In Vitro Antioxidants, Antidiabetic and Antiinflammatory Properties of Nigerian Matteucia Struthiopteris (Edible Fern)

Gregory Elayeche Oko
University of Calabar
Onyenweaku Eridiong Ogbonna
University of Calabar
Abdulhakeem Rotimi Agboola
agboolara@unical.edu.ng

University of Calabar
Udoh Ekaette Sunday
University of Calabar
Fatima Jummai Bala
University of Calabar

Research Article

Keywords: Matteuccia struthiopteris, antioxidant activity, antidiabetic properties, anti-inflammatory effects, ethanol extract, phytochemical screening

Posted Date: October 9th, 2025

DOI: https://doi.org/10.21203/rs.3.rs-7073209/v1

Additional Declarations: No competing interests reported.

Abstract

Matteuccia struthiopteris is an edible wild plant traditionally used for both nutritional and medicinal purposes across several continents, but its therapeutic relevance remains unexplored in Nigeria. This study investigated the antioxidant, antidiabetic, and anti-inflammatory properties of the ethanol extract of *M. struthiopteris* collected from Ugep, Cross River State, Nigeria. The plant was identified, air-dried, and extracted using ethanol. Phytochemical screening, proximate and mineral composition analyses were conducted. In vitro assays, including DPPH, FRAP, lipid peroxidation, were used to assess antioxidant potential. Antidiabetic activity was evaluated using α-amylase, α-glucosidase inhibition, and glucose uptake by yeast cells. Anti-inflammatory potential was measured through protein denaturation, proteinase inhibition, and membrane stabilization assays. The extract demonstrated a rich phytochemical profile, with the following reported levels of saponins (4.25 mg/100 g), flavonoids (2.73 mg/100 g), and terpenoids (2.23 mg/100 g), while proximate analysis showed high moisture (66.87%) and moderate carbohydrate, protein and fiber contents. Antioxidant, antidiabetic and anti-inflammatory assays showed dose-dependent activities, with the highest activities recorded at 500 µg/mL,. In conclusion, the ethanol extract of *M. struthiopteris* demonstrated therapeutic potential against oxidative stress, hyperglycemia, and inflammation, however at levels lower than the standard drugs. Although shown to be less potent than standard drugs, its natural origin and multifunctional properties make it a valuable candidate for functional foods or herbal therapies. Further research is recommended, including bioassay-guided fractionation, compound isolation and in vivo validation to enhance its pharmacological application.

INTRODUCTION

The growing prevalence of chronic diseases such as diabetes, inflammation-related disorders, and oxidative stress-induced conditions has intensified the search for therapeutic agents derived from natural sources. Medicinal plants have gained considerable attention due to their bioactive phytochemicals, which often possess multiple pharmacological properties with minimal side effects(1) Among these, ferns from the genus *Matteuccia* have been traditionally used for their purported health benefits.

Edible ferns have long been a significant component of vegetable consumption worldwide due to their high nutritional value (2). *Matteuccia struthiopteris* commonly known as ostrich fern, is one of the most widely consumed edible ferns globally, with a broad distribution across Asia, North America, and Europe(3) With a history deeply intertwined with both culinary and medicinal use, *M. struthiopteris* has been documented as a food source in North America as early as 1939 and remains popular in countries such as China, Canada, Korea, the United States, India, Russia, and Japan (4)The young fronds (fiddleheads), rhizome, and occasionally the entire plant are utilized as food. Additionally, *M. struthiopteris* has a long-standing role in traditional medicine, where it is valued for its diverse therapeutic properties, including antioxidant, anti-inflammatory, antibacterial, antiviral, and antidiabetic activities, as well as its potential to prevent influenza (5).

Oxidative stress, a key contributor to cellular damage and metabolic disorders, can be mitigated by antioxidants that neutralize reactive oxygen species (ROS) and reduce lipid peroxidation (6). Similarly, diabetes management increasingly focuses on agents that inhibit enzymes like α-amylase and α-glucosidase, thus regulating postprandial blood glucose levels (7)Furthermore, chronic inflammation, a central mechanism in diseases such as arthritis, cardiovascular disorders, and diabetes, can benefit from plant-derived anti-inflammatory compounds that modulate inflammatory pathways (8)

In vitro studies provide critical insights into the bioactivity of plant extracts by simulating biological processes under controlled conditions,(9) (10)Integrating these methodologies allows for a comprehensive evaluation of a plant's therapeutic potential, paving the way for the development of novel treatments.

Despite the widespread consumption of this vegetable globally and the scientific evidence from various studies, there is limited or no ethnobotanical research on this particular species in Nigeria. This study aims to offer novel insights into the medicinal properties of ostrich fern native to Nigeria.

This study evaluates the antioxidant, antidiabetic, and anti-inflammatory properties of *M. struthiopteris* in Nigeria using in vitro and in silico analysis, offering novel insights into it pharmaceutical and dietary applications.

Materials and Methods

Chemicals and reagents

The chemicals and reagents used for the antioxidant, antidiabetic and anti-inflammatory assays were procured from Sigma Chemical Co. (St. Louis, MO, USA) and were of analytical grades Acarbose, maltose (CP brand, Malaysia), Bovine serum albumin (Merck, Germany), Egg yolk, Phosphate buffer (Merck, Germany) were also utilized.

Collection and identification of plant samples

Fresh *M. struthiopteris* whole plants were collected from Ugep, Yakurr LGA, Cross River State, Nigeria in June 2024. The plant was identified and authenticated by Dr. Effah A. Effah from the Herbarium Unit, Department of Botany, University of Calabar, Cross River State with specimen voucher number: Botany/ Herbarium/UCC/546.

The plant was air-dried at room temperature for three days, followed by further drying in a heat oven to ensure complete desiccation. The dried leaves were then ground into a fine powder using a mini pulverizer, yielding approximately 300 g of material. Subsequently, 200 g of the powdered sample was soaked in 380 ml of ethanol for 72 hours. The mixture was filtered using Whatman filter paper into a sterile conical flask, and the filtrate was evaporated at room temperature. The resulting extract was stored in an airtight container under refrigeration. The mixture was filtered using Whatman filter paper into a sterile conical flask, and the filtrate was evaporated at room temperature. The resulting extract was stored in an airtight container under refrigeration until further use.

Proximate and Phytochemical screening

The moisture, ash, crude fiber, crude protein, and carbohydrate contents of the samples were determined using standard chemical methods outlined by the Association of Official Analytical Chemists (AOAC, 1998) and the outlined procedure by (11). Moisture content was assessed by drying 2 g of each sample at 105°C for 24 hours. Ash content was determined by incinerating 2 g of the sample in a muffle furnace at 500°C for 2 hours. The fat content was evaluated using the Soxhlet extraction method with petroleum ether (40–50°C), Crude protein content was measured using the Kjeldahl method. The presence of various phytoconstituents, including,

alkaloids, flavonoids, saponins, phenols, oxalate, anthraquinones, phytate, Tannins, Terpenoids, reducing sugars and phenolics, following well-established methods (12, 13)

In vitro antioxidant tests

DPPH ASSAY

The extract's ability to stabilize DPPH free radicals was assessed following standard protocols (14), as outlined by Tsado et al. (15). A 100 µL aliquot of DPPH solution in methanol (50 mg/mL) was mixed with 100 µL of the extract at concentrations of 31.25, 125, 250, and 500 µg/mL, and the mixture was incubated in the dark for 45 minutes. The reduction in absorbance was recorded at 517 nm against a blank, and the percentage inhibition of DPPH was determined.

FRAP ASSAY

The FRAP activity of the extract was evaluated using the method outlined by Oyaizu (14). A 1 mL sample containing varying concentrations of EEF (31.25, 125, 250, and 500) was incubated at 50 ºC for 20 minutes in 0.2 M sodium phosphate buffer with 1% potassium hexacyanoferrate (III). Following incubation, 10% trichloroacetic acid (TCA) was added, and the mixture was centrifuged. The resulting supernatant was combined with 0.1% ferric chloride for color development. Absorbance was measured at 700 nm, and the FRAP activity percentage was calculated.

Lipid peroxidation (LPO) assays

The thiobarbituric acid-reactive substance (TBARS) method described by Panjamurthy et al. (16)was followed for the LPO analysis.

Alpha-amylase inhibition assay

The α-amylase inhibitory activity of the extract was determined using the method described by Worthington.(17) Various concentrations of the acarbose standard (31–500 µg/mL) and the extract were prepared and incubated with a porcine pancreatic enzyme solution at 37°C for 10 minutes. The reaction was initiated by adding a starch solution, followed by further incubation at 37°C for 30 minutes. The reaction was then halted by adding 10 µL of 1 M HCl. Iodine was added for color development, and absorbance was measured at 580 nm.

Alpha-glucosidase inhibitory activity

To measure α-glucosidase inhibitory activity, we used a slight modification of the standardized procedure by Eyong et al (18). In a nutshell, we filled 96-well plates with 50 microliters of phosphate buffer (pH 6.8) containing an α-glucosidase solution (0.2 microliters per millilitre) and 60 microliters of the sample. The mixture was incubated at 37 degrees Celsius for twenty minutes. After pre-incubation for some time, a 50µl solution of P-nitrophenyl-α-D-glucopyranoside (PNPG) in 0.1M phosphate buffer (pH 6.8) was added to the wells. The combined mixtures were subjected to incubation at a temperature of 37°C for an extra duration of 20 minutes.

To stop the reaction, a 0.2M NaCO3 solution of 160µl was added to each well. The absorbance of the sample was taken at 405nm and compared to that of a standard sample.

Glucose uptake by yeast cells

Different concentrations of the standard drug or extract were incubated with a glucose solution at 37°C for 10 minutes. A yeast suspension was then added and incubated for 60 minutes at 37°C. After centrifugation, the percentage increase in glucose uptake was determined from the supernatant (19)

Inhibition of protein denaturation

The inhibition of protein denaturation was assessed using the method described by Mizushima and Kobayashi (20). Various concentrations of the extract or the standard drug aspirin were mixed with a 1% bovine serum albumin solution. The samples were heated at 55°C for 30 minutes and then allowed to cool. Turbidity was measured at 660 nm, and the percentage inhibition of protein denaturation was calculated.

Proteinase inhibitory assay

A proteinase inhibitory assay was conducted following the method of Oyedepo and Femurewa.(21) The reaction mixture (2 mL), consisting of 0.06 mg trypsin and 1 mL of Tris-HCl buffer, was incubated with 1 mL of the extract at 37°C for 5 minutes. Subsequently, 0.8% (w/v) casein was added, and the mixture was incubated for an additional 20 minutes. The reaction was terminated by adding 2 mL of 70% perchloric acid. After centrifugation, the absorbance of the supernatant was measured at 210 nm.

Results

Phytochemical, vitamin, and mineral composition of ethanol extract of M. struthiopteris

The qualitative and quantitative phytochemical composition of the ethanol extract of *M. struthiopteris* is presented in Tables 1 and 2. Preliminary qualitative phytochemical analysis of the plant extract revealed the presence of several bioactive constituents. Alkaloids, flavonoids, phenols, oxalates, and anthraquinones were detected in moderate amounts (denoted by +), while saponins were present in relatively higher concentrations (denoted by ++). The quantitative analysis of the phytochemical constituents in the plant sample revealed varying concentrations of bioactive compounds, expressed as mg/100g of the dry extract. The results are presented as mean ± standard deviation from replicate analyses. Saponins were the most abundant phytochemical, recorded at 4.25 ± 0.066 mg/100g. Flavonoids were also present in relatively high amounts (2.73 ± 0.095 mg/100g), Terpenoids were detected at 2.23 ± 0.23 mg/100g), reducing sugars, were found at 0.87 ± 0.16 mg/100g.Moderate levels of anthraquinones (0.72 ± 0.096 mg/100g), phytates (0.67 ± 0.11 mg/100g), tannins (0.60 ± 0.10 mg/100g), and oxalates (0.50 ± 0.80 mg/100g) were observed. Alkaloids were present at 0.37 ± 0.12 mg/100g, while phenols were quantified at 0.39 ± 0.70 mg/100g,

Proximate composition of ethanol extract of M. struthiopteris

The results of the proximate analysis of the ethanol extract of *Matteuccia struthiopteris* are presented in Table 3. The analysis quantified the relative concentrations of major nutritional constituents, expressed as percentages of the total composition. All values are reported as mean ± standard deviation based on replicate determinations. The extract exhibited a high moisture content, (66.87 ± 2.83%). carbohydrates were

predominant, accounting for 17.76 ± 2.79%. The crude protein content was measured at 6.58 ± 0.39%, while Crude fiber was present at 5.02 ± 0.40%, the ash content, indicative of total mineral content, was 2.13 ± 0.11% while The fat content was relatively low, at 1.34 ± 0.07%.

Mineral composition of ethanol extract of M. struthiopteris

The results of the mineral composition of *Matteuccia struthiopteris* are presented in Table 4, with concentrations expressed as percentages and values reported as mean ± standard deviation. Among the minerals analyzed, iron and manganese exhibited the highest concentrations, both recorded at 16.57% and 16.27% respectively. Zinc and magnesium were present in comparable amounts, measured at 6.31 ± 0.92% and 6.30 ± 0.090%, respectively. Calcium was the least abundant, with a concentration of 0.49 ± 0.070%.

In vitro Antioxidant potential of ethanol extract of M. struthiopteris

The antioxidant activities of the fern extract in comparison with ascorbic acid were evaluated using FRAP (Ferric Reducing Antioxidant Power), DPPH (2,2-diphenyl-1-picrylhydrazyl), and ILP (In vitro lipid peroxidation) assays across five concentrations (31.25–500 µg/mL). The results are presented in Table 6 as mean ± standard deviation. In all three assays, both the fern extract and ascorbic acid exhibited a concentration-dependent increase in activity. For the FRAP assay, the fern extract ranged from 23.04 ± 0.50 µg/mL at 31.25 µg/mL to 81.34 ± 0.77 µg/mL at 500 µg/mL, while ascorbic acid increased from 47.30 ± 0.26 µg/mL to 95.01 ± 0.57 µg/mL over the same range. In the DPPH assay, the fern extract values increased from 15.87 ± 0.78 µg/mL at the lowest concentration to 68.03 ± 0.79 µg/mL at the highest, whereas ascorbic acid ranged from 44.32 ± 0.69 µg/mL to 91.86 ± 0.38 µg/mL. Similarly, ILP values for the fern extract rose from 11.35 ± 0.71 µg/mL to 62.93 ± 0.56 µg/mL, and for ascorbic acid from 40.45 ± 0.84 µg/mL to 90.63 ± 0.82 µg/mL across the tested concentrations. The calculated IC_{50} values showed variation among the assays, with the fern extract displaying IC_{50} values of 158.97 µg/mL (FRAP), 248.62 µg/mL (DPPH), and 303.60 µg/mL (ILP). In contrast, ascorbic acid exhibited lower IC_{50} values of 14.65 µg/mL (FRAP), 45.69 µg/mL (DPPH), and 75.39 µg/mL (ILP).

In vitro antidiabetic potential of ethanol extract of M. struthiopteris

The inhibitory activities of the fern extract were assessed in comparison with the standard drug- acarbose against α-amylase and α-glucosidase enzymes. The assay was carried across a concentration range of 31.25–500 µg/mL. Results are expressed as mean percentage inhibition ± standard deviation and are presented in Table 7. For α-amylase inhibition, the fern extract showed a concentration-dependent increase, with inhibition values ranging from 16.15 ± 0.42% at 31.25 µg/mL to 74.81 ± 0.87% at 500 µg/mL. Acarbose followed a similar trend, increasing from 30.08 ± 0.89% to 88.96 ± 0.95% over the same concentration range. In the α-glucosidase inhibition assay, the fern extract exhibited inhibition values increasing from 15.11 ± 0.93% at the lowest concentration to 71.97 ± 0.68% at the highest. Acarbose inhibition ranged from 38.26 ± 0.77% at 31.25 µg/mL to 94.36 ± 0.41% at 500 µg/mL. The half-maximal inhibitory concentration (IC_{50}) values for the fern extract were 215.58 µg/mL for α-amylase and 238.98 µg/mL for α-glucosidase, while those for acarbose were 99.01 µg/mL and 55.04 µg/mL, respectively.

Glucose uptake by yeast cells

The result of the fern extract on glucose uptake by yeast extract is presented in table 7 below. The glucose uptake potential of the Fern extract was evaluated in comparison with Metformin using yeast cell models at varying glucose concentrations (5, 10, and 25 mM). both the Fern extract and Metformin stimulated glucose uptake in a dose-dependent manner across all glucose concentrations tested. At the highest concentration (500 µg/mL), the Fern extract significantly increased glucose uptake to 65.86 ± 0.21%, 59.89 ± 0.53%, and 47.99 ± 0.74% at 5, 10, and 25 mM glucose, respectively. In contrast, Metformin demonstrated markedly higher uptake at the same concentration, with values of 90.32 ± 0.42%, 73.37 ± 0.73%, and 62.05 ± 0.85%, respectively. This trend persisted across all tested concentrations, indicating that Metformin consistently outperformed the Fern extract. Furthermore, a reduction in glucose uptake was observed as the extracellular glucose concentration increased, The IC_{50} values further support the superior activity of Metformin. At 5, 10, and 25 mM glucose concentrations, the IC_{50} values for Metformin were 94.34, 171.44, and 275.60 µg/mL, respectively, compared to 279.51, 342.42, and 474.90 µg/mL for the Fern extract.

In vitro anti-inflammatory potential

The anti-inflammatory potential of the Fern extract was evaluated using three in vitro models: proteinase inhibitory assay, membrane stabilization assay, and inhibition of protein denaturation assay, with Diclofenac serving as the reference standard (Table 8). Across all assays, the Fern extract exhibited a concentration-dependent inhibition, though its activity remained consistently lower than that of Diclofenac at equivalent concentrations. In the proteinase inhibitory assay, the Fern extract showed strong inhibitory activity, reaching 83.98 ± 0.86% at 500 µg/mL compared to 86.79 ± 1.07% for Diclofenac. At decreasing concentrations (31.25 µg/mL), the inhibitory effect dropped to 29.02 ± 1.07% for Fern and 33.84 ± 0.59% for Diclofenac. The IC_{50} values revealed that Diclofenac (73.16 µg/mL) was more potent than Fern (118.63 µg/mL) in inhibiting proteinase activity. In the membrane stabilization assay, the Fern extract demonstrated moderate protective effects, with maximum stabilization of 59.94 ± 0.23% at 500 µg/mL, compared to 87.77 ± 0.86% for Diclofenac. At the lowest tested concentration, membrane stabilization decreased to 7.77 ± 0.83% for Fern and 29.74 ± 0.74% for Diclofenac. The calculated IC_{50} values was 350.00 µg/mL for Fern and 109.81 µg/mL for Diclofenac.

DISCUSSION

The increasing prevalence of Diabetes Mellitus and its associated complications, including hypertension and cardiovascular disease, continues to be a source of concern for researchers globally. Coupled with this is the challenge posed by the adverse effects of conventional drugs currently used for managing this condition (33). This has necessitated a more intense search for alternative medications with fewer side effects and greater therapeutic efficiency. Medicinal plants, rich in bioactive compounds, have gained widespread attention for their nutraceutical value and therapeutic versatility. This study explored the antioxidant, antidiabetic, and anti-inflammatory potentials of Matteuccia struthiopteris, an edible fern consumed in many parts of the world but yet to be pharmacologically evaluated in Nigeria. To the best of our knowledge, this is the first report focusing on the in vitro medicinal evaluation of this plant species in a Nigerian context.

Phytochemical screening of the ethanol extract of M. struthiopteris revealed several bioactive constituents, including saponins, flavonoids, terpenoids, alkaloids, phenols, oxalates, anthraquinones, tannins, and phytates. Quantitative analysis showed that saponins were the most abundant (4.25 mg/100 g), followed by flavonoids (2.73 mg/100 g) and terpenoids (2.23 mg/100 g). These compounds are widely recognized for their

pharmacological effects. Saponins have demonstrated antioxidant, hypocholesterolemic, and immune-boosting activities(22) while flavonoids possess strong free radical-scavenging properties that protect biological molecules from oxidative stress(23) Terpenoids are also notable for their anti-inflammatory and antimicrobial actions(24). Alkaloids and phenolic compounds further enhance the plant's therapeutic profile through their analgesic, antimicrobial, and antidiabetic effects (25, 26)While tannins and phytates are sometimes classified as antinutritional factors, their presence in moderate quantities may also contribute to antioxidant and chelating functions supporting their value in health promotion (27, 28). Although, there are no universal threshold or fixed amount for of phytochemicals that qualifies a particular plant as a good source, a plant should normally contain a biologically significant amount (usually in tens or several hundreds mg per 100g).

The proximate composition of M. struthiopteris showed a high moisture content (66.87%), consistent with characteristics of many edible fern species (2)Though moisture enhances freshness and palatability, it necessitates careful storage to prevent spoilage. The moderate levels of carbohydrate (17.76%), crude protein (6.58%), and fiber (5.02%) highlight the nutritional contribution of this plant. High-fiber and protein-containing diets are known to promote satiety, improve glycemic control, and enhance lipid metabolism(29). The fat content was relatively low (1.34%), aligning the plant with dietary recommendations for individuals managing obesity or cardiovascular risks. Mineral analysis revealed high iron content (16.27 mg/100 g), indicating its role in red blood cell formation and prevention of iron-deficiency anemia. Other essential minerals detected, such as magnesium, zinc, and calcium, though present in moderate amounts, are vital cofactors in enzymatic functions and antioxidant defense systems. This mineral profile supports the potential nutritional and therapeutic application of the plant in diet-based interventions.

The antioxidant capacity of M. struthiopteris extract was confirmed through DPPH, FRAP, and lipid peroxidation assays. In all the parameters tested, it was observed that the ethanol extract of fern had notable antioxidant activities but are relatively lower in comparison with the standard antioxidant (ascorbic acid) at the same concentrations. This was observed through the IC_{50} values obtained in this study. The IC_{50} is the concentration of drug that can achieve for 50% inhibition. It is an operational term based on the assay conditions(30) It is the most commonly utilized and effective measure of a drug's efficacy. It gives an accurate information on how much of a drug is required to inhibit a biological process by half, thus providing a measure of potency of an antagonist drug in pharmacological research(31). Therefore, the antioxidant activity obtained in the current study does not agree with earlier studies on M. struthiopteris and other fern species such as Diplazium esculentum and Pteridium aquilinum, where flavonoid-rich extracts were shown to modulate inflammation via multiple pathway and the extracts of these species showed a comparable antioxidant activity to standard antioxidant molecules.(32–34). The major reason for these discrepancies could be as a result of differences in the locations where the samples were collected.

Similarly, the antidiabetic activity of M. struthiopteris ethanol extract was assessed through inhibition of α-amylase and α-glucosidase and glucose uptake in yeast cells. The significance of this assay is to ascertain the potential of the extract in managing postprandial hyperglycemia (7)). Also, the glucose uptake assay is carried out to determine their potential to stimulate glucose uptake which indicates their potential in stimulating tissues for improved glucose utilization. By inhibiting these enzymes, the extract may slow down carbohydrate digestion and delay glucose absorption in the gastrointestinal tract(7). However, the IC_{50} obtained in the current study shows that the ethanol extract had a lower inhibitory activity against alpha amylase and alpha glucosidase when compared with the standard drug Metformin. These results do not correlate with previous

works on M. struthiopteris and related ferns. There are numerous reports affirming the potential of ferns to inhibit α-amylase and α-glucosidase (35–37). It is noteworthy that all of al the previously studied ferns, none is native to Nigeria where the present study is carried out. It is therefore possible that environmental and geographical differences could affect the phytochemical constituents of these species and by extension account for the difference in efficacy.

The potential of the ethanol extract of M. struthiopteris to counteract inflammation was carried out in the present study. Anti-inflammatory ability of a substance shows its potential ability to either treat or that reduces inflammation given that inflammation is the body's natural response to injury or infection, it is manifested as redness, swelling, pain, and heat. Agents or plants with anti-inflammatory potential or capabailities Anti-inflammatory agents, such as certain medications, help to reduce these symptoms and can by extension help delay or halt damage to tissues which are a product of inflammation. Proteolytic enzymes are involved in inflammatory tissue damage, and their inhibition is crucial in controlling the inflammatory cascade. The extract's anti-inflammatory potential was less demonstrated as compared to that of the standard drug (Diclofenac). Again, this is not in agreement with the findings reported in numerous previous studies. (38)

In general, the lesser activities of the extract in multiple assays in the current studies is likely due to several limitations. One of the limitations of this study is the use of ethanol as the only extraction solvent. While ethanol effectively extracts a broad range of polar and semi-polar phytochemicals, it may not isolate all classes of bioactive compounds, particularly very polar or non-polar ones. As such, certain active constituents may have been underrepresented or missed in the tested extract. Similarly, environmental and geographical factors could affect the phytochemical repositories of the specie studied when compared to those from other climes. This is only a preliminary study, therefore future studies should employ sequential solvent extraction using a gradient of polarities (e.g., hexane, ethyl acetate, methanol, and water) to obtain a broader and more comprehensive phytochemical profile. Comparing the activities of these extracts can help pinpoint the most bioactive fractions and direct compound isolation efforts. Additionally, the current study was limited to in vitro evaluations. While these provide essential baseline data, further in vivo studies are needed to assess pharmacokinetics, bioavailability, toxicity, and therapeutic efficacy in biological systems. In silico docking investigations may provide mechanistic insights into how molecules interact between separated compounds and their molecular targets.

CONCLUSION

The findings of this study highlight the first known investigation of *Matteuccia struthiopteris* in Nigeria, highlighting its rich phytochemical composition and notable biological activities. The ethanol extract demonstrated lesser antioxidant, antidiabetic, and anti-inflammatory properties in comparison to the standard agents utilized in the current study. While the extract showed lower efficacy compared to standard drugs, its potential as a natural origin and broad therapeutic should not be completely discountenanced. Further in silico, in vitro and in vivo studies, compound isolation, and solvent optimization are recommended to validate and enhance these findings for future nutraceutical or pharmaceutical applications.

Declarations

Declaration of Competing Interest

The authors declare that, there are no conflicting interests as far as this study is concerned.

Ethics approval: Ethics approval is not applicable

Availability of data and material: Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Funding source

No grant or any form of funding was received.

Consent to Publish Declaration: Not applicable

Consent to Participate Declaration: Not applicable

Declaration of Competing Interest

No potential conflict of interest was reported by the authors.

Permissions to collect the plants/plant parts:

Not Applicable

Clinical Trial: Not applicable

Ethics declaration: The plant sample was identified by Dr. Effah A. Effah from the Herbarium Unit, Department of Botany, University of Calabar, Cross River State with specimen voucher number: Botany/ Herbarium/UCC/546.

Author's Contribution

Gregory Elayeche Oko: conceptualization, lab analysis, Drafting, reviewing, editing, and finally approving the final version of the manuscript.

Onyenweaku Eridiong Ogbonna: Conceptualization, supervision, revising the final version of the manuscript.

Abdulhakeem Rotimi Agboola: Conceptualization, lab analysis, supervision, drafting and revising the manuscript.

Ekaette Udoh Sunday: lab analysis and revision of manuscript

Fatma Jummai Bala: Lab analysis, analysis of data, and revision of final draft

All authors have read and approved the final version of the manuscript submitted for publication and are responsible for the final content.

References

1. Prasathkumar M, Anisha S, Dhrisya C, Becky R, Sadhasivam S. Therapeutic and pharmacological efficacy of selective Indian medicinal plants – A review. Phytomedicine Plus [Internet]. 2021 May 1 [cited 2025 Jun 20];1(2):100029. Available from: https://www.sciencedirect.com/science/article/pii/S2667031321000117
2. Chettri S, S. Manivannan, Venkata Ramana Muddarsu. Nutrient and Elemental Composition of Wild Edible Ferns of the Himalaya. American Fern Journal . 2018;108(3):95–106.
3. Wang X, Guo J, Zang S, Liu B, Wu Y. Comparison of Flavonoid Content, Antioxidant Potential, Acetylcholinesterase Inhibition Activity and Volatile Components Based on HS-SPME-GC-MS of Different Parts from Matteuccia struthiopteris (L.) Todaro. Molecules [Internet]. 2024;29(5). Available from: https://www.mdpi.com/1420-3049/29/5/1142
4. Eduard V. Nekrasov, Lyudmila A. Shelikhan, Vasily I. Svetashev. Fatty acid composition of gametophytes of Matteuccia struthiopteris (L.) Tod. (Onocleaceae, Polypodiophyta). Botanica Pacifica A journal of plant science and conservation. 201AD;8(1):63–6.
5. Kimura T, Suzuki M, Takenaka M, Yamagishi K, Shinmoto H. l-O-Caffeoylhomoserine from Matteuccia struthiopteris. Phytochemistry [Internet]. 2004 Feb 1 [cited 2025 Jun 20];65(4):423–6. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0031942203006320?via%3Dihub
6. Jena AB, Samal RR, Bhol NK, Duttaroy AK. Cellular Red-Ox system in health and disease: The latest update. Biomedicine & Pharmacotherapy [Internet]. 2023 Jun 1 [cited 2025 Jun 20];162:114606. Available from: https://www.sciencedirect.com/science/article/pii/S0753332223003943
7. Tundis R, Loizzo MR, Menichini F. Natural products as alpha-amylase and alpha-glucosidase inhibitors and their hypoglycaemic potential in the treatment of diabetes: an update. . Mini Rev Med Chem. 2010;10(4):315–31.
8. Rangel-Galván M, Pacheco-Hernández Y, Lozoya-Gloria E, Villa-Ruano N. Dietary natural products as inhibitors of α-amylase and α-glucosidase: An updated review of ligand-receptor correlations validated by docking studies. Food Biosci [Internet]. 2024 Dec 1 [cited 2025 Jun 20];62:105456. Available from: https://www.sciencedirect.com/science/article/abs/pii/S221242922401887X
9. Zafar I, Imtiaz S, kanwal F, Abbas Z, Azmat M, Unar A, et al. In silico and in vitro study of bioactive compounds from Allium sativum with PTEN: A novel target and promising source for cancer diagnostic potentials. J King Saud Univ Sci [Internet]. 2024 Sep 1 [cited 2025 Jun 20];36(8):103281. Available from: https://www.sciencedirect.com/science/article/pii/S1018364724001939#bb0025
10. Agarwal A, D'Souza P, Johnson TS, Dethe SM, Chandrasekaran C V. Use of in vitro bioassays for assessing botanicals. Curr Opin Biotechnol [Internet]. 2014 Feb 1 [cited 2025 Jun 20];25:39–44. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0958166913006344
11. Nguyen VT, Ueng JP, Tsai GJ. Proximate composition, total phenolic content, and antioxidant activity of Seagrape (Caulerpa lentillifera). J Food Sci. 2011;(76):C950–6.
12. Ragesh KD, Vasantha S, Rajesh NV, Panneerselvam A. Qualitative and quantitative phytochemical analysis in four pteridophytes. Int J Pharm Sci Rev Res. 2014;27(2):408–12.
13. Sorescu AA, Nuta A, Ion RM, Ion Iancu L. Qualitative Analysis of Phytochemicals from Sea Buckthorn and Gooseberry. https://www intechopen.com/chapters/61610 . 2018;
14. M. Oyaizu. Studies on products of browning reaction antioxidative activities of products of browning reaction prepared from glucosamine. Jpn J Nutr Diet. 1986;44(6):307–15.

15. N. Tsado BLPOAJGNKJAGAUJO. Antioxidants and antimicrobial activities of methanol extract of Newbouldia laevis and Crateva adansonii. J Pharm Allied Health Sci. 2016;6:14–9.
16. K. Panjamurthy SMCRR. Lipid peroxidation and antioxidant status in patients with periodontitis. Cell Mol Biol Lett. 2005;10(2):255–64.
17. K. Worthington. Alpha Amylase Worthington Enzyme Manual. Worthington Biochemical Corporation, Lakewood, NJ . 1993;36–41.
18. Eyong ED IIAEAAUDOW. In vitro and in silico pharmaco-nutritional assessments of some lesser-known Nigerian nuts: Persea americana, Tetracarpidium conophorum, and Terminalia catappa. PLoS ONE . 2025;20(4).
19. Hogan IA, Kuo YC, Abubakar AN, Lawal B, Agboola AR, Lukman HY, et al. Attenuation of hyperglycemia-associated dyslipidemic, oxidative, cognitive, and inflammatory crises via modulation of neuronal ChEs/NF-κB/COX-2/NOx, and hepatorenal functional deficits by the Tridax procumbens extract. Biomedicine & Pharmacotherapy [Internet]. 2023 Feb 1 [cited 2025 Jun 21];158:114114. Available from: https://www.sciencedirect.com/science/article/pii/S0753332222015037#bib48
20. Y. Mizushima, M. Kobayashi. Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. J Pharm Pharmacol. 1968;20(3):169–73.
21. O. Oyedapo, A.J. Famurewa. Antiprotease and membrane stabilizing activities of extracts of Fagara zanthoxyloides, Olax subscorpioides and Tetrapleura tetraptera. Int J Pharmacogn. 1995;33(1):65–9.
22. Khan MI, Karima G, Khan MZ, Shin JH, Kim JD. Therapeutic Effects of Saponins for the Prevention and Treatment of Cancer by Ameliorating Inflammation and Angiogenesis and Inducing Antioxidant and Apoptotic Effects in Human Cells. . Int J Mol Sci. 2022 Nov 14;23((18):10665).
23. Frandsen JR, Narayanasamy P. Neuroprotection through flavonoid: Enhancement of the glyoxalase pathway. Redox Biol. 2018 Apr 1;14:465–73.
24. Masyita A, Mustika Sari R, Dwi Astuti A, Yasir B, Rahma Rumata N, Emran T Bin, et al. Terpenes and terpenoids as main bioactive compounds of essential oils, their roles in human health and potential application as natural food preservatives. Food Chem X [Internet]. 2022 Mar 30 [cited 2025 Jun 21];13:100217. Available from: https://www.sciencedirect.com/science/article/pii/S2590157522000153
25. Hilal B, Khan MM, Fariduddin Q. Recent advancements in deciphering the therapeutic properties of plant secondary metabolites: phenolics, terpenes, and alkaloids. Plant Physiology and Biochemistry [Internet]. 2024 Jun 1 [cited 2025 Jun 21];211:108674. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0981942824003425
26. Letchuman S, Madhuranga HDT, Kaushalya MBLN, Premarathna AD, Saravanan M. Alkaloids Unveiled: A Comprehensive Analysis of Novel Therapeutic Properties, Mechanisms, and Plant-Based Innovations. Intelligent Pharmacy [Internet]. 2024 Sep 27 [cited 2025 Jun 21]; Available from: https://linkinghub.elsevier.com/retrieve/pii/S2949866X24001047
27. López-Moreno M, Garcés-Rimón M, Miguel M. Antinutrients: Lectins, goitrogens, phytates and oxalates, friends or foe? J Funct Foods [Internet]. 2022 Feb 1 [cited 2025 Jun 21];89:104938. Available from: https://www.sciencedirect.com/science/article/pii/S1756464622000081
28. Singh P, Pandey VK, Sultan Z, Singh R, Dar AH. Classification, benefits, and applications of various anti-nutritional factors present in edible crops. J Agric Food Res [Internet]. 2023 Dec 1 [cited 2025 Jun 21];14:100902. Available from: https://www.sciencedirect.com/science/article/pii/S266615432300409X

29. Riccardi G, Rivellese AA. Effects of dietary fiber and carbohydrate on glucose and lipoprotein metabolism in diabetic patients. Diabetes Care. 1991 Dec 14;12(115–25).
30. Ugwu DI, Conradie J. Anticancer properties of complexes derived from bidentate ligands. J Inorg Biochem. 2023 Sep 1;246.
31. Aykul S, Martinez-Hackert E. etermination of half-maximal inhibitory concentration using biosensor-based protein interaction analysis. Anal Biochem. 2016 Sep 1;508:97-103.
32. Junejo J, Gogoi G, Islam J, Rudrapal M, Mondal P, Hazarika H, et al. Exploration of antioxidant, antidiabetic and hepatoprotective activity of Diplazium esculentum-A wild edible plant from North Eastern India. Future Journal of Pharmaceutical Sciences, . 2018;4(1):93–101.
33. Roy S, Dutta S, Chaudhuri TK. In vitro assessment of anticholinesterase and NADH oxidase inhibitory activities of an edible fern, Diplazium esculentum. Journal of Basic and Clinical © 2024 JETIR November 2024, Volume 11, Issue 11 www.jetir.org (ISSN-2349-5162) JETIR2411020 Journal of Emerging Technologies and Innovative Research (JETIR) www.jetir.org a182 Physiology and Pharmacology, . 2015;26(4):395–401.
34. Roy S, Roy S, Hazra B, . Mandal N, Chaudhuri TK. Assessment of the antioxidant and free radical scavenging activities of methanolic extract of Diplazium esculentum. Int J Food Prop. 2013;13(3):1351–70.
35. Basnet P, Kadota S, Shimizu M, Xu HX, Namba T. 2'-Hydroxymatteucinol, a new C-methyl flavanone derivative from Matteccia orientalis; potent hypoglycemic activity in streptozotocin (STZ)-induced diabetic rat. Chem Pharm Bull (Tokyo) . 1993;41:1790–5.
36. Kim NR, Chi LW, Lee CH. Alpha-glucosidase inhibition activity of methanol extracts obtained from nine pteridophyte species native to Korea. Korean J Plant Resour . 2013;26:411–6.
37. Li X, Zhu L-J, Chen J-P, Shi C-Y, Zhang X, Yao X-S. C-Methylated flavanones from the rhizomes of Matteuccia intermedia and their α-glucosidase inhibitory activity. Fitoterapia. C-Methylated flavanones from the rhizomes of Matteuccia intermedia and their α-glucosidase inhibitory activity Fitoterapia. 2019;136(104147.).
38. Dion C, Haug C, Guan H, Ripoll C, Spiteller P, Coussaert A, et al. Evaluation of the anti-inflammatory and antioxidative potential of four fern species from China intended for use as food supplements. Nat Prod Commun. 2015;10:597–603.

Tables

PHYTOCHEMICAL COMPOUNDS	PRESENCE
Alkaloids	+
Flavonoids	+
Saponins	++
Phenols	+
Oxalate	+
Anthraquinone	+

Table 1: Qualitative phytochemical composition of *M. struthiopteris* ethanol extract

PHYTOCHEMICAL COMPOUNDS	CONCENTRATION mg/100g
Alkaloids	0.37±0.12
Flavonoids	2.73±0.095
Saponins	4.25±0.066
Phenols	0.39±0.70
Oxalate	0.50±0.80
Anthraquinone	0.72±0.096
Phytate	0.67±0.11
Tannins	0.60±0.10
Terpenoids	2.23±0.23
Reducing sugar	0.87±0.16

Table 2: Quantitative phytochemical composition of *M. struthiopteris* ethanol extract

Proximate Analysis	Concentration %
Moisture content	66.87±2.83
Ash content	2.13±0.11
Fat content	1.34±0.07
Crude fiber content	5.02±0.40
Crude protein content	6.58±0.39
Carbohydrate content	17.76±2.79

Table 3: Proximate composition of *M. struthiopteris* ethanol extract

Mineral composition	Concentration %
Calcium	0.49±0.070
Zinc	6.31±0.92
Magnesium	6.30±0.090
Iron	16.57±0.57
Manganese	16.27±0.057

Table 4: Mineral composition of *M. struthiopteris* ethanol extract

Concentration (µg/mL)	FRAP		DPPH		ILP	
	Fern	Ascorbic acid	Fern	Ascorbic acid	Fern	Ascorbic acid
500	81.34±0.77^{e}	95.01±0.57^{e}	68.03±0.79^{e}	91.86±0.38^{e}	62.93±0.56^{e}	90.63±0.82^{e}
250	73.06±0.51^{d}	91.13±0.99^{d}	60.39±1.05^{d}	88.69±0.43^{d}	55.28±0.87^{d}	86.19±0.73^{d}
125	57.12±0.38^{c}	81.31±0.75^{c}	49.31±0.70^{c}	78.12±0.48^{c}	42.40±0.89^{c}	71.03±0.70^{c}
62.5	35.39±0.52^{b}	77.01±0.61^{b}	29.11±0.49^{b}	69.20±0.94^{b}	22.59±0.88^{b}	67.08±1.18^{b}
31.25	23.04±0.50^{a}	47.30±0.26^{a}	15.87±0.78^{a}	44.32±0.69^{a}	11.35±0.71^{a}	40.45±0.84^{a}
IC_{50}	158.97	14.65	248.62	45.69	303.60	75.39

Table 5: *in vitro* antioxidant potential of *M. struthiopteris* ethanol extract

Concentration (µg/mL)	Alpha Amylase		Alpha glucosidase	
	Fern	Acarbose	Fern	Acarbose
500	74.81±0.87^{e}	88.96±0.95^{e}	71.97±0.68^{e}	94.36±0.41^{e}
250	61.55±0.76^{d}	77.38±0.95^{d}	60.33±1.23^{d}	88.00±0.65^{d}
125	50.96±0.72^{c}	62.12±0.71^{c}	47.30±0.43^{c}	74.05±0.96^{c}
62.5	34.71±1.06^{b}	45.23±0.62^{b}	30.93±0.85^{b}	56.82±0.70^{b}
31.25	16.15±0.42^{a}	30.08±0.89^{a}	15.11±0.93^{a}	38.26±0.77^{a}
IC_{50}	215.58	99.01	238.98	55.04

Table 6: *in vitro* Antidiabetic effects of *M. struthiopteris* ethanol extract

Concentration (µg/mL)	Glucose uptake by yeast cells at 5 mM		Glucose uptake by yeast cells at 10 mM		Glucose uptake by yeast cells at 25 mM	
	Fern	Metformin	Fern	Metformin	Fern	Metformin
500	65.86±0.21^{e}	90.32±0.42^{e}	59.89±0.53^{e}	73.37±0.73^{e}	47.99±0.74^{e}	62.05±0.85^{e}
250	57.75±0.82^{d}	79.90±0.65^{d}	48.90±0.81^{d}	61.93±0.70^{d}	36.19±0.72^{d}	56.74±0.63^{d}
125	40.85±0.99^{c}	56.98±0.26^{c}	37.29±0.73^{c}	49.88±0.74^{c}	30.44±1.99^{c}	44.21±0.74^{c}
62.5	27.89±0.26^{b}	44.66±1.11^{b}	22.89±1.10^{b}	42.32±0.80^{b}	15.27±0.59^{b}	31.00±0.97^{b}
31.25	14.76±0.91^{a}	35.14±0.94^{a}	9.76±0.74^{a}	31.57±1.16^{a}	3.83±0.30^{a}	24.82±0.83a
IC_{50}	279.51	94.34	342.42	171.44	474.90	275.60

Table 7: Effect of M. *struthiopteris* ethanol extract on glucose uptake by yeast cells

Concentration (µg/mL)	Proteinase inhibitory assay		Membrane stabilization assay		Inhibition of protein denaturation assay	
	Fern	Diclofenac	Fern	Diclofenac	Fern	Diclofenac
500	83.98±0.86^{e}	86.79±1.07^{e}	59.94±0.23^{e}	87.77±0.86^{e}	62.89±0.83^{e}	89.73±0.47^{e}
250	72.78±0.96^{d}	78.22±0.81^{d}	47.39±0.61^{d}	75.17±0.95^{d}	54.33±0.86^{d}	77.75±0.93^{d}
125	57.21±0.72^{c}	63.03±0.93^{c}	34.16±0.79^{c}	59.10±0.43^{c}	41.95±0.85^{c}	59.99±1.52^{c}
62.5	46.09±0.36^{b}	49.11±0.36^{b}	23.98±0.32^{b}	45.23±0.53^{b}	26.34±0.70^{b}	48.04±1.40^{b}
31.25	29.02±1.07^{a}	33.84±0.59^{a}	7.77±0.83^{a}	29.74±0.74^{a}	9.90±0.93^{a}	36.50±1.11^{a}
IC_{50}	118.63	73.16	350.00	109.81	304.07	77.44

Table 8: *In* vitro anti-inflammatory activities of M. *struthiopteris* ethanol extract

Figures

Figure 1

Whole Plant of M. struthiopteris Collected from Ugep, Cross River State, Nigeria