



Extraction and preliminary investigation of the essential oil from *Adiantum lunulatum* Burm.f. via GC-HRT-MS: Exploring its antioxidant properties, potential inhibition of diabetes, and colorectal cancer

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Abstract

Adiantum lunulatum Burm.f. is a rich source of essential oils (EO) with various medicinal properties, including antimicrobial, anti-inflammatory, antioxidant, and wound healing potentials. The plant has also been suggested to exhibit anticancer and antiviral activities, although the efficacy and mechanism of action are very limited. This work is set out to first investigate the chemical constituents present in the *n*-hexane fraction (EO) obtained from a 50% aqueous-ethanol crude extract using GC-HRT-MS analytical techniques, and secondly to present an updated and categorized summary on the potential biological activities of the (EO) when compared to the crude ethanolic extract. Analysis shows 2,4-decadienal isomers and *n*-hexadecanoic acid are the most abundant chemical constituents in this fraction, with a total of 43.32% for the (E,E) isomers and 6.19% for the *n*-hexadecanoic acid, respectively. Antidiabetic, antioxidant, and cytotoxicity tests of the crude ethanolic extract (AEE) and *n*-hexane fraction (EO) revealed that AEE has far better bioactivity than the *n*-hexane fraction. Both extracts demonstrated lower activity against standard ascorbic acid for α -amylase and α -glucosidase. In addition, both extracts show moderate inhibitory effects on Caco-2 cells, a lower toxicity profile toward normal embryonic cells (Hek293-T), and significant efficacy against the HCT116 cells. The extracts also showed a non-toxicity property on HEK293-T cells in contrast to the positive control, doxorubicin.

Introduction

Over the past years, various biological and pharmacological activities like antifertility, anti-ulcerative, antipyretic, and antimicrobial properties are linked to the use of Adiantaceae plant family (a family of fern 5 allies) based on the presence

of diverse phytochemically active metabolites. Among the active phytochemicals previously isolated from this plant family are phenylpropanoids, carotenoids, alicyclic acids, lipids, essential oils, steroids, phenolic compounds, tannins, triterpenoids, and flavonoids. Pteridophytes, also referred to as Ferns (Fern 5 allies) of which the plant family Adiantaceae belongs in general, are common vascular plants which are known to be underused and scantily explored when compared to other vascular plants [1]. Major research work over the last two decades on pteridophyte plants shows quite a very few plants in the group have been examined for phytochemical constituents and a further few compounds were isolated.

Among the few studied pteridophytes is *Adiantum lunulatum* Burm.f. The plant has been used in traditional medicine to treat various human illnesses such as asthma, abdominal cramps, flu, and other respiratory problems. Other reports have shown the usefulness of the plant to have antioxidant, anti-inflammatory, hepatoprotective effects, wound healing effect, anti-diarrheal, antipyretic (fever-reducing), and hypotensive

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effects [2–6]. *A. lunulatum* is a chemically rich plant containing diverse variety of secondary metabolites which support its medicinal use. Phytochemical compounds such as tannins, saponins, alkaloids, phenolic flavonoids, glycosides, triterpenoids, phenyl propanoids, steroids, alicyclic acids, alicyclic acids, lipids, and long-chain have all been identified or isolated from this plant [7].

Colorectal cancer is the third most frequently diagnosed cancer globally and serves as the second leading cause of cancer-related deaths [8, 9]. There has been a significant increase in incidence rates, particularly in developing countries such as Africa [10]. Recently, a concerning trend has emerged, the co-infection of colorectal cancer and diabetes, specifically type 2 diabetes mellitus (T2DM). Research indicates that individuals with T2DM face a 20–40% higher risk of developing colorectal cancer (CRC) and tend to experience a worse prognosis upon diagnosis compared to those without diabetes [11]. In Africa, T2DM represents a significant portion of the disease burden, and various lifestyle factors have been associated with elevated risks of both colorectal cancer and diabetes. These factors include overweight and obesity, alcohol consumption, a high intake of red and processed meats, low dietary fiber, tobacco use, and insufficient physical activity [12]. Despite these connections, the specific role of type 2 diabetes in early-onset CRC remains inadequately understood. Thus, investigating the mechanisms by which diabetes and colorectal cancer interact is crucial for developing effective therapies. Colorectal cancer originates in the tissues of the colon. While advancements in treatment have been made, current therapies often have limited benefits or have adverse side effects [13, 14]. Therefore, there is an urgent need for innovative strategies to address both colorectal cancer and diabetes.

The increasing prevalence of diabetes is becoming a significant global health challenge, particularly due to its potential impact on colorectal cancer risk. Given the importance of understanding any differences in this association, this study aimed to explore the effects of *A. lunulatum* aqueous-ethanolic extract (AEE) and its *n*-hexane fraction (EO). We assessed the ability of these fractions to inhibit key enzymes, specifically α -amylase and α -glucosidase, to evaluate their antidiabetic activity. Additionally, we investigated their anticancer properties on colorectal adenocarcinoma (CaCo-2) and human colorectal carcinoma (HCT116) cell lines, alongside the cytotoxicity of the (AEE), and (EO) was determined to validate their safety in normal somatic (HEK293) cells. Their antioxidant capabilities using DPPH and ABTS free radical test methods were also evaluated.

Materials and method

Reagents and chemicals

The chemicals used in this study were purchased from Merck (Darmstadt, Germany). Aqueous solutions were prepared using ultra-pure water from a Milli-Q water purification system (Millipore, Molsheim, France). The human colorectal adenocarcinoma (Caco-2) and human embryonic kidney (HEK293-T) cells were purchased from Cellonex Separation Scientific SA (Pty) Ltd. (Roodepoort, South Africa), and Dulbecco's modified eagle's medium, fetal bovine serum, and penicillin–streptomycin were obtained from Celtic Molecular Diagnostics SA (Pty) Ltd. (Cape Town, South Africa). Dimethyl sulfoxide (DMSO) was purchased from Sigma-Aldrich® Darmstadt, Germany.

Instrumentation

The *A. lunulatum*, *n*-hexane fraction (EO), was analyzed by LECO Pegasus 4D GC-HRT-MS (High Resolution Time-of-Flight Mass Spectrometer) equipped with a modulator, a secondary oven (LECO Corporation, St Joseph, USA), and an Agilent 7890A gas Chromatograph (Agilent Technologies, Inc., Wilmington, DE, USA). Peaks were identified with the assistance of spectra contained in the mass spectral database (RepLib and MATLAB). VarioScan Flash (Thermo Fisher Scientific, Vantaa, Finland) was used for biological evaluation of the antioxidant and antidiabetic assays. A Scepter 3.0™, Merck, Burlington, MA, USA was used for the evaluation of the cytotoxicity experiment.

Preparation and extraction of plant material

From its natural habitat, the plant material was collected on Obafemi Awolowo University campus, Ile-Ife, Nigeria in the month of April, 2024, and a voucher specimen with herbarium number FPI 2499 was deposited at the Herbarium of the Faculty of Pharmacy. The extraction method followed as reported by Adeloye et al. [15]. The leaf of *A. lunulatum* collected was dried in the oven for two weeks and later ground to fine powder to obtain 253 g. A 60 g of the dried *A. lunulatum* leaves was then soaked in 50% aqueous-ethanol (2.0 L) for 72 h, while occasional agitation of the jar took place. The extraction was carried out at room temperature. The green solution obtained was filtered using cotton wool and then concentrated to dryness in a rotary evaporator to afford the crude extract weighing 5.20 g. The crude extract (3.50 g) was redissolved in 200 mL water and successively extracted in 300 mL volume of the following solvents: *n*-hexane, dichloromethane, ethyl acetate and *n*-butanol. Each of the

solvent filtrates was concentrated *in vacuo* to afford the *n*-hexane fraction (0.23 g), dichloromethane (0.27 g), ethyl acetate (0.28 g), and *n*-butanol (0.51 g). A sample of the crude ethanolic extract and the *n*-hexane was taken for further analysis and biological work as reported in this work.

Analysis of *A. lunulatum* *n*-hexane fraction (EO)

A LECO Pegasus GC-HRT-MS equipment on an Rxi®-5Sil MS (30 m × 0.25 mm × 0.25 µm) column (Restek, Bellefonte, PA, USA) was used for the analysis of the *n*-hexane fraction (EO) of *A. lunulatum*. Helium was used as the carrier gas with a flow rate of 1 mL/min and an inlet temperature of 250 °C. The temperature of the oven used was initially set at 40 °C and held for 2 min after which it was ramped at 5 °C/min up to 120 °C initially and held for 4 min. Later, the temperature was ramped further for 2 min at a temperature of 5 °C/min up to 280 °C. Parameters such as retention time, peak detection, and peak matching were carried out using ChromaTOF software (LECO, St Joseph, USA).

Biological activities

α-amylase and α-glucosidase inhibitory activity

The procedure of assaying the α-amylase and α-glucosidase inhibitory activities of the crude aqueous-ethanolic extract (AEE) and *n*-hexane (EO) fraction of *A. lunulatum* is as follows: a buffer solution at 127 mM containing different concentrations of the samples 0.78, 1.56, 3.12, 6.25, 12.5, 25, 50, and 100 µg/mL was prepared in a 96-well plate while maintaining the buffer pH at 6.8. The reaction mixture containing α-glucosidase (0.5 µg/mL, 17 µL), phosphate buffer (100 mM, 50 µL), and the test sample in DMSO (17 µL) was added to the 96-well plate. The α-glucosidase and α-amylase standard inhibitor (acarbose) were used as a positive control at the same concentrations. The mixture was incubated at 37 °C for 10 min. 17 µL of 2 mM PNP-G was further added to each well, and the absorbance was measured at 400 nm [16].

Antioxidant

DPPH radical scavenging activity A 1,1-diphenyl-2-picrylhydrazyl (DPPH) in methanol dilution general method was used to evaluate the antioxidant free radical scavenging activity of the aqueous-ethanolic extract (AEE) and *n*-hexane fraction (EO) of *A. lunulatum*. The extracts (AEE) and (EO) were prepared in a 96-well plate at the following concentrations: 0.78, 1.56, 3.12, 6.25, 12.5, 25, 50, and 100 µg/mL. The solutions were kept in the dark for 30 min before measurements were taken. Experiment was carried out in triplicates, while absorbance was taken at 517 nm using a

microplate reader. For this particular experiment, ascorbic acid was used as standard positive control, while % DPPH inhibitory activity [17] of the extract and fraction was calculated using the formula below:

$$\% \text{ DPPH Inhibitory} = [(Ac - As)/Ac] \times 100$$

where *Ac* = absorbance (control); *As* = absorbance (extracts).

ABTS radical scavenging activity According to the method as reported by Dzoyem et al. [18] with slight modifications to the preparation of the ABTS radical cation, the aqueous-ethanolic extract (AEE) and *n*-hexane fraction (EO) were subjected to the ABTS radical cation decolorization assay to determine the free radical scavenging properties of the two samples. Accordingly, the preparation of ABTS involves mixing of 10 mg ABTS reagent and 2 mg potassium persulfate in (20 mL) water at room temperature. The solution was allowed to stay in the dark for 12–16 h before taken out for use. To use this solution in the form of a DPPH assay (“DPPH radical scavenging activity” Sect.), 1 mL ABTS solution was prepared in 60 mL methanol and absorbance taken at 734 nm. Measurement was carried out in triplicates.

Using the general equation to calculate the effective concentration (IC₅₀) value:

$$\% \text{ ABTS Inhibitory} = [(Ac - As)/Ac \times 100],$$

where *Ac* = absorbance of the radical ABTS in methanol and *As* = absorbance of the radical ABTS and samples.

Cytotoxic activities

Mossman et al. [19] reported a method which employed the use of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) technique on Human Embryonic Kidney (HEK293-T), human colorectal adenocarcinoma (Caco-2) and human colorectal carcinoma (HCT116) cell lines was used to evaluate the cytotoxicity properties of *A. lunulatum* aqueous-ethanolic (AEE) extract and *n*-hexane fraction (EO). Accordingly, the cell culture was suspended in 100 µL glassware and then added by automation into 96-well cup plate of outside diameter 1 × 10⁵ cells/well determined by a handheld cell counter after which they were incubated for 24 h at 37 °C while treated in 5% carbon dioxide. The cell was further treated with the aqueous-ethanolic extract (AEE) and *n*-hexane fraction (EO) at different concentration range of 0.5, 1.0, 2.5, 5.0, 10.0, 25.0, 50.0, and 100.0 µg/mL. Due to its toxic nature, doxorubicin was used at the same concentrations as positive control for the experiment. A 20 µL of MTT solution containing 5 mg/mL PBS was then added to each plate and further incubated for 4 h. In order to dissolve any formazan that may be present as crystals,

dimethyl sulfoxide (100 μ L) was added and further incubated for another 1 h. To have a negative control result for the experiment, dissolution of untreated cells in 5% DMSO was prepared. Using an ELISA plate reader with a reference wavelength at 630 nm, each of the experiment was carried out in triplicates and the wavelength of plates each determined at 570 nm.

The cell viability percentage was determined by calculation using the general formula below:

$$\text{Cell viability \%} = At/Ab \times 100,$$

where At = absorbance value of test compound, and Ab = Absorbance value of the blank. A one-way ANOVA method was used to compute the dose–response curve from the IC_{50} calculated values.

Statistical analysis

Three replicates of the experimental absorbances were analyzed using a microplate reader (VarioSkan Flash, Thermo Fisher Scientific, Vantaa, Finland). The analysis of the data was conducted with GraphPad Prism version 8.2 software (GraphPad Software, CA, USA). A One-Way Analysis of Variance was utilized to compare the means, and the results were presented as the Mean $\pm$ SD.

Results and discussion

GC–MS analysis and structural activity relationship of *A. lunulatum* n-hexane fraction components

The *n*-hexane fraction (EO) of *A. lunulatum* was subjected to GC–MS analysis to obtain the chemical constituents present in the sample as depicted in Fig. 1, and a clear identification of about 60 different compounds was obtained (See Table 1 and SS1). Similarity index of the identified compound was found to be between 70 and 99%. It could be observed that 2,4-decadienal (E,E) with the total highest peak area was found to be the most abundant of the compounds at (36.9%), followed by 2,4-decadienal (E,Z) (2.44%) and 2,4-decadienal (E,E) (3.98%); this is followed by *n*-hexadecanoic acid at (6.19%). There are two isomers of 2-undecenal at (E) 4.14% and (Z) (3.16%); 8-heptadecene (1.84%), hexadecane (1.49%), hexadecenoic acid, ethyl ester (1.45%), and decanal (1.24%). The 2,4-dicadienal isomers were found to be the most abundant in the oil at percentage area of 43.32%. Both 2,4-dicadienal and *n*-hexadecanoic acid have been reported to have antioxidant properties [20–22]. 2,4-decadienal as a compound has been used as a flavoring agent as it possesses a citrus fruit like odor. Meanwhile, other compounds that were identified have percentage peak areas of less than 1%. In the total analysis, the identified compounds add up a percentage peak area of 72.97%. Hexadecanoic acid, ethyl ester; *n*-hexadecanoic acid, and neophytadiene were previously reported in the same species [23]. We are unable to identify other peak areas due to software limitation.

However, literature showed that 2,4-decadienal even though does not in particular has anticancer property, it is considered to be a potential pro-carcinogen and tumor

Fig. 1 Histogram of % chemical compositions of *n*-hexane fraction of *A. lunulatum* Burm.f

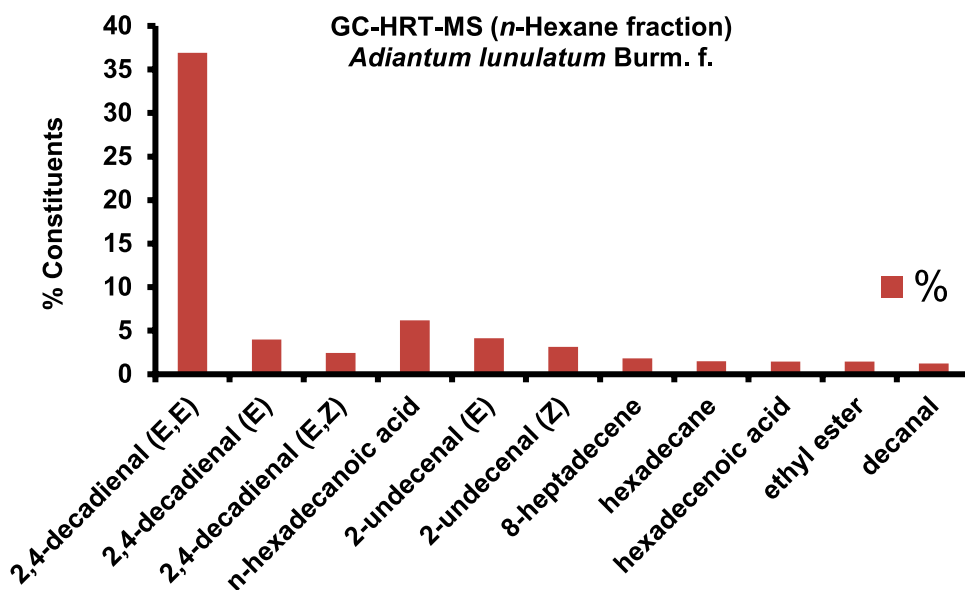


Table 1 GC-HRT-MS profile of the identified compounds, similarity index, retention time, formula and peak area percentage

R.T. (min:sec)	Name	Similarity	Formula	Area %
19:36,6	1-Butanamine	999	C4H11N	0,01
21:48,3	2-Oxetanone, 4-methyl-	999	C4H6O2	0,08
03:23,2	Heptane	956	C7H16	0,33
12:39,9	3H-1,2,4-Triazol-3-one, 1,2-dihydro-	955	C2H3N3O	0,01
32:37,8	8-Heptadecene	944	C17H34	1,84
33:37,6	Pentadecanal-	921	C15H30O	0,37
17:58,3	2-Undecenal	920	C11H20O	4,14
27:06,6	Hexadecane	913	C16H34	1,49
37:43,9	13-Octadecenal, (Z)-	905	C18H34O	0,63
05:18,8	Propargyl alcohol	897	C3H4O	0,04
26:07,3	Coumarin	896	C9H6O2	0,03
19:05,7	2-Undecenal	894	C11H20O	3,16
30:51,5	Pentadecanal-	887	C15H30O	0,18
27:20,9	Tridecanal	884	C13H26O	0,15
15:22,4	2,4-Decadienal, (E,E)-	883	C10H16O	36,9
22:45,6	1, 1, 5-Trimethyl-1, 2-dihydronaphthalene	862	C13H16	0,12
32:18,4	2,2'-Bipyrrolidine	854	C8H16N2	0,22
18:09,7	Trifluoroamine oxide	853	F3NO	0,27
03:21,4	DL-Lactamide, methyl ether	847	C4H9NO2	0,20
35:31,2	cis-9-Hexadecenal	847	C16H30O	0,38
33:13,3	Hexadecane	844	C16H34	0,89
17:35,2	2-Acetyl-2-methyltetrahydrofuran	843	C7H12O2	0,38
29:22,8	Benzeneethanol, α -methyl-	832	C9H12O	0,08
39:14,7	n-Hexadecanoic acid	831	C16H32O2	6,19
04:39,1	Benzeneethanol, α -methyl-	824	C9H12O	0,04
12:00,2	2-Aminocyanoacetamide	823	C3H5N3O	0,04
20:53,6	Propanoic acid, 2-methyl-, anhydride	819	C8H14O3	0,03
23:51,9	1-Methoxy-3-methyl-3-butene	814	C6H12O	0,20
36:01,8	Pentadecanal-	810	C15H30O	0,16
32:26,3	Cyclodecene, (Z)-	807	C10H18	0,69
09:54,1	2-Heptenal, (E)-	803	C7H12O	0,05
26:12,6	1-Tetradecene	800	C14H28	0,16
37:21,8	9-Octadecene, 1,1-dimethoxy-, (Z)-	800	C20H40O2	0,59
33:28,4	Benzenehexanenitrile, β,β -dimethyl- ϵ -oxo-	793	C14H17NO	0,08
29:36,7	Acetic acid, (dodecahydro-7-hydroxy-1,4b,8,8-tetramethyl-10-oxo-2(1H)-phenanthrenylidene)-,2-(dimethylamino)ethyl ester	775	C24H39NO4	0,04
38:13,0	Pentadecanal-	771	C15H30O	0,21
26:40,7	3,4-Hexanedione, 2,2,5-trimethyl-	765	C9H16O2	0,09
39:44,8	Hexadecanoic acid, ethyl ester	764	C18H36O2	1,45
24:27,8	Oxalic acid, allyl dodecyl ester	759	C17H30O4	0,17
11:10,1	Furan, 2-propyl-	758	C7H10O	0,06
35:22,2	Diazenecarboximidoyl bromide	758	CH2BrN3	0,19
33:04,1	Hexane, 3-ethyl-3-methyl-	754	C9H20	0,20
17:13,1	1H-Tetrazol-5-amine	747	CH3N5	0,08
20:23,8	2,4-Decadienal	741	C10H16O	2,44
39:59,1	Propanal, propylhydrazone	739	C6H14N2	0,09
30:47,0	3,5-Dimethyl-4-octanone	729	C10H20O	0,12
36:31,0	Neophytadiene	729	C20H38	0,13
25:42,8	Propanoic acid, 2-methyl-, anhydride	728	C8H14O3	0,19
25:15,6	2-Nonenal, (E)-	725	C9H16O	0,20

Table 1 (continued)

R.T. (min:sec)	Name	Similarity	Formula	Area %
32:23,8	8-Heptadecene	723	C17H34	0,28
27:45,5	2-Methyl-8-hydroxyquinoline, 2-methylpropionate	721	C14H15NO2	0,12
37:47,9	3H-1,2,4-Triazol-3-one, 1,2-dihydro-	721	C2H3N3O	0,12
26:52,8	Octane, 2,7-dimethyl-	717	C10H22	0,17
27:47,5	Methoxyflurane	715	C3H4Cl2F2O	0,20
37:53,0	2-Hexanone, 4-methyl-	714	C7H14O	0,28
19:23,4	2,4-Decadienal, (E,E)-	713	C10H16O	3,98
32:31,3	Benzene, decyl-	707	C16H26	0,18
28:07,6	1-Octadecyne	706	C18H34	0,43
35:38,8	2-Butanone, 3,3-dimethyl-1-thiocyanato-	706	C7H11NOS	0,18
31:48,9	Decanal	705	C10H20O	1,24

promoting agent based on inductive oxidative stress mechanisms and can as well be involved in inflammation, DNA damage, and promotion of uncontrolled cell proliferation at a higher concentration [24, 25]. Meanwhile, *n*-hexadecanoic acid, a common saturated fatty acid ubiquitous to both plants and animals, has been employed in human metabolism and cell membranes. Contradictory results have been recorded regarding its anticancer property, with reports citing both: (1), the induction of apoptosis and inhibition of various cancers (including prostate, gastric, breast, human colon (HCT-116), and human neuroblastoma cells), and (2), the cellular pathway modulation, which has been implicated in cell cycle arrest (predominantly in the G0/G1 phase) and the suppression of DNA replication and cell division [26].

Biological activity results

α -amylase and α -glucosidase inhibitory activity

The aqueous-ethanolic extract (AEE) and *n*-hexane (EO) of *A. lunulatum* were, respectively, screened for α -amylase and α -glucosidase inhibitory activities. As reported in Table 2, the AEE extract shows appreciable inhibitory activity with IC₅₀ values of 18.84 ± 0.011 μ g/mL and 10.94 ± 0.023 mM better than the *n*-hexane fraction (EO) at 50.01 ± 0.020 μ g/mL and 25.98 ± 0.022 μ g/mL. These results were compared to the standard reference which displayed high IC₅₀ = 2.90 ± 0.002 mM and 1.30 ± 0.002 μ g/mL. Based on

these results, literature shows that polarity of a chemical compound alone does not relate directly to the inhibitory activity of either α -amylase and α -glucosidase but are due to several factors of which the key point may be the specific molecular interactions between the inhibitor and the amino acid residues present in the enzyme's active site which is dictated by the structural patterns found in a specific molecule [27]. Thus, it has been reported that plants containing complex phytochemicals show excellent postprandial regulation of blood glucose level and possess the potential to inhibit α -amylase and α -glucosidase—two key important enzymes in the human digestion of starch [28].

Antioxidant activities

The antioxidant properties of the aqueous-ethanolic extract (AEE) and the *n*-hexane fraction (EO) of *A. lunulatum* were established using the DPPH and ABTS free radicals method of analysis, and values are compared to the standard ascorbic acid (Vitamin C)—a natural antioxidant agent used as the positive control. The dose–response curves used to calculate the IC₅₀ values against the DPPH and ABTS radicals are presented in Fig. 2. Table 2 summarizes the results obtained. It is observed that (AEE) gave higher inhibitory values for the DPPH and ABTS radical scavenging activity when compared to the *n*-hexane fraction (EO) at IC₅₀ values of 9.76 ± 0.083 and 7.71 ± 0.029 μ g/mL against (IC₅₀ = 18.48 ± 0.012 μ g/mL)

Table 2 IC₅₀ values of *A. lunulatum* aqueous ethanolic extract and essential oil on α -amylase, α -glucosidase inhibition, DPPH, ABTS free radical scavenging activities

Samples	IC ₅₀ values (μ g/mL $\pm$ SD)			
	α -amylase	α -glucosidase	DPPH	ABTS
<i>A. lunulatum</i> crude	18.84 ± 0.011	10.94 ± 0.023	9.76 ± 0.083	7.71 ± 0.029
<i>A. lunulatum</i> EO	50.01 ± 0.020	25.98 ± 0.022	18.48 ± 0.012	9.16 ± 0.017
Acarbose	2.90 ± 0.002	1.30 ± 0.002	–	–
Ascorbic Acid	–	–	4.01 ± 0.073	5.30 ± 0.019

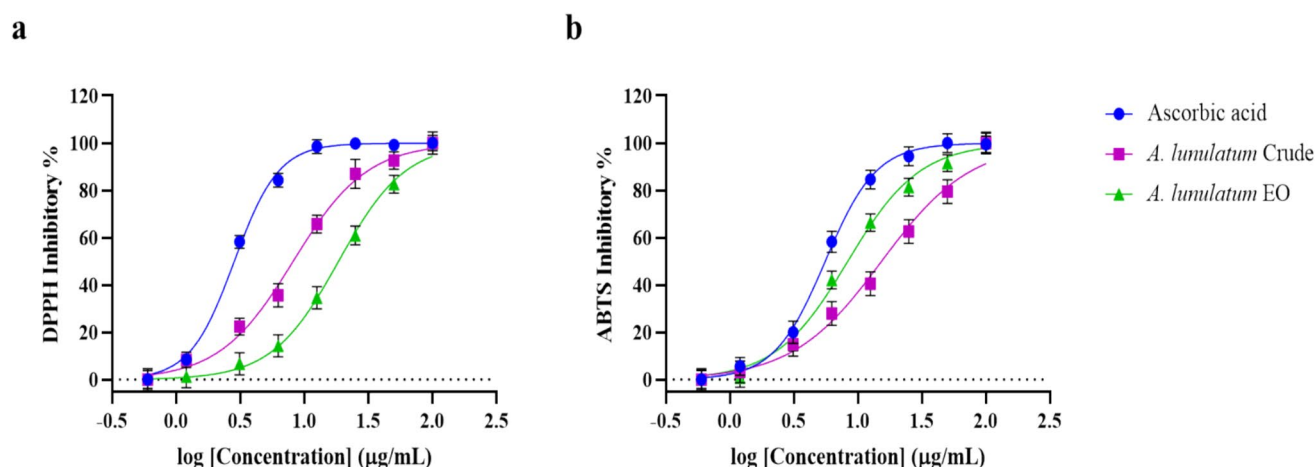


Fig. 2 The dose–response curves used to calculate the IC_{50} values representing the antioxidant activity of *A. lunulatum* against DPPH (a) and ABTS (b) free radical

and $9.16 \pm 0.017 \mu\text{g/mL}$, respectively. Both extracts (AEE) and (EO) showed lower activity against standard ascorbic acid ($\text{IC}_{50} = 4.01 \pm 0.073 \mu\text{g/mL}$ and $5.30 \pm 0.019 \mu\text{g/mL}$). Although ABTS assay works best for both hydrophilic and hydrophobic extracts, ascorbic acid being a polar compound is capable of releasing electron to become radical; we hypothesize that the moderate inhibitory property of EO toward DPPH may be due to the presence of certain polar chemical substituent groups inducing EO toward DPPH. In fact, the occurrence of lower percentage of compounds like coumarin (0.03%), 2-Oxetanone, 4-methyl- (0.08%), Trifluoroamine oxide (0.27%), 2-Aminocynoacetamide (0.04%) 2-Methyl-8-hydroxyquinoline, and 2-methylpropionate (0.12%) may have contributed to the slight antioxidant property shown by the fraction. In a similar experiment carried out on methanol and chloroform extracts of *A. lunulatum*,

against DPPH scavenging activity with IC_{50} values of 61.63 and 128.54 mM, respectively, the importance of extractant (solvent) favors the maximum extraction of polar compounds being better as antioxidant agents compared to the hexane, which had no or weak activity [29–31].

Cytotoxic activities

The MTT assay was employed to evaluate cell viability, specifically focusing on the effects of the aqueous-ethanolic extract (AEE) and the *n*-hexane fraction (EO) on Caco-2 and HCT116 cancer cells. The dose–response curves used to calculate the IC_{50} values against the DPPH and ABTS radicals are presented in Figs. 3. The results indicated that both extracts exhibited moderate inhibitory effects on Caco-2 cells, while demonstrating a reduced toxicity profile

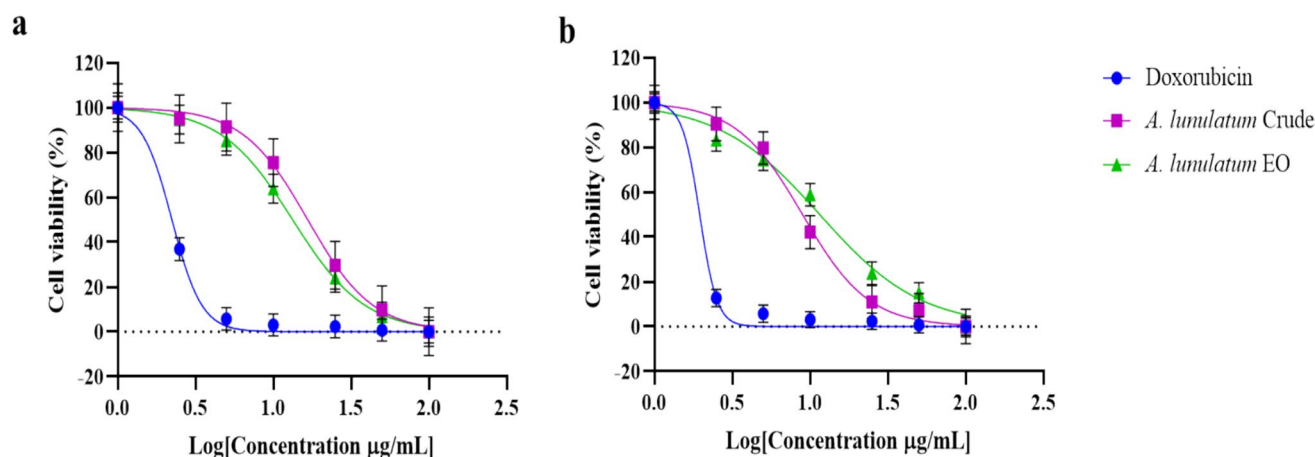


Fig. 3 The dose–response curves used to calculate the IC_{50} values representing the anticancer properties of *A. lunulatum* against Caco-2 (a) and HCT116 (b) cancer cell lines

Table 3 IC₅₀ values of *A. lunulatum* aqueous ethanolic extract (AEE) and essential oil (EO) evaluated for their cytotoxicity on HEK293-T and Caco-2 activities

Samples	IC ₅₀ values (μg/mL ± SD)	
	HEK293-T	Caco-2
<i>A. lunulatum</i> extract	28.59 ± 0.62	14.46 ± 0.19
<i>A. lunulatum</i> EO	40.36 ± 0.80	17.49 ± 0.22
Doxorubicin	1.30 ± 0.02	2.69 ± 0.13

toward normal embryonic cells (Hek293-T), as detailed in Table 3. When compared to the positive control, doxorubicin, which displayed an IC₅₀ value of 2.69 ± 0.13 μg/mL, the AEE and EO extracts were found to have IC₅₀ values of 14.46 ± 0.19 μg/mL and 17.49 ± 0.22 μg/mL, respectively, highlighting their relative cytotoxicity against Caco-2 cells. Additionally, both AEE and EO exhibited notable potency against the HCT116 cells, with IC₅₀ values recorded at 8.93 ± 0.09 μg/mL and 11.53 ± 0.15 μg/mL, respectively. In contrast, doxorubicin demonstrated a significantly lower IC₅₀ of 0.95 ± 0.02 μg/mL. Conversely, when assessing the toxicity of AEE and EO on HEK293-T cells, the extracts proved to be non-toxic, with IC₅₀ values of 28.59 ± 0.62 μg/mL and 40.36 ± 0.80 μg/mL, respectively. This is in contrast to the positive control doxorubicin, which had an IC₅₀ value of 1.30 ± 0.02 μg/mL. These findings underscore the selective inhibitory capacity of AEE and EO against cancerous cells while sparing normal embryonic cells from significant toxicity. It has been found out that extracts may be considered toxic at IC₅₀ value below 20 mM or safe at a higher value [32]. Therefore, the extracts tested in our study did not show toxicity toward the HEK293-T cells [33]. Previous literature revealed that *Adiantum philippense* aqueous and ethanolic extracts have cytoprotective effect against hydrogen peroxide (H₂O₂), where the viability of normal pancreatic islets was shown to be 73.33 and 76.66%, respectively, at 0.5 μg/mL concentration of H₂O₂, while treated with 0.5 mM H₂O₂ only showed 63.33% viability [34].

Conclusion

This study investigates the phytochemical constituents of the *n*-hexane fraction (EO) of the fern *A. lunulatum* and biological properties compared to the crude ethanolic extract. Previous studies have established that the plant contains significant phytoconstituents that contribute to its medicinal values. Extracts prepared using solvents, fraction including ethanol, methanol, chloroform, and distilled water, revealed that the plant contains critical phytochemicals such as phenol, tannins, terpenoids, saponins, glycosides, and proteins. These compounds support its traditional use

as anti-inflammatory, antimicrobial, and antioxidant agent, with potential anticancer activities. In this work, our findings show that *A. lunulatum* exhibits appreciable antidiabetic activity in its reaction with the carbohydrate-hydrolyzing enzymes, α-amylase and α-glucosidase. Furthermore, the crude aqueous-ethanolic extract (AEE) and the *n*-hexane fraction (EO) showed a competitive type of inhibition for DPPH and ABTS radicals, suggesting an antioxidative mode of interfering with possible diabetic and cytotoxic pathways. Since both plant samples did not induce cell death to Hek293-T cells, they are considered safe. The findings in this work advocate for more in-depth studies focused on the chemical constituents and clinical trials to explore the full potentials of the *n*-hexane fraction. In conclusion, the exploration of lesser-known plant species like this fern is essential. This work revealed that the biological activities expressed by the *A. lunulatum* AEE extract and the EO fraction are possibly hinged on the synergistic effects of the various chemical components present in the samples. The GC-HRT-MS profiles showed these fractions as sources of new pharmacophores, thus presenting better opportunities in drug development and their exploration at the highest therapeutic efficacy for safe drugs.

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Author contributions AOA conceptualized the research work and carried out the plant preparation, extraction and solvent–solvent partitioning of the crude extract. RCC carried out the GC-HRT-MS experiment. GKM carried out all biological experiments. AOA, RCC, and GKM involved in the manuscript preparation, writing, and editing.

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Data availability All data generated or analyzed during this study are included in this published article [and other information files provided in the supplementary section].

Declarations

Conflict of interests The authors hereby declare no conflict of interest in this work.

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