of varying concentrations (12.5 to 100 µg/mL) was mixed with 0.25 mL phosphate buffer (0.2 mM, pH 6.6) and 1% potassium ferricyanide (0.25 mL). The reaction system was closed and incubated at 50 °C (it's an o then superscripted, never use zero) in a water bath for 20 min. After the incubation

period, 10% trichloroacetic acid (0.25 mL) was added to the assay system, and the contents were mixed thoroughly. The mixture was then centrifuged at 5000 rpm for 5 min. The supernatant (0.5 mL) was mixed with equal volumes of distilled water and ferric chloride solution (0.1 mL, 0.1%, *w*/*v*). The developed color was read at 700 nm using a UV-visible spectrophotometer, and the results were compared with those of ascorbic acid as a standard reference. The readings were expressed as the mean absorbance value.

### *2.8. In-Vitro Antidiabetic Activity*

#### 2.8.1. α-Amylase Inhibitory Activity

An α-amylase inhibition assay was performed using the 3, 5-dinitrosalicylic acid (DNSA) method [29]. The α-amylase solution (200 µL, 5 mg/100 mL) was mixed with of the extract (200 µL) and incubated for 10 min at 30 °C. Next, the starch solution (200 µL, 1%) was added to each tube and incubated for 3 min. The reaction was terminated by adding DNSA (200 µL) reagent and boiling for 10 min in a water bath at 85–90 °C. The mixture was cooled to ambient temperature, diluted with distilled water (5 mL), and the absorbance was measured at 540 nm using a UV-visible spectrophotometer. A blank with 100% enzyme activity was prepared by replacing the plant extract with buffer (200 µL, mention buffer details, pH). The plant control was similarly prepared using the plant extract at each concentration in the absence of the enzyme solution. A positive control sample was prepared using acarbose (50 µg/mL, 100 µg/mL, 250 µg/mL, 500 µg/mL, 1000 µg/mL), and the reaction was performed similarly to the reaction with the plant extract, as mentioned above. The α-amylase inhibitory activity was expressed as a percentage inhibition and calculated using the following equation: % α-amylase inhibition was plotted against the extract concentration, and the $IC_{50}$ values were obtained from the graph.

#### 2.8.2. Acute Toxicity Studies

The Organization for Economic Co-operation and Development Guidelines (OECD) No. 425 were used to test the acute oral toxicity of *D. coronans* extracts [30]. Healthy non-pregnant and nulliparous female Swiss albino rats (180–240 g) were used for this purpose (Figure S4). All rats were fasted for 18 h with free access to water before and 3–4 h after the administration of the ethanolic extract. For the oral acute toxicity study, *D. coronans* ethanolic extract was dissolved in distilled water and treated as three doses at 2000 mg/kg, 3000 mg/kg, and 5000 mg/kg dose orally for three female healthy Swiss albino rats in the first phase by oral gavage tube. The treated rats were observed continuously for 4 h at half-hour intervals and then for 2 weeks at 1 d for general signs and symptoms of toxicity (diarrhea, weight loss, lethargy, tremors, and paralysis), food and water consumption, and mortality. In the second phase for the confirmation of the further toxicity study *D. coronans* ethanolic extract at a dose of 5000 mg/kg was administered to two female rats and observed for the signs and symptoms. If no mortality is observed in any of the tested animals, the $LD_{50}$ of the test substance is said to be greater than 5000 mg/kg and hence has a high degree of safety

#### 2.8.3. Statistical Analysis

A standard calibration curve for gallic acid and quercetin was created using the regression line equation in Microsoft Excel (2019) to estimate the concentrations of the phenolic and flavonoid compounds. All the in vitro and in vivo study was performed in triplicate and all results data are presented as mean ± standard error of mean.

## 3. Results

### *3.1. Phytochemical Screening*

Numerous phytoconstituents, including tannins, flavonoids, saponins, carbohydrates, alkaloids, phenol, and glycosides, were found in the ethanolic extract using qualitative phytochemical screening (Table 1).

Phenol, flavonoids, and antioxidants were confirmed by applying extracts and chemicals to TLC plates, as shown in Figure 1, Figure S2, Figure S3

**Table 1.** Phytochemical Screening of Hydro-ethanolic extract of *D. coronans*.

| Phytochemical Test | | Inference |
|---|---|---|
| Alkaloid | Wagner test | + |
| Flavonoid | Alkaline reagent test | + |
| Phenol | Sodium hydroxide test | + |
| Tannin | Ferric chloride Test | + |
| Glycoside | Salkowski's Test | + |
| Saponin | Lead acetate test | − |
| Carbohydrate | Fehling test | + |
| '+' and '−' sign indicates the presence and absence, respectively | | |

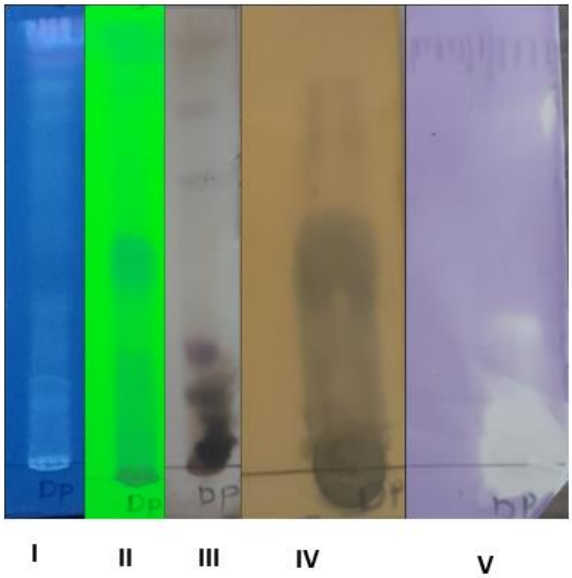


**Figure 1.** TLC plate observation. *Drynaria coronans* rhizome extract TLC spot observation under (I) short UV 254 nm, (II) long UV 365 nm, and TLC spot sprayed with (III) 10% $H_2SO_4$ /Heat, (IV) 10% $FeCl_3$, (V) 100 µM DPPH.

### *3.2. Total Phenolic and Flavonoid Content Estimation*

The extract obtained from the rhizome of *D. coronans* was a golden yellow solid (11.94%). The phenol content for the *D. coronans* ethanolic extract was (56.38 ± 0.09) mg GAE/gm dry extract weight while flavonoid content was obtained as (202.54 ± 0.22) mg QE/gm dry extract weight.

### *3.3. Antioxidant Assay*

#### 3.3.1. DPPH Radical Scavenging Activity

Compared with conventional ascorbic acid, the ethanol extracts of *D. coronans* rhizome ($IC_{50}$ = 43.59 µg/mL) demonstrated good DPPH free radical scavenging activity (Table 2).

**Table 2.** Antioxidant activity of hydroethanolic extract of *D. coronans* using DPPH, NO, $H_2O_2$, and FRAP assays.

| S.N. | Antioxidant Assay | Sample | Concentration (µg/mL) | | | | | $IC_{50}$ (µg/mL) |
|---|---|---|---|---|---|---|---|---|
| | | | 2.5 µg/mL | 5 µg/mL | 15 µg/mL | 30 µg/mL | 60 µg/mL | |
| 1 | DPPH scavenging (%) | *D. coronans* | 1.57 ± 0.04 | 3.86 ± 0.09 | 27.04 ± 2.44 | 36.86 ± 1.26 | 63 ± 2.43 | 43.59 |
| | | Ascorbic acid | 22.2 ± 0.5 | 48.4 ± 2.1 | 61.34 ± 0.34 | 76.28 ± 2.09 | 98.25 ± 0.13 | 3.96 |
| | | | 10 µg/mL | 100 µg/mL | 250 µg/mL | 500 µg/mL | 1000 µg/mL | |
| 2 | NO scavenging (%) | *D. coronans* | 3.7 ± 0.06 | 9.28 ± 0.6 | 22.11 ± 0.6 | 37.33 ± 2.9 | 62.49 ± 0.1 | 758.94 |
| | | Curcumin | 33.89 ± 0.74 | 43.23 ± 0.68 | 61.66 ± 1.75 | 81.74 ± 0.64 | 91.09 ± 1.34 | 162.79 |
| | | | - | 100 µg/mL | 250 µg/mL | 500 µg/mL | 1000 µg/mL | |
| 3 | $H_2O_2$ Scavenging (%) | *D. coronans* | - | 22.7 ± 0.1 | 29.89 ± 0.3 | 42.29 ± 2.07 | 61 ± 6.03 | 715.6 |
| | | Ascorbic acid | - | 28.7 ± 0.2 | 42.12 ± 0.3 | 61.89 ± 4.4 | 94.3 ± 1.7 | 353.96 |
| | | | 12.5 µg/mL | 25 µg/mL | 50 µg/mL | 75 µg/mL | 100 µg/mL | |
| 4 | FRAP ($Abs_{700\ nm}$) | *D. coronans* | 0.005 ± 0.008 | 0.084 ± 0.005 | 0.127 ± 0.006 | 0.145 ± 0.008 | 0.164 ± 0.011 | - |
| | | Ascorbic acid | 0.234 ± 0.007 | 0.388 ± 0.008 | 0.545 ± 0.005 | 0.797 ± 0.006 | 1.07 ± 0.006 | - |

All experiments were performed in triplicate (n = 3) and expressed as the mean ± standard error of the mean.

3.3.2. NO Free Radical Scavenging Activity

Compared with conventional curcumin, the ethanol extract of *D. coronans* rhizome ($IC_{50}$ = 758.94 µg/mL) demonstrated good NO free radical scavenging activity (Table 2).

3.3.3. Hydrogen Peroxide Scavenging Activity

Compared to conventional ascorbic acid, the ethanol extracts of *D. coronans* rhizomes ($IC_{50}$ value 715.60 µg/mL) demonstrated better hydrogen peroxide scavenging activity (Table 2).

3.3.4. Ferric ion Reducing Antioxidant Assay (FRAP)

The *D. coronans* extract demonstrated moderate ferric-reducing activity at 100 µg/mL (0.164 ± 0.011) compared to the ascorbic acid standard (Table 2).

3.3.5. α-Amylase Inhibitory Activity

Compared to conventional drugs acarbose, *D. coronans* rhizome extract performed comparable α-amylase inhibitory activity ($IC_{50}$ = 889.84 µg/mL) as shown in (Table 3, Figure S5).

**Table 3.** The α-amylase inhibitory activity of hydro-ethanolic extract of *D. coronas*.

| S. No. | SampleName | % α-Amylase Inhibitory Activity | | | | | $IC_{50}$ Value (µg/mL) |
|---|---|---|---|---|---|---|---|
| | | 50 µg/mL | 100 µg/mL | 250 µg/mL | 500 µg/mL | 1000 µg/mL | |
| 1 | *D. coronans* | 11.83 ± 6.98 | 14.31 ± 0.36 | 21.88 ± 0.58 | 35.04 ± 0.248 | 53.75 ± 1.39 | 889.84 |
| 2 | Acarbose | 16.06 ± 0.49 | 24.84 ± 0.15 | 29.47 ± 0.34 | 50.86 ± 0.27 | 83.08 ± 0.31 | 511.51 |

*All data are expressed as mean ± standard error of the mean (SEM) (n = 3).*

*3.4. Oral Acute Toxicity Study*

Oral administration of the ethanolic extract of *D. coronans* in rats did not cause any behavioral changes such as locomotion, activity, hair texture, pupil size, or feeding. No morbidity or mortality was observed at 5000 mg/kg. Hence, the $LD_{50}$ of both ethanolic extracts was estimated to be greater than 5000 mg/kg and was considered the Globally Harmonized System (GHS) category 5.

## 4. Discussion

It is generally acknowledged that using natural products to identify potential lead compounds for drug development is beneficial [31]. Isolation of morphine for the first time in 1806 paved the way for the development of many natural lead compounds such as strychnine, atropine, and colchicine [32]. Plant-derived natural products have been essential to the world's healthcare systems for thousands of years. They offer various biological activities and active components, making them useful for developing complementary or alternative therapies in contemporary medicine [33]. Based on traditional and ethno-medicinal usage and a scant scientific study of the plant, *D. coronans* rhizomes were chosen for this study. The *D. coronans* rhizome extract was subjected to various antioxidant tests, α-amylase inhibitory activity tests, and oral acute toxicity studies.

Phytochemical analysis of the *D. coronans* extract showed the presence of phytochemicals, such as phenols, flavonoids, tannins, saponins, alkaloids, terpenoids, coumarins, anthracene, and glycosides (Table 1). The presence of several *D. coronans* compounds was confirmed by separation of several bands in the chromatograms visualized under UV light at 254 and 365 nm (Figure 1, Figure S2, S3). The chromatogram after spray/heating with sulfuric acid (10%) showed yellow, black, and reddish-brown spots, suggesting the presence of flavonoids, carbohydrates, and terpenoids, respectively [34]. The presence of phenolic compounds in the chromatogram could also be visualized by spraying with ferric chloride (10%) [35]. The spots at the bottom of the *D. coronans* chromatogram suggested the presence of a phenolic compound. Similarly, the presence of antioxidants was confirmed by a white/yellow band on a violet or purple background on the *D. coronans* chromatogram [34]. Because the plant contains phenols, flavonoids, and antioxidants, they can favorably correlate with antidiabetic activity [36].

The TPC (mg/g) was determined from the regression equation of the calibration curve ($y = 0.01x + 0.093$, $R^2 = 0.9976$) and expressed as GAE 56.38 ± 0.09. TFC (mg/g) was determined from the regression equation of the calibration curve ($y = 0.0008x + 0.0324$, $R^2 = 0.99$) and expressed as QE 202.54 ± 0.22. The ethyl acetate and methanol extract of *D. coronans* carried out by Koirala et al., showed a phenolic content of 292.14 ± 1.82 and

37.48 ± 5.41 mg GAE/g extract respectively while the flavonoid content of the ethyl acetate and methanol fraction was obtained as 322.90 ± 3.94 and 9.87 ± 1.00 mg CE/g extract respectively [37,38]. Because we experimented with the ethanolic extract, the data may vary.

The $IC_{50}$ values for the free radical scavenging activities were determined using the linear regression method, which involves plotting the percentage of the radical scavenging activity against the tested drug concentrations. The DPPH scavenging assay was carried out following the method described in the literature [38]. It showed a higher value ($IC_{50}$ = 93.3 µg/mL) than the experimental result ($IC_{50}$ = 43.59 µg/mL). Similarly, DPPH activity carried in ethyl acetate and methanol extract of the same species showed a DPPH scavenging activity with $IC_{50}$ values (37.54 ± 1.25 µg/mL) and (80.25 ± 1.37 µg/mL) [37]. The ethanolic extract showed DPPH scavenging value similar to that of the ethyl acetate extract of *D. coronans.* This could be due to polar compounds in the ethanolic and ethyl acetate extracts.

A free radical scavenging method using NO, $H_2O_2$, and FRAP was performed for the first time using an ethanolic extract of *D. coronans.* NO or reactive species are responsible for altering the structural and functional nature of many cellular components [39]. Plant antioxidants can mitigate or inhibit the chain reactions triggered by excessive NO production, which is detrimental to human health. [40]. The NO radical scavenging activity in the *D. coronans* rhizome extract was $IC_{50}$ value of 758.94 µg/mL against the standard curcumin with $IC_{50}$ value of 162.79 µg/mL. The NO-scavenging activity of the extract was lower than that of the standard, suggesting that the extract may not be able to scavenge reactive species. The hydrogen peroxide scavenging activity of the extract showed a pattern similar to that of the NO scavenging activity. *D. coronans* showed moderate scavenging activity against $H_2O_2$, with an $IC_{50}$ Value of 715.60 µg/mL. The reducing capacity of a sample is an important indicator of its antioxidant activity. FRAP was used to determine the reducing potential of the extract. The reducing value of *D. coronans* extract was 0.164 ± 0.011 at a concentration of 100 µg/mL. The reducing capacity of the extract also increased with increasing extract concentrations (Table 2).

An α-Amylase inhibitory assay was performed according to the modified 3,5-Dinitrosalicyclic Acid (DNSA) method to determine the in vitro antidiabetic activity of the ethanolic extract of *D. coronans* rhizome. An inhibition assay was performed for the first time in this species. In this study, the extract showed α-amylase inhibitory activity ($IC_{50}$ Value 889.84 µg/mL), while we compared it with standard acarbose ($IC_{50}$ Value 511.51 µg/mL). The enzyme-inhibitory activity of plant extracts is attributed to various classes of phytochemicals such as alkaloids, flavonoids, tannins, and phenolic acids [34]. The α-amylase inhibitory activity of *D. coronans* rhizome extracts depend on the total phenol content, as phenols have been reported as potential antidiabetic agents. Data obtained on the phenol content of the extract also suggest that the α-amylase inhibitory activity could be due to the highest amount of phenolic compounds in this plants [41]. Since α-amylase activity and total phenolic and flavonoid contents and their respective antioxidant activity were correlated.

None of the treatment groups showed any mortality or notable behavioral changes related to acute toxicity. The ethanolic extract of *D. coronans* had an oral $LD_{50}$ of greater than 5000 mg/kg. It is unlikely that the plant will kill people because anything with an $LD_{50}$ above this threshold is thought to be harmless [42]. The limitation of thisstudy was the inability to perform the in vivo antidiabetic activity and evaluation of the biochemical parameters and histopathology examination for the further mechanism of the antidiabetic properties.

## 5. Conclusions

In conclusion, the ethanolic extract of *D. coronans* yielded significant amounts of extract and a considerable quantity of flavonoids and phenolic compounds, exhibiting antioxidant properties due to its high free radical scavenging activity in terms of DPPH, NO, FRAP, and $H_2O_2$. This study also demonstrates that *D. coronans* extracts shows high α-amylase inhibitory activity, since such plant could be potential hypoglycemic agents. The ethanolic extract of *D. coronans* rhizome did not show any toxicity effect up to 5000 mg/kg, which confirms its safety for oral use. The findings of this study warrant further scientific validation, exploration, and analysis to ascertain possible therapeutic applications of the *Drynaria coronans* ethanolic extract as oral hypoglycemic agents.

**Supplementary Materials:** The following are available online at https://www.sciltp.com/journals/jmnp/2025/1/748/s1, Figure S1: *Drynaria coronans* grown as an epiphytic plant in *Shorea robusta*; Figure S2: TLC of Drynaria coronans spot (10% $H_2SO_4$/Heat sprayed); Figure S3: Observation of Phenolic and antioxidant activity using the TLC method; Figure S4: Swiss albino rats used for toxicity study; Figure S5: Alpha-amylase activity of standards used for the research; Figure S6: Filtrate of *Drynaria coronans* rhizome; Figure S7: Ethical approval letter.

**Author Contributions:** P.K.J. and S.P. (Sushil Panta) conceived and designed the experiments. P.K.J., SK, R.K.Y., S.P2. (Sandesh Paudel) and R.K. performed experiments. P.K.J., B.P. and S.P2. (Sandesh Paudel) analyzed the data. P.K.J., B.P., R.K.Y., and K.S. wrote the manuscript. P.K.J., B.P., K.S. and S.P. (Sushil Panta) have reviewed the manuscript. S.P2. (Sandesh Paudel) and B.P. critically revised the manuscript and provided intellectual support. S.P. (Sushil Panta) supervised the project. All authors have read and agreed to the published version of the manuscript

**Funding:** No funding was allocated for this project.

**Institutional Review Board Statement:** The study was conducted with extreme care and showed no signs of endangering people or the environment according to an ethical clearance certificate obtained from the Institute Review Committee of Pokhara University, Nepal (Reference Number: 105-079/80). The documentation for ethical approval is attached in a supplementary file.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** All relevant data are in the paper, and upon request, the corresponding author can answer any questions regarding this study's findings.

**Acknowledgments:** The authors are grateful to the School of Health and Allied Sciences, Pokhara University, Kaski, Nepal, for providing the laboratory and research facilities during the study. We sincerely thank Hari Prasad Devkota, Graduate School of Pharmaceutical Sciences, Kumamoto University, Japan and Nabin Pathak, Madan Bhandari Academy of Health Sciences, Nepal for constructive feedback on the manuscript. The manuscript writing part has been assisted by the AI software Grammarly. It was only used for grammar correction and the necessary selection of words.

**Conflicts of Interest:** The authors declare no conflicts of interest.

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