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# GC–MS Characterization of Bioactive Metabolites in the Ethanolic Extract of *Matteuccia struthiopteris* (Edible Fern) Harvested in Nigeria: Potential Nutraceutical Applications

**Gregory Elayeche Oko[1]*, Abdulhakeem Rotimi Agboola[1], Eridiong Ogbonna Onyenweaku[2] Chibuzor Onyinye Okonkwo[1], Linda Ubong Umoh[2]**
[1] Department of Biochemistry, University of Calabar, Nigeria
[2] Department of Human Nutrition and Dietetics, University of Calabar, Nigeria.
* Corresponding author : oko@unical.edu.ng

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**ABSTRACT**
*Matteuccia struthiopteris*, commonly referred to as the ostrich fern, is an edible and medicinal fern valued for its nutritional and pharmacological properties. Despite extensive studies in temperate regions, limited data exist on its chemical profile in tropical environments. This study aimed to characterize the bioactive metabolites present in the ethanolic extract of *M. struthiopteris* harvested in Nigeria and to evaluate its potential nutraceutical applications. Gas Chromatography–Mass Spectrometry (GC–MS) analysis was performed using an Agilent Technologies 8890A GC coupled with a mass selective detector under optimized analytical conditions. A total of twenty-two (22) metabolites were detected, encompassing fatty acids, fatty acyl esters, terpenoids, alcohols, ketones, epoxides, and anthraquinones. The major constituents were cholesteryl palmitate (32.10%), citronellol (28.76%), isopropyl stearate (6.33%), and n-hexadecanoic acid (3.71%). Minor components included phytol, squalene, oleic acid, and 1,15-pentadecanediol. The predominance of sterol esters and terpene alcohols indicates that *Matteuccia struthiopteris* has strong antioxidant and lipid-regulatory potential, while the coexistence of fatty acids and acyl esters suggests protective roles in membrane stabilization and oxidative stress mitigation. These findings confirm that *M. struthiopteris* grown in Nigeria accumulates nutraceutically valuable metabolites with antioxidant, anti-inflammatory, and metabolic regulatory relevance.



## Introduction

*Matteuccia struthiopteris* commonly referred to as the ostrich fern, is a perennial, deciduous pteridophyte belonging to the family Onocleaceae. The species thrives in temperate regions of the Northern Hemisphere, particularly in moist forested wetlands, where it reaches a height of approximately 30–100 cm (Huh, 2025; Zhao et al., 2025). It features an upright rhizome densely covered with membranous scales measuring 10–15 mm in length and produces two morphologically distinct fronds: sterile and fertile. The sterile fronds have petioles ranging from 6 to 10 cm, reddish-brown scales, and leaf blades measuring 40–60 cm long and 15–25 cm wide. The fertile fronds are shorter, with lanceolate leaf blades measuring 15–30 cm, whose pinnae curl backward to form

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clusters that enclose sporangia. Spore release generally occurs between September and November (Huh, 2025). The young unfurled fronds, known as fiddleheads, are traditionally consumed as seasonal vegetables or preserved as a delicacy, especially in East Asia and North America. Aside from its nutritional value, the species has long been valued in ethnomedicine for its pharmacological properties, which include antiviral, antibacterial, anti-inflammatory, and hypoglycemic effects (Basnet et al., 1993, 1995; Li et al., 2019). These traditional uses have stimulated phytochemical investigations to identify the bioactive constituents responsible for its medicinal benefits. Phytochemical studies on *M. struthiopteris* have revealed an extraordinary diversity of secondary metabolites, including flavonoids, flavonoid glycosides, stilbene derivatives, isocoumarins, phthalides, chromone derivatives, lignan glycosides, and phenolic compounds (Huh, 2025; Zhang et al., 2008; Zhu et al., 2016; Kimura et al., 2004). Among these, flavonoids and their glycosides are the most abundant and biologically significant. Many of these compounds are C-methylated at the C-6 and/or C-8 positions and often conjugated with (S)-3-hydroxy-3-methylglutaryl (HMG) moieties, structural features that enhance their pharmacological potency (Huh, 2025; Kadota et al., 1994; Hattori et al., 2007). The presence of such C-methyl and HMG-linked derivatives distinguishes *M. struthiopteris* from many other medicinal ferns and contributes to its unique chemical fingerprint. These metabolites have demonstrated multiple therapeutic activities, including antiviral, antioxidant, α-glucosidase inhibitory, and aldose reductase inhibitory properties (Basnet et al., 1993; Basnet et al., 1995; Li et al., 2019; Kimura et al., 2004). For example, chlorogenic acid and L-O-caffeoylhomoserine isolated from its rhizome exhibit potent antioxidant activity (Kimura et al., 2004). Similarly, matteflavoside G and kaempferol-3-O-β-D-glucopyranoside display antiviral effects against the H1N1 influenza virus (Li et al., 2015). The cumulative evidence underscores the pharmacological importance of *M. struthiopteris* as a reservoir of bioactive metabolites with significant health-promoting potential.

Further investigation into the biosynthetic pathways of *M. struthiopteris* suggests that the C-methylation and HMG-conjugation patterns characteristic of its flavonoid and stilbene constituents may reflect complex enzymatic modifications comparable to those observed in other Onocleaceae members (Huh, 2025; Kadota et al., 1994; Bergot et al., 1979). These structural variations enhance solubility and cellular permeability, thereby improving the biological availability of the compounds. For instance, 2′-hydroxymatteucinol, matteuorienate A, and matteuorienate B, isolated from the related species *M. orientalis*, exhibit strong aldose reductase inhibitory and hypoglycemic activities (Basnet et al., 1993; Kadota et al., 1994). The discovery of these analogs reinforces the hypothesis that *M. struthiopteris* shares similar biochemical pathways, providing a basis for further comparative metabolomic studies. Additionally, compounds such as pinosylvin and its derivatives possess significant cytotoxic and antiproliferative activities against cancer cells, highlighting the chemopreventive potential of stilbene-type molecules (Park et al., 2004; Lee et al., 2005; Fang et al., 2002; Simard et al., 2008). The growing interest in these natural molecules reflects a broader scientific movement toward identifying plant-based therapeutics capable of modulating metabolic and degenerative diseases. However, despite the accumulating data on its chemical constituents, *M. struthiopteris* remains underexplored relative to other medicinal ferns. Comprehensive analyses employing advanced instrumental

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techniques are necessary to characterize its full spectrum of metabolites and elucidate their pharmacological relevance (Huh, 2025; Wang et al., 2016; Pang et al., 2024).

Gas Chromatography–Mass Spectrometry (GC–MS) represents a robust and versatile analytical tool for the separation, identification, and quantification of volatile and semi-volatile compounds in complex plant matrices. By integrating chromatographic separation with mass spectrometric detection, GC–MS provides both qualitative and quantitative data essential for characterizing phytochemical constituents (Jayaprakasha et al., 2001; Sultana et al., 2009; Swamy et al., 2017). The technique is particularly effective for profiling low-molecular-weight metabolites such as fatty acids, terpenoids, and phenolic derivatives. The extraction solvent has a significant impact on the yield and diversity of detectable compounds; hence, using ethanol, a polar solvent capable of dissolving a wide range of phytochemicals, enhances the recovery of bioactive metabolites with antioxidant and therapeutic potential (Sultana et al., 2009).

When applied to *M. struthiopteris* harvested in Nigeria, GC–MS analysis offers an opportunity to identify region-specific variations in chemical composition that may result from environmental factors, soil chemistry, or climatic conditions. Such analyses not only contribute to chemotaxonomic understanding but also to the identification of novel compounds with nutraceutical relevance. The characterization of ethanolic extracts using GC–MS supports the evaluation of bioactive metabolites linked to antioxidant, antimicrobial, and metabolic regulatory properties (Baeshen et al., 2023; Mondal et al., 2019; Rosales et al., 2020). Consequently, this analytical approach strengthens the pharmacognostic value of *M. struthiopteris* and facilitates its development as a potential source of nutraceutical agents. The present study aims to characterize the bioactive metabolites in the ethanolic extract of *M. struthiopteris* harvested in Nigeria using GC–MS, thereby providing a scientific basis for its nutraceutical applications and contributing to the broader understanding of fern-derived natural products.

## Materials and Methods

### Collection and Identification of Plant Samples

Fresh plants of *Matteuccia struthiopteris* were obtained from Ugep, Yakurr Local Government Area, Cross River State, Nigeria, in June 2024. The plant was taxonomically identified and verified at the Herbarium Unit of the Department of Botany, University of Calabar, where a voucher specimen was deposited under the reference number Botany/Herbarium/UCC/546.

### Preparation of Plant Extract

The collected plant material was initially air-dried at ambient room temperature for three days and subsequently oven-dried to achieve complete moisture removal. The dried leaves were pulverized into a fine powder using a laboratory-grade mini pulverizer, yielding approximately 300 g of powdered material. From this, 200 g was macerated in 380 ml of ethanol for 72 hours with intermittent agitation. The resulting mixture was filtered through Whatman No. 1 filter paper into a sterile conical flask, and the filtrate was left to evaporate at room temperature to obtain a concentrated extract. The dried extract was stored in an airtight container and kept under refrigeration until further analysis.

### Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

The chemical composition of the ethanolic extract of *Matteuccia struthiopteris* was analyzed using a Gas Chromatography–Mass Spectrometry (GC–MS) system (Agilent Technologies

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8890A GC coupled to a Mass Selective Detector, Agilent Technologies, USA). The GC–MS system was equipped with an autosampler and operated under the following optimized conditions. High-purity helium (99.999%) was employed as the carrier gas at a constant flow rate of 1.0 mL/min. The injector was operated in split mode, with a split ratio of 1:10 and an injection volume of 1 µL. The inlet temperature was maintained at 300°C, while the ion source and interface temperatures were set at 250°C and 300°C, respectively. The system pressure was held at 16.2 psi, with an out time of 1.5 min. The GC oven temperature was programmed as follows: an initial temperature of 50°C, held isothermally for 5 minutes, followed by a ramp at 20°C per minute to 250°C, where it was maintained for an additional 5 minutes. The total runtime was approximately 20 minutes. Electron ionization (EI) was performed at 70 eV, and mass spectra were recorded over an m/z range of 50–600 in full-scan mode. Compound identification was achieved by comparing the acquired mass spectra with reference spectra from established databases, including the NIST Mass Spectral Library (Version 2.3, NIST 20; National Institute of Standards and Technology, USA) and the Wiley Registry of Mass Spectral Data, 12th Edition (John Wiley & Sons, USA). Additional spectral interpretation and verification were conducted using Chromatec Analytic Software (Chromatec, Russia) and the NIST MS Search Program (Version 2.3).

## Results and Discussion

Table 1. Identified chemical constituents of the ethanolic extract of *Matteuccia struthiopteris*

| S/N | Compound Name | Retention Time (Min) | Concentration (% Area) | Molecular Formula | Compound Class |
|---|---|---|---|---|---|
| 1 | n-Hexadecanoic acid (Palmitic acid) | 3.849 | 3.709 | $C_{16}H_{32}O_2$ | Fatty acid |
| 2 | Octadecanoic acid (Stearic acid) | 3.913 | 1.454 | $C_{18}H_{36}O_2$ | Fatty acid |
| 3 | Isopropyl stearate | 5.013 | 6.331 | $C_{21}H_{42}O_2$ | Fatty acyl ester |
| 4 | Cholesteryl palmitate | 7.550 | 32.096 | $C_{43}H_{76}O_2$ | Sterol ester |
| 5 | Hexadecanoic acid, 1,2-ethanediyl ester | 8.022 | 1.338 | $C_{34}H_{66}O_4$ | Fatty acyl ester |
| 6 | Phytol | 8.569 | 1.800 | $C_{20}H_{40}O$ | Terpene alcohol |
| 7 | Citronellol | 9.651 | 28.762 | $C_{10}H_{20}O$ | Terpene alcohol |
| 8 | Oxirane, decyl- | 10.012 | 3.144 | $C_{12}H_{24}O$ | Epoxide |
| 9 | Benzene, undecyl- | 12.905 | 1.261 | $C_{17}H_{28}$ | Alkylbenzene |
| 10 | Oleic acid | 13.906 | 1.585 | $C_{18}H_{34}O_2$ | Fatty acid |
| 11 | Oleyl oleate | 15.826 | 1.441 | $C_{36}H_{68}O_2$ | Fatty acid ester |
| 12 | 9-Octadecenoic acid, 1,2,3-propanetriyl ester (E,E,E)- | 16.374 | 1.266 | $C_{57}H_{104}O_6$ | Fatty acyl ester |
| 13 | Squalene | 16.746 | 1.414 | $C_{30}H_{50}$ | Triterpenoid |
| 14 | 1-Eicosyne | 17.398 | 2.284 | $C_{20}H_{38}$ | Alkyne |
| 15 | 1,15-Pentadecanediol | 18.527 | 2.113 | $C_{15}H_{32}O_2$ | Fatty alcohol |
| 16 | 1-Hexadecanol | 18.638 | 1.447 | $C_{16}H_{34}O$ | Fatty alcohol |
| 17 | 3-Heptadecanol | 19.010 | 1.355 | $C_{17}H_{36}O$ | Fatty alcohol |
| 18 | 2-Ethoxyethyl acetate | 19.196 | 1.312 | $C_6H_{12}O_3$ | Ester |
| 19 | 2-Ethoxyethyl isobutyrate | 21.751 | 1.639 | $C_8H_{16}O_3$ | Ester |

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|---|---|---|---|---|---|
| **20** | 9,10-Anthracenedione, 1,2,4-trihydroxy- | 22.648 | 1.409 | $C_{14}H_8O_5$ | Anthraquinone |
| **21** | Tricosane-2,4-dione | 24.103 | 1.525 | $C_{23}H_{44}O_2$ | Ketone |
| **22** | 3-Isopropyl-5-methylhexan-2-one | 25.348 | 1.315 | $C_{10}H_{20}O$ | Ketone |

GC–MS analysis of the ethanolic extract of *Matteuccia struthiopteris* revealed twenty-two (22) phytochemical constituents comprising diverse classes of bioactive compounds, including fatty acids, esters, terpenoids, alcohols, epoxides, ketones, and anthraquinones. The most abundant constituents were cholesteryl palmitate (32.10%) and citronellol (28.76%), followed by isopropyl stearate (6.33%) and n-hexadecanoic acid (3.71%).

## Discussion

The GC–MS characterization of the ethanolic extract of *Matteuccia struthiopteris* harvested in Nigeria revealed a complex profile of twenty-two bioactive metabolites encompassing diverse chemical classes such as fatty acids, fatty acyl esters, terpenoids, alcohols, ketones, epoxides, and anthraquinones. The most prominent compounds were cholesteryl palmitate (32.10%), citronellol (28.76%), isopropyl stearate (6.33%), and n-hexadecanoic acid (3.71%), which together accounted for the majority of the extract composition. The high relative abundance of cholesteryl palmitate and citronellol suggests that *M. struthiopteris* is particularly rich in sterol esters and terpene alcohols, compounds often linked with antioxidant and lipid-regulatory activities. Fatty acids such as palmitic, stearic, and oleic acids were also identified in significant proportions, supporting their role as structural and metabolic components with potential health-promoting effects. These long-chain fatty acids are key constituents of biological membranes and play integral roles in energy storage, signaling, and modulation of inflammatory responses (Ferreri et al., 2022; Shramko et al., 2020). The detection of phytol and squalene further emphasizes the biochemical versatility of the species, as both compounds are widely recognized for their contributions to antioxidant, anti-inflammatory, and membrane-stabilizing functions in plants. Phytol, a chlorophyll-derived diterpenoid alcohol, has been reported to exhibit strong anti-inflammatory and metabolic regulatory properties through its participation in lipid metabolism and oxidative balance (Olofsson et al., 2014). Likewise, squalene functions as a triterpenoid hydrocarbon with potent antioxidant and membrane-protective capacities, serving as a biochemical precursor in sterol biosynthesis and an important agent in mitigating oxidative stress (Du et al., 2024). Collectively, the co-occurrence of these fatty acids, terpene alcohols, and triterpenoids suggests that Matteuccia struthiopteris possesses a robust phytochemical framework that may underpin its nutraceutical and pharmacological potential.These findings align with previous observations on *M. struthiopteris* and related Onocleaceae members, which have been shown to accumulate a variety of bioactive lipophilic metabolites capable of conferring medicinal and nutraceutical benefits (Li et al., 2013; Delong et al., 2011).

The diversity of metabolites observed in this study indicates that *M. struthiopteris* maintains a biosynthetic pattern consistent with its global distribution while also reflecting region-specific environmental influences. The presence of both saturated and unsaturated fatty acids, including n-hexadecanoic acid and oleic acid, is characteristic of edible and medicinal ferns, contributing to their nutritional value and antioxidant stability

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(Wang et al., 2024). Fatty acyl esters such as isopropyl stearate, hexadecanoic acid 1,2-ethanediyl ester, and oleyl oleate are derivatives formed through enzymatic esterification, serving as lipid storage and energy-reserve molecules within plant tissues (Zienkiewicz & Zienkiewicz 2020). These compounds have been associated with enhanced resistance to oxidative degradation of cellular lipids, as evidenced by improved physicochemical stability of fatty acid esters compared to their parent acids (Esipovich et al., 2024). Similarly, the detection of triterpenoids like squalene and terpenoid alcohols such as phytol supports the hypothesis that *M. struthiopteris* synthesizes metabolites involved in defense responses and photoprotection. Citronellol, a dominant monoterpene alcohol identified in this study, possesses a characteristic aromatic profile and contributes to the plant physiological functions, including antimicrobial and repellent activities. The co-occurrence of sterol esters and terpenoids suggests a synergistic biochemical adaptation that enhances membrane integrity and resilience under environmental stress. Comparable compound classes have been reported in *M. struthiopteris* extracts analyzed in other regions, indicating a conserved metabolic capability within the species irrespective of geographic origin (Chettri, 2018; Giri & Uniyal, 2022).

The metabolite composition observed also supports previously documented pharmacological properties of *M. struthiopteris*. According to Delong et al. (2011) and Wang et al. (2024), the fern rhizome and fronds contain significant amounts of phenolic and flavonoid compounds that confer antioxidant, antimicrobial, and anti-inflammatory potential. While the current GC–MS analysis primarily identified nonpolar compounds, such as lipids and alcohols, these molecules are often integral to plant defense systems and metabolic regulation. For instance, fatty acids like palmitic and oleic acids act as precursors for signaling molecules, while sterol esters such as cholesteryl palmitate play crucial roles in maintaining structural lipid homeostasis. The presence of anthraquinone derivatives in small proportions further enhances the plant chemical diversity, contributing to potential antitumor and antimicrobial activities as reported in earlier phytochemical studies (Li et al., 2013). Irrespective of region and environmental factors such as soil composition, temperature, and humidity most compound align with previous finding, suggesting that factors may modulate metabolite accumulation without altering the core phytochemical framework of *M. struthiopteris*.

From a nutraceutical perspective, the high abundance of cholesteryl palmitate, citronellol, and related lipophilic compounds in the ethanolic extract of *M. struthiopteris* underscores its potential as a source of bioactive molecules for functional food and therapeutic applications. Cholesteryl esters are known to contribute to lipid metabolism and membrane fluidity, while terpenoid alcohols such as citronellol and phytol exhibit antioxidant and anti-inflammatory properties beneficial for maintaining cellular homeostasis (Dufourc, 2008; Rogowska & Szakiel, 2020; Santos et al., 2013; Proshkina et al., 2020). The mixture of long-chain fatty acids, esters, and alcohols may enhance the extract antioxidant potential by stabilizing reactive oxygen species and preventing lipid peroxidation (Santos et al., 2013). Additionally, compounds such as squalene and 1,15-pentadecanediol are known intermediates in the biosynthesis of sterols and vitamins (Micera et al., 2020), which may further augment the nutritional value of this fern. The presence of epoxides and ketones, although in lower concentrations may also contributes to the extract chemical stability and biological functionality. Together, these constituents portray *M.*

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*struthiopteris* as a phytochemically balanced edible fern with multiple nutraceutical advantages, supporting its traditional use as both a food source and medicinal plant. The findings from the Nigerian sample provide valuable insights into the chemical variability of *M. struthiopteris* across environments and demonstrate its continued relevance in the search for natural, plant-derived bioactives for human health promotion.

## Conclusion

The GC–MS analysis of *Matteuccia struthiopteris* ethanolic extract revealed a rich composition of lipophilic bioactives dominated by sterol esters, fatty acids, and terpenoids. These metabolites contribute to the fern antioxidant and metabolic functions, highlighting its promise as a nutraceutical source and supporting its ethnomedicinal utilization in tropical regions.

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