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Allelopathic Investigations On Free Fatty Acids And Ester Components From The Rhizome Of Pteridium Aquilinum

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Abstract
Allelopathic has a potential to attribute in competing, hindering of native vegetation and alters the ecosystem dynamics. Pteridium aquilinum, commonly known as eagle fern or bracken fern distributed and considered as invasive species globally, exhibits the strong allelopathic properties in rhizome along with the other parts which has significant role in ecological dominance. Our study aims to identify and investigate the allelochemicals and their effects on the other species. Methanol crude extraction showed the inhibitory effect on wheat germination subsequently fractionised using GC-MS analysis which resulted in fatty acids and FAME'S namely Pentadecanoic acid, 14-methyl - methyl ester, 11-Octadecenoic acid, methyl ester, 9-Octadecenoic acid, (Z)-methyl ester, 9-Octadecenoic acid, (E)-methyl ester, trans-13-Octadecenoic acid, methyl ester and 6-Octadecenoic acid, (Z)-methyl ester, cis-13-Octadecenoic acid, methyl ester, Tridecanoic acid, methyl ester, n-Hexadecanoic acid and Hexadecanoic acid, methyl ester, and Pentadecanoic acid, methyl ester and followed by the HPLC analysis highlighted the presence of secondary metabolite ptaquiloside, this combination of allelochemicals have a significant impact in inhibition of seed germination and early seedling growth ranging from -71667% to -100% (P< 0.0001). The results contribute in understanding the allelopathic impact of rhizome of Pteridium aquilinum and their role in suppressing the neighbouring vegetation, which offers valuable insights for the control and management of grassland ecosystem.
Keywords: *Pteridium aquilinum, rhizome, fatty acids and FAME'S, Ptaquiloside, Bioassay,*

1. INTRODUCTION

Biodiversity loss is a major concern in the present world, due to anthropogenic activities and invasive species driven by climate change. The rapid distribution and establishment of invasive alien species across globally, pose a severe threat to the structure and function of natural ecosystems and loss of biodiversity (Roy et al. 2023). "Invasive alien species (IAS) are animals, plants, or other organisms introduced beyond their natural range, having adverse effect on native vegetation, ecosystem services, or human well-being" (IUCN 2021). The intensification of invasive species dispersal is occurring at an alarming rate due to one of their major contributing factors like allelopathy which plays a pivotal role in altering the landscape patterns (Ganiee et al.2024; Balah et al. 2024). The release of allelochemicals through rhizome secretion, volatilization, degradation of leaf and fronds (Jabran et al. 2015) hinder the germination, growth and development of the native species and alters the soil characteristics leading to potential invasion and successful colonization of invasive species (Noguchi 2024; Zhu et al. 2021; Qu et al. 2021; Kumar and Garkoti 2022). The Montane Grassland of the Western Ghats is observed at a higher altitude and supports diverse flora and fauna species with high endemism. Grassland is one of the most important ecosystems not devoid of invasion from alien invasive species (Balaguru et al. 2016). The distribution of invasive species has significantly affected the grassland habitat (Thomas and Palmer 2007).

Pteridium aquilinum (bracken fern) is an aggressive invasive species belonging to the family Dennstaedtiaceae, widely distributed across temperate and tropical regions (Marrs and Watt, 2006). It is recognized as one of the five most abundant plant species globally and is notoriously difficult to eradicate (Bardon et al. 2018). The fern typically colonizes disturbed habitats such as post-fire landscapes, deforested areas, pastures, and agricultural lands (Schneider, 2006; Silva and Matos, 2006; Miatto et al. 2011). Its remarkable adaptability is attributed to its broad tolerance to climatic and edaphic conditions, enabling it to thrive under high sunlight, acidic soils, and even waterlogged environments (Mira et al. 2021).

The dominance and persistence of P. aquilinum are largely facilitated by its extensive rhizome system,

which can extend up to 2 meters, serving as a reservoir of carbohydrates and essential nutrients that support continuous growth and regeneration (Jatoba et al. 2016). In addition, the fern exhibits several ecological attributes, including high spore production, resistance to fire and drought, rapid vegetative spread, and notable allelopathic potential (Butnariu et al. 2015). Previous studies have identified phenolics, terpenoids, flavonoids, coumaric acids, and quinones as key allelochemicals influencing its invasive behavior, with ptaquilosides being particularly linked to its phytotoxic effects (Mira et al. 2021). Several earlier studies have also reported the occurrence of allelopathic compounds in different plant species, which exhibit strong inhibitory effects on seed germination and seedling growth. The supporting literature consulted in this work indicates comparable chemical profiles to those identified in the rhizome extract of P. aquilinum, thereby reinforcing the potential allelopathic nature of the metabolites detected in this study. Such consistency across studies provides additional evidence that these compounds may contribute to the competitive dominance and invasive capability of P. aquilinum.

Despite the extensive attention given to the fern's secondary metabolites, the composition and ecological significance of fatty acids and their methyl esters in the rhizome of P. aquilinum remain poorly understood. These compounds are known to play essential roles in allelopathic interactions, interspecific signaling, and environmental stress tolerance among invasive plants. Therefore, the present study focuses on characterizing the bioactive compounds present in the rhizome extract of P. aquilinum using Gas Chromatography–Mass Spectrometry (GC–MS) and evaluating their allelopathic potential through a wheat coleoptile bioassay. This integrated chemical ecological approach aims to elucidate the mechanistic basis underlying the ecological dominance and invasive success of P. aquilinum.

2. MATERIALS AND METHODOLOGY

The Rhizome sample of Pteridium aquilinum were collected across the Kudremukh National Park, stands at an elevation of 1,892 meters above sea level, the park is situated at the meeting point of the Dakshina Kannada, Udupi, and Chikmagalur districts (75°01' to 75°25' E longitude and 13°01' to 13°29' N latitude) Western Ghats, India. The sample were processed as below.

2.1 Crude Extraction

The collected rhizome samples of Pteridium aquilinum were thoroughly cleaned to remove adhering soil particles and debris, followed by a three-step washing process using normal water, distilled water, and finally sterilized water to ensure complete surface decontamination. The cleaned rhizomes were air-dried at room temperature, pulverized into a fine powder, and stored in airtight containers until further analysis. For extraction, 50 g of the powdered rhizome was homogenized with 100 mL of methanol in a 1:2 ratios and incubated at 22 ± 2 °C for 48 hours under dark conditions to prevent photodegradation of sensitive compounds. The resultant mixtures were filtered, and the combined extracts were concentrated under reduced pressure using a rotary evaporator maintained at 40 °C to obtain the crude methanolic extract. The purified extract was then subjected to Gas Chromatography–Mass Spectrometry (GC–MS) analysis to identify and characterize the secondary metabolites, following the protocol described by Shanab et al. (2010).

2.2 GC-MS analysis

For Gas Chromatography–Mass Spectrometry (GC–MS) analysis, a PerkinElmer Clarus 680 Gas Chromatograph coupled with a Clarus SQ 8C Mass Spectrometer was employed. The instrument was fitted with an Elite-5MS capillary column (30 m length, 0.25 mm internal diameter, and 1 μm film thickness) composed of 100% dimethyl polysiloxane. The analysis was performed under electron ionization (EI) mode at 70 eV. High-purity helium gas (99.99%) served as the carrier gas, maintained at a constant flow rate of 2 mL min^{-1}. A 2 μL aliquot of the methanolic extract was injected into the system using a split ratio of 10:1 to ensure optimal resolution. The injector temperature was maintained at 250 °C, while the ion source was set to 230 °C to achieve efficient volatilization and ionization of the analytes. The oven temperature was initially held at 80 °C for 2 minutes, followed by a linear increase of 10 °C min^{-1} up to 150 °C, and subsequently raised at a rate of 15 °C min^{-1} until reaching 250 °C. The total analytical run time was 26.6 minutes. Data acquisition and processing of chromatograms and mass spectra were carried out using TurboMass software. The relative abundance of each detected compound was calculated by comparing individual peak areas with the total chromatographic area. The identification of metabolites was performed based on mass spectral matching with standard libraries, following the protocol described by Shanab et al. (2010).

2.3 Bioassay on wheat coleoptile

To analyse whether the extract of rhizome has negative impact on other species, a bioassay test on wheat coleoptile was carried out. According to the protocol (Miranda et al. 2015), Wheat (Triticum aestivum) seeds were sown, moistened with water, and germinated in controlled culture laboratory in the dark at 25 ± 1 °C for three days. The roots and caryopses were removed from the shoots, the apical 2 mm of the shoots were cut off and discarded, and the next 4 mm of the coleoptiles were collected for bioassays. All procedures were performed under a green safelight.

Crude extracts or pure compounds were dissolved in 0.1% DMSO and diluted in a phosphate-citrate buffer containing 2% sucrose at pH 5.6 to achieve the final bioassay concentrations of 0.8, 0.4, and 0.2 mg/mL for extracts. Parallel controls were also conducted. The commercial herbicide glyphosate, N-(phosphonomethyl) glycine, was used as an internal reference with the similar concentrations and conditions as mentioned earlier. To measure the phytotoxic activities of fractions on wheat coleoptile were conducted. Five coleoptiles and 2 mL of prepared solutions were placed in each test tube (three tubes per dilution) and rotated at 6 rpm in a roller tube apparatus for 24 hours at 25 °C in the dark. In addition, we also had control. Coleoptile lengths were measured by digitizing their images. Data generated were statistically analysed using linear regression and are presented as percentage differences from the control. Positive values indicate stimulation, while negative values indicate inhibition (Miranda et al. 2015).

2.4 High-Performance Liquid Chromatography (HPLC) analysis

The powdered rhizome of Pteridium aquilinum was extracted using HPLC-grade methanol and subjected to continuous shaking for approximately 72 hours to ensure complete dissolution of its chemical constituents. The resulting mixture was filtered through Whatman No. 1 filter paper to obtain a clear filtrate. The crude extract was analyzed using an HPLC system equipped with a Shim-pack Solar C18 column (particle size 5 μm, internal diameter 4.6 mm, and length 250 mm; P/N 227-30600-02). The chromatographic separation was carried out using a mobile phase composed of water and methanol (80:20 v/v) at a flow rate of 1.0 mL/min, with the column temperature maintained at 40°C to ensure stable and reproducible separation. Caffeine was used as the calibration standard for system standardization and retention time verification to ensure accurate quantification of analytes. To eliminate dissolved gases and prevent bubble formation, the mobile phase was passed through the degasser unit integrated into the system. A 1.0 μL aliquot of the supernatant solution from the crude extract was carefully injected into the instrument to achieve precise quantification, and detection was performed at a wavelength of 254 nm, ensuring reliable and sensitive analysis of the extract components. The analytical conditions were optimized to obtain sharp, well-resolved peaks for accurate identification of the metabolites, and each run was closely monitored to maintain consistency in retention time and chromatographic response across replicates.

3. RESULTS

3.1 Fatty acids and FAMES identified in crude extract of Rhizome of Pteridium aquilinum

3.1.1. Gas Chromatography-Mass Spectrum analysis and Characterization of Fatty Acids and FAMEs

The GC–MS analysis of the rhizome extract of Pteridium aquilinum revealed a diverse profile of bioactive compounds, predominantly consisting of fatty acids and their methyl esters (FAMEs), which are known to possess allelopathic and phytotoxic properties influencing interspecific plant interactions. Based on the interpretation of the mass spectral data, a total of eleven major fatty acid and FAME compounds were identified. Among these, Pentadecanoic acid, 14-methyl-, methyl ester exhibited the highest peak area (10.2%), indicating its dominance in the chemical composition of the rhizome. Other prominent constituents included 11-Octadecenoic acid, methyl ester (7.09%), 9-Octadecenoic acid (Z)-methyl ester (6.81%), 9-Octadecenoic acid (E)-methyl ester (6.55%), trans-13-Octadecenoic acid, methyl ester, and 6-Octadecenoic acid (Z)-methyl ester (6.29%), which together contribute substantially to the total fatty acid content. Additionally, cis-13-Octadecenoic acid, methyl ester (4.82%), Tridecanoic acid, methyl ester (2.36%), n-Hexadecanoic acid and Hexadecanoic acid, methyl ester (1.25%), along with Pentadecanoic acid, methyl ester (0.54%), were also detected in measurable quantities.

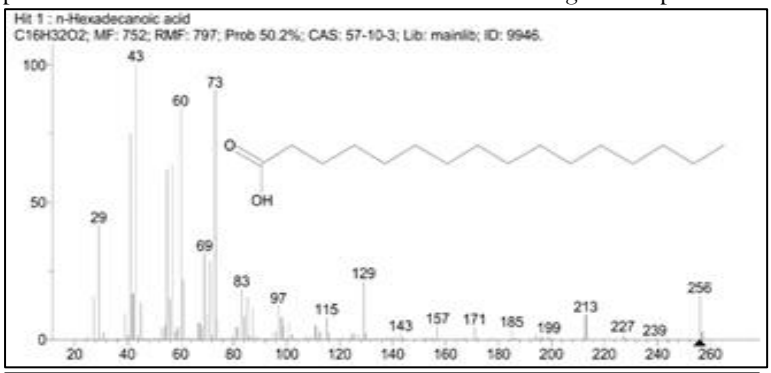
The predominance of unsaturated fatty acid methyl esters, particularly the octadecenoic derivatives, suggests their potential involvement in the allelopathic mechanisms of P. aquilinum, possibly contributing to its competitive dominance and invasiveness in various ecosystems. These compounds are known to interfere with seed germination and growth of neighboring plant species by altering membrane permeability, enzymatic activities, and hormonal balance. Thus, the chemical composition of P.

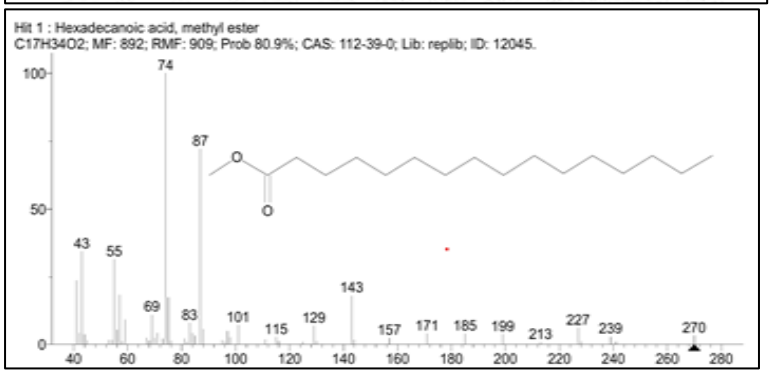
aquilinum rhizomes underscores its adaptive strategy and ecological success as a potent allelopathic invasive species. The following table represents the identified FAME compounds and their relative abundances as derived from GC–MS spectral data.

Table 1: GC–MS Characterization of Bioactive Phytoconstituents from the Rhizome of Pteridium aquilinum

Sl.No	Name of the compound	Molecular formula	MW	Peak Area %
1	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	1.25
2	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	1.25
3	Pentadecanoic acid, 14-methyl - methyl ester	$C_{17}H_{34}O_2$	270	10.2
4	Tridecanoic acid, methyl ester	$C_{14}H_{28}O_2$	228	2.36
5	Pentadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	256	0.54
6	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	7.09
7	9-Octadecenoic acid, (Z)-methyl ester	$C_{19}H_{36}O_2$	296	6.81
8	9-Octadecenoic acid, (E)-methyl ester	$C_{19}H_{36}O_2$	296	6.55
9	trans-13-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	6.29
10	6-Octadecenoic acid, (Z)-methyl ester	$C_{19}H_{36}O_2$	296	6.29
11	cis-13-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	4.82

The structural representations of the identified fatty acids and their corresponding fatty acid methyl esters (FAMEs) are illustrated below, depicting their molecular configurations as characterized through GC–MS analysis of the Pteridium aquilinum rhizome extract. These structural models provide valuable insight into the molecular diversity and chemical architecture of the compounds detected, reflecting the complex biochemical profile of the species. The GC–MS chromatogram revealed distinct peaks corresponding to various saturated and unsaturated fatty acids, along with their methyl ester derivatives, confirming the presence of both low and medium molecular weight compounds.





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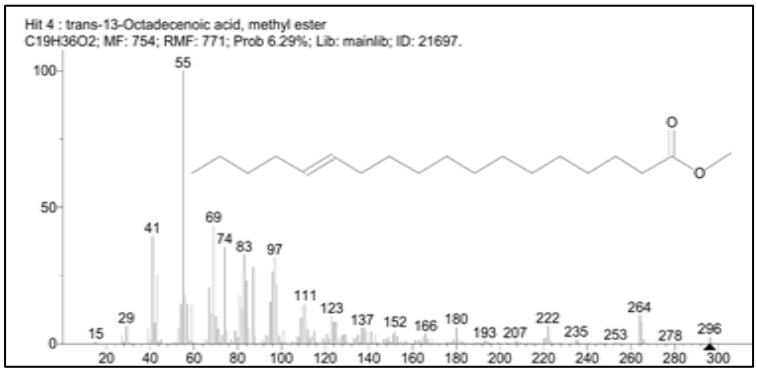

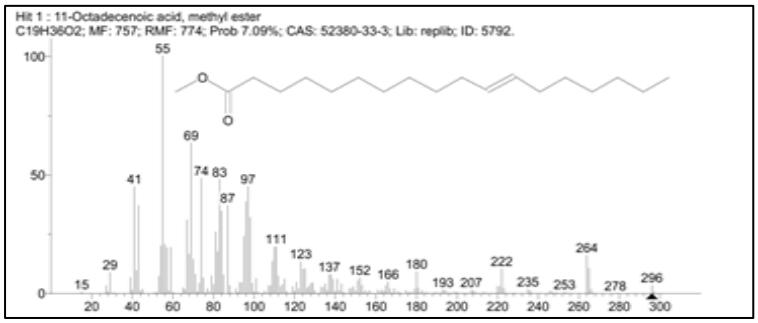

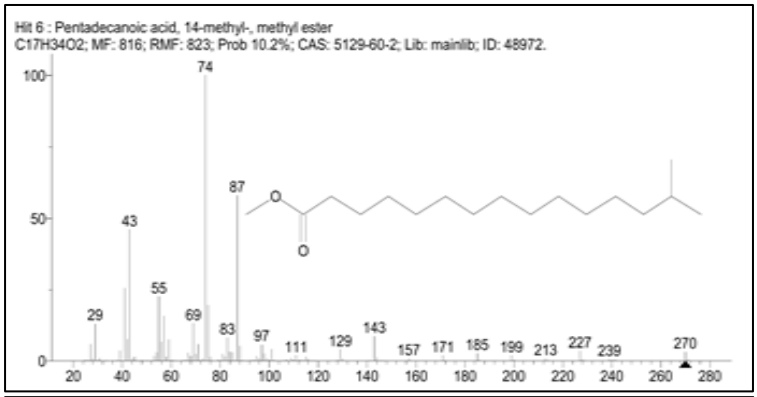

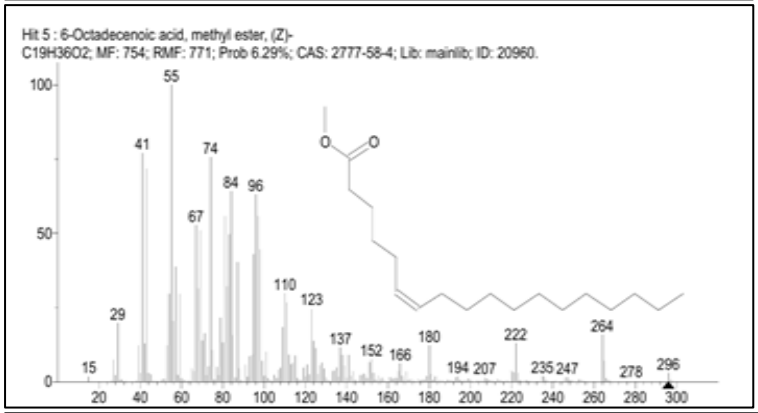

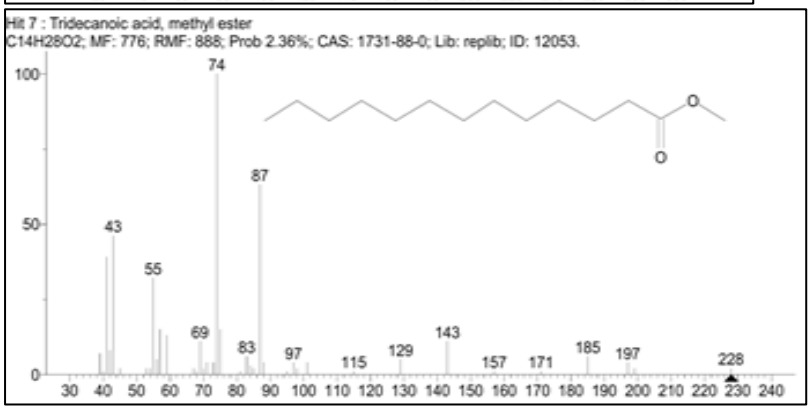

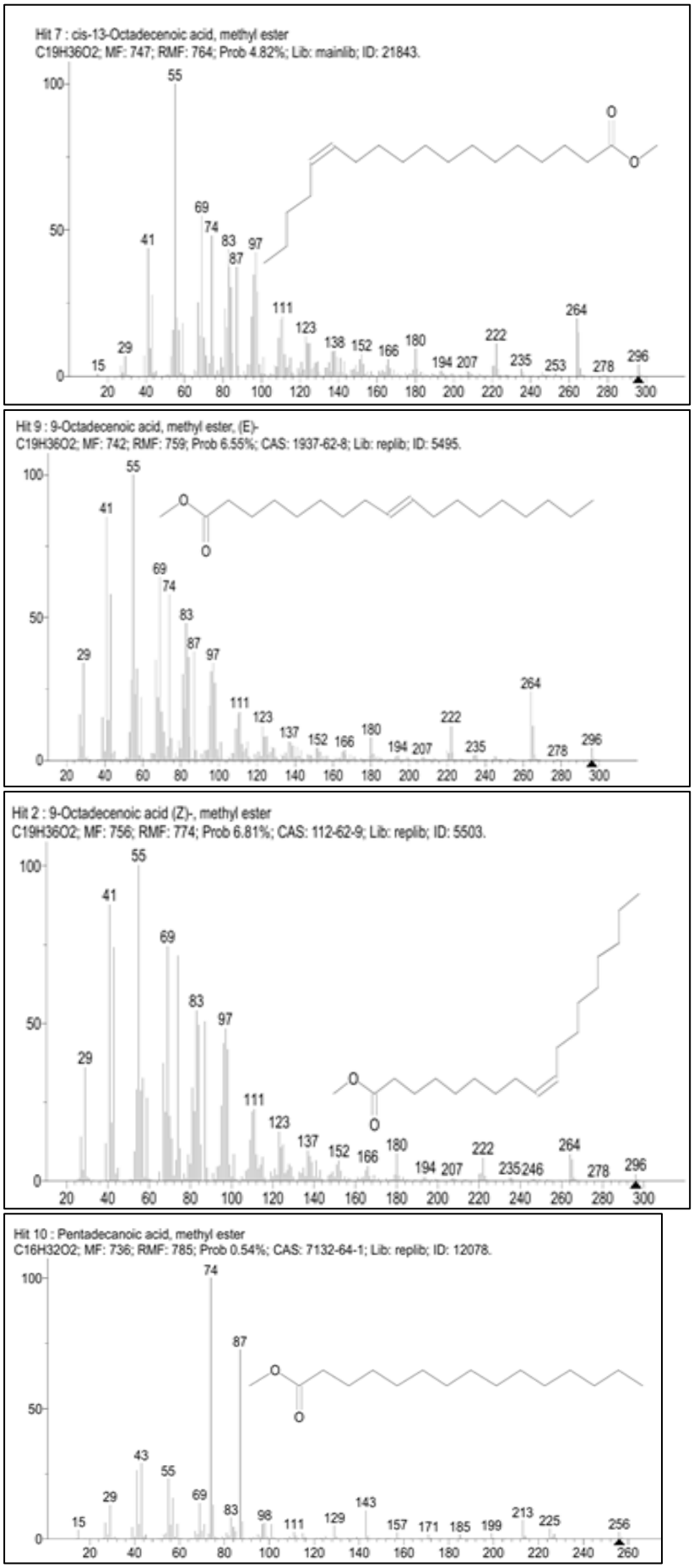

Fig 1: GC–MS mass spectra and corresponding structural formulas of the fatty acids and fatty acid methyl esters (FAMEs) identified in the rhizome extract of Pteridium aquilinum.

3.3. Bioassay test on Wheat coleoptile

Complementing to the GC-MS analysis our study recorded a significant inhibitory effect on wheat coleoptile growth wherein the phytotoxic activity primarily attributed to fatty acids and their methyl esters, acted as allelochemicals inhibiting the coleoptile growth with inhibition range of -71.667% to -100% ($P<0.0001$). This result clearly indicates that both the rhizome extract and herbicide extract exerted strong negative impact on wheat coleoptile development and growth under the laboratory condition. The inhibition pattern suggests a possible **disruption of cell elongation processes**, likely through **alterations in membrane integrity, interference with hormonal regulation and oxidative stress induction**, mechanisms commonly reported for fatty acid-derived allelochemicals (Charoenying et al. 2022). These key findings were consistent with those of (Jatoba et al. 2016) who investigated Pteridium arachnoideum and demonstrated similar bioassay test using isolated extract of Selligueain A reinforcing the role of bracken-derived secondary metabolites in suppressing plant growth.

3.3.1. Evaluation of Wheat Coleoptile Bioassay

The allelopathic potential of the crude rhizome extract of Pteridium aquilinum was quantitatively assessed using a wheat coleoptile bioassay. The degree of growth inhibition or stimulation was expressed as a percentage relative to the untreated control, enabling the evaluation of the extract's phytotoxic intensity. The percentage response was computed using the following relationship:

$$\% \text{ Inhibition} = ((Cr - Ct) / (Cr - Cc)) \times 100$$

where

Cr = reference coleoptile length (4 mm)

Ct = mean coleoptile length obtained from each treatment

Cc = mean coleoptile length from the control group

This computation provided a reliable estimate of the inhibitory effect produced by each concentration of the allelochemical constituents present in the crude extract.

To determine the statistical significance of variations among treatments, a one-way Analysis of Variance (ANOVA) was conducted using GraphPad Prism software. The experimental design included three graded concentrations (0.2, 0.4, and 0.8 mg mL^{-1}) of both the rhizome extract and a reference herbicide, along with an untreated control. The analysis revealed a highly significant inhibitory effect ($P < 0.0001$) for both the rhizome and herbicide treatments across all concentrations tested, confirming the reproducibility and strength of the allelopathic response.

The one-way Analysis of Variance (ANOVA) were performed to evaluate the statistical significance of differences in coleoptile length among treatments in wheat seedlings. Treatments included three concentrations of rhizome extract and herbicide extract (0.2, 0.4 and 0.8mg mL^{-1}) with an untreated control for comparison. The results revealed a highly significantly inhibitory effect ($P<0.0001$) for both rhizome and herbicide extracts across all tested concentrations.

Table 2: Growth inhibition of Wheat Coleoptile by the application of rhizome extract of Pteridium aquilinum and Herbicide with different concentrations

Sl. No	Concentration mg/ml	Control Length in mm	Herbicide extract Length in mm	Rhizome extract Length in mm	% of inhibition
1	0.2	5.8	4.1	4.51	-71.667
2	0.4	5.7	4.0	4.35	-79.41
3	0.8	5.7	4.0	4.0	-100

The mean coleoptile length recorded for the control seedlings was **5.8 ± 0.05 mm**. Upon treatment with the rhizome extract of Pteridium aquilinum at **0.2 mg mL^{-1}**, the average coleoptile length declined markedly to **4.51 ± 0.02 mm**, representing an inhibition of **-71.67%**, while the herbicide extract produced a comparable reduction with a mean length of **4.10 ± 0.03 mm**. At the intermediate concentration of **0.4 mg mL^{-1}**, coleoptile elongation further decreased to **4.35 ± 0.02 mm** under the rhizome extract, corresponding to an inhibition of **-79.41%**, whereas the herbicide treatment yielded an average length of **4.00 ± 0.01 mm**. At the highest concentration tested (**0.8 mg mL^{-1}**), complete suppression of coleoptile elongation was observed in both treatments, resulting in **100% inhibition**, thereby confirming a strong dose-dependent phytotoxic response.

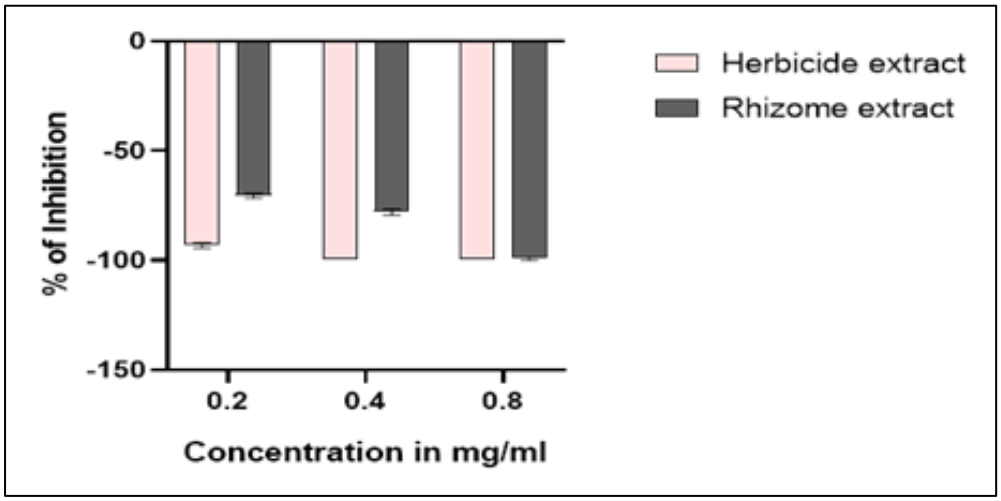

Fig 2: Inhibitory Effects of Allelopathic Compounds from Pteridium aquilinum and Glyphosate on Wheat Coleoptile Growth

A clear dose-dependent inhibition pattern was observed, indicating progressive suppression of coleoptile elongation with increasing concentrations of both extracts. The rhizome extract demonstrated a comparable inhibitory potential to the herbicide, highlighting the strong phytotoxic nature of P. aquilinum. This effect can be attributed to the presence of bioactive compounds such as fatty acids and their methyl esters (FAMEs), which may interfere with hormonal regulation and cell elongation processes. The pronounced inhibition recorded across treatments confirms that Pteridium aquilinum possesses a high degree of allelopathic potential. These findings suggest that its secondary metabolites play a significant ecological role in limiting the growth of neighboring species, thereby contributing to its dominance and invasiveness in natural ecosystems.

3.3.2. Evidence supporting Fatty acids and FAMES contributes as allelopathic compounds

A review of relevant literature supports the observation that the presence of **fatty acids and their methyl esters (FAMEs)** plays a crucial role in inducing growth inhibition in target plant species. Numerous studies have demonstrated that these compounds exhibit strong **allelopathic activity**, influencing various physiological and biochemical processes of susceptible plants. Compound-specific investigations further confirm that such allelochemicals contribute significantly to the invasive potential of certain species by **interfering with seed germination, seedling growth, and overall development** of the species.

Table 3: Validation of Reported Allelopathic Activities of Bioactive Compounds from Pteridium aquilinum through Literature Evidence

Sl.No	Compound	Identified from	Allelopathic effect on	Method of Identification	References
01.	n-Hexadecanoic acid, 9-Octadecenoic acid, (Z)-methyl ester, Hexadecanoic acid, methyl ester	Humulus scandens	Alternanthara philoxeroides	Petroleum ether extract (PE), ethyl acetate extract (EE) and n-butanol extract (NE)	Wang et al. (2021)
02.	Methyl palmitate (MP) and methyl linolenate (ML)	Humulus scandens, Ipomoea b atatas, and Phrag mites aust ralis	Alternanthara philoxeroides	Hydrponic experiment	Hua et al. (2023)
03.	Linoelaidic acid, Glycidyl oleate,18-Nonadecenoic acid, Palmatic acid,	Neanotis montholo nii	Vigna radiata and Oryza sativa	Aqueous extract	Torawane and Mokat (2020)

	Glycidyl palmitate				
04.	Hexadecanoic acid, Palmitic Acid, Methyl linolelaidate, Linolenic acid, Stearic acid, Hexadecanoic acid, methyl ester	Partheniu m hysteroph orus	Vigna radiata	Aqueous extract and bioassay	Lalita et al. (2020)
05.	eicosane, gamma-sitosterol, l-(+)-ascorbic acid 2,6-dihexadecanoate, octadecanoic acid, methyl 11,14,17-eicosatrienoate, and octadec-9-enoic acid	Moringa oleifera	Triticum aestivum	Hexane extraction of plant, GC-MS analysis, Statistical analysis-ANOVA	Tahir et al. (2018)
06.	n-hexadecanoic acid	Solidago canadensis	Triticum aestivum and Raphanus sativus	Volatiles, leachates, root exudates and decomposed residue studies, Bioassay and Isolation and identification of the compounds	Zhang et al. 2014
07.	Hexadecanoic acid, octadecanoic acid, dibutyl phthalate, (adipic acid, isohexyl methyl ester) and (adipic acid, di (oct-4-yl ester))	Spartina alterniflor a Loisel	Scirpus mariqueter	Soil extraction, Bioassay and GC-MS analysis	Cq et al. 2011
08.	methyl 8,9-octadecadienoate, n-hexadecanoic acid, phytol, loliolide, stigmasta-7,16-dien-3-ol, catechol borane, ellagic acid	Amaranth us caudatus and Amaranth us blitum	Raphanus sativus	Petri dish bioassay followed by a pot experiment	Ganiee et al., 2024
09.	Essential oil-29 constituents, with phytol, cholesterol trimethylsilyl ether, adipic acid, bis (2-ethylhexyl) ester, and muurola-4, 10(14)-dien-1β-ol being abundant in the oil	S. rostratum	2 dicot plants, Medicago Sativa and Amaranthus retroflexus, and a monocto plant, Poa annua.	Essential oil extraction, GC-MS analysis, Phytotoxic activity of essential oil, statistical analysis	Shixing et al. 2020
10.	Neophytadiene, 2-Pentadecanone, 6,10,14-trimethylHexadecanoic acid, methyl ester, Dibutyl phthalate, nHexadecanoic acid, 9,12-Octadecadienoic	Alternant hera philoxeroi des	Experiment was conducted for the same species	aqueous extract, ethanol extract, GC-MS analysis	Ashwini et al. 2020

	acid (Z,Z)-, methyl ester, Hexadecanoic acid, ethyl ester, 8-Octadecenoic acid, methyl ester, 12(Z)-Conjugated linoleic acid, (Z)- 9- Octadecenoic acid, methyl ester10(E)-, Phthalic acid, and butyl 2-pentyl ester.				

3.4. HPLC identification of Ptaquiloside in crude extract of P. aquilinum

HPLC analysis of the crude extract of Pteridium aquilinum rhizome revealed the presence of a major secondary allelopathic metabolite identified as **ptaquiloside**, a non-sesquiterpene compound detected at a **retention time of 15.771 minutes** with a **peak area percentage of 1.570** (Trendafilova et al., 2012). Along with ptaquiloside, several **fatty acids and their ethyl esters** were also detected (details provided in Supporting Table 2), confirming the chemical complexity of the extract. Although ptaquiloside is widely recognized for its **carcinogenic potential**, it also plays a crucial role in **allelopathic interactions**, significantly inhibiting the germination and growth of native plant species and contributing to **soil acidification** (Jatobá et al., 2016).

The combined presence of these allelochemicals provides Pteridium aquilinum with a strong ecological advantage by enhancing its **colonization, persistence, and spatial dominance**. These bioactive compounds modify the surrounding soil environment, creating conditions unfavorable for native vegetation while promoting the fern's own proliferation. Consequently, this biochemical interference poses a **serious threat to biodiversity**, disrupting ecosystem balance through alterations in **nutrient cycling and trophic interactions**, ultimately leading to **grassland degradation** and the **loss of vital habitats** that support herbivores, nocturnal fauna, and medicinally important plant species.

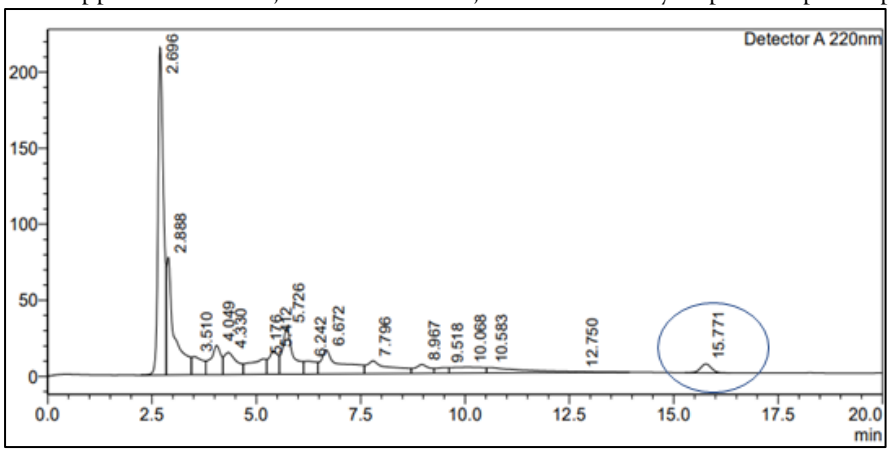

Fig 3: Quantitative Determination of Ptaquiloside in the Rhizome of Pteridium aquilinum Using HPLC

4. DISCUSSION

The findings of the present study are strongly supported by previous research highlighting the allelopathic effects of similar bioactive compounds identified through GC–MS analyses in various invasive and weedy species. Multiple studies (Table X) have reported fatty acids, fatty acid methyl esters (FAMEs), and other lipid-derived metabolites as major contributors to allelopathic interactions, consistently associating compounds such as n-hexadecanoic acid, 9-octadecenoic acid methyl esters, linoleic and palmitic acid derivatives with inhibitory effects on seed germination and seedling growth of target plants. These compounds, extracted through aqueous, organic solvent, or essential oil-based methods, have exhibited broad-spectrum phytotoxicity across diverse bioassay systems including hydroponic, soil, and petri dish experiments, significantly reducing growth in species like Vigna radiata, Triticum aestivum, Raphanus sativus, and Oryza sativa. The detection of these same compounds in the rhizome extract of P. aquilinum, together with the presence of ptaquiloside identified through HPLC, provides a comprehensive chemical basis for its pronounced allelopathic potential. The concurrence between this study and existing literature suggests a synergistic interaction between fatty acid-based compounds and ptaquiloside, enhancing the inhibitory effects on seedling growth and facilitating ecological dominance and successful colonization of

P. aquilinum in disturbed habitats. This comparative validation reinforces the view that the chemical composition of P. aquilinum is ecologically functional, acting as a potent biochemical suppressor of competing vegetation and thereby contributing to its invasive success.

5. CONCLUSION

Invasive species are recognized as one of the major drivers of global biodiversity loss, and Pteridium aquilinum exemplifies this threat through its strong chemical and ecological adaptability. The GC–MS analysis of the rhizome extract revealed eleven fatty acids and fatty acid methyl esters (FAMEs), which exhibited strong inhibitory effects on wheat coleoptile growth, ranging from −71.667% to −100% ($P < 0.0001$). Complementing this, the presence of ptaquiloside in low concentration further contributed to the inhibitory response (Jatoba et al. 2016). The combined action of fatty acids, FAMEs, and ptaquiloside indicates the potent allelopathic potential of P. aquilinum, enabling it to suppress native vegetation effectively.

As evident from these findings, P. aquilinum exerts a significant ecological impact by inhibiting native species regeneration, leading to grassland degradation and facilitating grassland-to-woodland conversion. This shift alters trophic interactions and ecosystem stability, ultimately resulting in the loss of habitats for herbivores, nocturnal fauna, and medicinal herbs. The study provides valuable insights into the mechanistic role of allelochemicals in the invasiveness of bracken and emphasizes the urgent need for its control and management to protect the biodiversity and ecological integrity of montane grasslands in the Western Ghats.

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