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Chemical characterization, *in vitro* anticancer effect of *Asplenium aethiopicum* (Burm. f.) Becherer

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ABSTRACT

Human interests for ferns and fern allies will be not only ornamental but also it can be used as medicinal plants. Previous studies on *Asplenium aethiopicum* (Burm. f.) Becherer determined the antioxidant, larvicidal and cytotoxic potential using brine shrimp biolethality assay. The present study was intended to reveal the chemical constituents and anticancer properties of *Asplenium aethiopicum* (Burm. f.) Becherer using A549 (lung adenocarcinoma) and African Green Monkey Kidney cell lines. The preliminary phytochemical, UV–Vis, FT-IR analysis were performed using various extracts of *A. aethiopicum.* The GC-MS and anticancer activity was performed in the ethanolic extracts of *A. aethiopicum*. Anticancer potential of *A. aethiopicum* ethanolic extracts on both A549 (lung adenocarcinoma) and African Green Monkey Kidney cells was determined by the 3-[4,5-ethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide assay. *A. aethiopicum* acetone, ethanolic and aqueous extracts were showed the presence of five metabolites. FT-IR analysis confirmed the presence of various functional groups in the *A. aethiopicum* ethanolic extracts. GC-MS analysis showed the presence of two major components viz., Bicyclo [4.1.0] heptan-2-one (13.92%) and Tridecane (10.50%). The *A. aethiopicum* ethanolic extracts showed the anticancer activity against human A549 lung adenocarcinoma in a dose and time-dependent inhibitory effect. Anticancer activity of *A. aethiopicum* ethanolic extracts may be due to the presence of bioactive compounds viz., Bicyclo[4.1.0]heptan-2-one,Cyclohexanaone,5-ethyl-4-(1-methylethenyl)-2-(1methylethylidene), cis-, 5,8,11-Eicosatriyonic acid, methyl ester, 1,5,5-Trimethyl-6-methylene-cyclohexane in the ethanolic extracts of *A. aethiopicum*. Further studies are needed to isolate the active principle responsible for anticancer activity.

1. Introduction

Cancer is currently a leading cause of death and growing evidence relates its occurrence to the oxidative damage to DNA, proteins and lipid in the body (Halliwell, 1996). Chemoprevention is recognized as an important approach to control malignancy and have focused on the search for desirable chemo-preventive agents. Natural products, particularly dietary substances, have played an important role in creating new chemo-preventive agents (Surh, 2003). Much research has been geared towards the evaluation of plant extracts as prophylactic agents, which offer great potential to inhibit the carcinogenic process. Simultaneously, the synergistic effects of the cocktail of plant metabolites and the multiple points of intervention offer higher efficacy during chemoprevention regimens (Guilford and Pezzuto, 2008). Regassa et al. (2022) listed some plant derived anticancer agents viz., campothecin derivatives, homoharringtonine, vinca alkaloids, podophyllotoxin derivatives, and taxanes and enumerated the angiospermic anticancer plants. Shukla et al. (2022) documented the cancer properties of orchids. Janakiraman and Johnson (2016) studied the anticancer potential of *Cyathea* species using MCF cell line.

It has been estimated that approximately two-thirds of anticancer drugs approved worldwide up to 1994 were derived from plant sources (Vickers, 2002). In the recent years, advancement in chromatographic and spectral fingerprints plays an important role in the quality control of complex herbal medicines. Pteridophytes are

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wonderful group of plants with fascinating foliage architecture and have drawn special attention of the plant lovers, researchers and horticulturists. Many pteridophytes are medicinal and ornamentals, in addition, they play an important role in the ecological niches of forest ecosystem as integral part of biogeochemical cycling of minerals. Since ferns and fern allies have survived from Paleozoic times, they have adapted with many changes of environment than the other primitive vascular plants (Wallace et al., 1991). Therefore, ferns are expected to have many useful secondary metabolites than other plants. Due to an increased concern about human health, longevity and eco-friendly life style, the health supplement markets are expanding rapidly.

Synthetic compounds were popular due to their cheap price and quick efficacy in the past. However many studies reported their side effects, such as carcinogenesis (Branen, 1975). The preference of ferns with potential pharmacological properties has increased rapidly worldwide (Hum et al., 2008; Lee et al., 2009). Pan et al. (2001) reported a 52-year-old female patient with cholangiocarcinoma who developed severe bone marrow suppression after taking *Selaginella doederleinii* as an alternative anticancer treatment. In these circumstances, human interests for ferns and fern allies will be not only ornamental but also it can be used as medicinal plants. Johnson et al. (2014) determined the total phenolics, flavonoid contents, antioxidant, larvicidal and cytotoxic potential of various extracts of *Asplenium aethiopicum* (Burm. f.) Becherer. Johnson et al. (2020) studied the phenolic, flavonoids, alkaloids and tannins profile of the *Asplenium aethiopicum* (Burm. f.) Becherer using HPTLC. But there is no report on the phytoconstituents and anticancer properties of *Asplenium aethiopicum*. Hence the present study was intended to examine the anticancer and phytoconstituents of *Asplenium aethiopicum* (Burm. f.) Becherer.

2. Materials and methods

2.1. Collection of materials

Healthy, disease free ferns of *Asplenium aethiopicum* (Burm. f.) Becherer (Aspleniaceae) was collected near Venkatraman Bridge, Ooty - Cudalore road, Nilgiris, Tamil Nadu, India and authenticated by Dr. M. Johnson, Fellow of Indian Fern Society, India and the specimens voucher were deposited in the centre for Plant Biotechnology Herbarium (CPB 23) for further reference. *Asplenium aethiopicum* (Burm. f.) Becherer is an epiphytic/ terrestrial spleenworts fern. It grows along roadsides, on partially / fully shade regions, near the stream banks and forest edges. *Asplenium aethiopicum* showed its distribution in India, Sri Lanka, Tropical America, Polynesia, Cape colony, Canary Islands etc. The whole plants of *A. aethiopicum* were brought to the laboratory and washed well with running tap water for 10 min to remove the soil particles and adhered debris and washed thoroughly with distilled water. The washed plant samples were blotted on the blotting paper and spread out at room temperature under shade for a period of fifteen days. The shade dried samples were ground to fine powder using tissue blender. The powdered samples were then stored in refrigerator at 4 °C for further use.

2.2. Preparation of extracts

The dried and powered whole plant materials (30 g) were extracted successively with 180 ml of petroleum ether, chloroform, acetone, ethanol and aqueous by using Soxhlet extractor for 8 hr at a temperature not exceeding the boiling point of the solvent. After 8 h of extraction, the slurry is kept in the petriplate at room temperature to evaporate the excess solvents. After evaporation, the extracts were stored in the refrigerator at 4□C for the further phytochemical and biological analysis.

2.3. Preliminary phytochemical analysis

The different extracts were tested for steroids, terpenoids, alkaloids, phenolic compounds, saponins, tannins, flavonoids, cardiac glycosides, sterols and aminoacids according to the standard method described by Harborne (1998).

2.4. UV-Visible spectral analysis

The different extracts were centrifuged at 3000 rpm for 10 min and filtered through Whatman No. 1 filter paper by using high pressure vacuum pump. The sample is diluted to 1:10 with the same solvent. The extracts were scanned in the wavelength ranging from 200 to 1000 nm using Shimazdu Spectrophotometer. The prominent characteristic peaks were detected and their absorbance was recorded.

2.5. FT-IR analysis

FT-IR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks and their functional groups. Ethanolic, acetone and chloroform extracts of *A. aethiopicum* were passed into the FT-IR and the peak values were recorded.

Based on the preliminary phytochemical analysis results and previous studies report on the methanolic extracts of *A. aethiopicum* (Johnson et al., 2014; Johnson et al., 2020), in the present study the GC-MS analysis and *in vitro* anticancer activity studies are performed in the ethanolic extracts.

2.6. GC-MS analysis

The Clarus 500 GC used in the analysis employed a fused silica column packed with Elite-1 [100% diethyl poly siloxane, 30 nm × 0.25 nm ID × 1 μm df] and the components were separated using helium as carrier gas at a constant flow of 1 mL/min. The 2 μl ethanolic extract of *A. aethiopicum* injected into the instrument was detected by the Turbo gold mass detector (Perkin Elmer) with the aid of the Turbo mass 5.1 software. During the 36th min GC extraction process, the oven was maintained at a temperature of 110 °C with 2 min holding. The injector temperature was set at 250 °C (mass analyser). The different parameters involved in the operation of the Clarus 500 MS, were also standardized (Inlet line temperature: 200 °C; Source temperature: 200□ C). Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450 Da.

2.7. Identification of components

Interpretation of mass spectrum of GC-MS was conducted using the database of National Institute Standard and Technique (NIST 08 s), WILEY 8 and FAME having more than 65,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST 08 s, WILEY 8 and FAME library. The name, molecular weight, molecular formula and structure of the component of the test material was ascertained. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a GC MS solution ver. 2.53. The biological activities are predicted based on Dr. Duke's Phytochemical and Ethnobotanical Databases (https://phytochem.nal.usda.gov/phytochem/search) and PASS online software (Lagunin et al., 2000)

2.8. In vitro *anti-cancer activity*

2.8.1. *Cell morphological studies*

To verify the morphological changes of cells DAPI staining was performed as described by Sandra et al. (2002). Briefly the A549 and VERO cells were seeded on glass slide and treated with ethanolic extracts of *A. aethiopicum* for 24 h. Untreated and treated cells were rinsed with phosphate buffered saline, fixed with ice- cold 10% trichloroacetic acid and further washed with cold 70%, 80% and 90% of ethanol. The cells were stained with 1 μg/ml 4–6-diamidino-2-phenylindole (DAPI) for 3 min, cover slipped with 90% glycerol and observed under fluorescence microscope.

2.8.2. *Evaluation of* in vitro *anticancer activity by MTT assay*

An anticancer of *A. aethiopicum* ethanolic extracts on both A549 (lung adenocarcinoma) and African Green Monkey Kidney (VERO) cells was determined by the MTT (3-[4,5-ethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) assay (Selvakumaran et al., 2003). Cells (3×10^3/well) were plated in 100 μl of medium/well in 96-well plates. After incubation overnight, the extracts were added in various concentrations (5, 6.25, 10, 25, 50, 100, 125 250, 500 and 1000 μg/ml); 5 wells were included in each concentration. After treatment with ethanolic extracts for 24 h, 20 μl of 5 mg/ml MTT (pH 4.7) was added per well and cultivated for another 4 h, the supernatant fluid was removed, 100 μl DMSO was added per well and shaken for 15 min. The absorbance at 570 nm was measured with a UV spectrophotometer, using wells without cells as blanks. All experiments were performed in triplicates. The absorbance of untreated cells was considered as 100%. Each and every experiment was repeated thrice with three replicates. The IC_{50} value was determined graphically. The conventional anti-cancer drug adriamycin was used as a positive control. The effect of *A. aethiopicum* ethanolic extracts on the proliferation of human A549 (lung adenocarcinoma) and African Green Monkey Kidney (VERO) was expressed as the% cytoviability, using the following formula:% cytoviability = A570 of treated cells / A570 of control cells × 100%.

3. Results and discussion

3.1. *Phytochemical analysis*

To reveal the metabolites present in the petroleum ether, acetone, chloroform, ethanolic and aqueous extracts of *A. aethiopicum* the following qualitative tests for ten different chemical compounds were carried out. Except petroleum ether and chloroform extracts all other studied extracts of *A. aethiopicum* showed the maximum presence of five metabolites. An acetone extracts of *A. aethiopicum* demonstrated the occurrence of steroids, phenolics groups, cardiac glycosides, flavonoids and sterols. An ethanolic extracts of *A. aethiopicum* illustrated the existence of Steroids, alkaloids, phenolics, cardiac glycosides and flavonoids. The metabolites phenolics, cardiac glycosides, flavonoids, saponins and tannins displayed their presence in aqueous (water) extracts of *A. aethiopicum*. Next to that chloroform extracts of *A. aethiopicum* showed the occurrence of steroids, cardiac glycosides, flavonoids and sterols. The petroleum ether extracts showed only the presence of sterols. Aminoacids and terpenoids failed to show their presence in any of the extracts of *A. aethiopicum*.

3.2. *UV-Vis analysis*

The UV-Visible spectrum of *A. aethiopicum* petroleum ether extract showed the metabolites and functional groups presence in the nm of 1029, 993, 919, 888, 663, 605, 535, 425 and 266 with absorption 0.011, 0.011, 0.006, 0.004, 0.991, 0.321, 0.479, 3.675 and 0.640 respectively. The UV-Visible spectrum of *A. aethiopicum* chloroform extract showed the metabolites and functional groups existence in the nm of 1022, 964, 664, 605,534 and 338 with the absorption 0.005, 0.008, 1.378, 0.370, 0.557 and 4.0 respectively. The UV-Visible spectrum of *A. aethiopicum* acetone extract showed the metabolites and functional groups occurrence in the nm of 1029, 993, 919, 888, 663, 605, 535,425 and 266 with absorption 0.110, 0.11, 0.006, 0.004, 0.991, 0.321, 0.479, 3.675 and 0.640 respectively. The UV-Visible spectrum of *A. aethiopicum* ethanolic extract showed the metabolites and functional groups presence in the nm of 1072, 1020, 945, 909, 877, 667,607,534,505,410 and 315 with absorption 0.002, 0.003, 0.004, 0.713, 0.154, 0.207, 0.257, 1.964 and 1.202. The aqueous extract of *A. aethiopicum* does not show any peak detection.

3.3. *FT-IR analysis*

The FT-IR results revealed the functional constituents of different extracts of *A. aethiopicum* based on the absorption spectrum (Table 1). The results of FT-IR analysis of *A. aethiopicum* confirmed the presence of alkanes, esters, primary amines, alkyl halides, aliphatic amines, carboxylic acids and secondary amines with different peak values.

Table 1
FT-IR peak values with functional groups in *A. aethiopicum*.

Peak values	Bond	Functional group	Range for Functional Groups in cm-1.	Extracts		
				E	A	C
2924	(C-H) Stretch	Alkanes	2850–3000	+	+	+
2854	(C-H) Stretch	Alkanes		+	+	+
1743	C=O stretching	Esters, Saturated aliphatic	1750–1735	-	+	+
1635	(N-H) Bend	Primary amines	1650–1580	+	-	-
1465	(C-H) Bend	Alkanes	1480–1440	-	-	+
1458	(C-H) Bend	Alkanes		+	+	-
1265	(C-H) Wag (-CH2X)	Alkyl halides	1300–1150	+		
1165	(C-H) Wag (-CH2X)	Alkyl halides		-	-	+
1072	(C-N) Stretch	Aliphatic amines	1250–1020	+	-	-
1064	(C-N) Stretch	Aliphatic amines		-	+	-
925	(O-H) Bend	Carboxylic acids	950–910	-	+	-
748	(=C-H) Bend, (N-H) Wag, (C-Cl) Stretch	Alkanes, Primary-Secondary amines, Alkyl halides	850–550	-	-	+

https://chem.libretexts.org/Bookshelves/Organic_Chemistry/Map%3A_Organic_Chemistry_(Wade)/11%3A_Infrared_Spectroscopy_and_Mass_Spectrometry/11.05%3A_Infrared_-Spectra_of_Some_Common_Functional_Groups accessed on 3rd January 2023.
https://www.sigmaaldrich.com/IN/en/technical-documents/technical-article/analytical-chemistry/photometry-and-reflectometry/ir-spectrum-table accessed on 3rd January 2023.
https://orgchemboulder.com/Spectroscopy/irtutor/alkhalidesir.shtml#:~:text=IR%20Spectroscopy%20Tutorial%3A%20Alkyl%20Halides&text=In%20general%2C%20C%E2%80%93X%20vibration,690%2D515%20cm%2D1. accessed on 3rd January 2023.
https://orgchemboulder.com/Spectroscopy/irtutor/carbacidsir.shtml accessed on 3rd January 2023.
E - Ethanol; A - Acetone; C – Chloroform.

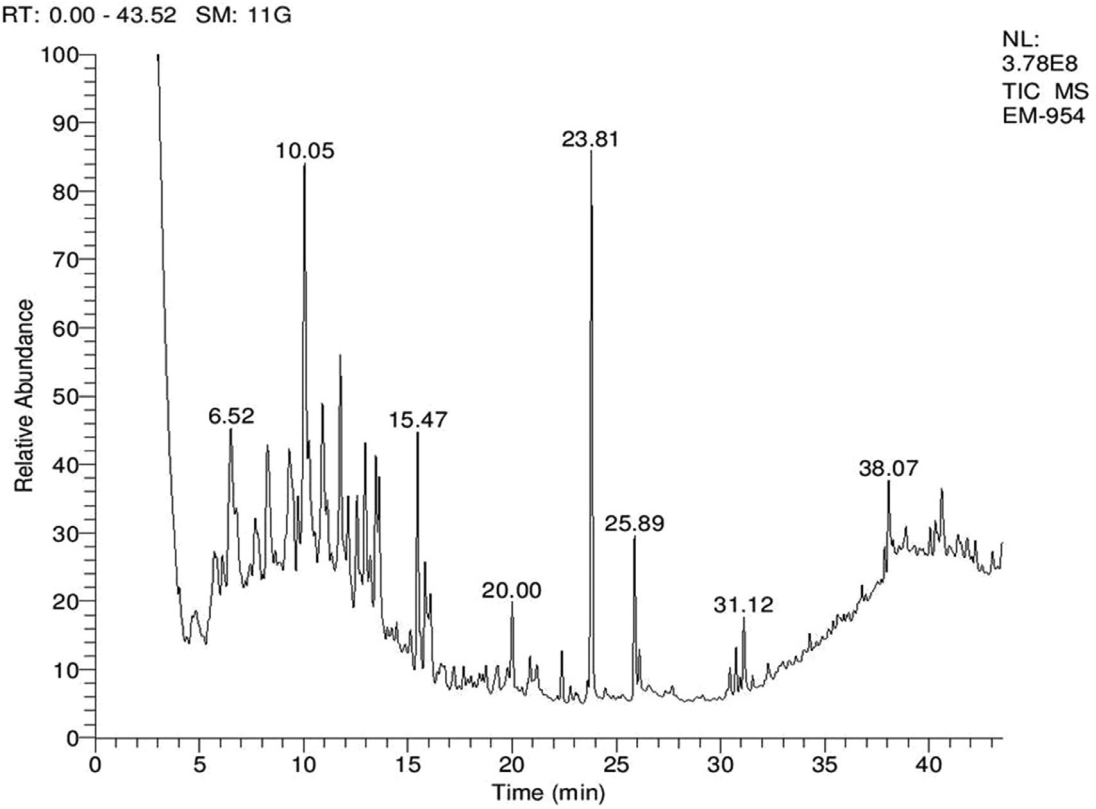

Fig. 1. Gas Chromatogram of *A. aethiopicum* ethanolic extracts.

3.4. GC-MS analysis

The results pertaining to GC-MS analysis leads to the identification of number of compounds from *A. aethiopicum* ethanolic extracts (Fig. 1). The results revealed the presence of 28 different compounds and were illustrated in Table 2. The major components were Bicyclo [4.1.0] heptan-2-one (13.92%) and Tridecane (10.50%). These compounds possess various biological activities such as antieczematic, antineoplastic, mucomembranous protector, cardiovascular analeptic, sclerosant, urethanase inhibitor and antiseborrheic properties. In addition to this, some minor compounds were also present viz., Benzne,1-ethyl-4-(1,2,2-triethylcyclopentyl (5.35%), Tetradecene (4.99%), 1-Propene,3-[2-(2-ethoxyethoxy)ethoxy]- (4.97%), Dodecane (4.11%), Pentadecane,2,6,10,14-tetraethyl (4.09%), Napthalene,1-ethyl- (4.09%) and Napthalene,1,7-diethyl- (4.08%). The PASS prediction revealed the different properties such as phobic disorders treatment, omptin inhibitor, fibrinolytic, antidyskinetic, antineurotic, dextranase inhibitor, sarcosine oxidase inhibitor, creatininase inhibitor, venombin AB inhibitor, glutathione thiolesterase inhibitor, urethanase inhibitor, antibacterial, antineoplastic, antioxidant etc. The PASS prediction analysis confirmed the antineoplastic properties of *A. aethiopicum* ethanolic extracts by the presence of chemical constituents viz., Bicyclo[4.1.0]heptan-2-one,Cyclohexanaone,5-ethyl-4-(1-methylethenyl)−2-(1 methylethylidene) *cis*, 5,8,11-Eicosatriyonic acid, methyl ester, 1,5,5-Trimethyl-6-methylene-cyclohexane. These observations may be due to the nature of biological active components and the stronger extraction capacity of ethanol could have been produced number of active constituents responsible for biological activities.

3.5. In vitro *anticancer activity*

To know the *A. aethiopicum* ethanolic extracts induced changes in cell structures of Human A549 lung adenocarcinoma cancer and African Green Monkey Kidney cells (VERO) the DAPI staining was performed. *A. aethiopicum* ethanolic extracts induced morphological alterations such as cytoplasmic shrinkage and loss of normal nuclear architecture of the tested cell lines were confirmed via fluorescence microscopy. 1000 μg/mL ethanolic extracts of *A. aethiopicum* demonstrated highest frequency of inhibitory effect on cell proliferation than the control. The percentage of inhibitory effect on the cell proliferation was directly proportional to the increase in the concentration of the extract (Fig. 2).

An anti-cancer activity of *A. aethiopicum* ethanolic extracts against human A549 lung adenocarcinoma and African Green Monkey Kidney (VERO) showed a dose and time-dependent inhibitory effect (Fig. 2). An IC_{50} 381.68 μg/mL and 714.28 μg/mL were observed in the *A. aethiopicum* ethanolic extracts against human A549 lung adenocarcinoma cells and African Green Monkey Kidney (VERO) respectively (Fig. 3). The positive standard adriamycin showed the IC_{50} with 23 μg/mL. The crude ethanolic extracts of *A. aethiopicum* showed potential inhibitory effect as compared to the positive standard (Fig. 4).

4. Discussion

Phytochemical standards are generally used for determining the identity, purity and quality of the drug source. These characters are also used to check the genuine nature of the crude drug, thus it plays an important role in preventing the possible steps of adulteration. In the present study, phytochemical analysis was conducted on different extracts of *A. aethiopicum* and the results revealed the presence of steroids, alkaloids, phenolic groups, cardiac glycosides, flavonoids, saponins, tannins and sterols in the crude with varied degree. The curative properties of medicinal plants are due to the presence of various classes of secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols, etc. (Lalnundanga et al., 2012). Tannins are dietary anti-nutrients with strong astringent property

Table 2
Chemical Constituents of *A. aethiopicum* ethanolic extracts.

Name of the compound	RT	Peak area%	Molecular formula	Molecular weight	Biological activity
7-Hexadecenal,(Z)-	4.82	2.31	$C_{16}H_{30}O$	238	Skin irritation, Antisecretoric, Antiinfective.
1,5,5-Trimethyl-6-methylene-cyclohexane	5.71	3.50	$C_{10}H_{16}$	136	Antineoplastic, Carminative, Dermatologic,
1-Propene,3-[2-(2-*methoxyethoxy*)*ethoxy*]-	6.50	4.97	$C_8H_{16}O_3$	160	Mucomembranous protector, Phobic disorders treatment
m-Toluic acid,4-pentadecyl ester	7.68	2.40	$C_{23}H_{38}O_2$	346	Vasoprotector, Acetylesterase inhibitor
Dodecane	8.26	4.11	$C_{12}H_{26}$	170	Sclerosant, Fibrinolytic, Antidyskinetic, Antineurotic,
Pentadecane,2,6,10,14-tetramethyl	9.32	4.09	$C_{19}H_{40}$	268	Antiseborrheic, Sclerosant, Omptin inhibitor
2-Butyl-3−4-(4-a-diethyl aminoethoxybenzoyl) benzoyl furan	9.72	1.48	$C_{25}H_{31}NO_3$	393	Cutinase inhibitor, Fragilysin inhibitor, Testosterone 17 beta-dehydrogenase (NADP+) inhibitor
Tridecane	10.05	10.50	$C_{13}H_{28}$	184	Cardiovascular analeptic, Sclerosant, Urethanase inhibitor, Antiseborrheic,
Glycine,N-(N-glycyl-L-leucyl)-	10.27	0.97	$C_{10}H_{19}N_3O_4$	245	Cardiovascular analeptic, Urethanase inhibitor, Antiseborrheic
Napthalene	10.91	3.91	$C_{10}H_8$	128	Fragilysin inhibitor, Thioredoxin inhibitor, IgA-specific metalloendopeptidase inhibitor.
Tetradecene	11.76	4.99	$C_{14}H_{30}$	198	Dextranase inhibitor, Sarcosine oxidase inhibitor, Creatininase inhibitor.
2-Pentanone,4-cyclohexylidene-3,3-diethyl	12.14	2.07	$C_{15}H_{26}O$	222	Cutinase inhibitor, Lysase inhibitor, Carminative.
Tetradecane,2,6,10-trimethyl-	12.56	2.72	$C_{17}H_{36}$	240	Chymosin inhibitor, Cutinase inhibitor, Polyporopepsin inhibitor
6-Oxododecanedioic	13.20	1.12	$C_{12}H_{20}O_5$	244	Mucomembranous protector, Dextranase inhibitor, Levanase inhibitor, Creatininase inhibitor
Napthalene,1-methyl-	13.46	4.09	$C_{11}H_{10}$	142	Feruloyl esterase inhibitor, Tryptophanamidase inhibitor, Thioredoxin inhibitor
Benzne,1-methyl-4-(1,2,2-trimethylcyclopentyl	15.46	5.35	$C_{15}H_{22}$	202	Venombin AB inhibitor, Glutathione thiolesterase inhibitor, Urethanase inhibitor
Di-epi-a-cedrene-(I)	15.82	2.78	$C_{15}H_{24}$	204	Prostaglandin E1 antagonist, Acylcarnitine hydrolase inhibitor
Napthalene,1,7-dimethyl-	16.09	4.08	$C_{12}H_{12}$	156	2-Hydroxymuconate-semialdehyde hydrolase inhibitor, IgA-specific serine endopeptidase inhibitor, tRNA-pseudouridine synthase I inhibitor
5,8,11-Eicosatriyonic acid, methyl ester	16.76	1.07	$C_{23}H_{36}O_2Si$	372	Antineoplastic, Acrocylindropepsin inhibitor, Angiogenesis inhibitor, Antiinflammatory
Napthalene,2,3,6-trimethyl-	18.75	1.53	$C_{13}H_{14}$	170	Antiseborrheic, Glutathione thiolesterase inhibitor, Carminative, Urethanase inhibitor, Mucomembranous protector
Napthalene,1,6,7-trimethyl-	19.20	1.24	$C_{13}H_{14}$	170	Taurine dehydrogenase inhibitor, Glutathione thiolesterase inhibitor, Nitrate reductase (cytochrome) inhibitor, Kidney function stimulant
Cyclohexanaone,5-ethyl-4-(1-methylethenyl)−2-(1-methylethylidene)-,cis-	20.00	2.17	$C_{15}H_{22}O$	218	Carminative, Antieczematic, Antineoplastic, Antiinflammatory, Dermatologic
Bicyclo[4.1.0]heptan-2-one,	23.81	13.92	C15H22O	218	Antieczematic, Antineoplastic, Mucomembranous protector
Cinoxate	25.87	3.81	$C_{14}H_{18}O_4$	250	Antieczematic, Eye irritation, Antisecretoric, Respiratory analeptic,

(*continued*)

Table 2 (*Continued*)

Name of the compound	RT	Peak area%	Molecular formula	Molecular weight	Biological activity
Phytol	30.75	1.08	$C_{20}H_{40}O$	296	Antipruritic, Vasodilator Antiparasitic, Antihelmintic, Antifungal, Immunosuppressant, Antiprotozoal, Hepatoprotectant, Antioxidant, Anticarcinogenic, Antituberculosic, Antimycobacterial
9,11-Octadecadienoic acid,methyl ester,(E,E)-	31.12	1.43	$C_{19}H_{34}O_2$	294	Cutinase inhibitor, Preneoplastic conditions treatment, Gastrin inhibitor, Platelet aggregation stimulant
Octadecanoic acid,2,3-dihydroxypropyl ester	36.06	1.82	$C_{21}H_{42}O_4$	358	Eye irritation, Skin irritation, Antihypoxic, Radioprotector, Antiseptic, Antiinfective, Choleretic, Reductant, Antithrombotic
Butyl9,12-octadecadienoate	40.60	1.26	$C_{22}H_{40}O_2$	336	Skin irritation, Vasoprotector, Antiinflammatory, Antipruritic, Sclerosant

and are known to be useful in the treatment of inflamed or ulcerated tissues, cancer, mild anti-septics, diarrhea as well as to check small hemorrhages. Flavonoids have a membrane permeability effect and are considered as potential antioxidants and have protective action against allergies, inflammation, free radical, platelet aggregation, microbes, ulcers, hepatoxins, viruses and tumor (Aiyegoro and Okoh, 2010; Eleazu et al., 2012). The plant extract contain saponins, which have a potential anti-inflammatory, coagulant, antidiabetic, antioxidant, aldose reductase inhibitory activity, haemolytic and cholesterol binding properties (Patel et al., 2012). Phenols and phenolic compounds are greatly used in skin infections, wound healing, inflammation; antioxidant, immune enhancers, anti-clotting and hormone modulators (Hussain et al., 2012). Alkaloids can work on the nervous system of the human body and used for analgesic, antispasmodic and bacterial effects (*Okwu and Josiah, 2006*). The presence of various secondary metabolites in the present study viz., alkaloids, flavonoids, phenolic groups, saponins, tannins and steroids suggest that *A. aethiopicum* possess various therapeutic values. Johnson et al. (2014) confirmed the antioxidant, larvicidal and cytotoxic properties of *A. aethiopicum*. FT-IR spectrum is a most credible method to validate and identify the mix-substance systems such as traditional medicine and herbal medicine (Liu et al., 2007). The results of the present study FT-IR spectrum revealed the functional constituents present in the different extracts of *A. aethiopicum* and it confirmed the presence of

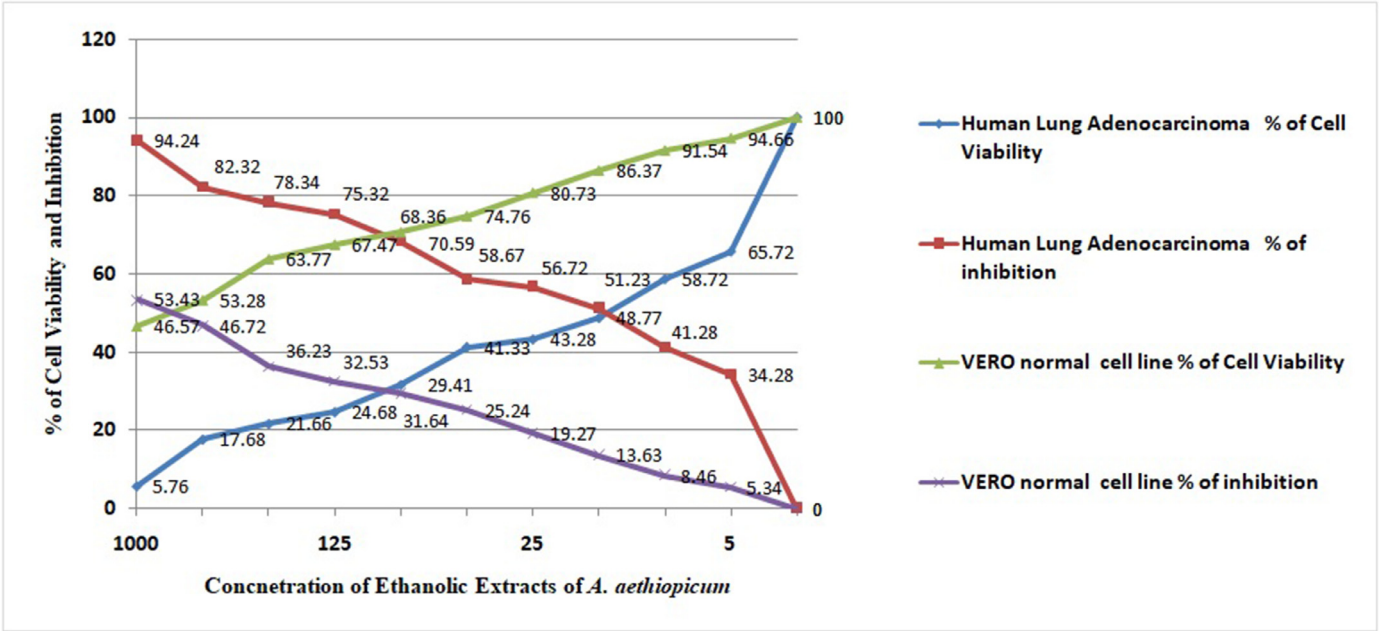

Fig. 2. Anticancer activity of *A. aethiopicum* ethanolic extracts.

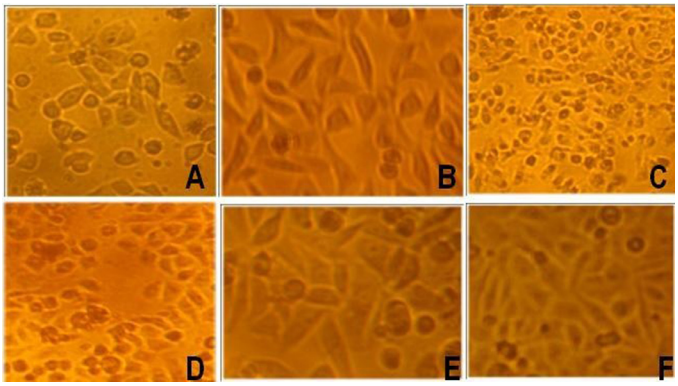

Fig. 3. Anticancer activity of *A. aethiopicum* ethanolic extracts against human A549 lung adenocarcinoma cells (A - C) and African Green Monkey Kidney -VERO (D - F).

alkanes, esters, primary amines, alkyl halides, aliphatic amines, carboxylic acids and secondary amines with different peak values.

Epidemiological and experimental studies have implicated oxidative cellular damage arising from an imbalance between free radical generating and scavenging systems as the primary cause of cardiovascular disease, cancer and aging (Halliwell 1996; Rajesh and Perumal, 2013). Due to risk of adverse effects encountered with the use of synthetic antibiotics, medicinal plants may offer an alternative source for anticancer agents with significant activity (Lee et al., 2009). Lai et al. (2010) tested cytotoxic activity of the fern Blechnum orientale against four cancer cell lines and a non-malignant cell using MTT assay. The results showed best cytotoxic effect on human colon cancer cell HT-29 (IC_{50} 27.5–42.8 μg/ml). In the present study also, MTT (3-(4,5-dimethyl-thiazol-2-yl)–2,5-diphenyltetrazolium bromide) assay was employed to detect the anti-cancer potential of *A. aethiopicum* in A549 lung adenocarcinoma and African Green Monkey Kidney (VERO) and observed that ethanolic extracts of *A. aethiopicum* caused marked cell growth inhibition in the A549 lung adenocarcinoma cells. Morphological studies of *A. aethiopicum* ethanolic extract also confirmed the potential anticancer properties. An anticancer activity of *A. aethiopicum* showed a dose and time-dependent inhibitory effect. This may be due to the presence of bioactive compounds viz., Bicyclo [4.1.0]heptan-2-one, Cyclohexanaone,5-ethyl-4-(1-methylethenyl)–2-(1-methylethylidene)-,cis-, 5,8,11-Eicosatriyonic acid, methyl ester, 1,5,5-Trimethyl-6-methylene-cyclohexane present in the ethanolic extracts of *A. aethiopicum*.

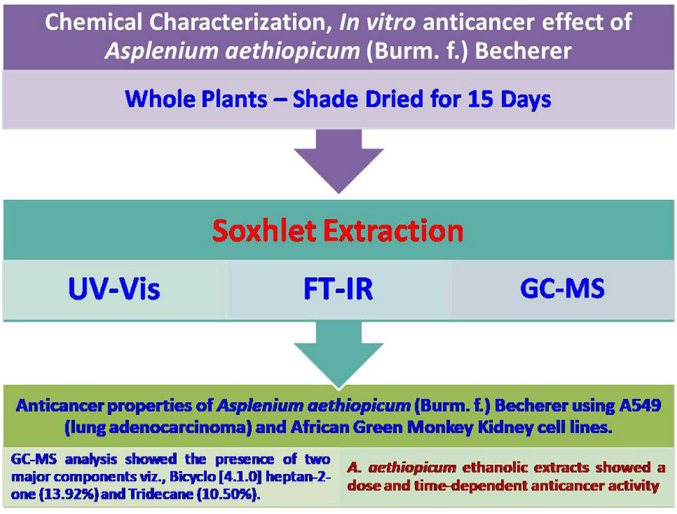

Fig. 4. Chemical Characterization, *In vitro* Anti-cancer effect of *Asplenium aethiopicum* (Burm. f.) Becherer.

5. Conclusions

The preliminary phytochemical analysis confirmed the presence of phenolics groups, cardiac glycosides, flavonoids in the acetone, ethanolic and aqueous extracts of *A. aethiopicum*. The existence of the metabolites may be responsible for the best cytotoxic effect. An anticancer activity of *A. aethiopicum* showed a dose and time-dependent inhibitory effect. The GC-MS analysis revealed the presence of Bicyclo [4.1.0]heptan-2-one, Cyclohexanaone,5-ethyl-4-(1-methylethenyl)–2-(1-methylethylidene)-,cis-, 5,8,11-Eicosatriyonic acid, methyl ester, 1,5,5-Trimethyl-6-methylene-cyclohexane in the ethanolic extracts of *A. aethiopicum* and the predicted compounds may be responsible for the toxicity. The results of the present study suggest that the ethanolic extract of *A. aethiopicum* may be used as anti-cancer drug in the near future. UV –Vis and FTIR spectroscopic profile not only revealed the metabolites and functional groups existence in the *A. aethiopicum* extracts and these profiles will be used as pharmacognostical marker to identify the medicinal fern *A. aethiopicum*. Further research on *Asplenium aethiopicum* may help to isolate therapeutically potent compounds which can be finally be subjected to pharmacological activities and clinical trials, thus leading to open up new avenues in the use of natural products for therapeutic purposes.

Declaration of Competing Interest

No conflict of interest to disclose.

CRediT authorship contribution statement

Johnson Marimuthu alias Antonysamy: Conceptualization. **Gowtham Janarthanan:** Methodology. **Janaina Esmeraldo Rocha:** Project administration. **Ray Silva de Almeida:** Software. **Henrique D. M. Coutinho:** Supervision.

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Supplementary materials

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