

PHYTOCHEMICAL ANALYSIS, *IN VITRO* ANTIOXIDANT POTENTIAL AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY STUDIES OF *DICRANOPTERIS LINEARIS*

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ABSTRACT

Objective: The aim of the study was to analyze qualitative and quantitative phytochemicals, evaluate *in vitro* antioxidant properties and determine the bioactive compounds in extracts of *Dicranopteris linearis* (Burm.f.) Underw. collected from Western Ghats of Kanyakumari district.

Methods: The qualitative, quantitative phytochemical, and *in vitro* antioxidant analysis were performed using standard procedures. The bioactive compounds were analyzed using gas chromatography-mass spectrometry (GC-MS) instrument.

Results: The qualitative phytochemical analysis studied in aqueous, acetone, chloroform, ethanol, and petroleum ether solvent extract showed acetone had strong positivity to express the 12 phytoconstituents studied except anthocyanin when compared to other solvent extracts. The quantitative phytochemistry revealed considerable amount of terpenoids (97.0±1.15 mg/g), tannins (30.8±0.44 mg tannic acid equivalents/gram), phenols (28.6±0.33 mg gallic acid equivalents/gram), and flavonoids (8.50±0.29 mg quercetin equivalent/g) in decreasing order of concentrations. The *in vitro* antioxidant activity of aqueous, ethanol, acetone, chloroform, and petroleum ether suggested that the extract of DL has prominent antioxidant prospective against various free radicals such as 2,2-diphenyl-1-picrylhydrazyl while butylated hydroxy toluene being the standard antioxidant used. The GC-MS analysis displayed the presence of 11 bioactive compounds each belonging to various categories of phytochemicals such as terpenoids, flavonoids, phenols, and fatty acid derivatives.

Conclusion: The results indicate that *D. linearis* (Burm.f.) Underw. present in the Western Ghats of Kanyakumari is an effective scavenger of free radicals and has the potential to be used as a natural antioxidant which is attributed to the rich presence of secondary metabolites.

Keywords: *Dicranopteris linearis* (Burm.f.) Underw., Phytochemistry, Antioxidant activity, Gas chromatography-mass Spectrometry.

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INTRODUCTION

Medicinal plants are very ancient and are true natural medicines which are useful for the treatment of different diseases. Plants are eminent source of new therapeutic agents that helps to alleviate human ailments and promote health. The noteworthy preventive and protective properties of these substances are related to their strong antioxidative, antimutagenic and anticarcinogenic potential [1]. They can be used directly or in extracted forms for the management of various ailments due to the presence of various secondary metabolites [2]. Many plants contain a variety of phytochemical property found to be significant in the fields of agriculture, human and veterinary medicine. Natural products play a dominant role in the development of novel drug leading to treatment and prevention of diseases [3]. A sufficient number of plants have been proven to be effective against ailments and massively screened for their therapeutic compounds. Pteridophyta has been known for its medicinal and therapeutic values, gaining importance in plant-based novel drug therapy. Many species of this plant division are highly ignored and are determined to have potential secondary metabolites that act against various diseases [4]. The limited knowledge of these medicinal plants for disease control and their weed habitat make these ferns to be destroyed by human. The ferns had an important role in folklore medicine and are being used as valuable sources of food and medicine for the prevention of illness and maintenance of human and animal health. *Dicranopteris linearis* (Burm.f.) Underw. is a terrestrial pteridophyte covered with scales or hairs. Leaves monomorphic, large, scrambling or trailing, one to many times forked. Literature study reveals that the

plant possesses significant antioxidant activity with high flavonoid content [5], antimicrobial [6], gastroprotective [7], antinociceptive, anti-inflammatory and antipyretic [8], and anthelmintic [9]. The above-mentioned properties of the plant could be credited to the presence of various primary and secondary metabolites in significant quantity.

Vegetables and plants consumed as food or medicines are widely accepted to provide new sources of antioxidants because of their potential biological and pharmacological activities. In recent times, research activities on antioxidants from plants sources have attracted a wide range of interest across the world. Antioxidants have great importance because they can reduce oxidative stress which could cause damage to biological molecules [10]. Various research activities on antioxidant property of Pteridophytes have been reported [11-13]. In recent years, there is an increasing trend of screening medicinal plants for bioactive compounds as a basis for further pharmacological studies. Several studies have shown that plant derived antioxidants scavenge free radicals and modulate oxidative stress. The chemistry of free radical is complicated and it caused a major limitation in the identification of free radical scavenging activity. To withstand this problem the potential antioxidant substance is tested in *in vitro* model and such approaches expand the scope of antioxidant activity [14]. Research on relationship between antioxidants and prevention of non-communicable disease such as cardiovascular disease, neoplastic, and diabetic condition has attain awareness in recent years. Epidemiological and *in vitro* studies strongly suggest that plant food containing phytochemicals with antioxidants have potent protective effects against these diseases. However, there is widespread agreement that some synthetic antioxidants such as

butylated hydroxyanisole, butylated hydroxy toluene (BHT), and tert-butyl hydroquinone need to be replaced with natural antioxidants owing to their hazardous health risks and toxicity [15]. Therefore, it is utmost need to find out new sources of safe and economy antioxidants of natural origin. Phenolic compounds are the natural antioxidants acting such as chelating metal ions, preventing radical formation, and improving the antioxidant endogenous system. Probably the most important natural phenolics are flavonoids. Being widespread across the plant kingdom, medicinal plants have gained tremendous interest as potential therapeutic agents against a wide range of biological actions such as antibacterial, antiviral, anticancer, anti-inflammatory, and anti-allergic activities [16]. Higher plants have developed different adaptive mechanisms to reduce oxidative damage resulting from salt stress, through the biosynthesis of a cascade of antioxidants [17]. Bearing this in mind, the present work was designed to investigate the presence of secondary metabolites, *in vitro* antioxidant properties and identification of bioactive compounds through gas chromatography-mass spectrometry (GC-MS) analysis.

METHODS

Plant collection and identification

Fresh samples of *D. linearis* (Burm.f.) Underw. were collected from Kodhayar hills, Western Ghats of Kanyakumari district and authenticated from Scott Christian College, Nagercoil, Kanyakumari District and the specimen (Voucher No SPCH/1005) preserved in A.V.V.M Sri Pushpam College, Thanjavur, India.

Preparation of fern extract

About 1 g of dried powder of whole plant, *D. linearis* (Burm.f.) Underw. was extracted with 20 mL ethanol 75%, acetone, chloroform, aqueous, and petroleum ether (Merck, extra pure) for 1 minute using an ultra turax mixer (13,000 rpm) and soaked overnight at room temperature. The sample was then filtered through Whatman No. 1 paper in a Buchner funnel and evaporated under vacuum in a rotavator at 40°C to a constant weight and then dissolved in respective solvents. The dissolving rate of the crude extracts was approximately 100%. The solution was stored at 18°C until use [18].

Qualitative phytochemistry

The phytochemical screening was assessed as per standard method [19]. Phytochemical screening was performed using aqueous, acetone, chloroform, ethanol, and petroleum ether solvents to identify the major natural chemical groups such as tannins, saponins, flavonoids, phenols, terpenoids, alkaloids, glycosides, cardiac glycosides, coumarins, steroids, anthocyanin, and betacyanin.

Quantification of total terpenoids

Total terpenoid content in the leaf extracts was assessed by standard method [20]. 1 g of plant powder was taken separately and soaked in alcohol for 24 hrs. Then filtered, the filtrate was extracted with petroleum ether; the ether extract was treated as a total terpenoid.

Quantification of total tannins

Tannins content in the leaf extracts was estimated by following the method [21]. The sample extracts (1 ml) were mixed with Folin-Ciocalteu reagent (0.5 mL), followed by the addition of saturated sodium carbonate (Na_2CO_3) solution (1 mL) and distilled water (8 mL). The reaction mixture was allowed to stand for 30 minutes at room temperature. The supernatant was obtained by centrifugation and absorbance was recorded at 725 nm using ultraviolet visible (UV-VIS) spectrophotometer. Different concentrations of standard tannic acid were prepared, and the absorbance of various tannic acid concentrations was plotted for a standard graph. The tannin content was expressed as μg tannic acid equivalent (TAE)/g of the sample.

Quantification of total phenols

Total phenolic content (TPC) in leaf the extracts was determined by the Folin-Ciocalteu colorimetric method [22]. For the analysis, 0.5 ml of an aliquot of the sample was added to 0.5 ml of Folin-Ciocalteu

reagent (0.5 N) and the contents of the flask were mixed thoroughly. Later 2.5 ml of Na_2CO_3 (2%) was added, and the mixture was allowed to stand for 30 minutes after mixing. The absorbance was measured at 760 nm in a UV-VIS spectrophotometer. The total phenolics contents were expressed as mg gallic acid equivalents (GAE)/g extract.

Quantification of total flavonoids

Total flavonoids content in plant extracts was determined by the aluminum chloride colorimetric method [23]. 0.5 ml of plant extracts at a concentration of 1 mg/ml was taken, and the volume was made up to 3 ml with methanol. Then, 0.1 ml AlCl_3 (10%), 0.1 ml of potassium acetate, and 2.8 ml distilled water were added sequentially. The test solution was vigorously shaken. Absorbance was recorded at 415 nm after 30 minutes of incubation. A standard calibration plot was generated at 415 nm using known concentrations of quercetin. The concentrations of flavonoid in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent (QE)/g of sample.

In vitro free radical scavenging assays

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The antioxidant activities determined using DPPH (Sigma-Aldrich) is a free radical. Scavenging activity. 100 μl of leaf extracts were mixed with 2.7 ml of methanol, and then 200 μl of 0.1% methanolic DPPH was added. The suspension was incubated for 30 minutes in dark condition. Initially, absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured as a control [24]. Subsequently, at every 5 minutes interval, the absorption maxima of the solutions were measured using a UV double beam spectra scan (Chemito, India) at 517 nm. The antioxidant activity of the sample was compared with known synthetic standard of 0.16% BHT. The experiment was carried out in triplicates. Using the below-mentioned equation, the percentage inhibition was calculated:

$$\% \text{ Inhibition} = [\text{Abs control} - \text{Abs sample} / \text{Abs control}] \times 100$$

Where Abs is absorbance.

GC-MS analysis

Based on the preliminary phytochemical results, methanolic extract of DL was chosen for the analysis of possible bioactive compounds by GC and MS technique. The trace GC ultra and DSQII model MS from Thermo Fisher Scientific Limited were engaged for analysis [25]. The instrument was set as follows, injector port temperature set to 250°C, interface temperature set as 250°C, and source kept at 200°C. The oven temperature programed as a variable, 70°C for 2 minutes, 150°C at 8°C/minutes, up to 260°C at 10°C/minutes. Split ratio set as 1:50 and the injector used was splitless mode. The DB-35 MS nonpolar column was used whose dimensions were 0.25 mm OD \times 0.25 μm ID \times 30 m length procured from Agilent Co., USA. Helium was used as the carrier gas at 1 ml/minutes. The MS was set to scan from 50 to 650 Da. The source was maintained at 200°C and < 40 motor vacuum pressure. The ionization energy was -70 eV. The MS was also having inbuilt pre-filter which reduced the neutral particles. The data system has two inbuilt libraries for searching and matching the spectrum. NIST4 and WILEY9 each contain more than 5 million references. Only those compounds with spectral fit values equal to or >700 were considered positive identification.

RESULTS

Qualitative phytochemistry

Qualitative analysis indicated the presence of 12 phytoconstituents, except anthocyanin. Out of 5 solvent extracts, acetone extract performed well to show positivity of phytoconstituents than other 4 solvent extracts (Table 1).

Quantitative phytochemistry

The results of the quantitative analysis indicated the presence of significant quantities of terpenoids (97.0 \pm 1.15 mg/g), tannins

Table 1: Qualitative phytochemistry of various solvent extract of *Dicranopteris linearis* (Burm. f.) Underw.

Phytoconstituents	Aqueous	Acetone	Chloroform	Ethanol	Petroleum ether
Tannins	-	++	-	+	-
Saponins	++	+	+	+	+
Quinones	++	++	-	++	-
Terpenoids	+	++	-	++	+
Steroids	+	++	+	++	+
Flavonoids	++	++	-	+	-
Phenol	++	++	+	+	+
Alkaloids	-	+	-	+	-
Glycosides	-	+	-	-	-
Cardiac glycosides	+	+	-	+	-
Coumarins	++	+	-	++	-
Anthocyanin	-	-	-	-	-
Betacyanin	+	+	-	+	-

+: Positive, ++: Strong positive, -: Negative

(30.8±0.44 mg TAE/g), phenols (28.6±0.33 mg GAE/g), and flavonoids (8.5±0.29 mg QE/g) in decreasing order of concentrations (Table 2).

In vitro antioxidant activities

The DPPH free radical scavenging activity for antioxidant property showed acetone extract had strongest antioxidant potential (96.0%), and petroleum ether extract had least activity (52.7%) (Table 3).

GC-MS analysis

The GC-MS chromatogram and mass spectrum of methanolic extracts of *D. linearis* (Burm.f.) Underw. are displayed in Fig. 1. GC-MS analysis resulted in the identification of 7 chemical compounds. The retention time, molecular formula, and the area % are presented in Table 4 and the nature of compounds and medicinal uses are mentioned in Table 5.

DISCUSSION

Examination for evidence of phytoconstituents showed acetone extract performed well to express the phytochemicals in the fern studied. Tannin, phenol, terpenoid, flavonoid, quinones, and saponins showed strong positivity in acetone fern extract which was the best solvent to express phytoconstituents in this study [26] performed phytochemical screening with acetone, benzene, chloroform, ethanol, petroleum ether, and aqueous extracts of whole plants of *Blechnum orientale*, *Ceratopteris thalictroides*, *Dracocephalum heterophyllum*, *D. linearis*, *Hexastylis arifolia*, *Inula ensifolia*, *Nephrolepis multiflora*, *Pellaea calomelanos*, *Partulina confusa*, and leaves and rhizomes of *Drynaria quercifolia* revealed that the presence or absence of the phytoconstituents depends on the solvent medium used for extraction and the physiological property of individual taxa. This study on the phytochemical analysis of *D. linearis* (Burm.f.) Underw. was in confirmation with the study of Mithraja *et al.* [27] stated that tannin containing drugs are used in medicine as an astringent and have been found to possess antiviral, antibacterial, and anti-parasitic effects for possible therapeutic applications. Since tannin was present in the acetone and ethanolic extract of all the fern under study. Similarly [28] ascertain the presence of different Phytoconstituents in the water, methanol, ethanol and acetone extracts of 34 species of pteridophytes by qualitative screening methods and stated water and ethanol revealed maximum number of phytochemicals than methanol and acetone.

Plant-derived secondary metabolites such as alkaloids, polyphenols, saponins, tannins, terpenoids, and flavonoids are gaining much importance in recent years due to their imperative medicinal activities such as antioxidant, antitumor, antimicrobial, antidiabetic, and anthelmintic activity. The results of this study suggest that the acetone extract of *D. linearis* (Burm.f.) Underw. contains considerable quantities of terpenoids, tannins, phenols, and flavonoids in a decreasing order of concentrations.

GC-MS analysis showed the presence of 11 phytoconstituents belonging to varied nature of chemical compounds and possess various biological activity. Hexadecanoic acid, dioctyl ester (CAS) one of the

Table 2: Quantitative phytochemical analysis of *Dicranopteris linearis* (Burm. f.) Underw. extract

Parameters	Contents
Total terpenoids (mg/g sample)	97.00±1.15
Total tannins (mg/g of TAE)	30.80±0.44
Total phenols (mg/g of GAE)	28.60±0.33
Total flavonoids (mg/g of QE)	8.50±0.29

TAE: Tannic acid equivalent, GAE: Gallic acid equivalents, QE: Quercetin equivalent

major compounds detected through GC-MS analysis is a palmitic acid derivative exhibit various biological function such as antioxidant, flavouring agent, pesticide, lubricant, antiandrogenic, haemolytic, alpha reductase inhibitor, anti-inflammatory, hypocholesterolemic, nematocide, insectifuge, and anti-bacterial activity [29]. The results of the phytochemical screening and quantitative estimation of the chemical constituents of plant sample have indicated the high content of terpenoids, total tannin, total phenol, and flavonoids. The abundance of flavonoids which are hydroxylated phenolics substances might be responsible for their therapeutic effectiveness against a wide array of micro-organisms, probably due to their ability to complex with extracellular and soluble proteins and to complex with the bacterial cell wall [30]. Flavonoids and other phenolic compounds are potent water-soluble antioxidants and free radical scavengers, which prevent oxidative cell damage, have strong anticancer activity and anthelmintic activity [31]. QUERCETIN 7,3,4-TRIMETHOXY, a potent flavonoid (polyphenolic group) present in these fern extract under GC-MS analysis might be attributed to significant antioxidant property [24]. PHYTOL, a terpenoids derivative also attribute to significant antioxidant property in *D. linearis* (Burm.f.) Underw. [32].

Swamy *et al.* [33] drawn much attention to natural antioxidant and their associations with health benefits. Plants are potential sources of natural antioxidants. They produce various antioxidant compounds to counteract reactive oxygen species to survive. Some of the antioxidant compounds, namely, flavan - 4-ol glycosides, abacopterins, huperzine A, isoquercetin, di-E-caffeoyl-mesotartaric acid, flavaspidic acid PB, flavaspidic acid AB, flavan-3-ol, kaempferol, A-type proanthocyanidins, and afzelechin were isolated from few pteridophytes such as *Abacopteris penangiana*, *Huperzias elago*, *Equisetum arvense*, and *Dryopteris crassirhizoma*. From the previous research, it has been given that only 36 numbers of pteridophyte plants species were examined for antioxidant activity study. This paper highlights the antioxidant property of *D. linearis* (Burm.f.) Underw. which is important to this group of plants since their remedial activity against different diseases is remain incomplete. Hence, an exclusive study is essential for better understanding and exploration of the potentiality of antioxidant from pteridophytes.

Lam and Lim [34] explore new and natural sources of antioxidant among some ferns in Malaysia. 15 fern species were screened. TPC was

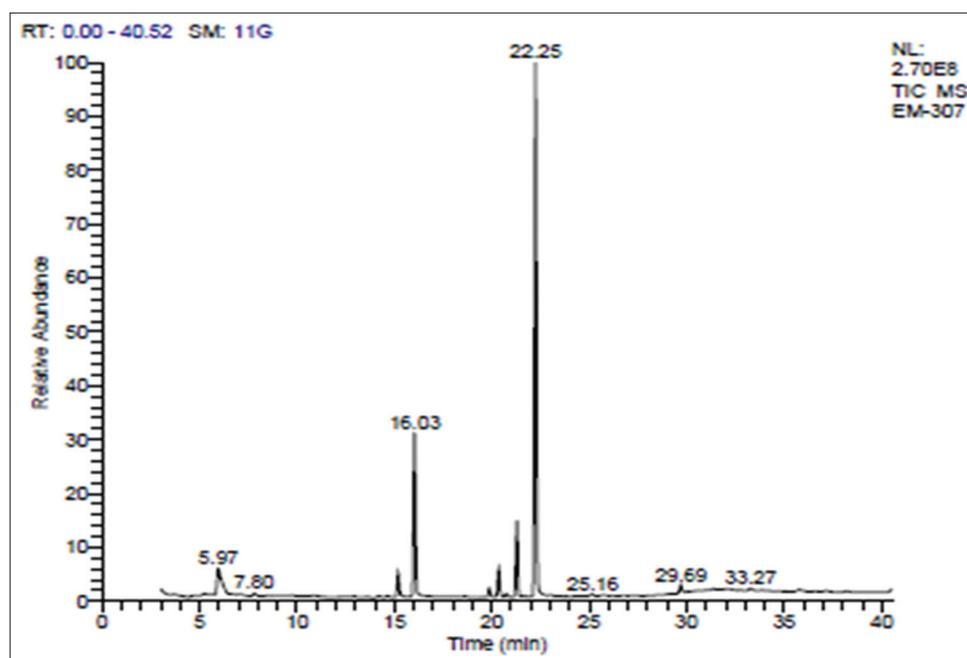


Fig. 1: Gas chromatography-mass spectrometry chromatogram of methanol extract of whole fern of *Dicranopteris linearis* (Burm. f.) Underw.

Table 3: DPPH free radical scavenging activity

Solvent(%)	0 minute	5 minutes	10 minutes	15 minutes	20 minutes	25 minutes	30 minutes
Aqueous (OD)	0.11	0.10	0.10	0.09	0.09	0.09	0.09
	91.3	92.1	92.1	92.9	92.9	92.9	92.9
Acetone (OD)	0.13	0.10	0.09	0.09	0.07	0.07	0.05
	92.1	92.1	92.9	92.9	94.4	94.4	96.0
Chloroform (OD)	0.63	0.60	0.58	0.53	0.51	0.50	0.49
	50.3	52.7	54.3	58.2	59.8	60.6	61.41
Ethanol (OD)	0.36	0.17	0.11	0.09	0.09	0.08	0.08
	71.6	86.6	91.3	92.9	92.9	93.7	93.7
Petroleum ether (OD)	0.64	0.62	0.61	0.60	0.60	0.60	0.60
	49.6	51.1	51.9	52.7	52.7	52.7	52.7
BHT (OD)	0.14	0.11	0.09	0.07	0.06	0.04	0.02
	88.9	91.3	92.9	94.4	95.2	96.8	98.4
Control	1.27						

BHT: Butylated hydroxy toluene, DPPH: 2,2-diphenyl-1-picrylhydrazyl

Table 4: Secondary metabolite detected in *Dicranopteris linearis* (Burm. f.) Underw.

No	RT	Name of the compound	Molecular formula	MW	Peak area %
1	5.97	Furan (CAS)	C_4H_4O	68	5.38
		5,5-dimethyl furan-2 (5H)-one	$C_6H_8O_2$	112	5.38
2	7.80	Dodecane	$C_{12}H_{26}$	170	0.45
		1,2,3-propanetricarboxylic acid, 2-hydroxy-, triethyl ester (CAS)	$C_{12}H_{20}O_7$	276	16.97
3	16.03	Triethyl citrate	$C_{12}H_{20}O_7$	276	16.97
		1,2-benzenedicarboxylic acid, dibutyl ester (CAS)	$C_{16}H_{22}O_4$	278	55.85
5	25.16	PHYTOL	$C_{20}H_{40}O$	296	0.37
6	29.69	QUERCETIN 7,3',4'-TRIMETHOXY	$C_{18}H_{16}O_7$	344	0.20
		Hexadecanoic acid, dioctyl ester (CAS)	$C_{22}H_{42}O_4$	370	0.99
7	33.27	1,2-Diphenyl-5-(t-butyl) acephenanthrylene	$C_{30}H_{50}$	410	0.40
		Fern-8-ene	$C_{30}H_{50}$	410	0.40

measured using the Folin-Ciocalteu method. Antioxidant properties were determined via the DPPH radical scavenging, ferric reducing power (FRP), and β -carotene bleaching assays. Results showed five

ferns with the very high total phenolic content of above 2000 mg GAE/100 g fresh leaves. These ferns exhibited strong antioxidant activity based on the DPPH radical scavenging activity, FRP and

Table 5: Activity of phytochemicals in *Dicranopteris linearis* (Burm. f.) Underw.

Name of the compound	Compound nature	Biological activity
Furan (CAS) 5,5-dimethyl furan-2 (5H)-One	Heterocyclic organic compound	Antioxidant, antimicrobial
Dodecane	Alkane	Antifungal, Antitumor
1,2,3-Propanetricarboxylic acid, 2-hydroxy-, triethyl ester (CAS)	Fatty acid derivative Fatty acid derivative	Anti-microbial Anti-ulcer, Anti-inflammatory
Triethyl citrate	Plasticizer compound	Antimicrobial, Anti-inflammatory, Anti-coronary activity
1,2-benzenedicarboxylic acid, dibutyl ester (CAS)	Fatty acid derivative	Neuro-degenerative disorder, anti-fouling, antimicrobial
Dibutyl Phthalate		
PHYTOL	Diterpene	Antimicrobial, Anticancer, Diuretic, Antioxidant, anti-inflammatory
QUERCETIN	Flavonoids	Antioxidant, anthelmintic, antimicrobial, antileishmanial, antiplasmodial
7,3',4'-TRIMETHOXY	Palmitic acid	Antioxidant, flavouring agent, pesticide, lubricant, antiandrogenic, haemolytic, alpha reductase inhibitor; Anti-inflammatory, Hypocholesterolemic, Nematicide, Insectifuge and Anti-bacterial activity
Hexadecanoic acid, dioctyl ester (CAS)		
Fern-8-ene	Triterpenoids	Antioxidant, Anti-inflammatory and Anthelmintic
1,2-Diphenyl-5-(t-butyl) acephenanthrylene	Phenol	Antioxidant, Antimicrobial

inhibition of lipid peroxidation. The ferns with strong antioxidant properties were *Cyathea latebrosa*, *Cibotium barometez*, *Drynaria quercifolia*, *B. orientale*, and *D. linearis*. In conclusion, the methanol extracts of *C. latebrosa*, *C. barometez*, *D. quercifolia*, *B. orientale*, and *D. linearis* showed very high total phenolic content are potent antioxidants which were in opinion with this study in *D. linearis* (Burm.f.) Underw.

Soare et al. [35] performed antioxidant analysis in methanolic extracts obtained from leaves of *Athyrium filix-femina*, *Dryopteris affinis* and *Dryopteris filix-mas* ferns have shown a good antioxidant activity. A positive correlation was obtained between the antioxidant activity and the total phenolic compounds. The tested ferns could be used as cosmetic ingredients, as preservatives in food or in antimicrobial therapy. Similarly, the tested *D. linearis* (Burm.f.) Underw. in this study could be a good antioxidant potential and is used in wound healing, anthelmintic drugs, anti-inflammatory agents, etc.

Chai et al. [36] stated promising source of phenolic antioxidants, although the effectiveness of the specific phenolic mixture in each leaf extract varied according to the type of leaf used. High contents of phenolic constituents, including anthocyanins, and high specific metal chelating activity in the edible young sterile frond of *Stenochlaena palustris* highlight the potential of the fern as a functional food. On the other hand, the mature sterile frond, with its high phenolic contents, potent effectiveness as reductants and year-round availability, is a potential source of phenolic antioxidants for further exploitation. Based on our findings, future studies to characterise the phenolic profiles of both young and mature sterile fronds of *D. linearis* (Burm.f.) Underw. are warranted.

Gupta et al. [37] collected eight different ferns from Mawsynram of the state of Meghalaya in India were evaluated for their *in vitro* antioxidant activity as well as associated phytochemical contents to explore the natural source of antioxidants. The *in vitro* antioxidant activity of the methanolic extract of the fern specimens was determined spectrophotometrically following DPPH, ABTS, FRP, and metal chelating methods. Other phytochemical contents such as phenol, flavonol, and orthodihydric phenol were also evaluated following standard methodology. A positive correlation between the antioxidant activities and phytochemical contents was observed. The findings suggest that all these ferns are the good source of natural antioxidants and could be used as therapeutic agents in preventing the disorders associated with oxidative stress.

CONCLUSION

In conclusion, the acetone extracts of *D. linearis* (Burm.f.) Underw. showed very high terpenoids, total phenolic, and total tannin content and are potent antioxidants. QUERCETIN 7,3',4'-TRIMETHOXY, a potent flavonoid (polyphenolic group) present in these fern extract under GC-MS analysis is a strong antioxidant. The tested ferns could be used as cosmetic ingredients, as preservatives in food or in antiparasitic, antimicrobial therapy. The methods employed in this study are easily used and provide reproducible results. Much higher antioxidant activities of the acetone extracts have given evident assumption that the extracts are more potent than other solvent extracts from a medical point of view.

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