International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 22-09-2021, Accepted: 06-10-2021, Published: 23-10-2021
Volume 6, Issue 5, 2021, Page No. 1379-1381

Phytochemical analysis of the ethanolic extract of *Hypolepis glandulifera* brownsey *et* chinnock using UV-VIS, FTIR and HPLC

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Abstract
The aim of the present study was to screen the phytoconstituents composition of ethanol extract of *Hypolepis glandulifera* Brownsey *et* Chinnock collected from Kothiyar, located in Kanyakumari district, Tamil Nadu, India. The phytochemical screening of ethanol extract was estimated using the standard procedure for UV-Vis spectroscopic, HPLC and FTIR. The UV-Visible spectrum showed ten compounds separated at the wavelength of at the nm of 1024, 888, 664, 606, 532, 500, 450, 364, 312 and 236 with the absorption 0.247, 0.287, 0.886, 1.348, 1.456, 1.794, 2.268, 1.204, 1.755 and 1.650 respectively. The qualitative HPLC fingerprint profile displayed five compounds at different retention times. The profile displayed two prominent peaks at the retention time of 2.117min and 2.800min, followed by three moderate peaks were predicted at the retention time of 1.207min, 3.220min and 4.043min. The FTIR analysis recognized different peaks at 624.89, 670.22, 778.22, 1056.92, 1173.6, 1363.58, 1423.37, 1514.98, 1606.59, 1730.99, 2925.81, 3062.75 and 3381.95cm^{-1} with the presence of functional groups such as naphthalenes, aldehydes, 1,2,3-trisubst benzenes, alkyl sulfoxides, sulfonic acids, sulfonyl chlorides, carboxylic acids, triazine compounds, β-diketones, δ lactones, aliphatic compounds, aromatic and unsaturated compounds and alcohols and phenols.

Keywords: *Hypolepis glandulifera*, phytochemical, Kothiyar, UV-Vis, HPLC, FTIR

Introduction

Nature had provided in such a way that various secondary metabolites of pteridophytes had adaptability mechanism to their environment under varied climatic conditions (Bennett and Wallsgrove, 2006) [1]. The World Health Organization (WHO) estimates that 80% of the world's inhabitants rely mainly on traditional medicines for their health care. The tribal and rural people of various parts of India are highly depending on medicinal plant therapy for meeting their health care needs (Gurib, 2006; Pankajalakshmi and Taralakshmi, 1994) [2-3]. Plants are the major source of medicines and foods which play a vital role in maintenance of human health. The importance of plants in medicine remains even of greater relevance with the current global trends of shifting to obtain drugs from plant sources, as a result of which attention has been given to the medicinal value of herbal remedies for safety, efficacy, and economy (Glombitza *et al.*, 1993; Mahabir and Gulliford, 1997) [4-5].

The pteridophytes are the non-flowering, vascular and spore bearing plants including ferns and fern allies. They grow luxuriantly in moist tropical and temperate forests and their occurrence in different geographically threatened regions from sea level to the mountains are of much interest (Dixit, 2000) [6]. Phytochemical characterization of plant material is important as it relates to the therapeutic actions. It is perhaps obvious that different species of plants would have different chemical constituents. They are nonessential nutrients, meaning that they are not required by the human body for sustaining life. It is well-known that plant produces these chemicals to protect them but recent research demonstrates that they can also protect humans against diseases. Hence the present study was undertaken to screen various secondary metabolites present in *Hypolepis glandulifera* Brownsey *et* Chinnock using UV-VIS, FTIR and HPLC.

Materials and Methods

Collection of sample

The plant materials used in the present study was *Hypolepis glandulifera* Brownsey *et* Chinnock belonging to the family Dennstaedtiaceae. The plant materials for the present study were collected from Kothiyar, located in Kanyakumari district, Tamil Nadu, India and identified and confirmed by Pteridophyte flora of the Western Ghats - South India (Manickam and Irudayaraj, 1991) [7].

Preparation of extracts

For the preparation of ethanol extract, the plant specimens were washed thoroughly and placed on blotting paper and spread out at room temperature in the shade condition for drying. The shade dried samples were grounded to fine powder using a tissue blender. The powdered samples were then stored in the refrigerator for further use. 30g powdered

samples were packed in Soxhlet apparatus and extracted with ethanol for 12 h separately (Iniya Udhaya and John Peter Paul, 2017) [8].

UV-Vis spectral analysis

The ethanol crude extract containing the bioactive compound was analyzed UV-Vis spectroscopically for further confirmation. The ethanol crude extract of *Hypolepis glandulifera* Brownsey *et* Chinnock was scanned in a wavelength ranging from 200-1100nm using a Shimazdu spectrophotometer and characteristic peaks were detected (John Peter Paul and Shri Devi (2013) [9].

FTIR analysis

FTIR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks and their functional groups. The peak values of the FTIR were recorded. Each and every analysis was repeated twice and confirmed the spectrum (John Peter Paul and Yuvaraj, 2013) [10].

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 methanol (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 (Amster Regin Lawrence *et al.*, 2017) [11].

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 5µl C-18 and Phenomenex column (250 mm X 4.6mm) were used. The mobile phase components methanol: 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 and was injected by using a Rheodyne syringe (Model 7202, Hamilton).

Results and Discussion

UV-Visible spectrum analysis

The UV-Visible spectrum of the different solvent extracts of *Hypolepis glandulifera* Brownsey *et* Chinnock was performed at the wavelength range of 200nm to 1100nm due to the sharpness of the peaks and proper baseline. The ethanol extract displayed the compounds at the nm of 1024, 888, 664, 606, 532, 500, 450, 364, 312 and 236 with the absorption 0.247, 0.287, 0.886, 1.348, 1.456, 1.794, 2.268, 1.204, 1.755 and 1.650 (Fig.1).

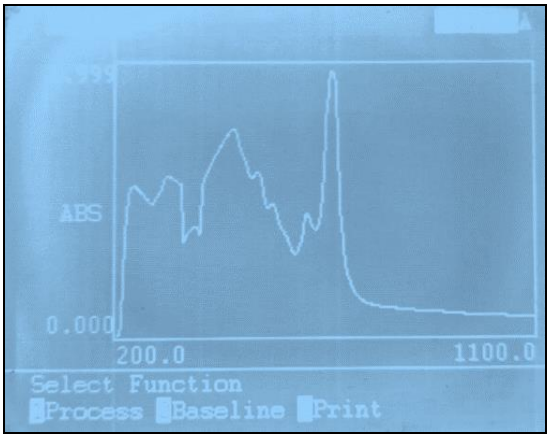

Fig 1: UV-Visible spectrum analysis of ethanolic extract of *Hypolepis glandulifera* Brownsey *Et* Chinnock

Fourier Transform Infrared (FTIR) spectrum

The FTIR spectrum of ethanol extract of *Hypolepis glandulifera* Brownsey *et* Chinnock displayed different peaks at 624.89, 670.22, 778.22, 1056.92, 1173.6, 1363.58, 1423.37, 1514.98, 1606.59, 1730.99, 2925.81, 3062.75 and 3381.95cm^{-1}. It was reported the presence of functional groups such as naphthalenes (in plane ring deformation), aldehydes (C-C-CHO bending), 1,2,3-trisubst benzenes (CH out of plane deformation), alkyl sulfoxides (S=O stretch), sulfonic acids (S=O stretch), sulfonyl chlorides (SO_2 antisym stretch), carboxylic acids (in plane OH bending), triazine compounds (ring stretch), β-diketones (C=O stretch), lactones (C=O stretch), aliphatic compounds (CH antisym and sym stretching), aromatic and unsaturated compounds (=C-H stretch) and alcohols and phenols (OH stretch) respectively (Table 1 and Fig.2).

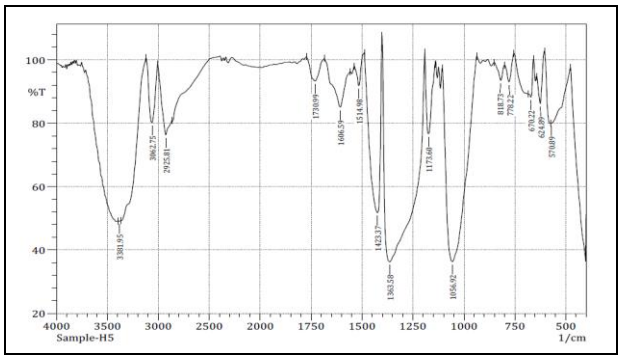

Fig 2: FTIR spectrum of ethanolic extract of *Hypolepis glandulifera* Brownsey *Et* Chinnock

Table 1: FTIR spectrum of ethanolic extract of *Hypolepis glandulifera* Brownsey *Et* Chinnock

Peak Value	Functional group	Assignments
624.89	Naphthalenes	in-plane ring deformation
670.22	Aldehydes	C-C-CHO bending
778.22	1,2,3-trisubst benzenes	CH out of plane deformation
1056.92	Alkyl sulfoxides	S=O stretch
1173.6	Sulfonic acids	S=O stretch
1363.58	Sulfonyl chlorides	SO_2 antisym stretch
1423.37	Carboxylic acids	in plane OH bending
1514.98	Triazine compounds	Ring stretch; sharp band
1606.59	β-diketones	C=O stretch
1730.99	Lactones	C=O stretch
2925.81	Aliphatic compounds	CH antisym and sym stretching
3062.75	Aromatic and unsaturated compounds	=C-H stretch
3381.95	Alcohols and phenols	OH stretch

HPLC Analysis

Five compounds were separated from the ethanol extract of *Hypolepis glandulifera* Brownsey *et* Chinnock at different retention time of 1.207min, 2.117min, 2.800min, 3.220min and 4.043min. The profile observed two prominent peaks at the retention time of 2.117min and 2.800min, followed by three moderate peaks were predicted at the retention time of 1.207min, 3.220min and 4.043min (Table 2 and Fig. 3).

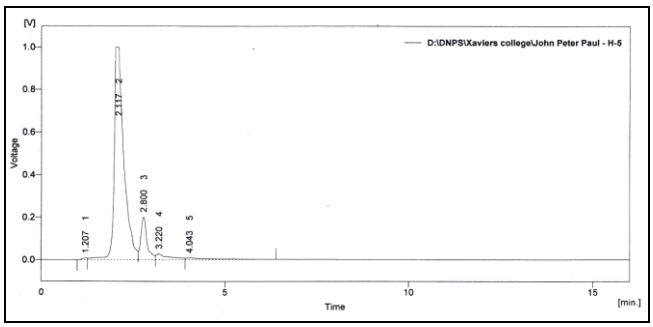

Fig 3: HPLC profile of ethanol extract of *Hypolepis glandulifera* Brownsey *et* Chinnock

Table 2: HPLC profile of ethanol extract of *Hypolepis glandulifera* Brownsey *et* Chinnock

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	1.207	74.068	7.787	0.3	0.6	0.16
2	2.117	18450.452	999.233	84.0	80.4	0.25
3	2.800	2358.009	200.078	10.7	16.1	0.16
4	3.220	678.149	27.204	3.1	2.2	0.27
5	4.043	415.198	8.451	1.9	0.7	0.62
	Total	21975.876	1242.752	100.0	100.0	

Conclusion

From the present study, it was concluded that The UV-Visible spectrum showed five compounds separated at the wavelength of 1024nm, 888nm, 664nm, 606nm, 532nm, 500nm, 450nm, 364nm, 312nm and 236nm with the absorption 0.247, 0.287, 0.886, 1.348, 1.456, 1.794, 2.268, 1.204, 1.755 and 1.650 respectively. The qualitative HPLC fingerprint profile displayed five compounds at different retention times. The profile displayed two prominent peaks at the retention time of 2.117min and 2.800min, followed by three moderate peaks were predicted at the retention time of 1.207min, 3.220min and 4.043min. The FTIR analysis recognized different peaks at 624.89, 670.22, 778.22, 1056.92, 1173.6, 1363.58, 1423.37, 1514.98, 1606.59, 1730.99, 2925.81, 3062.75 and 3381.95cm^{-1} with the presence of functional groups such as naphthalenes, aldehydes, 1,2,3-trisubst benzenes, alkyl sulfoxides, sulfonic acids, sulfonyl chlorides, carboxylic acids, triazine compounds, β-diketones, δ lactones, aliphatic compounds, aromatic and unsaturated compounds and alcohols and phenols.

Conflict of Interest

The author declares that they have no conflict of interest.

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