Journal of Pharmaceutical and Scientific Innovation

www.jpsionline.com (ISSN : 2277 -4572)

Research Article

ANALYSIS OF BIOACTIVE COMPOUNDS IN *DICRANOPTERIS LINEARIS* (BURM.F.) UNDERW.

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DOI: 10.7897/2277-4572.084142

Received on: 08/05/19 Revised on: 09/06/19 Accepted on: 13/06/19

ABSTRACT

The present study was carried out to characterize the bioactive constituents present in aerial parts of acetone and chloroform extract of *Dicranopteris linearis* (Burm.f.) Underw. using UV-VIS, FTIR and HPLC. UV, FTIR and HPLC spectroscopy is an established time-saving method to characterize and identify functional groups. There phytoconstituents were analyzed for their phytochemical properties, High Performance Liquid Chromatography (HPLC) assay, Fourier Transform Infra-Red (FTIR). The UV-VIS profile showed different peaks ranging from 330-800 nm with different absorption respectively. FTIR analysis and UV-VIS analysis showed the presence of flavonoids and a phenolic compound. The FTIR spectra had amply evidenced the occurrence of OH group together with the Terpenoids and Phenol. The FT-IR spectrum showed the presence of an amine (N-H), alkyl (C-H), nitrile (C≡N), halo formyl (C=O), alkenyl (C=C), hydroxyl (O-H), haloalkane (C-F), nitrates and carbonate compounds. Totally six phyto constituents were separated and identified in HPLC analysis. The results confirm the fact that this plant posse's important bioactive constituent, so further scientific investigation is needed to isolate the pure compounds from the plant.

KEYWORDS: UV-Vis Spectrum, FTIR, *Dicranopteris linearis*, HPLC and functional groups.

INTRODUCTION

Pteridophytes (vascular cryptogams or ferns) enjoy a ubiquitous distribution in India, but they are generally shade-loving plants. Ethnobotanical accounts of 20 ferns have been documented from Kumauni Himalayas[1]. Medicinal properties of 16 fern species of Western Ghats, India also have been recorded[2]. 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[3].

UV-visible spectroscopy uses light in the visible ranges or its adjacent ranges. Molecules undergo electronic transitions in these ranges of the electromagnetic Spectrum[4]. The Fourier Transform Infrared Spectrophotometer (FT-IR) was perhaps the most powerful tool for identifying the types of chemical bonds/functional groups present in the phytochemicals. It is an established time-saving method to characterize and identified functional groups[5]. High Performance Liquid Chromatography (HPLC) analysis is most widely used methodology and easily adapted for the flavonoid's quantification during last 20 years[6]. The aim of the present work was to study the presence of bioactive constituents in *D. linearis* plant extract.

MATERIALS AND METHODS

COLLECTION AND IDENTIFICATION OF PLANT

The plant *Dicranopteris linearis* (Burm.f.) Underw. (Gleicheniaceae) was collected from Marthandam, Kanyakumari district. Identification of plant the species have been confirmed with the help of pteridophytic floras[7].

PREPARATION OF THE EXTRACT

The plant was washed under running tap water, shade dried at room temperature and powdered. About 30gm of plant powder was taken in a digestion flask fitted to the Soxhlet apparatus and extracts were obtained separately with acetone and chloroform.

UV - VISIBLE SPECTRUM ANALYSIS

For UV-Vis spectrophotometer analysis, the acetone and chloroform 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 300-1000 nm using Shimazdu Spectrophotometer. The prominent characteristic peaks were detected, and their absorbance was recorded. Each and every analysis was repeated twice and confirmed the spectrum.

FT-IR SPECTROPHOTOMETER ANALYSIS

The wavelength of light absorbed is characteristic of the chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined. Dried powder of acetone and chloroform extracts of each plant materials were used for FTIR analysis. 10 mg of the dried extract powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample of each plant specimen was loaded in FTIR spectroscope (SJASCO FTIR 410 Spectrophotometer), with a scan range from 400 to 4000 cm with a resolution of 2 cm $^{-1}$.

HPLC ANALYSIS

The HPLC method was performed on a Shimadzu LC-10AT VP HPLC system, equipped with a model LC-10AT pump, UV-Vis detector SPD-10AT, a Rheodyne injector fitted with a 20µl loop and an auto injector SIL-10AT. A Hypersil® BDS C-18 column (4.6 × 250mm, 5µm size) with a C-18 guard column was used. The elution was carried out with gradient solvent systems with a flow rate of 1ml/min at ambient temperature (25-28°C). The mobile phase consisted of 0.1% v/v plant extract (solvent A) and water (solvent B). The mobile phase was prepared daily, filtered through a 0.45µm and sonicated before use. Total running time was 15min. The sample injection volume was 20µl while the wavelength of the UV-Vis detector was set at 254nm[8].

INSTRUMENTATION

An isocratic HPLC (Shimadzu HPLC Class *VP* series) with two LC- 0 AT *VP* pumps (Shimadzu), a variable wave length programmable photo diode array detector SPD-M10A *VP* (Shimadzu), a CTO- 10AS *VP* column oven (Shimadzu), a SCL-10A *VP* system controller (Shimadzu), a reverse phase Luna 5C18 (2) and Phenomenex column (250 mm X 4.6mm) were used. The mobile phase components plant extract: water (45:55) were filtered through a 0.2µ membrane filter before use and were pumped from the solvent reservoir at a flow rate of 1ml/min which yielded column backup pressure of 260-270kgf/cm^2. The column temperature was maintained at 27°C. 20µl of the respective sample was injected by using a Rheodyne syringe (Model 7202, Hamilton).

Table 1: UV- Vis analysis of acetone and chloroform extract of *Dicranopteris linearis*

S. No	Chloroform extract		Acetone extract	
	Wavelength	Absorption	Wavelength	Absorption
1.	414.50	1.094	349.50	1.407
2.	504.00	0.200	600.00	0.043
3.	536.00	0.174	647.50	0.022
4.	608.50	0.119	700.50	0.052
5.	668.00	0.299	-	-

Table 2: FT-IR spectral values and functional groups of acetone extract of *Dicranopteris linearis*

Peak Values (cm-1)	Functional Group	Class
602.71	Unknown	Unknown
656.72	Unknown	Unknown
678.9	NH2 & N-H	Amines
752.19	S-OR	Esters
952.77	Aliphatic, C-C	Amine oxide (N-O), Alkane
1081.03	C-O, C-O, O-C	Alcohol, Ether, Anhydrides
1163	P=O, P-H, P=O, C=S, C-N, C-O, C-O, C-O	Phosphine oxide, Phosphine, Phosphate, Thiocarbonyl, Amines, Alcohol, Ester, Carboxylic Acids
1198.68	C-O, C-O, P=O, P-H, P=O, C=S, C-N, C-O	Ester, Alcohol, Phosphine oxide, Phosphine, Phosphate, Thiocarbonyl, Amines, Alcohol
1390.58	S=O, nitro	Sulfate, N=O
1409.87	S=O	Sulfate
1511.12	N-H, C=C, nitro	Amides, Aromatic, N=O
1606.59	NH2	Amines
1649.99	NH2, C=C, C=O	Amines, Alkene, Amides
1697.24	Unknown	Unknown
2662.55	O-H	Carboxylic Acids
2872.77	C-H, C-H	Alkane, Aldehyde
3005.85	C-H, O-H	Aromatic, Carboxylic Acids
3192.94	O-H	Carboxylic Acids

Table 3: FT-IR spectral values and functional groups of chloroform extract of *Dicranopteris linearis*

Peak Values (cm-1)	Functional Group	Class
603.68	Unknown	Unknown
655.75	Unknown	Unknown
678.9	NH2 & N-H	Amines
752.19	S-OR	Esters
985.56	Aliphatic, C-C	Amine oxide (N-O), Alkane
1098.39	C-O, C-O, O-C	Alcohol, Ether, Anhydrides
1191.93	C-O, C-O, P=O, P-H, P=O, C=S, C-N, C-O	Ester, Alcohol, Phosphine oxide, Phosphine, Phosphate, Thiocarbonyl, Amines, Alcohol
1283.54	P-H	Phosphine
1406.97	S=O	Sulfate
1609.49	NH2	Amines
1736.78	C=O	Ester
2270.06	-N=C=O, -N=C=S, -N=C=N-, -N3, C=C=O	Isocyanates, Isothiocyanates, Diimides, Azides, Ketenes
2694.37	(O=) PO-H	Phosphonic acid
2850.59	C-H, C-H	Alkane, Aldehyde
2920.99	C-H	Alkane
3006.82	C-H, O-H	Aromatic, Carboxylic Acids

Table 4: HPLC chromatogram of acetone extract of *Dicranopteris linearis*

Peak	Retention Time [Min]	Area [Mv.s]	Height [Mv]	Area [%]
1.	2.859	21395	1223	2.856
2.	3.109	41512	2558	5.541
3.	3.642	686296	25620	91.603
Total	9.61	749204	29401	100

Table 5: HPLC chromatogram of chloroform extract of *Dicranopteris linearis*

Peak	Retention Time [Min]	Area [Mv.s]	Height [Mv]	Area [%]
1.	2.924	83638	4606	7.807
2.	3.174	747206	31086	69.749
3.	5.052	240442	3738	22.444
Total	11.152	1071287	39431	100

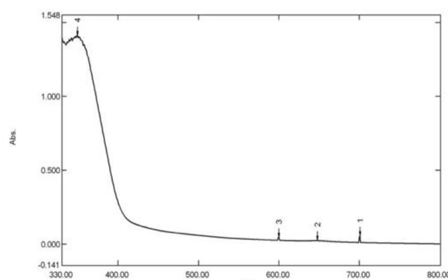

Figure 1: UV- Vis spectrum of acetone extract of *Dicranopteris linearis*

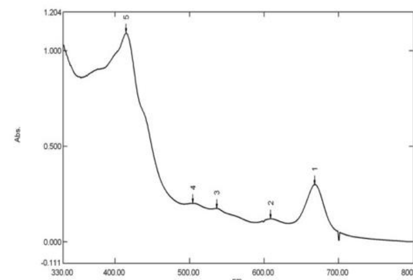

Figure 2: UV- Vis spectrum of chloroform extract of *Dicranopteris linearis*

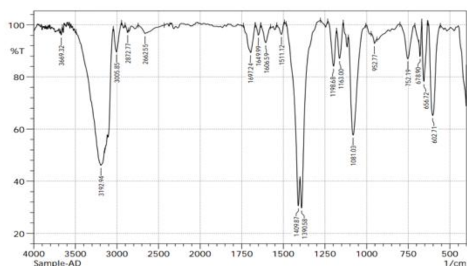

Figure 3: FT-IR spectrum and peak values of acetone extract of *Dicranopteris linearis*

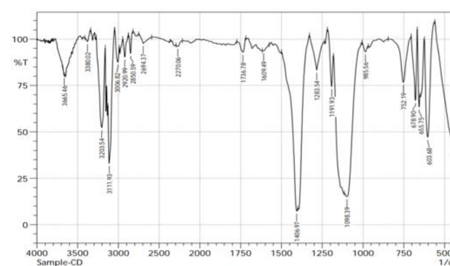

Figure 4: FT-IR spectrum and peak values of chloroform extract of *Dicranopteris linearis*

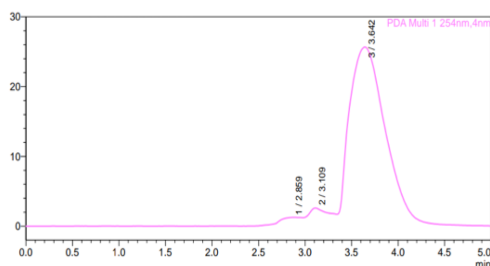

Figure 5: HPLC chromatogram of acetone extract of *Dicranopteris linearis*

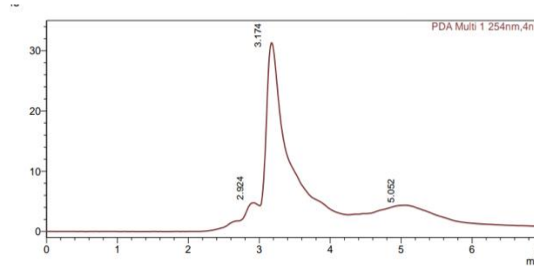

Figure 6: HPLC chromatogram of chloroform extract of *Dicranopteris linearis*

RESULT AND DISCUSSION

Characterization of secondary metabolite fingerprint by spectroscopy and chromatography revealed that two types of solvent (acetone and chloroform) extracts from the aerial part of *D. linearis.* Further secondary metabolites were confirmed by the spectral studies like UV-Visible Spectroscopy, FT-IR Spectroscopy and HPLC analytical techniques.

UV- VIS SPECTRUM ANALYSIS

The qualitative UV-VIS spectrum profile of acetone and chloroform extract of *D. linearis* was selected at wavelength from

330 to 800 nm due to sharpness of the peaks and proper baseline. The acetone extract profile showed the peaks at 349.50, 600, 647.50, 700.50 nm with the absorption of 1.407, 0.043, 0.174, 0.022 and 0.052 respectively. The chloroform extract profile showed the peaks at 414, 504, 536, 608 and 668 nm with the absorption of 1.094, 0.200, 0.174, 0.119 and 0.299 respectively (Figure1, 2 and Table 1).

FT-IR ANALYSIS

Fourier Transform Infrared Spectrophotometer (FTIR) is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds. FT-IR stands for Fourier Transform Infrared, the preferred method of infrared spectroscopy. FT-IR analysis peak values and functional groups of acetone and chloroform extracts of *D. linearis* were represented in following Figures 3,4 and Tables 2,3.

The FT-IR Spectrum of acetone extract of *D. linearis* confirmed the presence of functional groups such as, NH_2& N-H Amines, S-OR Esters, Aliphatic C-C Amine oxide (N-O), Alkane, C-O Alcohol, Ether, Anhydrides, P=O, C=S, C-N, C-O, C-O, Phosphine oxide, Thiocarbonyl, Amines, Alcohol, Ester, Carboxylic Acids, C-O Ester, Alcohol, Phosphine oxide, Phosphine, Thiocarbonyl, Amines, Alcohol, S=O nitroSulfate, N=O, N-H nitro Amides, Aromatic, N=O, NH_2 Amines, NH_2 C=C C=O Amines, Alkene, Amides, O-H Carboxylic Acids, C-H Alkane, Aldehyde, O-H Aromatic, Carboxylic Acids, O-H Carboxylic Acids in the peak values of 602.71, 656.72, 678.9, 752.19, 952.77, 1081.03, 1163,1198.68, 1390.58, 1409.87, 1511.12, 1606.59, 1649.99, 1697.24, 2662.55, 2872.77, 3005.85, 3192.94 and 3669.32 respectively (Figure 3 and Table 2).

The FT-IR Spectrum of chloroform extract of *D. linearis* confirmed the presence of functional groups such as, NH_2 & N-H Amines, S-OR Esters Aliphatic, C-CAmine oxide (N-O), Alkane, O-C Alcohol, Ether, Anhydrides, C-O, P=O, P-H, P=O, C=S, C-N, C-OEster, Alcohol, Phosphine oxide, Phosphine, Phosphate, Thiocarbonyl, Amines, Alcohol, P-H Phosphine, S=O Sulfate, NH2Amines, C=O Ester, -N=C=O, -N=C=S, -N=C=N-, -N3, C=C=O Isocyanates, Isothiocyanates, Diimides, Azides, Ketenes, (O=) PO-H Phosphonic acid, C-H, C-H, Alkane, Aldehyde, C-H Alkane, C-H O-H Aromatic, Carboxylic Acids in the peak values of 603.68, 655.75, 678.9, 752.19, 985.56, 1098.39, 1191.93, 1283.54, 1406.97, 1609.49, 1736.78, 2270.06, 2694.37, 2850.59, 2920.99, 3006.82, 3111.93, 3151.47, 3380.02 and 3665.46 respectively (Figure 4 and Table 3). Recent interest in these substances has been stimulated for the potential health benefits. Useful hydroxyl bunches in flavonoids intercede their cell reinforcement which impact by rummaging free radicals and by chelating metal particles[9].

FTIR offers a rapid and non-destructive investigation to fingerprint plant extracts or powders. The results revealed the presence of alkaloids due to N-H stretching, polyphenols and flavonoids due to O-H stretching, terpens due to CH group[10]. Fourier Transform Infrared Spectroscopy (FTIR) is a high-resolution analytical technique to identify the chemical constituents and elucidate the structural compounds[11]. FTIR offers a rapid and non-destructive investigation to fingerprint plant extracts or powders[12].

HPLC ANALYSIS

HPLC is also known as High- Pressure Liquid Chromatography. The acetone and chloroform extract prepared by hot extraction was subjected to HPLC for the separation and identification of constituents present in the *D. linearis.* Three following compounds were separated at different retention times of 2.859min, 3.109min and 3.642min. The profile displayed one prominent peak at the retention times of 3.642min followed by two moderate peaks were also observed at the retention times of 2.859min and 3.109min respectively (Figure 5 and Table 4).

Three following compounds were separated at different retention times of 2.924min, 3.174min and 5.052min. The profile displayed one prominent peak at the retention times of 3.174min followed by two moderate peaks were also observed at the retention times of 2.924min and 5.052min respectively (Figure 6 and Table 5).

In previous report the quantitative determination of flavonoid compound such as Quercetin in the seeds of ethanolic extract of Elaeocarpus ganitrus by using high performance thin layer chromatography was studied by Awan[13]. The concentration of quercetin in *Elaeocarpus ganitrus* seed was calculated based on calibration curve and the types of flavonoids such as astragarin, kaempferol glucoside and kaempferol rutinoside have been reported in *C. parasitica*[14].

CONCLUSION

In the present study UV-VIS spectrum, FTIR and HPLC analysis of *D. linearis* showed the presence of many secondary metabolites which are responsible for various medicinal properties of test plant. Further, this compound can be isolated further screened for different kinds of biological activities based on their therapeutic uses. The investigation concluded that the stronger extraction capacity of acetone and chloroform could have been produced number of active constituents responsible for many biological activities.

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How to cite this article:
J. Vijayakumari and T. Leon Stephan Raj. Analysis of bioactive compounds in *Dicranopteris linearis* (Burm.F.) Underw. J Pharm Sci Innov. 2019;8(4):136-140.
http://dx.doi.org/10.7897/2277-4572.084142

Source of support: Nil, Conflict of interest: None Declared

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