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PHYTOCHEMICAL PROFILING AND ANTIOXIDANT ACTIVITY OF *DRYOPTERIS FILIX-MAS* COLLECTED FROM BADAGRY, LAGOS STATE, NIGERIA.

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Abstract

Ferns are increasingly recognized as valuable sources of bioactive secondary metabolites with potential nutraceutical and pharmacological applications, yet information on how phenological stages influence their phytochemical composition and antioxidant capacity remains limited. This study investigated the phytochemical profile and antioxidant activity of *Dryopteris filix-mas* collected from Badagry, Lagos State, Nigeria, across six phenological stages: young fiddle head, mature fiddle head, mature vegetative frond, fertile frond, senesced frond, and root. The plant material was authenticated and extracted using aqueous, methanolic, and n-hexane solvents. Quantitative phytochemical analyses were conducted to determine major secondary metabolites, while gas chromatography–mass spectrometry was employed to characterize bioactive compounds. Antioxidant potential was evaluated using 2,2-diphenyl-1-picrylhydrazyl radical scavenging and ferric reducing antioxidant power assays. The results revealed marked stage- and solvent-dependent variations in phytochemical composition, with methanolic extracts generally yielding higher concentrations of polar and moderately polar compounds, while n-hexane selectively extracted non-polar constituents. Mature vegetative and fertile fronds consistently exhibited stronger antioxidant activity, reflected by lower IC₅₀ values and higher reducing power, compared to other phenological stages. GC–MS profiling confirmed the presence of diverse fatty acids, esters, alcohols, hydrocarbons, and nitrogen-containing compounds, suggesting dynamic metabolic regulation during plant development. The findings demonstrate that phenological stage plays a critical role in determining the phytochemical composition and antioxidant potential of *D. filix-mas*, highlighting the importance of developmental stage selection in phytochemical and bioactivity studies of ferns and providing a basis for future bioactive compounds isolation and utilization.

Keywords: Antioxidant activity, Bioactive compounds, GC-MS, *Dryopteris filix-mas*, Phytochemical profiling

1. Introduction

Medicinal plants continue to attract significant global interest owing to their crucial roles in disease management, health promotion, and their extensive applications in pharmaceutical, nutraceutical, and allied industries. Although primary metabolic pathways are largely conserved among plant taxa, variations in secondary metabolite biosynthesis underpin differences in medicinal efficacy, nutritional value, and ethnobotanical relevance among species (Akbar *et al.*, 2024; Hilal *et al.*, 2024). Consequently, the exploration of plant species rich in bioactive secondary metabolites has

intensified, particularly in the context of discovering novel natural antioxidants and therapeutic agents.

Pteridophytes, especially ferns, constitute one of the most ancient lineages of vascular plants and are widely distributed across tropical and subtropical ecosystems (Qian *et al.*, 2021). These plants occupy diverse ecological niches, ranging from forest understories to wetlands and disturbed habitats, where they contribute significantly to ecosystem stability. Ferns are also recognized for their notable resistance to microbial attack and environmental stressors, a trait largely attributed to their complex and

diverse secondary metabolite profiles (Castrejón-Varela *et al.*, 2022; Divekar *et al.*, 2022). Numerous studies have documented the presence of biologically active compounds in ferns, including alkaloids, flavonoids, phenolic compounds, terpenoids, steroids, fatty acids, amino acids, and distinctive metabolites such as pteridines, many of which are uncommon in angiosperms (Dubale *et al.*, 2023; Castrejón-Varela *et al.*, 2022; Dvorakova *et al.*, 2021). These phytochemicals have been associated with a wide range of pharmacological properties, including antioxidant, antimicrobial, anti-inflammatory, anticancer, and cytoprotective activities.

Among medicinally important fern species, *Dryopteris filix-mas* (male fern) (Plate 1) has received considerable attention in traditional medicine across Europe, Asia, and parts of Africa. Historically, extracts of *D. filix-mas* rhizomes and fronds have been used as anthelmintics and for the treatment of gastrointestinal disorders, wounds, and inflammatory conditions (Laudato *et al.*, 2013; Murthy and Joseph, 2011). Phytochemical investigations of the species have revealed the presence of phloroglucinol derivatives, phenolics, flavonoids, and other bioactive constituents responsible for its therapeutic effects (Singha *et al.*, 2023; Alam *et al.*, 2021). Despite these reports, comprehensive investigations into variation in phytochemical composition across phenological stages and its influence on antioxidant capacity, particularly in tropical fern species, remain limited (Osinaike *et al.*, 2026).

Oxidative stress, resulting from an imbalance between reactive oxygen species (ROS) production and antioxidant defense mechanisms, has been implicated in the pathogenesis of numerous degenerative diseases, including cardiovascular disorders, cancer, diabetes, and neurodegenerative conditions (Tüngen and Buruleanu, 2025; Houldsworth, 2024; Abeyrathne *et al.*, 2022). Plant-derived antioxidants play a vital role in mitigating oxidative damage by scavenging free radicals, chelating metal ions, and enhancing endogenous antioxidant systems (Tüngen and Buruleanu, 2025; Akbari *et al.*, 2022). Consequently, assessing the antioxidant potential of fern species such as *Dryopteris filix-mas* is essential for validating their ethnomedicinal uses and identifying novel sources of natural antioxidants.

In Nigeria, ferns are predominantly distributed within the rainforest belt, where climatic conditions favor their growth and persistence (Egbe *et al.*, 2025; Akomolafe *et al.*, 2022; Akinsoji *et al.*, 2016). Lagos State, situated within this ecological zone, harbors a diversity of fern

species, including *Dryopteris filix-mas*, *Acrostichum aureum*, *Nephrolepis biserrata*, and *Phymatosorus scolopendria*. These species are traditionally utilized for food, medicine, environmental remediation, and soil improvement (Lawal *et al.*, 2022; Giri *et al.*, 2021; Muhammed *et al.*, 2020; Emashogwe *et al.*, 2020). However, scientific investigations into the phytochemical composition and bioactivity of many Nigerian ferns remain sparse (Osinaike *et al.*, 2026), with *Dryopteris filix-mas* being particularly underexplored despite its medicinal relevance.



Plate 1: *Dryopteris filix-mas* (male fern)

In Badagry, Lagos State, Nigeria, *D. filix-mas* occurs in forest margins, fallow lands, and moist disturbed sites, yet it is often overlooked or destroyed due to limited awareness of its economic and medicinal value. Furthermore, there is a paucity of data on the phytochemical constituents and antioxidant properties of *D. filix-mas* populations growing in this region. To address this knowledge gap, the present study investigates the phytochemical composition, characterizes the bioactive compounds, and evaluates the antioxidant activity of *Dryopteris filix-mas* collected from Badagry, Lagos State, Nigeria. By integrating phytochemical screening, GC–MS profiling, and antioxidant assays, this study aims to provide a scientific basis for the potential nutraceutical and pharmacological applications of this medicinally important fern.

2. Materials and Methods

Area of study

Lagos State, Nigeria, located in southwestern Nigeria along the coast of the Bight of Benin, experiences a tropical savanna climate, with average temperatures often between about 25°C and 32°C and distinct wet and dry seasons (TimeandDate.com, 2026). The region's vegetation includes rainforest, swamp/saline marshland, and freshwater inland areas, with predominantly sandy soils along the coast and loamy soils inland.



Map of the sampling area

Sample collection and preparation

Plant materials of *Dryopteris filix-mas* were collected from Badagry in late July 2022, during the peak growth period for these ferns. The specimens were authenticated by Dr. Adebowale Shonde, Curator of the LASUSTECH Herbarium, Ikorodu Campus, Lagos State University of Science and Technology, and a voucher specimen was deposited under the herbarium number LSTH 045. Healthy plant parts at different growth and developmental stages were collected across various phenological stages (young fiddle head, mature fiddle head, mature vegetative frond, fertile frond, senesced frond, and root) in polyethylene bags, washed with distilled water, and air-dried at room temperature (~27°C) for one month until fully dried. Dried samples were grinded using a sterilized blender, transferred into sterilized containers, sealed, and appropriately labeled.

Preparation of extracts from the sampled phenological stages

Powdered samples from the different phenological stages were subjected to solvent extraction using methanol, n-hexane, and distilled water, following established procedures for plant phytochemical analysis. For each solvent, 50 g of the dried powder was weighed and soaked in 250 mL of the respective solvent at room temperature for 72 hours, with occasional shaking to ensure thorough extraction of phytochemicals. Each extraction was performed in triplicate to ensure reproducibility. The mixtures were filtered through Whatman No. 1 filter paper, and the filtrates were concentrated under reduced pressure using a rotary evaporator at 40°C (for methanol and n-hexane extracts) or freeze-dried (for aqueous extracts) to obtain the crude extracts. The resulting extracts were stored in airtight containers at 4°C until subsequent phytochemical screening, GC-MS analysis, and antioxidant assays.

Qualitative and Quantitative phytochemical analysis of secondary metabolites in plant parts

Qualitative phytochemical screening

Preliminary qualitative screening of the plant extracts was carried out to determine the presence of major secondary metabolites using standard procedures (Harborne, 1998; Sofowora, 2008; Evans, 2009). The analyses included tests for reducing sugars, terpenoids, steroids, phenolic compounds, flavonoids, tannins, alkaloids, saponins, anthraquinones, and cardiac glycosides. Phytochemical presence was indicated by characteristic colour changes or precipitate formation.

Quantitative phytochemical analysis

Quantitative phytochemical analyses were carried out using standard methods as previously described (Evans, 2009; Sofowora, 2008; Harborne, 1998; Singleton, 1965). The phytochemical constituents assayed included alkaloids, flavonoids, tannins, saponins, steroids, anthraquinones, cardiac glycosides, and reducing sugars. All determinations were performed in triplicate and results were expressed as Mean $\pm$ Standard error.

Gas chromatography mass spectrometry (GC-MS) profiling

GC-MS analysis was carried out on a GC Agilent technologies 7890A coupled with MS 5795 with an autosampler (Agilent Technologies, Santa Clara, CA, USA). The instrument was set to employ the following conditions: column HP5MS fused silica capillary column (30 x 0.32mm ID x 0.25 μ Mdf, composed of 100% Dimethylpolysiloxane), operating in electron impact mode at 70eV; hydrogen gas (99.999%) was used as carrier gas at a constant flow of 1 ml /min and an injection volume of 1 μ l was employed (splitless) injector temperature 250 °C; ion-source temperature 280 °C. The oven temperature was programmed from 80 °C (isothermal for 2 min), with an increase of 10 °C/min, to 240 °C, then held for 6 mins scan intervals from 50 to 550 Da. The relative percentage composition of each compound was calculated by comparing its average peak area with the total chromatographic peak area. Compound identification was performed by comparing the mass spectra of the detected compounds with those in the NIST/EPA/NIH Mass Spectral Library using Agilent ChemStation software. Only compounds with a similarity (match) index $\geq$ 80% were considered reliably identified. Further confirmation was achieved by comparing the fragmentation patterns and retention characteristics with published literature.

Antioxidant potential analysis of the sampled phenological stages

2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was performed according to the method of Shimada *et al.* (1992). 0.1 mM solution of DPPH in ethanol was prepared; 1 ml of the solution was added to 1 ml of the extract in water at different concentrations (25-100 µg/ml). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. The absorbance was then measured at 517 nm using a UV-Visible Spectrophotometer. The lower absorbance of the reaction mixture indicated a higher free radical scavenging activity. All assays were carried out in triplicate, and gallic acid was used as the reference standard. The percentage DPPH scavenging effect was calculated using the following equation.

$$\text{DPPH Scavenging effect (\%)} = \frac{(A_0 - A_1)}{A_0} \times 100$$

Where A_0 = Absorbance of the control, and A_1 = Absorbance in the presence of the standard sample or extract.

The IC_{50} value represents the concentration of the compounds that caused 50% inhibition of DPPH radical formation.

Ferric Reducing Antioxidant Power (FRAP) Assay

The ferric reducing antioxidant power of the extracts was evaluated according to Oyaizu (1986), and Ita (2020) with slight modifications. The reaction was carried out in a mixture containing 1 mL of sample (25-100 µg/ml), 2.5 mL of 0.1 M sodium phosphate buffer (pH 6.6), and 2.5 mL of 1%, w/v potassium ferrocyanide [$K_3Fe(CN)_6$] by incubating at 50 °C for 20 min. After the addition of 2.5 ml trichloroacetic acid (10 %, w/v), the mixture was centrifuged at 5000 rpm for 10 min. The upper layer (5 mL) was mixed with 0.5 mL of 0.1% (w/v) $FeCl_3$ and the absorbance was measured at 700 nm. All determinations were performed in triplicate, and gallic acid was used as the standard antioxidant.

Statistical Analysis

Data collected were subjected to one-way analysis of variance (ANOVA) using Statistical Packages for the Social Sciences (SPSS) version 23. Significant differences among means

were separated using Duncan's Multiple Range Test (DMRT) at a significance level of $p < 0.05$.

3. Results and discussion

3.1 Results

3.1.1 Preliminary Qualitative Phytochemical Screening

Preliminary qualitative phytochemical screening of *Dryopteris filix-mas* across phenological stages; young fiddle head (YFH), matured fiddle head (MFH), matured adult frond (MA), fertile frond (AS), senesced frond (S), and root (R), in aqueous, methanolic, and n-hexane extracts confirmed the presence of a broad spectrum of secondary metabolites, with distribution patterns that varied with both developmental stage and solvent type (Table 1).

Alkaloids, saponins, and reducing sugars were detected across all phenological stages in aqueous and methanolic extracts, while in n-hexane extracts they were present in most stages except fertile fronds. Terpenoids and anthraquinones were detected across several stages, with absence observed in fertile fronds under specific solvent conditions.

Phenolic compounds, including tannins and phenols, were present in matured adult, fertile, senesced, and root tissues across all solvent systems, but were absent in young and matured fiddle heads in aqueous and n-hexane extracts. In methanolic extracts, tannins and phenols were additionally present in young fiddle heads but absent in matured fiddle heads.

Flavonoids were not detected in matured fiddle heads across all solvent systems. They were present in all other stages in aqueous and n-hexane extracts, while in methanolic extracts they were present only in matured adult, fertile, senesced, and root tissues.

Steroids were absent in aqueous extracts across all phenological stages but were present in all stages in n-hexane extracts. In methanolic extracts, steroids were detected only in young and matured fiddle heads.

Phlobatannins were present in all stages except fertile fronds in aqueous and methanolic extracts, but absent in n-hexane extracts. Cardiac glycosides were present in methanolic extracts across all stages, while in aqueous extracts they were absent only in fertile fronds. In n-hexane extracts, they were detected only in matured fiddle heads and senesced fronds.

Table 1: Qualitative analysis of phytochemicals in *D. filix-mas*

Phytochemicals	Extracts	Phenological stages					
		YFHD	MFHD	MAD	ADS	SD	DR
Tannins	Aqueous	-	-	+	+	+	+
	Methanolic	+	-	+	+	+	+
Phenols	Hexane	-	-	+	+	+	+
	Aqueous	-	-	+	+	+	+
	Methanolic	+	-	+	+	+	+
	Hexane	-	-	+	+	+	+
Phlobatannins	Aqueous	+	+	+	-	+	+
	Methanolic	+	+	+	-	+	+
Alkaloids	Hexane	-	-	-	-	-	-
	Aqueous	+	+	+	+	+	+
	Methanolic	+	+	+	+	+	+
	Hexane	+	+	+	-	+	+
Saponins	Aqueous	+	+	+	+	+	+
	Methanolic	+	+	+	+	+	+
Flavonoids	Hexane	+	+	+	-	+	+
	Aqueous	+	-	+	+	+	+
	Methanolic	-	-	+	+	+	+
	Hexane	+	-	+	+	+	+
Steroids	Aqueous	-	-	-	-	-	-
	Methanolic	+	+	-	-	-	-
Anthraquinones	Hexane	+	+	+	+	+	+
	Aqueous	+	+	+	-	+	+
	Methanolic	+	+	-	-	+	-
	Hexane	+	+	+	-	+	+
Terpenoids	Aqueous	+	+	+	-	+	+
	Methanolic	+	+	+	-	+	+
Cardiac glycosides	Hexane	+	+	+	+	+	+
	Aqueous	+	+	+	-	+	+
	Methanolic	+	+	+	+	+	+
	Hexane	-	+	-	-	+	-
Reducing sugar	Aqueous	+	+	+	+	+	+
	Methanolic	+	+	+	+	+	+
	Hexane	-	+	-	-	+	-

3.1.2 Quantitative Phytochemical Analysis

The quantitative phytochemical profile of *Dryopteris filix-mas* across phenological stages (YFH, MFH, MA, AS, S, and R) showed variation in secondary metabolite composition across aqueous, methanolic, and n-hexane extracts (Table 2). The phytochemicals assessed included tannins, alkaloids, saponins, flavonoids, steroids, terpenoids, cardiac glycosides, and reducing sugars.

Methanolic extracts recorded higher concentrations of most polar and moderately polar phytochemicals, aqueous extracts showed higher levels of water-soluble constituents such as reducing sugars and glycosidic compounds, while n-hexane extracts contained higher levels of non-polar metabolites, including steroids and selected alkaloids.

Tannin content varied across phenological stages and solvents. Higher values were recorded in methanolic extracts, with fertile fronds showing 48.24 mg/100 g, followed by

matured adult fronds (36.41 mg/100 g) and roots (37.10 mg/100 g). In aqueous extracts, tannins were detected in fertile fronds (46.65 mg/100 g) and roots (27.85 mg/100 g), while no detection occurred in young and matured fiddle heads.

Alkaloids were present across most phenological stages. Higher values were recorded in methanolic extracts of roots (54.86 mg/100 g) and young fiddle heads (53.21 mg/100 g), while n-hexane extracts showed high levels in matured fiddle heads (52.79 mg/100 g) and young fiddle heads (50.42 mg/100 g). Alkaloids were not detected in n-hexane extracts of fertile fronds.

Saponins were detected across all stages. Higher values were recorded in aqueous extracts of young fiddle heads (60.08 mg/100 g) and senesced fronds (57.90 mg/100 g), while methanolic extracts showed higher values in roots (63.65 mg/100 g) and matured fiddle heads (57.58 mg/100 g). No detection occurred in fertile fronds under n-hexane extraction.

Flavonoids varied across stages and solvents, with the highest value recorded in aqueous extracts of fertile fronds (67.04 mg/100 g). No detection occurred in matured fiddle heads across all solvents.

Steroids were absent in aqueous extracts. Methanolic extracts contained steroids only in matured fiddle heads (28.08 mg/100 g), while n-hexane extracts showed presence across all stages, with higher values in senesced fronds (43.08 mg/100 g) and matured fiddle heads (42.99 mg/100 g).

Terpenoids were present across developmental stages. Higher values were recorded in methanolic extracts of matured fiddle heads (42.15 mg/100 g) and senesced fronds (40.20 mg/100 g), while aqueous extracts showed moderate levels mainly in young fiddle heads (27.36 mg/100 g).

Cardiac glycosides were detected in aqueous and methanolic extracts, with higher values in roots (44.09 mg/100 g) and young fiddle heads (45.91 mg/100 g). In n-hexane extracts, detection occurred only in matured fiddle heads and senesced fronds.

Reducing sugars were highest in aqueous extracts of fertile fronds (76.08 mg/100 g), followed by methanolic extracts (65.70 mg/100 g). Minimal levels were recorded in n-hexane extracts.

Table 2: Quantitative analysis of phytochemicals in *D. filix-mas*

Phytochemicals	Extracts (mg/100g)	Phenological ages (MEAN ± S.E.)					
		YFH	MFH	MA	AS	S	R
Tannins	Aqueous	0±0.00 ^h	0±0.00 ^k	21.79±0.15 ^{lm}	46.65±1.37 ^h	19.34±0.08 ^k	27.85±0.07 ^g
	Methanolic	36.27±0.23 ^e	0±0.00 ^k	36.41±0.12 ^g	48.24±0.44 ^g	28.29±0.08 ^{hi}	37.10±0.34 ^e
	Hexane	0±0.00 ^h	0±0.00 ^k	28.01±0.17 ^{ij}	12.61±0.11 ^o	31.62±0.14 ^{ah}	28.25±0.19 ^g
Phenols	Aqueous	0±0.00 ^h	0±0.00 ^k	29.78±0.11 ^{ij}	64.17±0.49 ^d	27.05±0.40 ^{hij}	38.17±0.12 ^e
	Methanolic	50.06±0.19 ^{bc}	0±0.00 ^k	50.17±0.09 ^{ab}	76.15±0.07 ^a	39.04±0.17 ^e	51.48±0.26 ^c
	Hexane	0±0.00 ^h	0±0.00 ^k	39.05±0.54 ^{ef}	27.29±0.18 ^k	43.49±0.21 ^{de}	35.87±3.49 ^e
Alkaloids	Aqueous	31.73±0.19 ^{ef}	39.07±0.18 ^f	29.03±0.15 ^j	52.29±0.62 ^f	43.01±0.30 ^{de}	37.80±0.27 ^e
	Methanolic	53.21±0.59 ^b	50.28±0.21 ^c	43.28±0.30 ^{cd}	33.14±0.28 ^j	39.99±0.25 ^e	54.86±0.39 ^b
	Hexane	50.42±0.16 ^{bc}	52.79±0.18 ^b	46.80±0.52 ^{bc}	0±0.00 ^p	49.77±0.16 ^c	45.08±0.21 ^d
Saponins	Aqueous	60.08±0.38 ^a	48.08±0.23 ^d	31.71±0.09 ^{hi}	36.16±0.08 ⁱ	57.90±2.25 ^a	35.59±0.35 ^e
	Methanolic	48.43±0.39 ^{bc}	57.58±0.30 ^a	43.16±0.42 ^{cd}	25.65±0.11 ⁱ	42.24±0.04 ^e	63.65±0.36 ^a
	Hexane	53.01±0.16 ^b	52.56±0.30 ^b	44.01±0.90 ^{cd}	0±0.00 ^p	55.06±0.35 ^{ab}	44.72±0.42 ^d
Flavonoids	Aqueous	48.26±0.39 ^{bc}	0±0.00 ^k	41.67±0.45 ^{de}	67.04±0.16 ^b	41.77±0.34 ^e	37.80±0.44 ^e
	Methanolic	43.29±0.25 ^d	0±0.00 ^k	52.20±0.29 ^a	56.16±0.16 ^e	37.98±0.17 ^{ef}	27.07±0.45 ^f
	Hexane	43.79±0.19 ^d	0±0.00 ^k	52.53±0.29 ^a	23.13±0.08 ^m	47.63±0.35 ^{cd}	47.29±0.08 ^d
Steroids	Aqueous	0±0.00 ^h	0±0.00 ^k	0±0.00 ^p	0±0.00 ^p	0±0.00 ⁱ	0±0.00 ^j
	Methanolic	0.00±0.00 ^h	28.08±0.19 ^j	0±0.00 ^p	0±0.00 ^p	0±0.00 ⁱ	0±0.00 ^j
	Hexane	35.49±0.58 ^e	42.99±0.35 ^e	35.73±0.23 ^{fg}	35.26±0.05 ^j	43.08±0.25 ^{de}	27.09±0.29 ^f
	Aqueous	27.36±0.46 ^f	19.52±0.22 ^j	19.00±0.95 ^m	0±0.00 ^p	23.01±0.49 ^{jk}	11.82±0.25 ^j

Terpenoids				n			
Cardiac glycosides	Methanol	17.86±0.12 ^g	42.15±0.14 ^e	18.13±0.44 ^m	0±0.00 ^p	40.20±0.20 ^e	26.10±0.40 ^g
	Hexane	19.48±0.24 ^g	32.14±0.19 ^h	23.08±0.16 ^{kl}	25.00±0.07 ^l	23.76±0.36 ^{jk}	17.03±0.32 ^h
	Aqueous	32.44±0.36 ^{ef}	20.53±0.10 ^j	34.09±0.31 ^{gh}	0±0.00 ^p	30.21±0.62 ^{gh}	44.09±0.18 ^d
	Methanol	45.91±0.21 ^{cd}	27.68±0.22 ⁱ	26.68±0.29 ^k	19.16±0.60 ⁿ	40.75±0.61 ^e	27.83±0.20 ^f
Reducing sugar	Hexane	0±0.00 ^h	35.63±0.28 ^g	0±0.00 ^q	0±0.00 ^p	52.02±0.16 ^{bc}	0±0.00 ^j
	Aqueous	36.08±0.19 ^e	20.60±0.20 ^j	9.35±0.14 ^o	76.08±0.40 ^a	29.10±0.25 ^{gh}	30.03±0.14 ^f
	Methanol	28.26±0.11 ^f	19.98±0.23 ^j	15.76±0.16 ⁿ	65.70±0.15 ^c	30.47±0.19 ^{gh}	27.05±0.39 ^f
	Hexane	0±0.00 ^h	26.92±0.09 ⁱ	0±0.00 ^q	0±0.00 ^p	33.51±0.20 ^{fg}	0±0.00 ^j

*Means followed by the same letters on the same column are not significantly different at 5% probability (DMRT).

*YFH = young fiddle head; MFH = matured fiddle head; MA = matured adult; AS = fertile frond; S = senesced frond; R = root

3.1.3 Phytochemical Compounds Profile and Identification by GC–MS Analysis

GC–MS analysis of *Dryopteris filix-mas* revealed a diverse array of phytochemicals, including fatty acids, alcohols, esters, hydrocarbons, nitrogen-containing compounds, and cyclic derivatives, with variations observed between methanol and n-hexane extracts.

In the young fiddlehead, methanol extracts were dominated by pentadecanoic acid, 14-methyl-, methyl ester (31.95%), while 3-cyclopentylcyclopentene (0.92%) occurred at the lowest proportion. In contrast, the n-hexane extract showed ethyl 2-octynoate as the most abundant compound (30.02%), whereas 5-chloro-1-pentyne (3.51%) was the least abundant (Table 3; Figure 1).

Table 3: Major bio-compounds identified from young fiddlehead of *D. filix-mas*

METHANOL				N- HEXANE		
PEAK#	RT(min)	COMPOUND	%TOTAL	RTmin)	COMPOUND	%TOTAL
1	12.525	2-Decanoic acid	1.28	14.616	Hexadecanoic acid, methyl ester	23.04
2	13.739	3-Cyclopentylcyclopentene	0.92	14.979	Ethyl 2-octynoate	30.02
3	14.159	Cyclohexadecane	1.07	16.131	3-Methylpyridazine	6.28
4	14.632	Pentadecanoic acid, 14-methyl-, methyl ester	31.95	16.297	5-Chloro-1-pentyne	3.51
5	15.015	n-Hexadecanoic acid	5.23	16.671	9,17-Octadecadienal, (Z)-	19.73
6	16.136	5-Octadecene, (E)-	3.00	18.020	5-Vinyl-pyrazole	17.42
7	16.250	7,10-Octadecadienoic acid, methyl ester	2.04			
8	16.313	9-Octadecenoic acid (Z)-, methyl ester	18.43			
9	16.370	9-Octadecenoic acid (Z)-, methyl ester	12.27			
10	16.546	Methyl stearate	15.58			
11	16.681	9-Octadecenoic acid	5.66			
12	16.878	Undec-10-ynoic acid, dodecyl ester	1.68			
13	18.300	Eicosanoic acid, methyl ester	0.89			

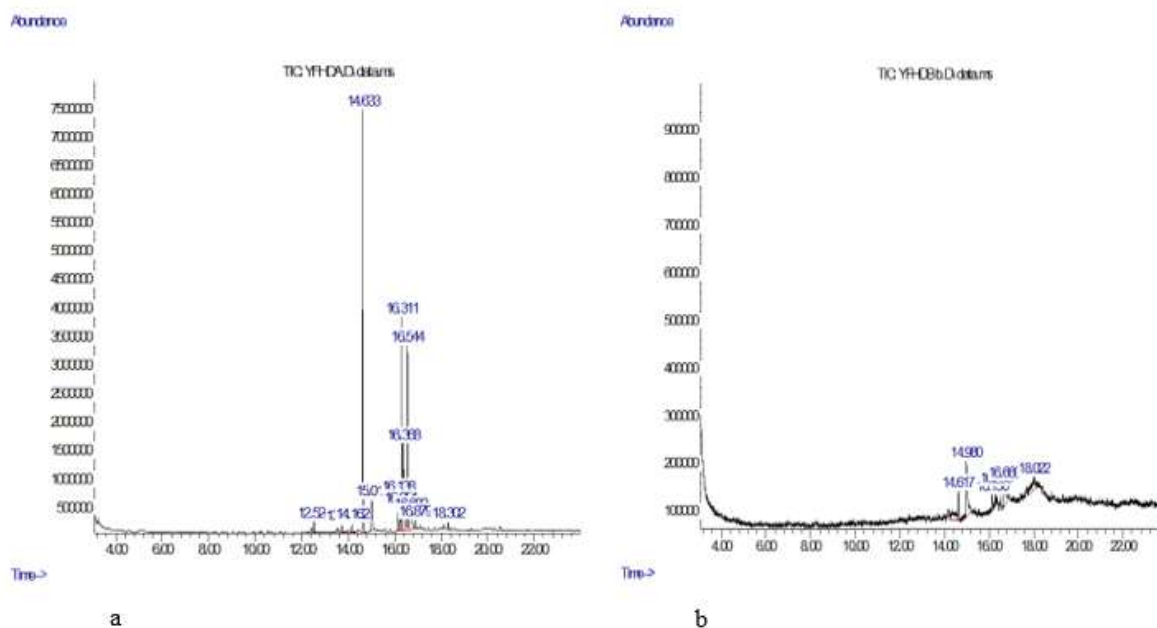


Figure 1: Chromatogram obtained from GC-MS with methanol (a) and n-hexane (b) extracts of *D. filix-mas* young fiddlehead

In the mature fiddlehead methanol extracts were dominated by exo-norbornyl alcohol (47.92%), while N-acrylonitrilaziridine was present at the lowest proportion (1.92%). In the n-hexane

extract, a 1H-indole-2-carboxylic acid derivative was the most abundant compound (23.45%), whereas 2-norbornyl acetate occurred at the lowest proportion (2.52%) (Table 4; Figure 2).

Table 4: Major bio-compounds identified from mature fiddlehead of *D. filix-mas*

METHANOL				N-HEXANE		
PEAK#	RT(min)	COMPOUND	%TOTAL	RT(min)	COMPOUND	%TOTAL
1	12.53	Tridecanoic acid, methyl-, methyl ester	0.75	10.117	3-Hydroxypyridine, trifluoroacetate	4.85
2	13.739	Neophytadiene	1.1	12.364	Borazine, 1-methyl-	4.27
3	14.165	3-Hexadecene, (Z)-	1.05	14.159	4-Heptafluorobutyryloxyhexadecane	7
4	14.632	Pentadecanoic acid, 14-methyl-, methyl ester	34.7	14.616	2-Decanoic acid	14.28
5	14.99	9-Tetradecenal, (Z)-	1.22	14.984	Cyclopentene,3-methoxy	20.65
6	16.136	7-Hexadecene, (Z)-	1.93	16.131	6-Nonynoic acid	12.17
7	16.251	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	3.14	16.416	3-Tridecen-1-yne, (E)-	6.16
8	16.308	9-Octadecenoic acid (Z)-, methyl ester	22.25	16.707	2-Norbornanemethanol, pentafluoropropionate	23.66
9	16.365	9-Octadecenoic acid (Z)-, methyl e	14.23	16.863	1-Cyclopenteneacetic acid, 5-oxo-	6.94
10	14.541	Methyl stearate	18.77			
11	18.298	Eicosanoic acid, methyl ester	0.86			

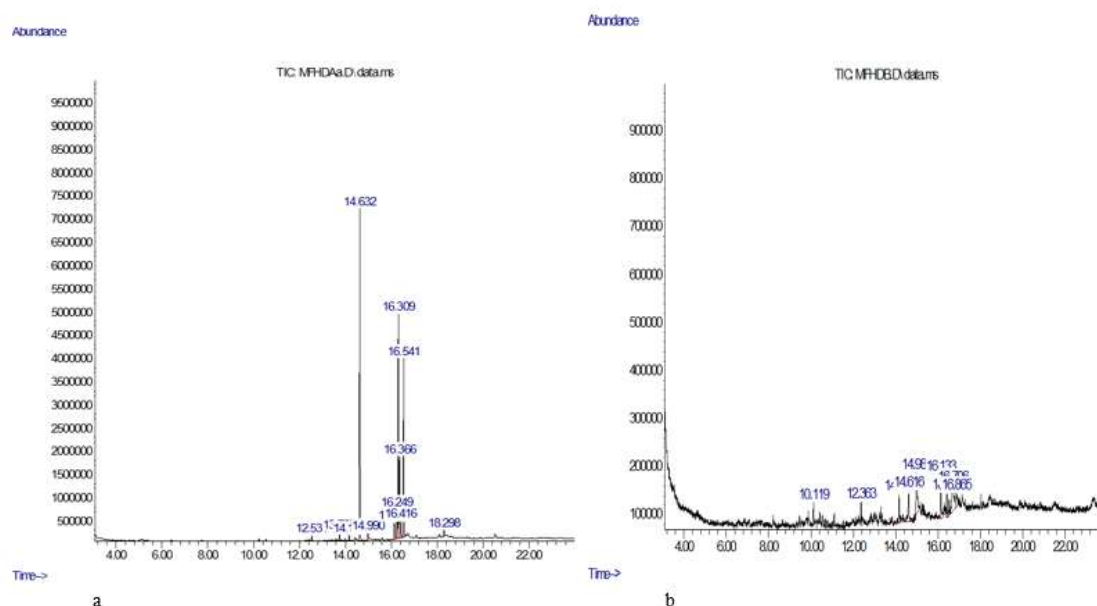


Figure 2: Chromatogram obtained from GC-MS with methanol (a) and n-hexane (b) extracts of *D. filix-mas* mature fiddlehead

In the mature vegetative frond of *Dryopteris filix-mas*, distinct phytochemical compositions were observed between methanol and n-hexane extracts.

Methanol extraction revealed hexadecanoic acid, methyl ester as the most abundant compound (35.29%), while 7-oxabicyclo[4.1.0]heptane, 1-methyl-

4-(1-methylethenyl)- occurred at the lowest proportion (0.06%). In contrast, the n-hexane extract was dominated by 1,2-epoxyundecane (14.52%), whereas 4,7-methanoisobenzofuran-1-ol, hexahydro- was the least abundant (4.85%) (Table 5; Figure 3).

Table 5: Major bio-compounds identified from mature vegetative frond of *D. filix-mas*

PEAK#	RT(min)	METHANOL		RT(min)	N-HEXANE	
		COMPOUND	%TOTAL		COMPOUND	%TOTAL
1	13.734	2-Norbornane Acetic acid	1.49	10.117	2,4-Di-tert-butylphenol	10.11
2	14.616	Hexadecanoic acid, methyl ester	35.29	10.413	Decane	5.31
3	16.126	7-Oxabicyclo[4.1.0]heptane, 1-methyl-4-(1-methylethenyl)-	0.06	12.836	Dodecane	7.62
4	16.297	trans-13-Octadecenoic acid, methyl ester	23.66	12.987	1-Undecene, 7-methyl-	7.5
5	16.354	11-Octadecenoic acid, methyl ester	13.07	13.08	4,7-Methanoisobenzofuran-1-ol, 1,3,3a,4,7,7a-hexahydro-	4.85
6	16.406	1,2-Epoxyundecane	7.87	13.241	17-Pentatriacontene	4.93
7	16.526	Heptadecanoic acid, 16-methyl-, methyl ester	16.71	14.159	7-Hexadecene, (Z)-	5.71
8	18.29	1,2,4-Triazol-4-amine, 5-methyl-3-(3,5-dimethylpyrazol-1-yl)-	1.85	14.616	Decanoic acid, methyl ester	9.97
9				15.01	Decane, 3-methyl-	12.09
10				15.265	7,11-Hexadecadienal	3.59
11				16.131	5-Octadecene, (E)-	6.04
12				16.411	1,2-Epoxyundecane	14.52
13				17.132	2-Decanoic acid	7.75

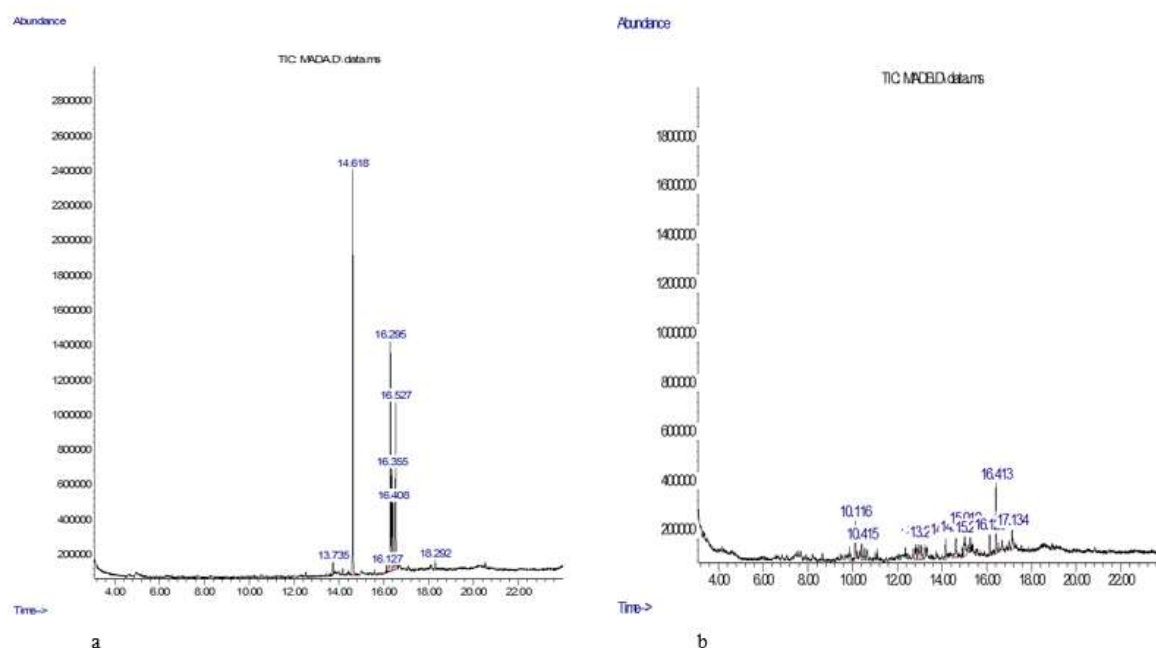


Figure 3: Chromatogram obtained from GC-MS with methanol (a) and n-hexane (b) extracts of *D. filix-mas* adult vegetative frond

In the fertile frond of *Dryopteris filix-mas*, methanol extracts were dominated by pentadecanoic acid, 14-methyl-, methyl ester (29.90%), while 3,4-octadiene, 7-methyl was present at the lowest proportion (1.04%). In contrast, the n-hexane extract showed 1-dodecanol,

2-methyl- (S)- as the most abundant compound (23.59%), whereas phenol, 2,5-bis(1,1-dimethylethyl) occurred at the lowest proportion (2.59%) (Table 6; Figure 4).

Table 6: Major bio-compounds identified from fertile frond of *D. filix-mas*

METHANOL				N-HEXANE		
PEAK#	RT(min)	COMPOUND	%TOTAL	RT(min)	COMPOUND	%TOTAL
1	13.739	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, (1.alpha.,2.beta.,5.alpha.)	5.861	10.117	Phenol, 2,5-bis(1,1-dimethylethyl)	2.593
2	13.993	3,4-Octadiene, 7-methyl	1.037	12.359	Heneicosane	3.237
3	14.18	1-Hexadecyne	1.855	12.246	1-Docosene	2.443
4	14.631	Pentadecanoic acid, 14-methyl-, methyl ester	29.898	14.164	Undec-10-ynoic acid, undecyl ester	2.11
5	15.015	n-Hexadecanoic acid	2.649	14.595	Hexadecanoic acid, methyl ester	11.517
6	16.25	Methyl 5,12-octadecadienoate	2.753	15.015	1-Dodecanol, 2-methyl-, (S)-	23.592
7	16.313	9-Octadecenoic acid (Z)-, methyl ester	21.847	15.27	Cyclododecane, ethyl	2.046
8	16.37	9-Octadecenoic acid, methyl ester	9.171	16.292	9-Octadecenal, (Z)-	3.056
9	16.422	Phytol	6.989	16.416	7-Oxabicyclo[4.1.0]heptane, 1,5-dimethyl	17.235
10	16.541	Methyl stearate	14.447	16.53	Heptadecanoic acid, 16-methyl-, methyl ester	5.244
11	16.686	9,12-Octadecadienoic acid (Z,Z)-	3.493	16.681	9,17-Octadecadienal, (Z)-	14.404
12				16.883	Undec-10-ynoic acid, nonyl ester	4.018
13				17.127	Undec-10-ynoic acid, undecyl ester	3.723
14				17.439	2-Decen-1-ol, (E)	4.781

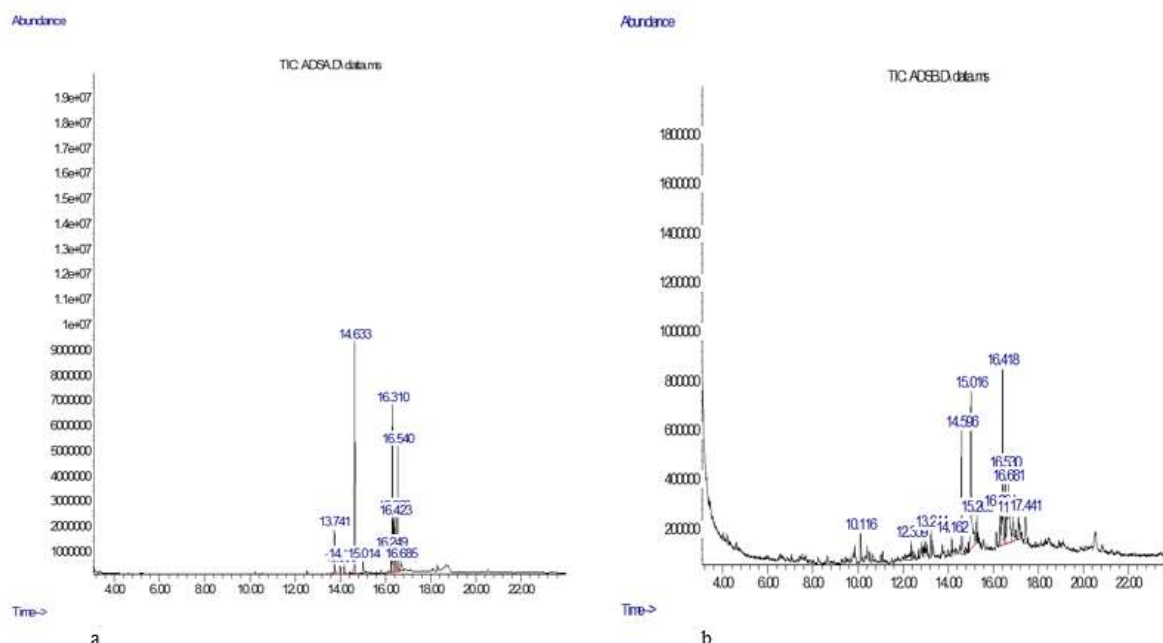


Figure 4: Chromatogram obtained from GC-MS with methanol (a) and n-hexane (b) extracts of *D. filix-mas* fertile frond

In the senesced frond of *Dryopteris filix-mas*, methanol extracts were dominated by pentadecanoic acid, 14-methyl-, methyl ester (25.68%), while 5-vinyl-pyrazole was present at the lowest proportion (0.64%). In contrast, the n-

hexane extract showed 9-oxabicyclo[6.1.0]nonane as the most abundant compound (21.98%), whereas a 1H-pyrrolo[2,3-d]pyrimidine derivative occurred at the lowest proportion (9.68%) (Table 7; Figure 5).

Table 7: Major biocompounds identified from senesced frond of *D. filix-mas*

METHANOL				N- HEXANE		
PEAK#	RT(min)	COMPOUND	%TOTAL	RT(min)	COMPOUND	%TOTAL
	5.744	benzenamine, 3-(5-chloro-1H-tetrazol-1-yl)-	1.89	14.164	1H-Pyrrolo(2,3-d)pyrimidine-2,4(3H,7H)-dione, 1,3-dimethyl-	9.68
2	10.289	2,4-Hexadiyne	0.88	14.621	Hexadecanoic acid, methyl ester	18.86
3	12.535	Carbamic acid, butylmethyl-, phenyl ester	1.28	14.984	9-Oxabicyclo[6.1.0]nonane	21.98
4	13.744	5-Vinyl-pyrazole	0.88	16.297	3-Phenoxypropionic acid	6.83
5	14.626	Pentadecanoic acid, 14-methyl-, methyl ester	25.68	16.411	Bicyclopentylidene	17.72
6	14.985	9-octadecanoic acid, 2,2,3,3,4,4,4-heptafluorobutyl ester	4.58	16.530	exo-Norbornyl alcohol	6.25
7	16.142	5-Vinyl-pyrazole	0.64	16.686	3-Isopropyl-5-(phenoxyethyl)-2-oxazolidinone	13.49
8	16.308	10-Octadecenoic acid, methyl ester	21.77	20.832	Bicyclo[4.1.0]hept-2-ene	5.18
9	16.365	trans-13-Octadecenoic acid, methyl ester	13.67			
10	16.541	Heptadecanoic acid, 15-methyl-, methyl ester	12.51			
11	16.707	3-Tridecen-1-yne, (E)-	5.46			
12	16.873	4,7-Methano-1H-inden-1-ol, octahydro-	1.91			
13	17.101	Metaraminol	1.01			
14	18.124	3,8-Dioxatricyclo[5.1.0.0(2,4)]octane, 4-ethenyl-	4.98			
15	18.305	N-Acrylonitrylaziridine	1.67			
16	20.542	Borazine, 2,4-dimethyl-	1.23			

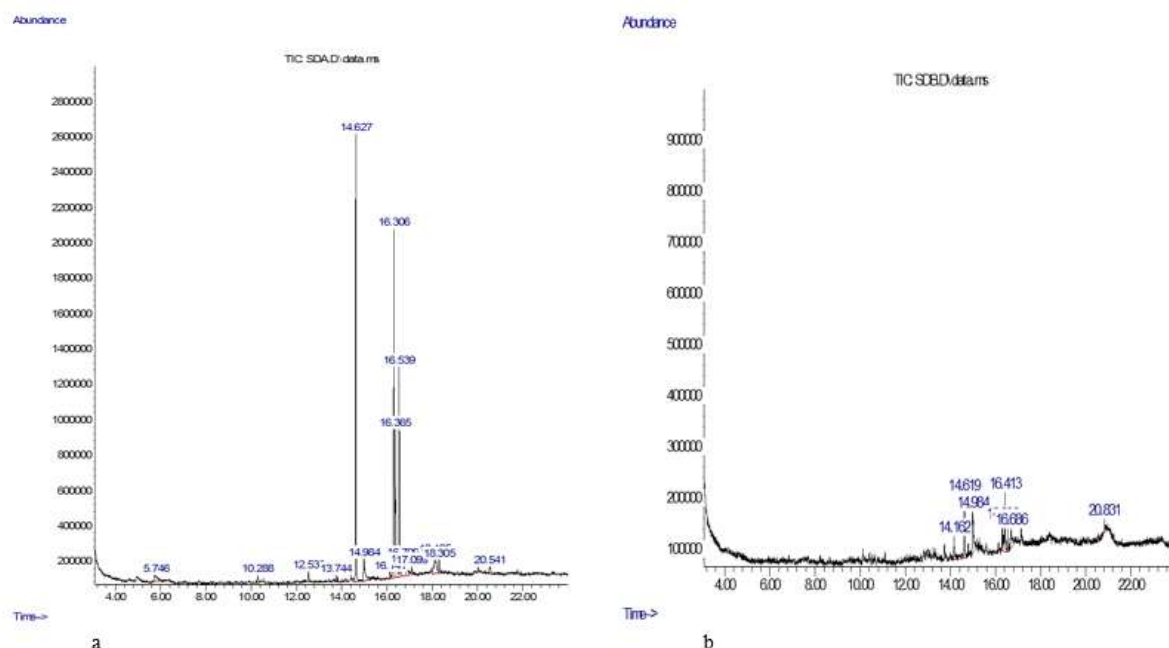


Figure 5: Chromatogram obtained from GC-MS with methanol (a) and n-hexane (b) extracts of *D. filix-mas* senesced frond

In the root of *Dryopteris filix-mas*, methanol extracts were dominated by pentadecanoic acid, 14-methyl-, methyl ester (31.53%), while 9,12-octadecadienoic acid (Z,Z)-, methyl ester occurred at the lowest proportion (4.62%). In

contrast, the n-hexane extract showed hexadecanoic acid, methyl ester as the most abundant compound (28.43%), whereas hentriacontane was present at the lowest proportion (1.66%) (Table 8; Figure 6).

Table 8: Major bio-compounds identified from *D. filix-mas* root

METHANOL				N-HEXANE		
PEAK#	RT(min)	COMPOUND	%TOTAL	RT(min)	COMPOUND	%TOTAL
1	14.637	Pentadecanoic acid, 14-methyl-, methyl ester	31.53	12.364	Hentriacontane	1.66
2	16.256	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	4.62	12.836	Nonyl tetradecyl ether	3.19
3	16.318	9-Octadecenoic acid (Z)-, methyl ester	26.75	13.246	5-Octadecene, (E)-	3.21
4	16.375	6-Octadecenoic acid, methyl ester,	12.55	13.314	1-Iodo-2-methylundecane	3.45
5	16.546	Methyl stearate	16.24	14.248	Nonanoic acid, 9-oxo-, methyl ester	2.86
6	17.838	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	8.31	14.621	Hexadecanoic acid, methyl ester	28.43
7				15.01	Carbonic acid, decyl undecyl ester	6.53
8				15.265	Cyclotetradecane	2.48
9				15.327	1-Decanol, 2-hexyl-	2.98
10				16.131	Undec-10-ynoic acid, dodecyl ester	2.26
11				16.245	9,12-Octadecadienoic acid, methyl	7.51
12				16.297	9-Oxabicyclo[6.1.0]nonan	9.42
13				16.411	endo-2-Methyl-2-norbornanol	2.46
14				16.531	Heptadecanoic acid, 16-methyl-, methyl ester	12.6
15				17.132	Cyclopropane, 1-hexyl-2-propyl-, cis-	4.57
16				17.267	9-Octadecene, (E)-	6.4

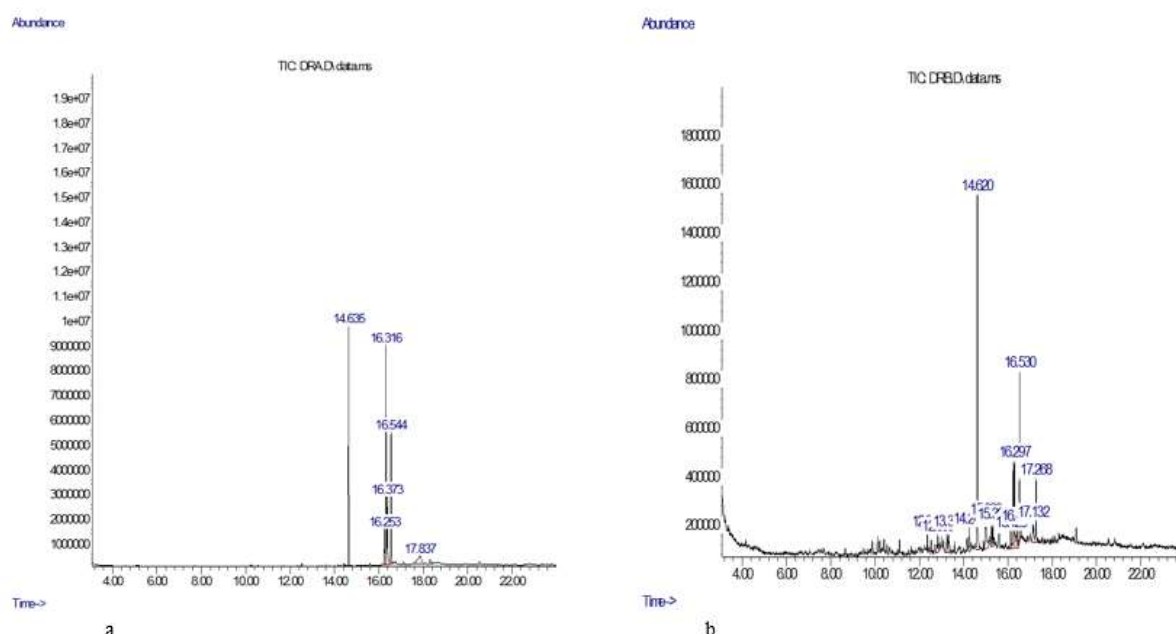


Figure 6: Chromatogram obtained from GC-MS with methanol (a) and n-hexane (b) extracts of *D. filix-mas* root

3.1.4 Antioxidant Activity

The antioxidant capacity of *D. filix-mas* was evaluated using DPPH radical scavenging (Table 9), and FRAP reducing power assays (Figures 9). The results demonstrated notable variability across phenological stages.

3.1.4.1 DPPH Radical Scavenging Activity

The DPPH assay revealed variations in antioxidant activity among the phenological stages of *Dryopteris filix-mas*, as indicated by their half-maximal inhibitory concentration (IC_{50}) values (Table 9) and percentage radical scavenging activity (%RSA) (Figure 7). Lower IC_{50} values correspond to stronger antioxidant activity.

Among the samples, the mature vegetative frond exhibited the lowest IC_{50} value ($\approx 24.40 \mu\text{g/mL}$), followed by the fertile frond ($\approx 26.53 \mu\text{g/mL}$), young fiddle head ($\approx 28.53 \mu\text{g/mL}$), senesced frond ($\approx 29.87 \mu\text{g/mL}$), root ($\approx 33.17 \mu\text{g/mL}$), and mature fiddle head ($\approx 37.07 \mu\text{g/mL}$). The ascorbic acid control recorded an IC_{50} value of $34.06 \mu\text{g/mL}$.

Percentage radical scavenging activity increased with concentration across all samples. At $100 \mu\text{g/mL}$, the mature vegetative frond recorded the highest %RSA (80.4%), followed by the fertile frond (79.0%), while ascorbic acid showed 74.6%. Other phenological stages recorded values ranging from 72.9% to 77.8%.

Table 9: IC_{50} values of As A (Control), and crude extracts of *D. filix-mas* sampled at different phenological stages and root against DPPH

SAMPLES	IC_{50} ($\mu\text{g/mL}$)
Ascorbic acid (Control)	34.06 ± 0.20
Young fiddle head	28.53 ± 0.08
Mature fiddle head	37.07 ± 0.72
Mature vegetative frond	24.40 ± 0.08
Fertile frond	26.53 ± 0.08
Senesce frond	29.87 ± 0.06
Root	33.17 ± 0.08

*The values are expressed as Mean $\pm$ SEM of triplicate determinations.

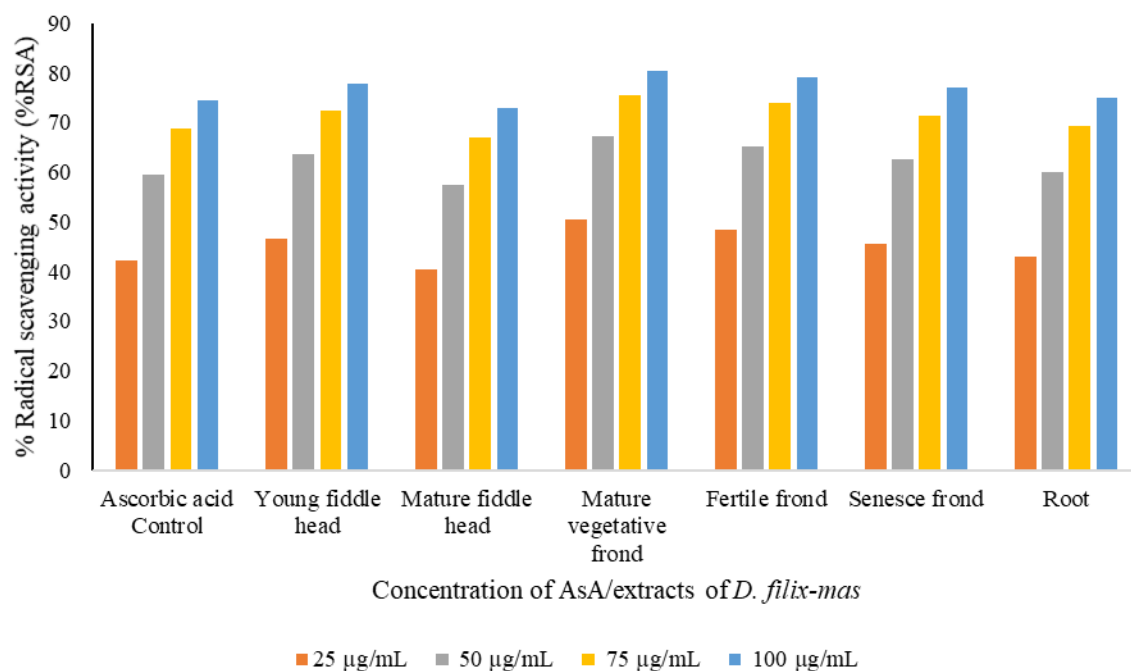


Figure 7: Percentage Radical Scavenging Activity (%RSA) of AsA (Control), and crude extracts of *D. filix-mas* sampled at different phenological stages and root

3.1.4.2 Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay showed that ferric reducing power increased with extract concentration across all phenological stages of *Dryopteris filix-mas*, indicating a dose-dependent response (Figure 8).

Among the samples, the fertile frond exhibited the highest reducing activity, reaching an absorbance value of 0.54 at 100 µg/mL. This was closely followed by ascorbic acid (0.55), the senesced frond (0.42), and the matured vegetative frond (0.35). The matured fiddle head showed intermediate activity, peaking at 0.40, while the roots (0.38) and young fiddle heads (0.32) exhibited comparatively lower reducing power.

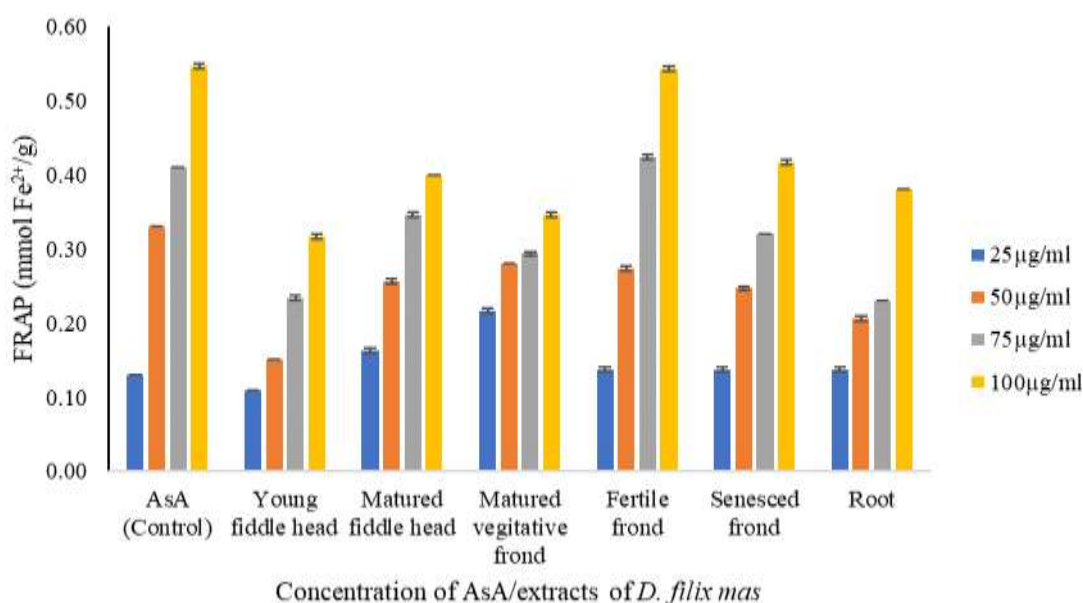


Figure 8: FRAP values of AsA (Control), and crude extracts of *D. filix-mas* sampled at different phenological stages and root

3.2 Discussion

3.2.1 Preliminary Qualitative Phytochemical Screening

Qualitative phytochemical screening of *Dryopteris filix-mas* across phenological stages and extraction solvents revealed the presence of a broad spectrum of secondary metabolites, with distribution patterns that were both developmentally regulated and solvent-dependent. Alkaloids, saponins, and reducing sugars were consistently present across all phenological stages and solvent systems, suggesting their involvement in plant metabolism and defense, as widely reported in medicinal plants (Sofowora, 2008; Harborne, 1998). Terpenoids and anthraquinones were also widely distributed, although their absence in fertile fronds under specific solvent conditions suggests possible metabolic shifts associated with reproductive development.

Phenolic compounds, including tannins and phenols, were largely confined to matured adult, fertile, senesced, and root tissues, while absent in early developmental stages (young and matured fiddle heads), particularly in aqueous and hexane extracts. This distribution suggests increased synthesis and accumulation of phenolic defenses in later developmental stages, consistent with their roles in protection against herbivory and oxidative stress (Barbehenn and Constabel, 2011; Treutter, 2005). Flavonoids showed selective occurrence, being absent in matured fiddle heads across all solvent systems, indicating developmental regulation of their biosynthesis, which has been linked to environmental adaptation and physiological function in plants (Agati *et al.*, 2012; Ferreyra *et al.*, 2021).

Steroids were not detected in aqueous extracts but were consistently present in hexane extracts across all phenological stages, reflecting their hydrophobic nature and preferential solubility in non-polar solvents, in agreement with established extraction principles (Azwanida, 2015). Phlobatannins and cardiac glycosides exhibited stage- and solvent-dependent occurrence, with absence observed in fertile fronds under certain extraction conditions, suggesting possible metabolic adjustments during reproduction. The qualitative profile indicates that metabolite occurrence is governed by both phenological stage and solvent polarity, supporting patterns widely documented in phytochemical studies of medicinal plants (Wink, 2010; Nwozo *et al.*, 2023; Osinaike *et al.*, 2026).

3.2.2 Quantitative Phytochemical Analysis

The quantitative phytochemical analysis revealed variation in secondary metabolite composition as influenced by both developmental stage and extraction solvent.

Methanolic extracts consistently yielded the highest concentrations of most polar and moderately polar phytochemicals, aqueous extracts favored highly water-soluble compounds such as reducing sugars and glycosidic constituents, while n-hexane extracts selectively recovered non-polar metabolites, notably steroids and certain alkaloids. This extraction pattern underscores the strong influence of solvent polarity on phytochemical recovery and aligns with established reports on medicinal plants and pteridophytes (Nwozo *et al.*, 2023; Azwanida, 2015).

Tannins content showed marked variation across phenological stages and solvents. Methanolic extracts recorded the highest tannins concentration, with fertile fronds exhibiting the maximum value, followed by matured adult fronds and roots. Aqueous extracts displayed moderate tannin levels, particularly in fertile fronds and roots, while tannins were absent in young and matured fiddle heads under aqueous extraction. This absence in early developmental stages may indicate limited synthesis or allocation of complex secondary metabolites during rapid tissue expansion, whereas their accumulation in matured and fertile fronds could reflect increased investment in chemical defense and protection against biotic stress. The reduced tannin levels observed in senesced fronds may suggest declining biosynthetic activity associated with tissue aging and nutrient remobilization (Tyagi *et al.*, 2020; Barbehenn and Constabel, 2011).

Alkaloids were widely distributed across most phenological stages and extraction solvents. Methanolic extracts of roots and young fiddle heads showed particularly high concentrations, while n-hexane extracts also recorded substantial alkaloid levels in matured fiddle heads and young fiddle heads. The absence of alkaloids in the n-hexane extract of fertile fronds suggests a developmental shift toward more polar alkaloid forms at the reproductive stage. High level of alkaloids in roots and young tissues are consistent with their defensive role against herbivores and pathogens and reflect enhanced biosynthesis during periods of active growth and physiological vulnerability (Ali *et al.*, 2019; Wink, 2010; Ziegler and Facchini, 2008).

Saponins were among the most abundant phytochemicals detected across all stages. Aqueous extracts showed exceptionally high saponin concentrations in young fiddle heads and senesced fronds, while methanolic extracts recorded maximum levels in roots and matured fiddle heads. Substantial quantities were also detected in n-hexane extracts, except in fertile fronds where saponins were absent. Their widespread distribution suggests multifunctional roles in antimicrobial defense, membrane stabilization, and deterrence of herbivores. Accumulation in senesced fronds may represent residual storage, whereas high root concentrations emphasize their protective role in subterranean tissues (Shakeel *et al.*, 2025; Augustin *et al.*, 2011).

Flavonoids content exhibited strong dependence on phenological stage and solvent type. The highest concentration was recorded in the aqueous extract of fertile fronds, followed by methanolic and n-hexane extracts of matured adult fronds. Flavonoids were completely absent in matured fiddle heads across all solvents, indicating strict developmental regulation. Their enrichment in fertile fronds suggests enhanced antioxidant and UV-protective functions during spore formation and dispersal, consistent with previous observations in ferns and higher plants (Ferreira *et al.*, 2021; Agati *et al.*, 2012; Treutter, 2005).

Steroids were not detected in aqueous extracts across any phenological stage, confirming their hydrophobic nature. Methanolic extracts revealed steroids only in matured fiddle heads, whereas n-hexane extracts consistently recorded high concentrations across all stages, with senesced fronds and matured fiddle heads showing peak values. This extraction trend is consistent with the hydrophobic nature of steroidal compounds and corroborates previous reports that plant steroids are preferentially extracted using non-polar solvents. The persistence of steroids in senesced tissues suggests limited degradation during aging and possible structural or signaling roles (Piironen *et al.*, 2002).

Terpenoids showed complex variation across developmental stages and solvents. Methanolic extracts of matured fiddle heads and senesced fronds exhibited the highest concentrations, while aqueous extracts showed moderate levels mainly in young fiddle heads. N-hexane extracts yielded moderate but consistent terpenoid levels across most tissues. These variations suggest dynamic regulation of terpenoid biosynthesis in response to developmental cues and physiological demands, with elevated levels in young and senescing tissues reflecting roles in defense, signaling, and stress adaptation (Shan and Jin,

2025; El Amerany, 2024; Gershenzon and Dudareva, 2007).

Cardiac glycosides were predominantly recovered in aqueous and methanolic extracts. The highest aqueous concentration occurred in roots, while methanolic extracts of young fiddle heads recorded the maximum value. N-hexane extracts showed detectable levels only in matured fiddle heads and senesced fronds. Their prevalence in roots and young tissues supports their role as potent chemical defenses, whereas reduced levels in fertile fronds may reflect metabolic trade-offs associated with reproductive development (Morsy, 2014).

Reducing sugar were most abundant in aqueous extracts, with fertile fronds recording the highest concentration, followed by methanolic extracts of the same stage. Moderate levels were observed in young fiddle heads and roots, while n-hexane extracts yielded negligible amounts. This distribution reflects the high water solubility of free sugars and their central role in energy metabolism. Elevated sugar levels in fertile fronds likely support sporangial development and spore maturation, consistent with the critical importance of carbohydrates in plant reproduction and stress tolerance (Taiz *et al.*, 2015).

3.2.3 Phytochemical Compounds Profile and Identification by GC–MS Analysis

GC–MS analysis of *Dryopteris filix-mas* across its phenological stages revealed a complex and diverse array of phytochemicals, encompassing fatty acids, alcohols, esters, hydrocarbons, nitrogen-containing compounds, and epoxy or cyclic derivatives. Methanol and n-hexane extracts exhibited distinct compound profiles, reflecting solvent polarity and selective extraction of bioactive constituents, as well as developmental regulation of metabolite accumulation (Nwozo *et al.*, 2023; Wink, 2010; Azwanida, 2015).

In the young fiddlehead, methanol extracts were dominated by pentadecanoic acid, 14-methyl-, methyl ester, while 3-cyclopentylcyclopentene was the least abundant. In the n-hexane extract, ethyl 2-octynoate was most abundant, with 5-chloro-1-pentyne as the least. These observations suggest that young fiddleheads accumulate fatty acid derivatives and aliphatic esters to support energy provision and chemical defense during rapid growth, while minor compounds may serve antimicrobial or signaling roles, consistent with patterns reported in ferns and other medicinal plants (Wink, 2010; Gershenzon and Dudareva, 2007; Treutter, 2005).

In mature fiddleheads, methanol extracts showed exo-norbornyl alcohol as the highest compound and N-acrylonitrilaziridine as the lowest. For n-hexane, a 1H-indole-2-carboxylic acid derivative dominated, whereas 2-norbornyl acetate was minimal. The enrichment of cyclic and nitrogen-containing compounds in mature fiddleheads reflects enhanced protective and signaling functions required for tissue maturation and defense against herbivory and oxidative stress (Wink, 2010; Gershenzon and Dudareva, 2007).

For mature vegetative fronds, methanol extraction revealed hexadecanoic acid, methyl ester as the most abundant compound, and 7-oxabicyclo[4.1.0]heptane, 1-methyl-4-(1-methylethenyl)- as the least abundant. The n-hexane extract was dominated by 1,2-epoxyundecane, with 4,7-methanoisobenzofuran-1-ol, hexahydro- as the lowest. These patterns indicate that mature vegetative fronds prioritize accumulation of structural fatty acids, epoxy derivatives, and hydrocarbons, likely contributing to membrane stability, energy storage, and defense against biotic stressors (Shiade *et al.*, 2024; Okazaki and Saito, 2014).

In fertile fronds, pentadecanoic acid, 14-methyl-, methyl ester was highest in methanol extracts, whereas 3,4-octadiene, 7-methyl was lowest. In n-hexane, 1-dodecanol, 2-methyl- (S)-predominated, while phenol, 2,5-bis(1,1-dimethylethyl) was minimal. This enrichment of fatty acids, alcohols, and methyl esters supports reproductive development, antioxidant protection, and sporangial maturation, aligning with observations in other ferns and angiosperms (Wang *et al.*, 2024; Agati *et al.*, 2012; Gershenzon and Dudareva, 2007).

In senesced fronds, methanol extracts were highest in pentadecanoic acid, 14-methyl-, methyl ester and lowest in 5-vinyl-pyrazole, while n-hexane extracts showed 9-oxabicyclo[6.1.0]nonane as the most abundant and a 1H-pyrrolo[2,3-d]pyrimidine derivative as the least abundant. The persistence of fatty acids and lipophilic compounds in senesced fronds reflects residual storage and protective functions during tissue aging, despite a decline in active biosynthesis (Wang *et al.*, 2024; He and Ding, 2020).

Root extracts in methanol were dominated by pentadecanoic acid, 14-methyl-, methyl ester, with 9,12-octadecadienoic acid (Z,Z)-, methyl ester being the least abundant. In n-hexane, hexadecanoic acid, methyl ester predominated, whereas hentriacontane

was the lowest. Roots serve as metabolic reservoirs and defensive tissues, storing fatty

acids and hydrocarbons that provide energy and protection against soil-borne pathogens (He and Ding, 2020; Wink, 2015).

3.2.4 Antioxidant Activity

The antioxidant capacity of *D. filix-mas* was evaluated using DPPH radical scavenging, and FRAP reducing power assays. The results demonstrated notable variability across phenological stages.

3.2.4.1 DPPH Radical Scavenging Activity

The DPPH assay revealed notable variations in antioxidant potential among the phenological stages of *D. filix-mas*, as reflected by differences in half-maximal inhibitory concentration (IC_{50}) values and percentage radical scavenging activity (%RSA). Lower IC_{50} values indicate stronger radical scavenging activity. Among the samples, the mature vegetative frond exhibited the strongest antioxidant activity, followed by the fertile frond, young fiddle head, senesced frond, root, and mature fiddle head. The ascorbic acid control showed a comparatively higher IC_{50} value, indicating that several phenological stages exhibited antioxidant capacities comparable to or greater than the standard antioxidant.

These trends were further supported by %RSA values across the tested concentrations, which increased progressively with concentration for all samples. The mature vegetative frond consistently demonstrated the highest radical scavenging activity at all concentrations, followed closely by the fertile frond. In comparison, ascorbic acid showed lower inhibition at equivalent concentrations. Other phenological stages exhibited moderate scavenging activity, with %RSA values showing relatively close ranges at the highest concentration.

The enhanced antioxidant activity observed in the mature vegetative and fertile fronds may be attributed to the accumulation of bioactive secondary metabolites, including fatty acids, terpenoids, epoxy compounds, and other polar and moderately polar constituents identified through GC-MS analysis. These compounds may act synergistically to mitigate oxidative stress, suggesting that antioxidant potential in *D. filix-mas* is developmentally regulated, with peak activity occurring in tissues supporting active growth and reproductive processes (Wang *et al.*, 2024; He and Ding, 2020; Agati *et al.*, 2012).

3.2.4.2 Ferric Reducing Antioxidant Power (FRAP)

FRAP values increased with extract concentration for all phenological stages,

confirming dose-dependent enhancement of reducing power. Among the samples, the fertile frond exhibited the highest ferric reducing activity, closely followed by the ascorbic acid control, senesced frond, and mature vegetative frond. Young fiddle heads and roots displayed comparatively lower reducing activity across concentrations, while matured fiddle heads showed intermediate reducing power at higher concentration levels.

These observations align with the DPPH results, suggesting that antioxidant potential in *D. filix-mas* is developmentally regulated and correlates with the accumulation of electron-donating bioactive compounds, such as fatty acid methyl esters, epoxy derivatives, and terpenoids (Edewor *et al.*, 2024; Akinyede *et al.*, 2022). The higher reducing power in fertile and mature vegetative fronds highlights their functional role in protecting actively growing and reproductive tissues against oxidative stress, whereas moderate FRAP values in senesced fronds reflect residual activity from previously synthesized metabolites.

4. Conclusion

This study demonstrates that *Dryopteris filix-mas* exhibits significant phenological stage-dependent variation in phytochemical composition and antioxidant activity. Quantitative phytochemical analysis showed that the accumulation of secondary metabolites is influenced by both developmental stage and extraction solvent, with

mature vegetative and fertile fronds generally exhibiting higher levels of several bioactive constituents. The observed distribution patterns of tannins, alkaloids, saponins, flavonoids, terpenoids, cardiac glycosides, and reducing sugars suggest that secondary metabolite biosynthesis in *D. filix-mas* is developmentally regulated and linked to physiological processes associated with growth, reproduction, and senescence. GC–MS profiling further confirmed qualitative differences in phytochemical constituents across phenological stages, reflecting dynamic metabolic adjustments during plant development. Antioxidant assays revealed marked variability among the stages, with lower IC₅₀ values and higher % radical scavenging and ferric reducing antioxidant power responses in mature vegetative and fertile fronds, indicating stronger antioxidant potential compared to younger or senesced tissues. The findings emphasize the importance of phenological stage selection in phytochemical and antioxidant evaluations of ferns and highlight the potential of *D. filix-mas* as a promising source of natural antioxidant compounds, supporting its prospective application in nutraceutical and pharmacological formulations. Further studies involving compound isolation, mechanistic antioxidant assays, and in vivo evaluations are recommended to better elucidate the functional significance and therapeutic potential of the identified metabolites.

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Conflict of Interest

The author declares that there is no conflict of interest.

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