Ignatius Ajuru University of Education
Journal of Applied Chemical Sciences
ISSN: 3156-3619

Print ISSN: 3156-3619 https://iauejournals.com **Volume 1; Issue 1; April 2026; Page No. 64-79.**

Phytochemical Composition and Multi-Target Bioactivities of *Telfairia occidentalis Root*: Anti-Inflammatory, Antidiabetic, and Enzyme-Modulating Potential

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Abstract
Telfairia occidentalis (fluted pumpkin) is widely recognized in traditional medicine for the management of inflammatory conditions and metabolic disorders. While its leaves have been extensively investigated, information on the phytochemical composition and biological activities of the root remains limited. This study evaluated the phytochemical constituents, anti-inflammatory, antidiabetic, and enzyme-modulating activities of T. *occidentalis* root extract and determined its heavy metal content. Fresh root samples were collected from Idu ogba Rivers State, extracted with ethanol, and analyzed using gas chromatography–flame ionization detection (GC-FID) for phytochemical profiling, while heavy metals were determined using atomic absorption spectrophotometry (AAS). The biological activities were assessed using standard in vitro anti-inflammatory and antidiabetic assays. Data were expressed as mean ± standard deviation of triplicate determinations and analyzed using one-way analysis of variance (ANOVA) at a significance level of $p < 0.05$. The phytochemical analysis revealed the presence of abundant flavonoids, phenolic acids, and terpenes, with limonene (16.30 ppm), butein (9.38 ppm), naringenin (4.30 ppm), and ferulic acid (3.58 ppm) identified as major constituents. The extract exhibited appreciable biological activities, including 66% inhibition of proteinase activity, 64% inhibition of protein denaturation, and approximately 60% antidiabetic activity, whereas α-glucosidase inhibition was relatively low (7%). The concentrations of lead (0.061 ppm), cadmium (0.086 ppm), mercury (0.022 ppm), chromium (0.027 ppm), arsenic (0.010 ppm), and copper (0.089 ppm) were all below the recommended permissible limits. The observed biological activities may be attributed to the combined effects of the phytochemical constituents present in the extract. These findings suggest that *T. occidentalis* root is a promising source of bioactive compounds with in vitro anti-inflammatory and antidiabetic potential. However, further studies on toxicity, animal models, and mechanistic investigations are required to establish its safety and therapeutic value.

Keywords: *Telfairia Occidentalis*, Flavonoids, Phenolic Acids, Anti-Inflammatory, Antidiabetic

Introduction

Telfairia occidentalis (fluted pumpkin) is a perennial vegetable widely cultivated across West Africa, where it serves as both a nutritious food source and an important medicinal plant. Different parts of the plant, including the leaves, seeds, and roots, have long been used in traditional medicine for the management of inflammatory disorders, diabetes, anaemia, and oxidative stress-related conditions (Obadire et al., 2026). Ethnomedicinal reports also describe the use of the root in the treatment of fever, joint pain, and hyperglycaemia, suggesting that it contains biologically active compounds with therapeutic potential (Alegbejo, 2012; Dendegt, 2024). These traditional applications have stimulated scientific interest in identifying the phytochemicals responsible for its medicinal properties. Medicinal plants owe

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their biological activities largely to secondary metabolites such as flavonoids, phenolic acids, terpenes, alkaloids, and saponins. Previous studies have shown that *T. occidentalis* contains flavonoids including butein, naringenin, and kaempferol, phenolic acids such as ferulic and vanillic acids, and terpenes including limonene and pinene (Bhandare & Malode, 2026). These compounds have been reported to possess antioxidant, anti-inflammatory, and antidiabetic properties through mechanisms involving free radical scavenging, modulation of inflammatory mediators, stabilization of cellular proteins, and regulation of carbohydrate-metabolizing enzymes (Orie et al., 2021; Thakur et al., 2024). Because the composition and concentration of these metabolites vary among different plant parts, each organ may exhibit distinct biological activities. Despite the extensive investigation of the leaves and seeds of *T. occidentalis*, comparatively little attention has been given to the root. Plant roots frequently accumulate unique secondary metabolites or different concentrations of bioactive compounds because they are directly involved in nutrient uptake, storage, and interactions with the surrounding soil environment (Weston & Mathesius, 2014; Asare et al., 2023). Consequently, the phytochemical profile and pharmacological properties of the root cannot be assumed to be identical to those of the aerial parts. In addition, medicinal plants may accumulate environmental contaminants, including heavy metals, which could influence their safety for therapeutic use. Therefore, evaluating both the beneficial phytochemicals and potential contaminants in the root is necessary to support its safe and effective utilization.

Although several studies have reported the antioxidant, anti-inflammatory, and antidiabetic properties of *T. occidentalis* leaves, comprehensive information on the phytochemical composition, enzyme-modulating activity, anti-inflammatory potential, antidiabetic properties, and heavy metal content of the root remains scarce. This lack of information limits the scientific validation of the root despite its widespread use in traditional medicine. The present study was therefore undertaken to characterize the phytochemical constituents of *T. occidentalis* root, evaluate its **in vitro** anti-inflammatory, antidiabetic, and enzyme-modulating activities, and determine the concentrations of selected heavy metals. The findings will contribute to existing knowledge on the medicinal value and safety profile of the root and provide baseline information for future pharmacological and toxicological investigations.

Materials and Methods

Plant Collection and Preparation of Extract

Fresh roots of *Telfairia occidentalis* were collected in October, 2025 from Idu Ogba, Ogba Egbema, Ndoni local Government Area, Rivers State, Nigeria. A botanist authenticated the plant sample, and a voucher specimen (No. UNIPORT/PSB/082) was placed in the Department of Botany's herbarium for future reference. The collected roots were thoroughly washed with distilled water to remove adhering soil particles and other contaminants before being air-dried at room temperature (25–28°C) for approximately 14 days until a constant weight was obtained. The dried samples were milled into a fine powder using a laboratory grinder and stored in airtight containers until extraction. Fifty grams (50 g) of the powdered root was extracted with 500 mL of 80% ethanol by cold maceration for 72 hours at room temperature with intermittent agitation. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure using a rotary evaporator (RE-52A, Yarong Biochemical Instrument Factory, Shanghai, China) at 40°C. The crude extract was transferred into sterile amber bottles and stored at 4°C until required for phytochemical and biological analyses (Nna et al., 2026).

Phytochemical Analysis

Qualitative and quantitative phytochemical analyses of the ethanolic root extract were carried out using Gas Chromatography coupled with Flame Ionization Detection (GC-FID) following the method of Massalha et al. (2017) with slight modifications. Approximately 1 mg of the crude extract was dissolved in 1 mL of HPLC-grade methanol and filtered through a 0.45 μm polytetrafluoroethylene (PTFE) membrane filter prior to analysis.

The analysis was performed using an Agilent 7890B Gas Chromatograph equipped with a Flame Ionization Detector (FID) (Agilent Technologies, Santa Clara, CA, USA). Separation was achieved on an HP-5 fused silica capillary column (30 m × 0.32 mm internal diameter × 0.25 μm film thickness). Nitrogen (or helium, depending on the carrier gas used) served as the carrier gas at a constant flow rate of 1.0 mL min^{-1}. The injector was operated in split mode (split ratio 20:1), with an injection volume of 1 μL. The injector and detector temperatures were maintained at 250°C and 280°C, respectively. The oven temperature program was set as follows: an initial temperature of 60°C was held for 2 min, increased to 200°C at 10°C min^{-1}, and then raised to 280°C at 5°C min^{-1}, where it was held for 10 min. The

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total run time was approximately 40 min. Identification of phytochemical constituents was based on comparison of their retention times with those of authenticated analytical standards analyzed under identical chromatographic conditions. Quantification was performed using external calibration curves prepared from certified reference standards, including butein, naringenin, kaempferol, ferulic acid, vanillic acid, limonene, and other available phytochemical standards. The concentration of each compound was expressed as parts per million (ppm) based on the corresponding calibration curve.

Heavy Metal Determination

The concentrations of lead (Pb), cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), and arsenic (As) in the powdered roots of Telfairia occidentalis were determined after wet acid digestion. Briefly, 2.0 g of the powdered sample was transferred into a digestion flask and treated with a mixture of concentrated nitric acid (HNO_3) and perchloric acid ($HClO_4$) in a 3:1 (v/v) ratio. The mixture was heated on a hot plate until complete digestion was achieved, as indicated by the formation of a clear solution. After cooling to room temperature, the digest was filtered through Whatman No. 42 filter paper into a 50 mL volumetric flask and made up to volume with deionized water following the procedure of Massalha et al. (2017), with minor modifications.

The digested samples were analyzed using a Buck Scientific Model 210 VGP Atomic Absorption Spectrophotometer (Buck Scientific Inc., Norwalk, CT, USA). Lead, cadmium, chromium, and copper were determined by flame atomic absorption spectrophotometry (FAAS), whereas mercury and arsenic were analyzed using the appropriate atomic absorption techniques recommended for these elements to ensure adequate analytical sensitivity. Instrument calibration was performed with certified standard solutions prepared over suitable concentration ranges for each metal. Reagent blanks and calibration standards were included throughout the analysis to verify instrument performance and minimize analytical error. Each sample was analyzed in triplicate, and metal concentrations were calculated from the respective calibration curves. Results were expressed as mean ± standard deviation (mg/kg dry weight), which is equivalent to parts per million (ppm).

Anti-Inflammatory Assays

Protein (Albumin) Denaturation Assay: Anti-inflammatory activity was evaluated by the inhibition of heat-induced albumin denaturation following the method of Williams et al. (2008), with slight modifications. Briefly, 0.5 mL of the ethanolic root extract at concentrations of 40, 60, and 80 mg/mL was mixed with 0.5 mL of 1% bovine serum albumin (BSA) prepared in phosphate-buffered saline (pH 6.4). The reaction mixtures were incubated at 37°C for 20 min, followed by heating at 70°C for 5 min to induce protein denaturation. After cooling to room temperature, the absorbance of each mixture was measured at 660 nm using a UV–Visible spectrophotometer. Aspirin was used as the reference anti-inflammatory drug, while a reaction mixture without the extract served as the control. The percentage inhibition of protein denaturation was calculated using the following equation:

$$\%\text{Inhibition}=\frac{A_{control}-A_{sample}\times 100}{A_{sample}}$$

Where: Acontrol = Absorbance of the control (albumin solution without extract)
Asample = Absorbance of the test sample (albumin solution with extract)

Heat-Induced Hemoglobin Denaturation Assay: The inhibition of heat-induced hemoglobin denaturation was determined according to the method described by Williams et al. (2008), with slight modifications. Briefly, 0.5 mL of the ethanolic root extract at concentrations of 40, 60, and 80 mg/mL was mixed with 0.5 mL of freshly prepared hemoglobin solution (1% w/v) in phosphate-buffered saline (pH 6.4). The reaction mixtures were incubated at 37°C for 20 min and subsequently heated at 70°C for 10 min to induce protein denaturation. The mixtures were allowed to cool to room temperature, and the absorbance was measured at 540 nm using a UV–Visible spectrophotometer. Aspirin served as the reference anti-inflammatory drug, while the reaction mixture without the extract was used as the control. The percentage inhibition of hemoglobin denaturation was calculated using the following equation:

$$\%\text{Inhibition}=\frac{A_{control}-A_{sample}\times 100}{A_{sample}}$$

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Where: Acontrol = Absorbance of the heated hemoglobin control
Asample = Absorbance of the heated hemoglobin solution containing the extract

Anti-Proteinase Activity: The anti-proteinase activity of the ethanolic root extract was determined using the method described by Leelaprakash and Dass (2011), with slight modifications. Briefly, 0.5 mL of the extract at concentrations of 40, 60, and 80 mg/mL was mixed with 0.5 mL of trypsin solution (0.06 mg/mL) prepared in 20 mM Tris-HCl buffer (pH 7.4). The reaction mixture was incubated at 37°C for 10 min, after which 1.0 mL of 0.8% (w/v) casein solution was added as the substrate. The mixture was further incubated at 37°C for 20 min to allow enzymatic hydrolysis. The reaction was terminated by adding 2.0 mL of 70% perchloric acid, followed by centrifugation at 3000 rpm for 10 min to remove the precipitated proteins. The absorbance of the supernatant was measured at 210 nm using a UV–Visible spectrophotometer. Aspirin was used as the reference anti-inflammatory drug, while the reaction mixture without the extract served as the control. The percentage inhibition of proteinase activity was calculated using the following equation:

$$\% \text{ Inhibition} = \frac{[(\text{Absorbance of control} - \text{Absorbance of sample})]}{\text{Absorbance of control}} \times 100.$$

Anti-Lipoxygenase Activity: The inhibitory effect of the ethanolic root extract on lipoxygenase activity was determined using the method described by Gunathilake et al. (2018), with slight modifications. Briefly, 0.5 mL of the extract at concentrations of 40, 60, and 80 mg/mL was mixed with 0.5 mL of soybean lipoxygenase solution (20,000 U/mL) prepared in 0.1 M borate buffer (pH 9.0). The reaction mixture was pre-incubated at 25°C for 5 min, after which 1.0 mL of linoleic acid substrate solution was added to initiate the reaction. Following incubation for 10 min at room temperature, the formation of conjugated dienes was monitored by measuring the absorbance at 234 nm using a UV–Visible spectrophotometer. Aspirin was used as the reference anti-inflammatory drug, while the reaction mixture without the extract served as the control. The percentage inhibition of lipoxygenase activity was calculated using the following equation:

$$\%\text{Inhibition} = \frac{A_{control} - A_{sample} \times 100}{A_{sample}}$$

Where: Acontrol = Absorbance of the control reaction (enzyme + substrate without extract)
Asample = Absorbance of the test reaction (enzyme + substrate + extract)

Antidiabetic Assays

- **α-Glucosidase Inhibitory Assay:** Extracts at 0–160 mg/mL were incubated with α-glucosidase enzyme and p-nitrophenyl-α-D-glucopyranoside substrate. Absorbance was measured at 405 nm, and % inhibition was calculated as:
  $$\%\text{Inhibition} = \frac{A_{control} - A_{sample} \times 100}{A_{sample}}$$
- **Glucose Adsorption Capacity:** A 1 g sample of extract was incubated with 25 mL of glucose solution for 6 h. Glucose adsorption (mM glucose/g extract) was calculated using the formula:
  $$\text{Glucose adsorption (mM glucose/g extract)} = \frac{[(G1 - G2) * \text{Volume of solution}]}{\text{Weight of sample}}$$

  G1 is the glucose concentration ppm of original solution
  G2 is the glucose Concentration ppm after 6 h incubation
- **General Antidiabetic Activity:** Extracts at 0, 40, 60, and 80 mg/mL were evaluated for inhibition of glucose formation in vitro, with ascorbose serving as a reference standard. Absorbance was measured spectrophotometrically, and % inhibition was calculated relative to control.
  $$\%\text{ inhibition} = \frac{(\text{control} - \text{test}) \times 100}{\text{Control}}$$

Statistical Analysis

All experiments were conducted in triplicate. Data are presented as mean ± standard deviation (SD). One-way

analysis of variance (ANOVA) followed by Tukey's post hoc test was used to assess statistical significance, with $p < 0.05$ considered significant. Data were analyzed using SPSS version 25

Results

Phytochemicals of *Telfairia occidentalis* root

The quantitative phytochemical constituents of *Telfaira occidentalis* root are illustrated in Table 1.

Table 1: Quantitative phytochemical composition of the ethanolic root extract of *Telfairia occidentalis*

Component	Concentration (ppm)	Class
Resveratrol	0.755187	Flavonoid (stilbene)
Butein	9.37956	Flavonoid (chalcone)
Naringenin	4.30289	Flavonoid (flavanone)
Luteolin	1.22061	Flavonoid (flavone)
Kaempferol	2.27941	Flavonoid (flavonol)
Epicatechin	1.52605	Flavonoid (flavanol)
Epigallocatechin	0.825854	Flavonoid (flavanol)
Quercetin	1.50980	Flavonoid (flavonol)
Gallocatechin 3-gallate	0.207978	Flavonoid derivative
Robinetin	0.931750	Flavonoid (flavonol)
Myricetin	2.83198	Flavonoid (flavonol)
Artemetin	0.414125	Flavonoid (flavone)
Nobiletin	0.397781	Flavonoid (polymethoxyflavone)
Tangeretin	0.614579	Flavonoid (polymethoxyflavone)
Naringin	0.543410	Flavonoid (flavanone glycoside)
Lunamarin	0.963802	Flavonoid derivative
Vanillic acid	1.43974	Phenolic acid
Cinnamic acid	0.272863	Phenolic acid (phenylpropanoid)
Coumaric acid	0.366539	Phenolic acid (hydroxycinnamic)
Ferrulic acid	3.58421	Phenolic acid (hydroxycinnamic)
Pinene	4.34933	Terpene (monoterpene)
Carene	2.81402	Terpene (monoterpene)
Limonene	16.29554	Terpene (monoterpene)
Myrcene	1.25910	Terpene (sesquiterpene)

The root of *Telfairia occidentalis*is rich in flavonoids, including stilbenes (resveratrol, 0.755 ppm), chalcones (butein, 9.38 ppm), flavanones (naringenin, 4.30 ppm; naringin, 0.543 ppm), flavones (luteolin, 1.22 ppm; artemetin, 0.414 ppm), flavonols (kaempferol, 2.28 ppm; quercetin, 1.51 ppm; myricetin, 2.83 ppm; robinetin, 0.932 ppm), polymethoxyflavones (nobiletin, 0.398 ppm; tangeretin, 0.615 ppm), and flavanol derivatives (epicatechin, 1.53 ppm; epigallocatechin, 0.826 ppm; gallocatechin-3-gallate, 0.208 ppm; lunamarin, 0.964 ppm)..

Phenolic acids in the root include ferrulic acid (3.58 ppm), vanillic acid (1.44 ppm), coumaric acid (0.367 ppm), and cinnamic acid (0.273 ppm). These compounds are key contributors to antioxidant capacity and can protect cellular proteins from thermal and oxidative damage. Hydroxycinnamic acids, especially ferulic acid, also strengthen cell walls and participate in redox balance, which can support anti-inflammatory and antidiabetic mechanisms.

Terpenes in the root profile—limonene (16.296 ppm), pinene (4.349 ppm), carene (2.814 ppm), and myrcene (1.259 ppm)—represent volatile compounds that contribute to the plant's aroma and chemical defense mechanisms. Limonene's high concentration suggests a dominant role in deterring herbivores and inhibiting microbial growth. Monoterpenes like pinene and carene may act synergistically with flavonoids and phenolic acids to enhance the root's bioactivity. Sesquiterpenes and related terpenoid compounds such as myrcene are often involved in long-term ecological interactions and may influence root-soil microbial communities.

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While flavonoids, phenolic acids, and terpenes confer beneficial bioactivities, the root may also contain minor potentially toxic compounds, such as saponins, alkaloids, or cyanogenic glycosides, which could contribute to toxicity if consumed in excessive amounts.

Heavy metal of *Telfaira occidentials* root

The heavy metals of *Telfaira occidentials* root is illustrated in Table 2.

Table 2 Heavy metal concentrations in the root of *Telfairia occidentalis*

Sample	***Teiferia occidentalis***
Lead ppm	0.061
Mercury ppm	0.022
Chromium ppm	0.027
Arsenic ppm	0.010
Cadmium ppm	0.086
Copper ppm	0.089

The concentrations of lead, mercury, chromium, arsenic, cadmium, and copper detected in the root of *Telfairia occidentalis* are presented in Table 2. These findings indicate that the analyzed root sample was not contaminated with heavy metals above recommended limits. However, because the samples were collected from a single location, the results should not be considered representative of *Telfairia occidentalis* grown under different environmental conditions.

Lead, mercury, arsenic, and chromium are non-essential metals that may produce adverse health effects when present at elevated concentrations or after prolonged exposure. In the present study, these metals occurred at relatively low concentrations, suggesting minimal contamination of the sampled roots. Nevertheless, the accumulation of heavy metals in medicinal plants is influenced by factors such as soil composition, irrigation water, agricultural practices, and environmental pollution.

Anti-inflammatory Activity of *Telfaira occidentials* root

Figure 1 demonstrates a clear dose-dependent increase in inhibition of albumin denaturation by *Telfairia occidentalis* root extract. At 0 mg/mL, there is no inhibition, but the activity rises steadily to approximately 64% at 80 mg/mL. This indicates that bioactive compounds in the extract, likely flavonoids and phenolic acids as identified, exert stabilizing effects on protein structure and reduce denaturation.

The increasing inhibition correlates with the abundance of bioactive flavonoids such as butein (9.38 ppm) and naringenin (4.30 ppm) in the root, suggesting these compounds contribute substantially to the observed anti-inflammatory effect. Phenolic acids such as ferulic acid (3.58 ppm) may also enhance protein stabilization and antioxidant activity. Butein has been reported to suppress inflammatory mediators and oxidative stress, while naringenin exhibits anti-inflammatory and immunomodulatory activities through inhibition of pro-inflammatory signaling pathways.

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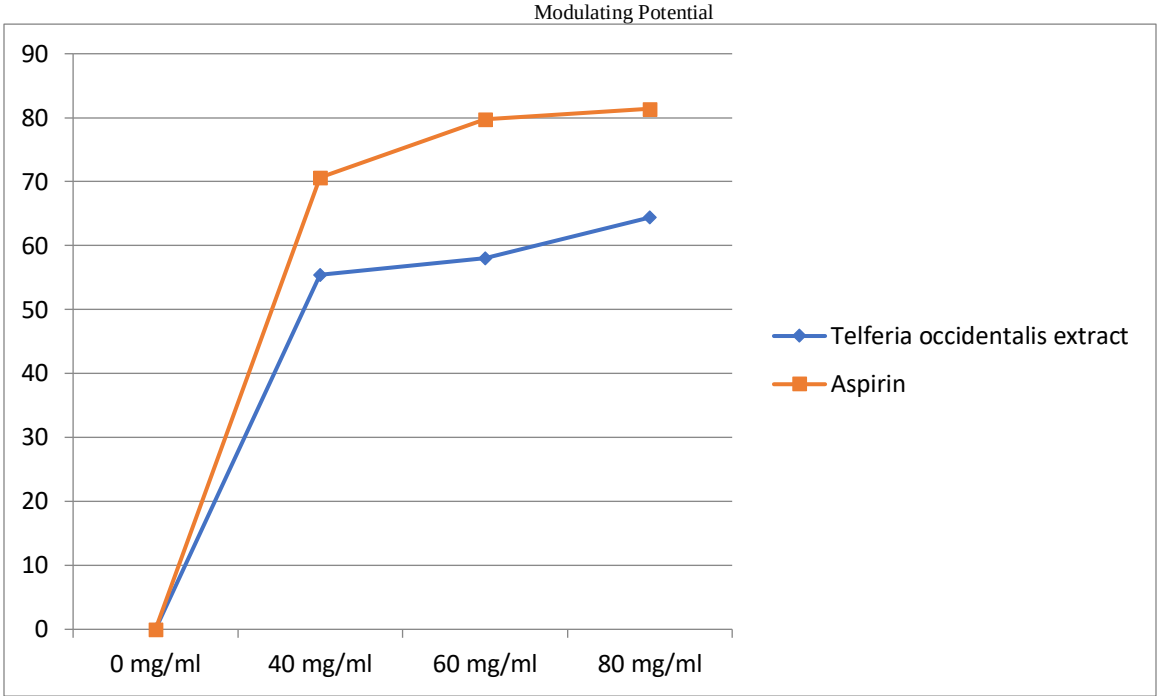

Figure 1: Ant-inflammation of *Telfaira occidentials* root

Heat-Induced Heamoglobin of **Telfaira occidentials root**

Figure 2 illustrates a dose-dependent inhibition of heat-induced hemoglobin denaturation by *Telfairia occidentalis*root extract. At 0 mg/mL, inhibition is negligible, but the activity rises steadily with increasing concentrations, reaching approximately 61% at 80 mg/mL. This trend demonstrates that bioactive phytochemicals, particularly flavonoids (butein 9.38 ppm, naringenin 4.30 ppm) and phenolic acids (ferulic acid 3.58 ppm), contribute to stabilising haemoglobin against thermal stress.

Compared to aspirin, which shows higher inhibition (approximately 75–85% across concentrations), the root extract demonstrates substantial but slightly lower activity. At higher concentrations (60–80 mg/mL), the extract approaches 61% inhibition, indicating significant efficacy of natural compounds in preventing protein denaturation. This suggests that while the extract is not as potent as a standard drug, its complex mixture of polyphenols and flavonoids acts synergistically to confer bioactivity, which is characteristic of crude plant extracts. For example, butein (9.38 ppm) and naringenin (4.30 ppm) likely contribute substantially to thermal protein stabilisation, while ferulic acid (3.58 ppm) and other phenolic acids enhance antioxidant effects. Butein has been reported to inhibit inflammatory mediators and oxidative damage, whereas naringenin possesses anti-inflammatory and immunomodulatory activities through suppression of pro-inflammatory signalling pathways. Ferulic acid further contributes through antioxidant, membrane-stabilising, and cytoprotective mechanisms.

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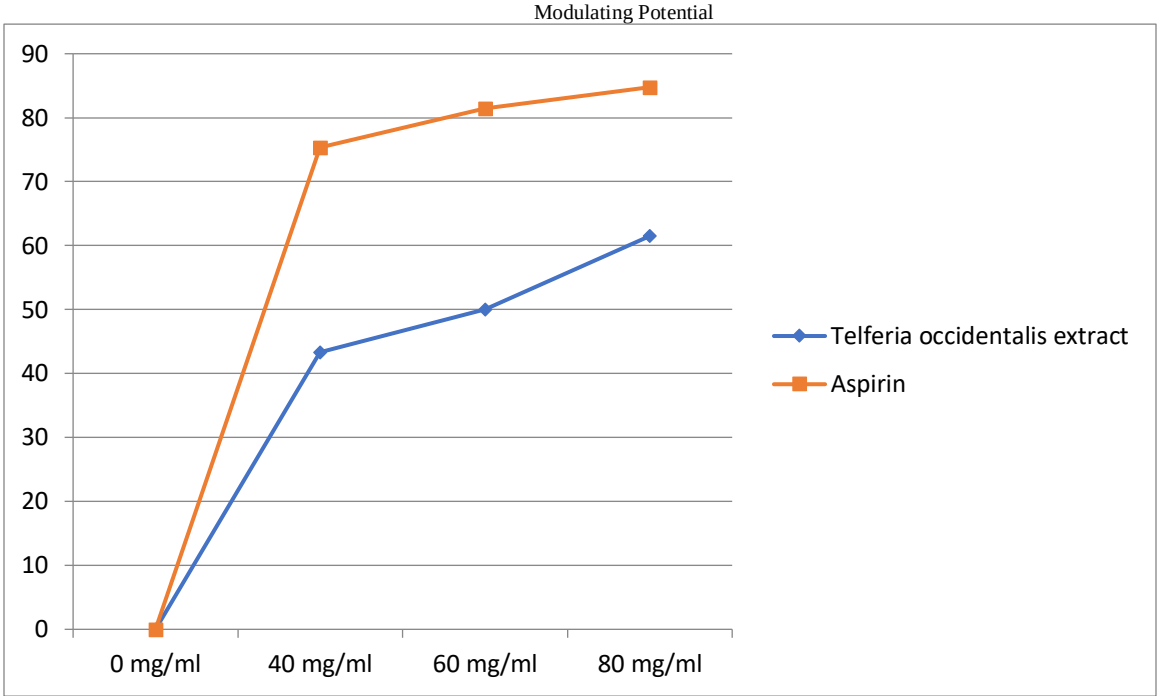

Figure 2: Heat-Induced Heamoglobin of *Telfaira occidentials* root

Anti-proteinase of *Telfaira occidentials* root

Figure 3 shows a clear dose-dependent increase in inhibition of proteinase activity by *Telfairia occidentalis root* extract, rising from 0% at 0 mg/mL to approximately 66% at 80 mg/mL. Proteinases are key mediators in inflammation, contributing to tissue degradation and promoting the release of pro-inflammatory cytokines. The observed inhibition suggests that the root extract stabilises protein structures and prevents excessive enzymatic activity. Flavonoids such as butein (9.38 ppm), naringenin (4.30 ppm), and kaempferol (2.28 ppm) likely contribute through both direct interaction with proteolytic enzymes and indirect antioxidant mechanisms that reduce oxidative stress-induced protease activation.

Aspirin, the reference standard, reaches approximately 81% inhibition at 80 mg/mL, showing higher potency than the root extract. However, the extract demonstrates comparable activity at higher concentrations, suggesting significant pharmacological potential. Unlike aspirin, which acts primarily by inhibiting cyclooxygenase enzymes and suppressing prostaglandin synthesis, *T. occidentalis* extract likely exerts multi-targeted effects, combining proteinase inhibition, antioxidant activity, membrane stabilisation, and modulation of inflammatory mediators. The inhibition of proteinase activity suggests that the extract may interfere with proteolytic processes involved in tissue inflammation.

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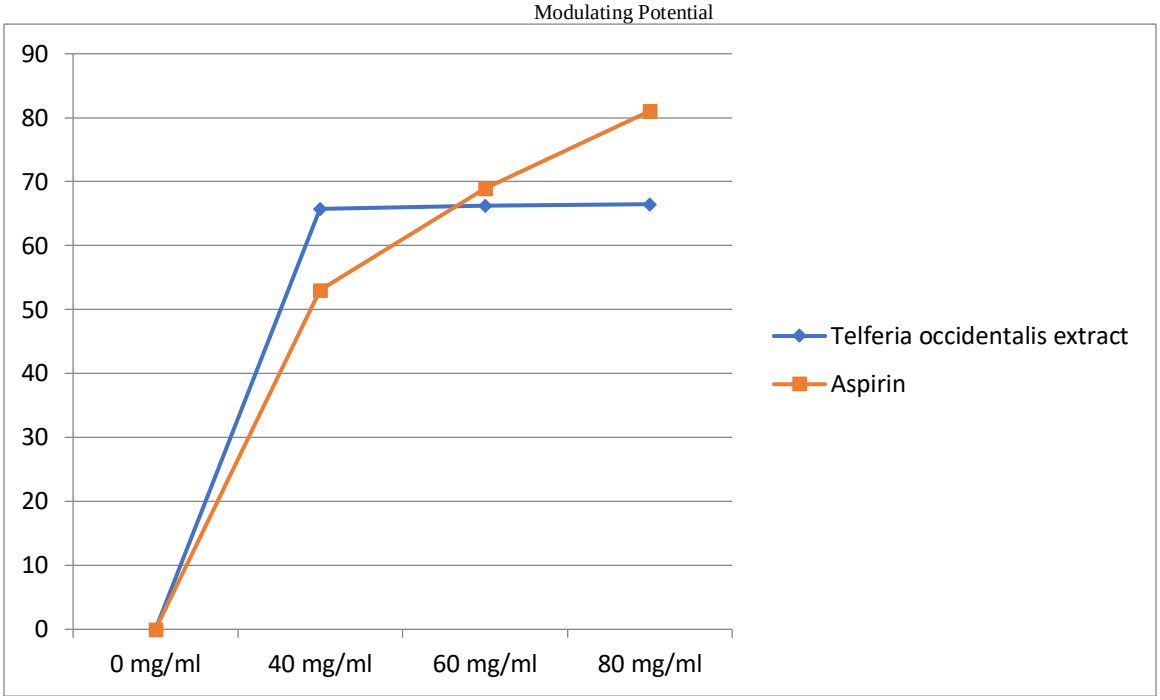

Figure 3: Anti-proteinase of Telfaira occidentials root

Anti-Lipoxygenase of *Telfaira occidentials* root

Figure 4 demonstrates that *Telfairia occidentalis root* extract inhibits lipoxygenase activity in a clear dose-dependent manner, increasing from 0% at 0 mg/mL to approximately 64% at 80 mg/mL. Lipoxygenases catalyze the oxidation of polyunsaturated fatty acids into leukotrienes and hydroperoxides, which are key pro-inflammatory mediators. The inhibitory effect suggests that bioactive phytochemicals—particularly flavonoids such as butein, naringenin, and kaempferol—can suppress lipid peroxidation and modulate enzyme activity through antioxidant and chelation mechanisms.

Figure 4 shows that the ethanolic root extract inhibited lipoxygenase activity in a concentration-dependent manner, reaching approximately 64% inhibition at 80 mg/mL. Lipoxygenase catalyses the formation of leukotrienes, which are important mediators of inflammation. Although aspirin produced greater inhibition than the extract, the results indicate that *Telfairia occidentalis* root possesses measurable lipoxygenase inhibitory activity.

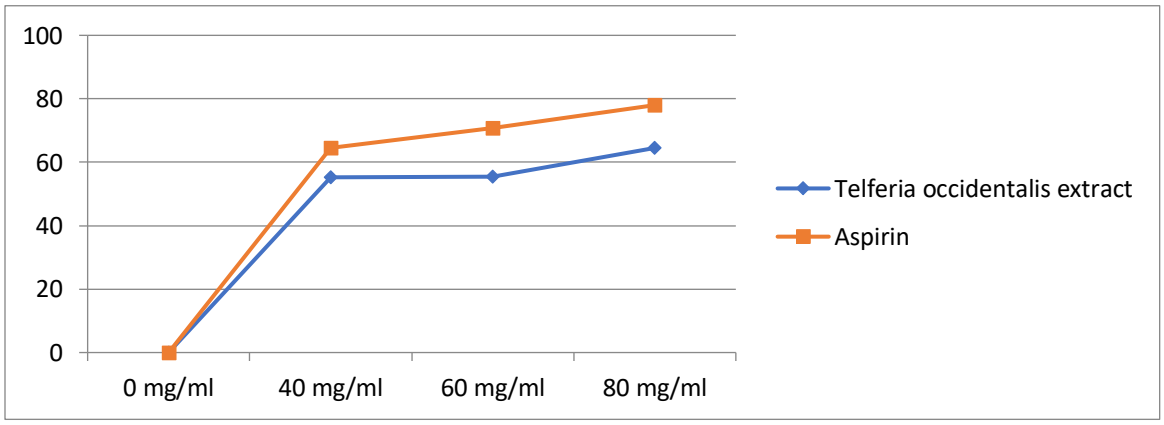

Figure 4: Anti-Lipoxygenase of *Telfaira occidentials* root (**%** Inhibition of Anti lipogenase)

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Antidiabetic Activities of *Telfaira occidentials* root

General Anti-diabetic Activity Antidiabetic of *Telfaira occidentials* root

The antidiabetic activity observed in this study may be associated with the phytochemical constituents identified in the root extract, particularly flavonoids such as butein, naringenin, and kaempferol, together with phenolic acids including ferulic acid. Figure 5 demonstrates that the ethanolic root extract exhibited measurable in vitro antidiabetic activity, with inhibition increasing to approximately 60% at the highest concentration tested. Although the extract was less active than the reference drug, the results indicate the presence of bioactive constituents capable of influencing glucose metabolism under the experimental conditions.

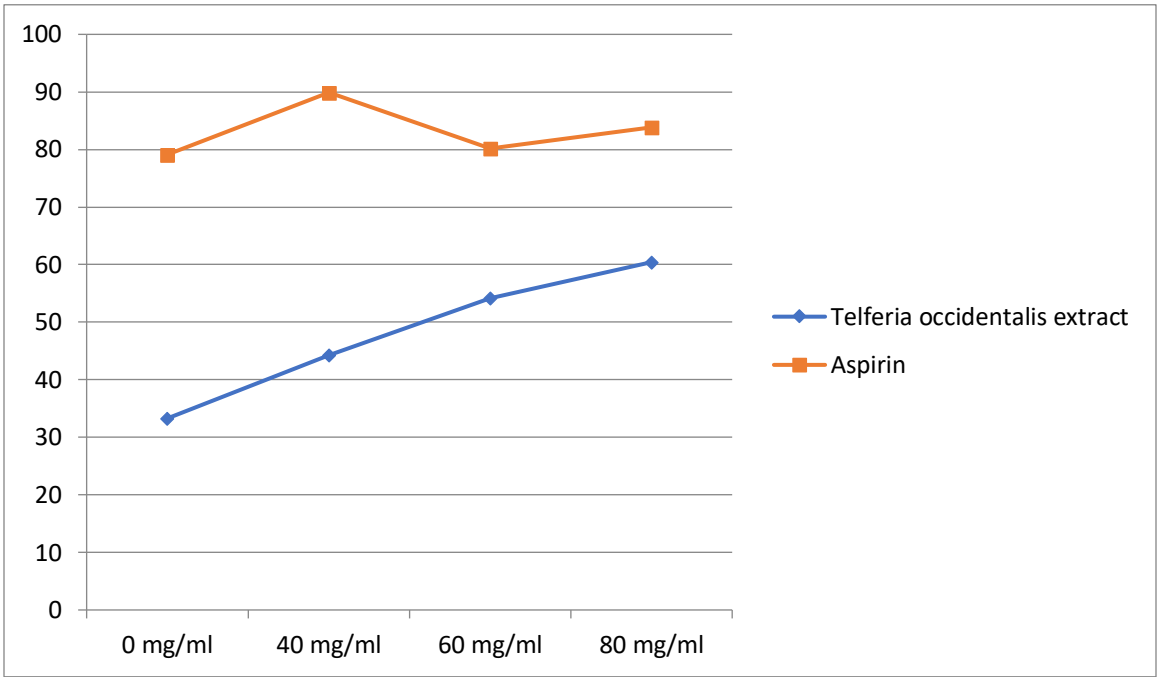

Figure 5: Anti-diabetic of *Telfaira occidentials* root

Glucose adsorption capacity assay

Figure 6 shows that the glucose adsorption capacity of *Telfairia occidentalis root* extract increased gradually with increasing extract concentration, ranging from approximately 0.25 to 0.65 mM glucose/g between 0 and 80 mg/mL. Although the adsorption capacity was lower than that of the reference treatment used in this study, the results indicate that glucose adsorption is unlikely to be the principal mechanism underlying the antidiabetic activity of the extract. This suggests that the extract may exert its biological effects through mechanisms other than direct glucose binding. The relatively low glucose adsorption capacity should be interpreted alongside the findings from the enzyme inhibition and anti-inflammatory assays. Therefore, the contribution of the identified phytochemicals to the observed biological activity can only be inferred from previous reports. Taken together, the findings suggest that the antidiabetic activity of *Telfairia occidentalis* root extract is unlikely to depend solely on glucose adsorption. Rather, the observed activity may result from the combined effects of several bioactive constituents acting through different biological pathways.

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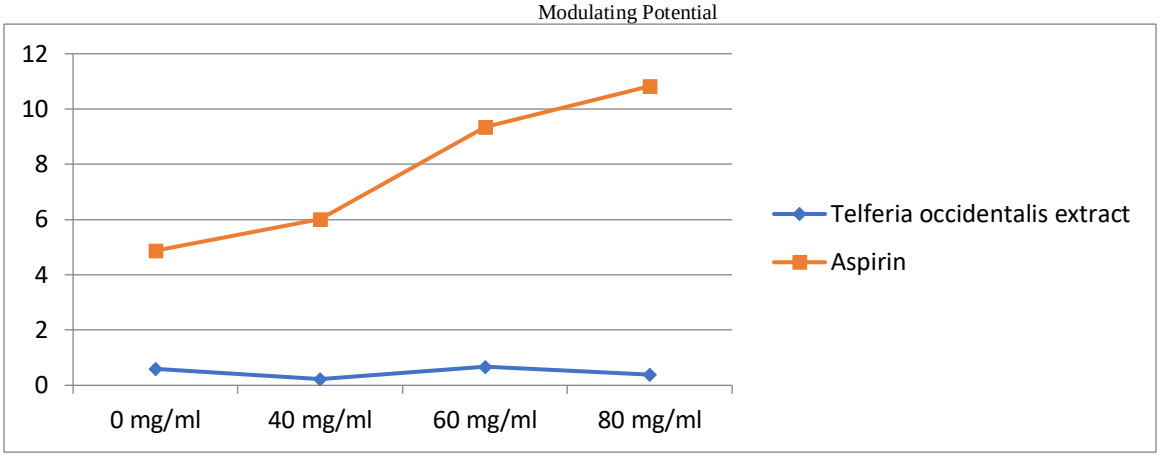

Figure 6: Glucose absorption Capacity assay (Glucose adsorption mMglucose/g)

Alpha-Glucosidase Inhibitory Test

Table 3 shows that the ethanolic root extract of *Telfairia occidentalis* exhibited weak inhibitory activity against α-glucosidase. The percentage inhibition remained below 10% across the tested concentrations, indicating that the extract possesses only limited α-glucosidase inhibitory activity under the experimental conditions.

Table 3: Alpha-Glucosidase Inhibitory of *Telfairia occidentalis*root extract

Concentration ppm	Absorbance	% inhibition
Telferia occidentalis		
0mg/ml	0.810	18.380
40mg/ml	0.274	4.785
80mg/l	0.244	4.271
120mg/ml	0.187	4.226
160mg/ml	0.309	7.133

The ethanolic root extract of *Telfairia occidentalis* exhibited relatively low inhibitory activity against α-glucosidase under the experimental conditions. Since α-glucosidase catalyses the hydrolysis of dietary carbohydrates into absorbable glucose, inhibition of this enzyme has been widely explored as a strategy for controlling postprandial hyperglycaemia in individuals with type 2 diabetes mellitus. However, the modest inhibition observed in the present study suggests that α-glucosidase inhibition alone may not fully account for the root extract's overall antidiabetic activity. The observed biological activity may be associated with the phytochemical constituents identified in the extract, particularly flavonoids such as butein, naringenin, and kaempferol, together with phenolic acids including ferulic acid. Nevertheless, the present study did not determine the contribution of individual phytochemicals or investigate their specific mechanisms of action. Consequently, any relationship between the identified compounds and the observed enzyme inhibition remains speculative. Compared with conventional α-glucosidase inhibitors, the ethanolic root extract showed only modest enzyme inhibitory activity. This finding suggests that the antidiabetic potential of *Telfairia occidentalis* root is unlikely to depend solely on α-glucosidase inhibition. required to identify the constituents responsible for the observed activity and to clarify their mode of action.

Discussion

Phytochemical Composition of *Telfairia occidentalis* Root

The phytochemical analysis demonstrated that the ethanolic root extract of *Telfairia occidentalis* contains a diverse mixture of flavonoids, phenolic acids, and terpenes. The relatively high concentrations of butein and naringenin are particularly noteworthy among the flavonoids, while limonene was the most abundant terpene identified. The diversity of flavonoid subclasses, including stilbenes, chalcones, flavanones, flavones, flavonols, flavanols, and polymethoxyflavones, indicates that the root contains chemically diverse compounds that can exert biological effects through different pathways.

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The presence of these flavonoids is important because flavonoids have been associated with free-radical scavenging, stabilization of proteins, and modulation of inflammatory enzymes. Kumar and Pandey (2013) and Panche et al. (2016) described the antioxidant and enzyme-modulating activities of flavonoids, while van Geem et al. (2016) linked their biological activities with modulation of inflammatory processes. The relatively high concentration of butein (9.38 ppm) and naringenin (4.30 ppm) in the present extract may therefore contribute to some of the biological activities observed in the subsequent assays.

The phenolic acid profile, particularly the presence of ferulic acid at 3.58 ppm, further strengthens the potential biological significance of the extract. Heleno et al. (2015) and Pandey and Rizvi (2009) identified phenolic acids as important contributors to antioxidant activity and cellular protection. The simultaneous presence of flavonoids and phenolic acids may provide complementary biological effects, with the different classes of compounds potentially acting on oxidative and inflammatory processes through different mechanisms.

The terpene profile was dominated by limonene (16.30 ppm), followed by pinene, carene, and myrcene. Bakkali et al. (2008), Miguel (2010), and Salehi et al. (2019) have associated plant terpenes with antimicrobial, antioxidant, anti-inflammatory, and other biological activities. The coexistence of these terpenes with flavonoids and phenolic acids suggests that the biological effects of the root extract may arise from the combined action of several phytochemical classes rather than from a single constituent. This is particularly relevant when interpreting the anti-inflammatory and antidiabetic results. However, the presence of bioactive phytochemicals does not automatically establish therapeutic safety. The existing interpretation notes that plant roots may also contain potentially toxic constituents such as saponins, alkaloids, or cyanogenic glycosides. Salehi et al. (2019) and Asare et al. (2023) therefore support the importance of appropriate preparation, dosage control, and safety assessment when considering medicinal applications.

Heavy Metal Status of the Root

The heavy metal analysis showed relatively low concentrations of lead, mercury, chromium, arsenic, cadmium, and copper, with the measured values reported as being below the permissible limits referenced in the existing interpretation. This finding suggests that the sampled root did not contain heavy metals at concentrations considered excessive under the stated standards.

The relatively low concentrations of lead, mercury, arsenic, and chromium are important because these metals are not essential nutrients and may produce adverse effects following sufficiently high or prolonged exposure. Tchounwou et al. (2012), Jaishankar et al. (2014), and Genchi et al. (2020) emphasized the health significance of heavy-metal exposure and the importance of monitoring their accumulation in biological materials. The present results therefore provide some support for the safety of the analyzed sample with respect to the metals investigated. Copper recorded the highest concentration (0.089 ppm). Unlike the non-essential metals, copper is an essential trace element and contributes to enzymatic reactions, antioxidant defence, and iron metabolism. Nevertheless, the safety of medicinal plant materials cannot be generalized from a single sample because metal accumulation can be affected by soil composition, irrigation water, agricultural practices, and environmental pollution. Consequently, the observation in the present sample should be interpreted as evidence concerning the analyzed root rather than all *T. occidentalis* roots. This limitation is consistent with the existing interpretation and with the concerns raised by Miguel (2010), Tchounwou et al. (2012), and Nna et al. (2024).

Anti-inflammatory Activity

The results from the albumin denaturation assay demonstrate that the ethanolic root extract possesses concentration-dependent anti-inflammatory activity. The progressive increase in inhibition from zero to approximately 64% indicates that greater concentrations of the extract produced greater protection against protein denaturation. This pattern is consistent with the established observation that protein-denaturation assays can provide an indication of anti-inflammatory potential.

The activity may be associated with the phytochemical composition of the root, particularly the relatively abundant butein and naringenin and the presence of ferulic acid. Williams et al. (2008), Gunathilake et al. (2018), and Panche et al. (2016) have associated polyphenols and flavonoids with antioxidant and anti-inflammatory effects. The present

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findings therefore provide experimental support for the possibility that the phytochemical-rich root extract contributes to inhibition of processes associated with inflammation.

Although aspirin produced greater inhibition, the difference does not eliminate the biological significance of the plant extract. Wagner (2011), Caesar and Cech (2019), and Salehi et al. (2020) have emphasized that crude plant extracts contain multiple constituents that may act simultaneously on different biological targets. Thus, the lower activity relative to aspirin may reflect the fact that the extract is a complex mixture rather than a purified pharmaceutical compound. Importantly, however, the present assay establishes in vitro activity, not clinical effectiveness. The heat-induced haemoglobin-denaturation assay produced a similar pattern. The extract reached approximately 61% inhibition at 80 mg/mL, again showing concentration-dependent activity, although aspirin was more effective. The consistency between the albumin and haemoglobin assays strengthens the evidence that the extract possesses protein-stabilizing activity under the experimental conditions. Williams et al. (2008), Panche et al. (2016), and Gunathilake et al. (2018) provide support for the relationship between polyphenolic compounds, antioxidant effects, and inhibition of protein denaturation.

Anti-proteinase Activity

The approximately 66% inhibition of proteinase activity at 80 mg/mL indicates that the root extract has appreciable anti-proteinase activity. Proteinases are involved in tissue degradation and inflammatory processes; consequently, their inhibition may contribute to limiting inflammatory damage. Leelaprakash and Dass (2011) identified proteinase inhibition as one mechanism relevant to anti-inflammatory activity. The activity observed may be related to the presence of butein, naringenin, kaempferol, and other polyphenolic constituents identified in Table 1. Panche et al. (2016) and Rathee et al. (2009) have associated polyphenol-rich plant extracts with anti-inflammatory and enzyme-inhibitory activities. The fact that the extract demonstrated activity but remained below aspirin suggests that it has measurable biological potential but is less potent than the pharmaceutical reference under the conditions employed.
Unlike a conventional drug acting predominantly through a defined target, the extract may exert effects through multiple pathways. Wagner (2011) and Salehi et al. (2020) have discussed the multi-target nature of phytochemical-rich extracts. Nevertheless, because the individual compounds were not isolated and tested separately in this study, the contribution of any particular constituent remains unconfirmed.

Anti-lipoxygenase Activity

The concentration-dependent inhibition of lipoxygenase, reaching approximately 64% at 80 mg/mL, provides additional evidence of anti-inflammatory potential. Lipoxygenase is involved in the conversion of polyunsaturated fatty acids into lipid mediators such as leukotrienes, which participate in inflammatory processes. Kühn et al. (2015) described the importance of lipoxygenase pathways in inflammation, while Panche et al. (2016) highlighted the ability of flavonoids and related polyphenols to influence inflammatory pathways. The observed inhibition may be associated with the combined presence of flavonoids, phenolic acids, and terpenes in the root extract. Kumar and Pandey (2013) and Salehi et al. (2019) have linked these phytochemical groups with antioxidant and anti-inflammatory properties. However, the present study did not isolate the individual compounds responsible for the lipoxygenase inhibition. Therefore, the result demonstrates activity of the extract as a whole, rather than establishing the activity of limonene, butein, naringenin, ferulic acid, or any other single constituent.

Antidiabetic Activity

The general antidiabetic assay showed measurable activity from the ethanolic root extract, with inhibition reaching approximately 60% at the highest concentration tested. Although the extract was less active than the reference treatment, the concentration-related response indicates that the extract contains constituents capable of influencing processes associated with glucose regulation. The phytochemical composition provides a plausible basis for this observation. Butein, naringenin, kaempferol, and ferulic acid have been associated with antioxidant activity and modulation of carbohydrate metabolism. Alam et al. (2012), Pandey and Rizvi (2009), and Salehi et al. (2019) provide support for the involvement of flavonoids and phenolic compounds in these biological processes. Elekofehinti (2015) and Williamson (2001) further indicate that medicinal plant extracts may influence glucose-related processes through multiple mechanisms. The glucose adsorption experiment, however, showed only a modest increase in adsorption capacity, from approximately 0.25 to 0.65 mM glucose/g, and the values remained below the reference treatment. This suggests that direct glucose adsorption may not be the major mechanism underlying the observed antidiabetic activity.

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Tundis et al. (2010) and Hanhineva et al. (2010) have highlighted the importance of other mechanisms, including modulation of carbohydrate-digesting enzymes and broader effects on glucose metabolism.

The α-glucosidase results provide further support for this interpretation. Inhibition was generally weak, remaining below 10% at the tested concentrations. Since α-glucosidase participates in the breakdown of dietary carbohydrates into absorbable glucose, its inhibition is one recognized strategy for reducing postprandial glucose elevation (Kim et al., 2005; Tundis et al., 2010). However, the weak inhibition observed in this study suggests that α-glucosidase inhibition alone cannot adequately explain the overall antidiabetic activity of the extract. The findings are therefore more consistent with a multifactorial interpretation of the extract's antidiabetic activity. Salehi et al. (2020), Williamson (2001), and Elekofehinti (2015) have emphasized that the biological activity of medicinal plant extracts may result from the combined effects of several phytochemicals operating through different pathways. The present study, however, did not directly establish these mechanisms or determine which individual compounds were responsible. Therefore, the antidiabetic findings should be regarded as preliminary in vitro evidence rather than proof of therapeutic efficacy.

Taken together, the findings show that *Telfairia occidentalis* root contains a chemically diverse mixture of flavonoids, phenolic acids, and terpenes and demonstrates measurable biological activities in several in vitro assays. The phytochemical findings provide a reasonable basis for the antioxidant, anti-inflammatory, and enzyme-modulating activities observed. In particular, butein, naringenin, ferulic acid, kaempferol, and the abundant terpene limonene are notable constituents within the chemical profile. The anti-inflammatory results were relatively consistent across the albumin-denaturation, haemoglobin-denaturation, proteinase, and lipoxygenase assays, with activity generally increasing as extract concentration increased. This convergence across different assays strengthens the evidence for anti-inflammatory potential. The antidiabetic findings were more complex: although the extract demonstrated measurable general antidiabetic activity, glucose adsorption was modest and α-glucosidase inhibition was weak. Therefore, the overall antidiabetic effect is unlikely to be explained by either mechanism alone. The heavy-metal results provide an additional positive observation because the concentrations detected in the analyzed sample were below the permissible limits cited in the existing interpretation. Nevertheless, geographical variation and environmental conditions can influence heavy-metal accumulation, so broader sampling would be necessary before generalizing the safety finding.

The results support the potential biological importance of *T. occidentalis* root extract, particularly its anti-inflammatory activity, while providing preliminary evidence of antidiabetic potential. The literature cited in the existing interpretation supports the possibility that these effects arise from the collective action of multiple phytochemicals rather than a single compound. However, the present findings remain primarily in vitro, and the study did not isolate individual active constituents or establish their specific mechanisms. As already indicated in the results, further bioassay-guided fractionation, compound isolation, mechanistic studies, toxicity assessment, and in vivo investigations are required before firm therapeutic conclusions can be made.

Conclusion

This study provides additional information on the phytochemical composition, heavy metal content, and biological activities of the ethanolic root extract of *Telfairia occidentalis*. The extract contained detectable amounts of flavonoids, phenolic acids, and terpenes, with butein, naringenin, ferulic acid, and limonene among the major constituents identified by GC-FID analysis. The extract demonstrated measurable in vitro anti-inflammatory activity by inhibiting protein denaturation, proteinases, and lipoxygenases, as well as moderate antidiabetic activity in the in vitro assays performed. However, the relatively weak inhibition of α-glucosidase suggests that the observed antidiabetic activity is unlikely to depend solely on this mechanism. The heavy metal concentrations measured in the analyzed root samples were below the recommended permissible limits, indicating that no evidence of heavy metal contamination was detected under the conditions of this study. These findings provide experimental support for the traditional use of *Telfairia occidentalis* root and contribute additional information on its phytochemical composition and biological properties. Nevertheless, the results are limited to in vitro investigations and should be interpreted with caution. Further studies are required to assess toxicity, bioassay-guided fractionation, identify active constituents, investigate mechanisms, and evaluate in vivo efficacy to establish the plant's safety and therapeutic potential.

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