UV-VIS AND FTIR SPECTROSCOPIC PROFILE OF GAMETOPHYTE AND SPOROPHYTE ETHANOLIC EXTRACT OF *ANEMIA SCHIMPERIANA* C. PRESL SUBSP. *WIGHTIANA* (GARDNER) FRASER-JENK. AND *CYATHEA GIGANTEA* (WALL. EX. HOOK.) HOLTT.

SILVIA JULIET IRUTHAYAMANI, M. JOHNSON**#*
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*Reg. No. 19211282262014, Affiliated to Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu, India 627012
**Centre for Plant Biotechnology, Department of Botany, St. Xavier's College (Autonomous), Palayamkottai, Tamil Nadu, India 627002, #e-mail: ptcjohnson@gmail.com, cpbsxc@gmail.com, Mob: +91 9786 9243 34

Abstract. In the present study, an attempt is made to reveal the functional groups and metabolites of gametophyte and sporophyte of two pteridophytes *Anemia schimperiana* C. Presl subsp. *wightiana* (Gardner) Fraser-Jenk. and *Cyathea gigantea* (Wall ex Hook.) Holtt using UV-Vis and FTIR analysis. For UV-Vis analysis, the ethanolic extracts of *A. schimperiana* subsp. *wightiana* and *C. gigantea* were centrifuged at 3000 rpm for 5 min and filtered using Whatman No. 1 filter paper. The ethanolic extracts were scanned and recorded using Shimadzu UV-Vis spectrophotometer at the absorbance of 200 to 1100 nm. For FTIR spectrometry about 1.0 mg of gametophyte and sporophyte ethanolic extracts of *A. schimperiana* subsp. *wightiana* and *C. gigantea* were separately made into very thin discs with potassium bromide and the pellets are measured in an automatic recording IR Spectrophotometer in the range of $400–4000\ \text{cm}^{-1}$. Based on the observed peak values, the existence of alkaloids, phenolics, tannin and carotenoid existence were confirmed. Terpenoids, flavonoid and chlorophyll existence were also validated in different peak values of the ethanolic extracts of gametophytes and sporophytes of *A. schimperiana* subsp. *wightiana* and *C. gigantea*. The FTIR analysis results showed various functional groups viz., aromatic compounds, alkene, fluoro compounds, anhydride, aliphatic ether, alcohol, secondary amine presence in the gametophytes and sporophytes of *A. schimperiana* subsp. *wightiana* and *C. gigantea.* The gametophyte and sporophyte stages expressed the existence of different functional groups and displayed the variations and similarities between the studied stages. The spectroscopic results confirmed the secondary metabolites existence in the gametophytes and sporophytes of *A. schimperiana* subsp. *wightiana* and *C. gigantea* ethanolic extracts. Further chromatographic and separation studies may bring out stage-specific bioactive principles from the gametophytes and sporophytes of studied ferns.

Key words: *Anemia schimeriana* subsp. w*ightiana*, *Cyathea gigantea*, functional group, gametophyte, sporophyte.

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INTRODUCTION

Pteridophytes are the first vascular plants on the earth and exist till now. Their sexual reproduction life cycle was defined by an alternation of two generations like gametophyte and sporophyte phases [24]. In India about 1200 pteridophytes species were reported, among that 414 species were comes under vulnerable, threatened endangered or rare species [14]. The selected two fern species *Anemia schimperiana* C. Presl subsp. *wightiana* (Gardner) Fraser-Jenk. [11] and *Cyathea gigantea* (Wall. Ex. Hook.) Holtt. [4] were noted as endemic, endangered and rare species. Plants have manufactured a diverse variety of low molecular natural components that are also called secondary metabolites [14]. Each secondary metabolite provides various health benefits such as anti-oxidant, anti-bacterial, anti-inflammatory, anti-HIV, anti-viral and anti-tumor [8, 13, 32]. Phenolic compounds phenol and polyphenols are used as medicine for muscle spasticity, ingrown toenails surgeries, anti-inflammatory [28]. Steroids are largely used in neurocritical care unit for their immune suppressive and anti-inflammatory effects [44]. Pteridophytes have been used for their medicinal values for long period and they contain a large number of secondary metabolites like lignin, tannin, flavonoids, phenols, terpenoids, and polyphenols [51]. Chettri *et al*. [6] reported the edible ferns proteins, crude fiber, minerals and vitamins and very few secondary metabolites like terpenoids, phenolic acids, steroids and flavonoids.

UV-Vis spectroscopy is an effective, simple and rapid test to find the phytocomponents [23]. It is one of the oldest instrumental techniques to determine micro and semi-micro quantities [15]. Alexander *et al.* [1] studied the secondary metabolites in *S. cylindrica* by using UV-Vis spectroscopic analysis. Number of studies have performed using UV-Vis spectroscopy on various marine algae and plants with different extracts and revealed the qualitative and quantitative metabolites existence [10, 19, 20, 36, 42, 52]. The Fourier transform infrared (FTIR) spectroscopy was considered as one of the effective ways to study the chemical (functional group) and understand the surface chemistry in various types of cells [5, 30]. In *Marsilea quadrifolia* and *Vittaria elongata* extract, the occurrence of alkyne, alcohol, nitro group, aromatic compounds, alkyl halides and carboxylic acids are confirmed by the FTIR analysis [40, 46]. FTIR spectroscopic profile of *Cyathea nilgirensis* [22, 38, 50], *C. gigantea* and *C. crinita* [25] and *C. latebrosa* [16] are studied. In addition, FTIR profiles are reported from algae and pteridophytes and flowering plants [9, 10, 27, 39, 48]. Most of the phytochemical studies on pteridophytes focused on sporophytes only, very few studies are focused on gametophytes. Vincent *et al*. [54] studied the anti-bacterial efficacy of *in vitro* cultured gametophyte of *Cyclosorus interruptus*. Živković *et al.* [55] performed comparative phytochemical and antioxidant studies on gametophytes and sporophytes of *Asplenium ceterach*. Recently, Vidyarani *et al.* [53] studied the existence of functional groups between the gametophyte and sporophyte of *Phlebodium aureum* using FTIR analysis.

A. schimperiana subsp. *wightiana* was used as anti-microbial agent to treat tuberculosis from the ancient times and also confirmed the presence of phenolic, tannin and flavonoid [49]. Samy *et al.* [45] noted the antioxidant properties of *A. schimperiana* subsp. *wightiana.* Nair *et al.* [33] identified thirty compounds from the essential oil of *A. schimperiana* subsp. *wightiana* and reported the anti-proliferative properties. The qualitative and quantitative profile of *Cyathea* sps secondary metabolites is reported and the hepatoprotective, anti-microbial and anti-cancer potential of *C. gigantea* are reported [17, 29, 34, 37–40, 42]. But there is no report on UV-Vis and FTIR spectroscopic profile of *Anemia schimperiana* subsp. *wightiana* and *Cyathea gigantea* gametophyte and sporophyte. Hence, the present study was intended to reveal the UV-Vis profile and predict the existence of secondary metabolites and reveal the functional group occurrence between gametophyte and sporophyte of *Anemia schimperiana* C.Presl subsp. *wightiana* (Gardner) Fraser-Jenk. and *Cyathea gigantea* (Wall. Ex. Hook.) Holtt using UV-Vis and FTIR spectroscopic analysis.

MATERIALS AND METHODS

PLANT EXTRACT PREPARATION

The *in vitro* spore culture derived gametophyte and sporophytes of *Anemia schimperiana* C. Presl subsp. *wightiana* (Gardner) Fraser-Jenk. and *Cyathea gigantea* (Wall. Ex. Hook.) Holtt. (Fig. 1) were collected. 10 g of gametophyte and sporophyte were collected, and 100 mL of ethanol was added and kept in the room temperature for 72 h (cold extraction).

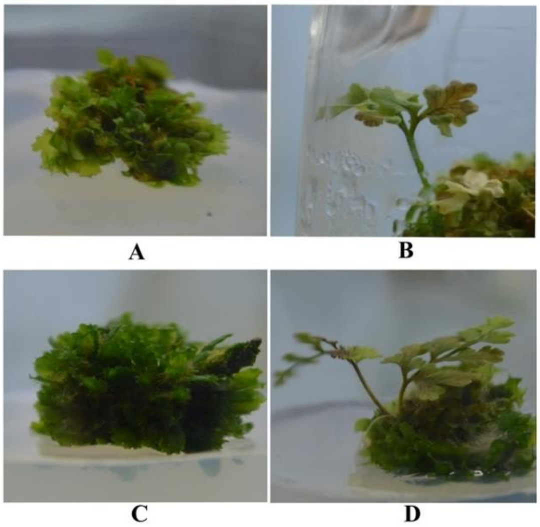

Fig. 1. *In vitro* gametophyte and sporophyte of *A. schimperiana* subsp *wightiana* (A and B) and *C. gigantea* (C and D).

After 72 h, the extract was collected and centrifuged at 3000 rpm for 5 min. The supernatant was collected and used for UV-Vis spectroscopic analysis. The supernatant was collected and kept in a Petri plate and at room temperature to evaporate the excess solvents. After evaporation, the gametophytes and sporophytes slurry were used for FTIR analysis.

UV-VIS SPECTROSCOPIC ANALYSIS

The UV-Vis analysis of *A. schimperiana* subsp *wightiana and C. gigantea* gametophyte and sporophyte extracts were recorded using Shimadzu UV-Vis spectrophotometer at the absorbance of 200 to 1100 nm. The extracts were centrifuged at 3000 rpm for 5 min and filtered using Whatman No. 1 filter paper. The extracts were scanned in the wavelength from 200–1100 nm with 1 nm interval. The observed absorbance peaks (Abs) UV-Vis spectra were constructed using MS-Excel 2007.

FTIR ANALYSIS

1 mg of the gametophyte and sporophyte of *Anemia schimperiana* C. Presl subsp. *wightiana* (Gardner) Fraser-Jenk. and *Cyathea gigantea* (Wall. Ex. Hook.) Holtt. extracts were separately made into a thin disc with potassium bromide (10–100 mg) using a mould and under anhydrous conditions pressed to prepare a translucent sample disc. The pellets were measured in an automatic recording with Fourier transform infrared spectroscopy (Shimadzu 8400S) in the range of 400 to 4000 cm^{-1}. The transmission percentages were recorded against the wave number. The FTIR peak values were record, and the functional groups were predicted using Aldrich and Sigma IR table [18, 22].

RESULTS

UV-VIS ANALYSIS

The UV-Vis spectra profile of *A. schimperiana* subsp. *wightiana* and *C. gigantea* sporophytes and gametophytes ethanolic extracts showed various peaks with different absorption values indicating the existence of varied metabolites with different quantities (Tables 1 and 2, Figs 2 to 5). Based on the observed peak values, the existence of alkaloids, phenolics, tannins, terpenoids, flavonoids, carotenoids and chlorophyll pigments were validated in the ethanolic extract of gametophytes and sporophytes of *A. schimperiana* subsp. *wightiana* and *C. gigantea* (Tables 1 and 2). The gametophytes of *A. schimperiana* subsp. *wightiana* ethanolic extract possess more amounts of metabolites than sporophytes (Table 1). On the contrary, the sporophytes of *C. gigantea* ethanolic extract showed more amounts of metabolites than gametophytes (Table 2).

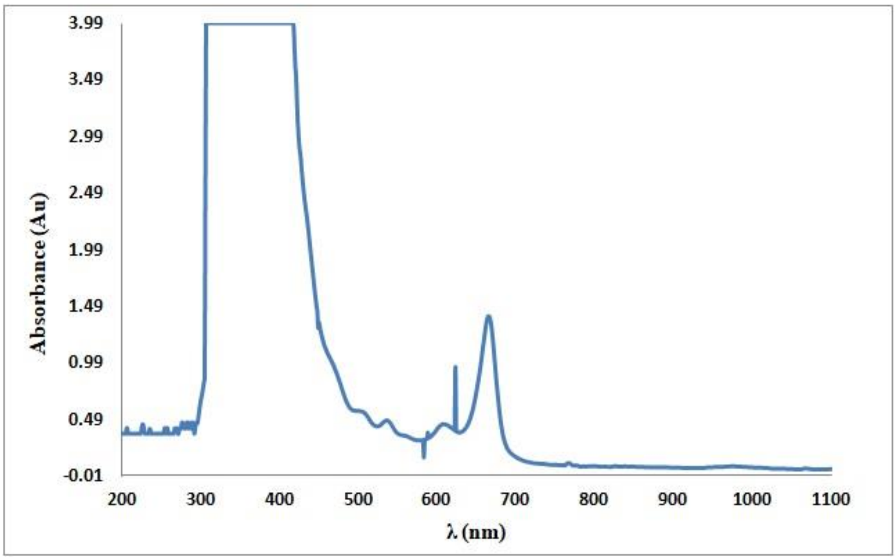

Fig. 2. UV-Vis spectrum of *A. schimperiana* subsp. *wightiana* gametophytes ethanolic extract.

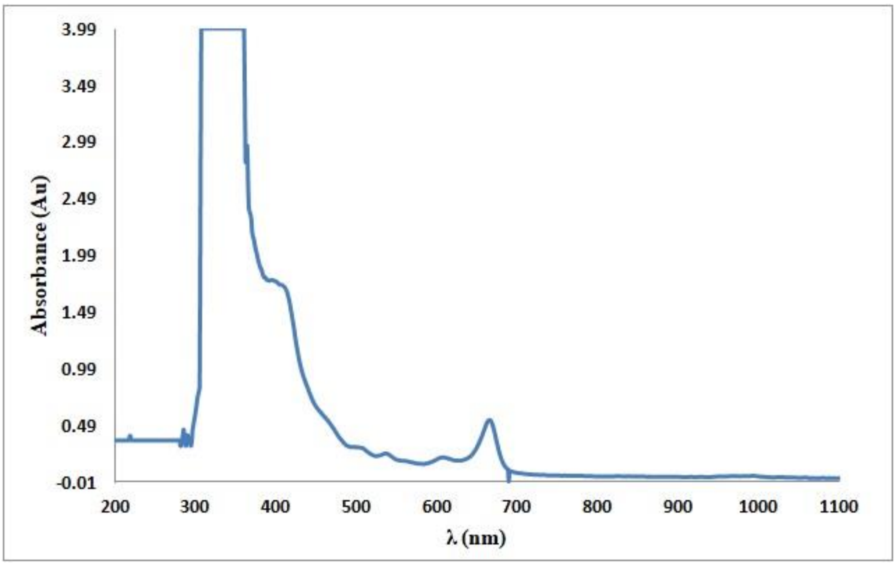

Fig. 3. UV-Vis spectrum of *A. schimperiana* subsp. *wightiana* sporophytes ethanolic extract.

Table 1

UV-Vis peak values of gametophytes and sporophyte of *Anemia schimperiana* subsp. *wightiana* ethanolic extract

λ_{max} (nm)	Gametophytes (Abs)	Sporophytes (Abs)	Predicted metabolites	Reference
333.0		2.297	Alkaloids, phenolics	[36]
417.0	3.135		Alkaloids, phenolics, terpenoids,	[42] [36]
507.0	0.712		Alkaloids, phenolics, flavonoids, terpenoids	[9] [42] [36]
537.0	0.666		Terpenoids, alkaloids, flavonoids	
607.0	0.547	0.249	Flavonoids, alkaloids, phenolics	
665.0	2.003	0.364	Chlorophyll	[42]

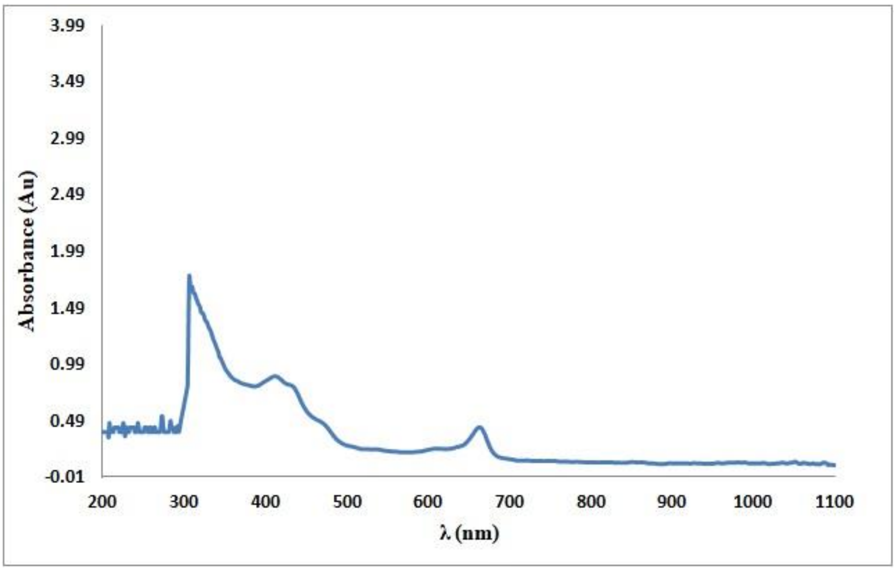

Fig. 4. UV-Vis spectrum of *C. gigantea* of gametophytes ethanolic extract.

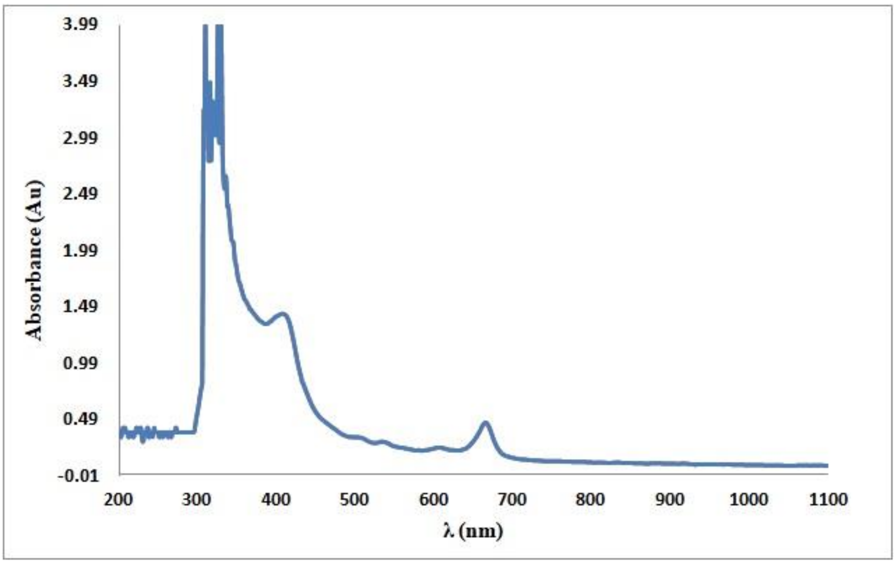

Fig. 5. UV-Vis spectrum of *C. gigantea* of sporophytes ethanolic extract.

Table 2

UV-Vis peak values of gametophytes and sporophyte of *C. gigantea* ethanolic extract

λ_{max} (nm)	**Gametophytes (Abs)**	**Sporophytes (Abs)**	**Predicted metabolites**	**Reference**
309.0		4.000	Alkaloids, phenolics	[36]
338.0	2.889		Alkaloids, phenolics	
409.0		2.219	Terpenoids, alkaloids, phenolics	[36] [42]
411.0	1.954		Terpenoids, alkaloids, phenolics	
506.0	0.238	0.254	Flavonoids, alkaloids, phenolics	[9] [36] [42]
537.0	0.224	0.338	Flavonoids, alkaloids, phenolics	
607.0	0.195	0.193	Chlorophyll	[42]
664.0	0.682		Chlorophyll	
665.0		0.753	Chlorophyll	

FTIR ANALYSIS

Anemia schimperiana C. Presl subsp. *wightiana* Fraser-Jenk

Nitro compound with N–O stretching was observed in gametophyte (1434.4 cm^{-1}) and sporophyte (1400.73 cm^{-1}). C=C bending with alkene was determined in gametophyte (644.46 cm^{-1}) and sporophyte (669.88 cm^{-1}). C–H bending with aromatic compound, 1,3-disubstituted and 1,2,4-trisubstituted was observed in gametophyte (878.85 cm^{-1}, 1667.11 cm^{-1}) and sporophyte (880.07 cm^{-1}, 1654.56 cm^{-1}). Carbondioxide with O=C=O stretching was determined in gametophyte (2088.13 cm^{-1}, 2350.79 cm^{-1}). N–H stretching with secondary amine was observed in gametophyte (2946.21 cm^{-1}) and sporophyte (2896.71 cm^{-1}, 2975.75 cm^{-1}). Alcohol with O–H stretching was found in gametophyte (3156.43 cm^{-1}) and sporophyte (3135.42 cm^{-1}, 3565.93 cm^{-1}). C–Br stretching and C–CI stretching with Halo compounds were illustrated in sporophyte (600.23 cm^{-1}, 808.63 cm^{-1}). C–H bending with 1,3-disubstituted, 1,2,4-trisubstitured and aromatic compounds were found in gametophyte (878.85 cm^{-1}, 1667.11 cm^{-1}) and sporophyte (880.87 cm^{-1}, 1654.56 cm^{-1}). Aliphatic ether with C–O stretching was observed in gametophyte (1068.85 cm^{-1}) and sporophyte (1086.87 cm^{-1}). O–H bending with phenol, and C–H stretching with alkyne were found in gametophyte (1375.49 cm^{-1}, 2541.2 cm^{-1}). C–F stretching with fluoro compound and allene with C=C=C stretching was observed uniquely in sporophyte extract (1273.47 cm^{-1}, 1928.26 cm^{-1}) (Table 3, Figures 6 and 7).

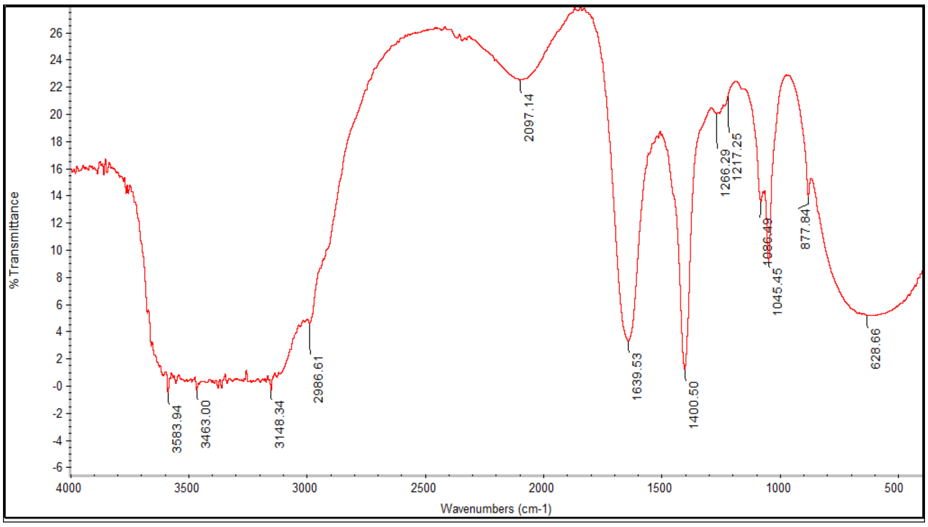

Fig. 6. FTIR spectra of *in vitro* spore derived gametophyte *of Anemia schimperiana* subsp. *wightiana* ethanolic extract.

Table 3

FTIR peak values with functional groups of gametophyte and sporophyte of *Anemia schimperiana* subsp. *wightiana* ethanolic extract

Absorption rate (cm^{-1})	**Appearance**	**Group**	**Compound class**	**Gametophyte**	**Sporophyte**
600.23	Weak, broad	C–Br stretching	Halo compound	–	+
644.46	Medium, broad	C=C bending	Alkene	+	–
669.88	Weak	C=C bending	Alkene	–	+
808.63	Weak	C–CI stretching	Halo compound	–	+
878.85	Strong	C–H bending	1,3-disubstituted	+	–
880.07	Strong	C–H bending	1,2,4-trisubstituted	–	+
1029.28	Strong, broad	C–F stretching	Fluoro compound	+	–
1048.75	Strong	CO–C–CO stretching	Anhydride	–	+
1068.85	Strong, broad	C–O stretching	Primary alcohol	+	–
1086.87	Strong	C–O stretching	Aliphatic ether	–	+
1273.47	Weak	C–F stretching	Fluoro compound	–	+
1375.49	Strong	O–H bending	Phenol	+	–
1400.73	Strong	N–O stretching	Nitro compound	–	+
1434.4	Strong	N–O stretching	Nitro compound	+	–
1654.56	Strong, broad	C–H bending	Aromatic compound	–	+
1667.11	Strong	C–H bending	Aromatic compound	+	–
1928.26	Weak	C=C=C stretching	Alkene	–	+
2088.13	Medium, broad	O=C=O stretching	Carbondioxide	+	–
2350.79	Strong	O=C=O stretching	Carbondioxide	+	–
2541.2	Weak	C–H stretching	Alkyne	+	–
2896.71	Weak	N–H stretching	Secondary amine	–	+

2946.21	Medium, broad	N–H stretching	Secondary amine	+	–
2975.75	Medium, broad	N–H stretching	Secondary amine	–	+
3135.42	Weak	O–H stretching	Alcohol	–	+
3156.43	Weak	O–H stretching	Alcohol	+	–
3565.93	Weak	O–H stretching	Alcohol	+	–

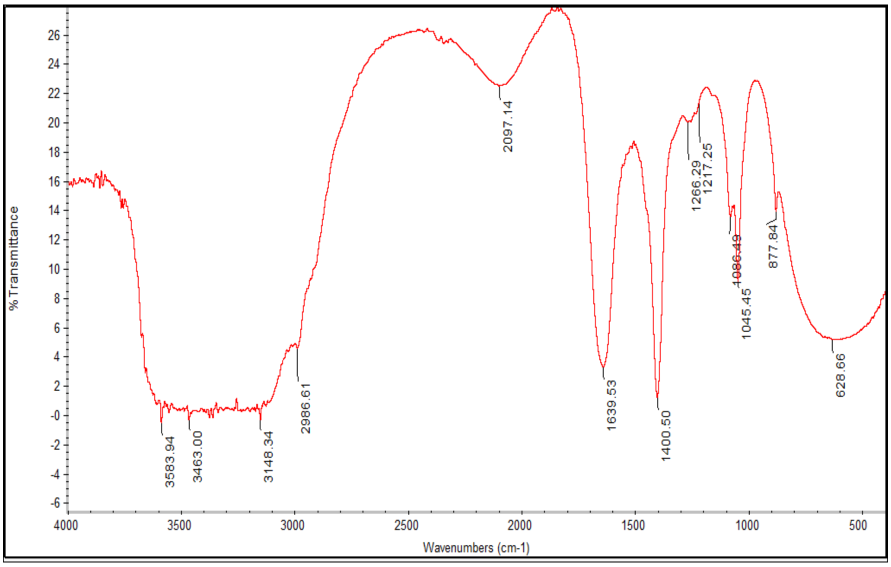

Fig. 7. FTIR spectra of *Anemia schimperiana* subsp. *wightiana* sporophyte ethanolic extract.

Cyathea gigantea **(Wall. Ex. Hook.) Holtt**

Halo compound with C–Br stretching was illustrated in gametophyte (628.66 cm^{-1}) and sporophyte (669.44 cm^{-1}). Alcohol with O–H stretching was found in gametophyte extract (3148.34 cm^{-1}, 3463 cm^{-1}, 3583.94 cm^{-1}) and sporophyte (3260.68 cm^{-1}). C–C stretching with cyclic alkene was illustrated in gametophyte extract (1639.53 cm^{-1}) and sporophyte extracts (1654.57 cm^{-1}). Anhydride compound with CO–O–CO stretching was observed in gametophyte (1045.45 cm^{-1}) and sporophyte (1047.48 cm^{-1}). Ester with C–O stretching was found in Gametophyte (1217.25 cm^{-1}), primary alcohol in gametophyte (1086.49 cm^{-1}), sporophyte (1086.9 cm^{-1}) and alkyl aryl ether was illustrated in gametophyte (1217.25 cm^{-1}, 1266.29 cm^{-1}). C–F stretching with fluoro compound was observed in gametophyte (1400.5 cm^{-1}) and sporophyte (1400.65 cm^{-1}). Aromatic compound with C–H bending was found in sporophyte (1928.26 cm^{-1}) and 1,2,4 trisubstituted was found in gametophyte (877.84 cm^{-1}) and sporophyte (880.13 cm^{-1}) (Table 4, Figs 8, 9). C=C bending with alkene and N–H stretching with aliphatic primary amine was uniquely observed in sporophyte (804.54 cm^{-1}, 3377.18 cm^{-1}). N=C=S stretching with isothiocyanate was found in gametophyte (2097.14 cm^{-1}).

Table 4

FTIR peak values with functional groups of gametophyte and sporophyte stages of *Cyathea gigantea* ethanolic extract

Absorption rate (cm^{-1})	**Appearance**	**Group**	**Compound class**	**Gametophyte**	**Sporophyte**
628.66	Medium, broad	C–Br stretching	Halo compound	+	–
669.44	Weak, broad	C–Br stretching	Halo compound	–	+
804.54	Weak	C=C bending	Alkene	–	+
877.84	Weak	C–H bending	1,2,4-trisubstituted	+	–
880.13	Strong	C–H bending	1,2,4-trisubstituted	–	+
1045.45	Strong	CO–O–CO stretching	Anhydride	+	–
1047.48	Strong	CO–O–CO stretching	Anhydride	–	+
1086.49	Weak	C–O stretching	Primary alcohol	+	–
1086.90	Strong	C–O stretching	Primary alcohol	–	+
1217.25	Weak, broad	C–O stretching	ester	+	–
1266.29	Weak	C–O stretching	Alkyl aryl ether	+	–
1274.00	Weak	C–O stretching	Alkyl aryl ether	–	+
1400.50	Strong	C–F stretching	Fluoro compound	+	–
1400.65	Strong	C–F stretching	Fluoro compound	–	+

1639.53	Strong	C–C stretching	alkene	+	–
1654.57	Strong, broad	C–C stretching	Cyclic alkene	–	+
1928.26	Weak	C–H bending	Aromatic compound	–	+
2097.14	Broad	N=C=S stretching	Isothiocy anate	+	–
2930.98	Weak	C–H stretching	alkane	–	+
2978.51	Medium	C–H stretching	alkane	–	+
2986.61	Weak	C–H stretching	alkane	+	–
3148.34	Weak	O–H stretching	alcohol	+	–
3260.68	Weak	O–H stretching	alcohol	–	+
3377.18	Weak	N–H stretching	aliphatic primary amine	–	+
3463.00	Weak	O–H stretching	Alcohol	+	–
3583.94	Weak	O–H stretching	Alcohol	+	–

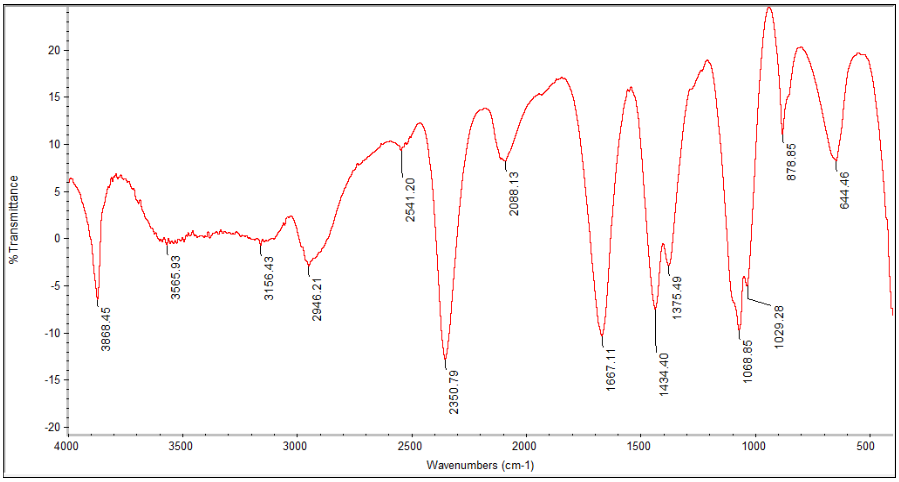

Fig. 8. FTIR spectra of *Cyathea gigantea* (Wall. Ex. Hook.) Holtt gametophyte ethanolic extract.

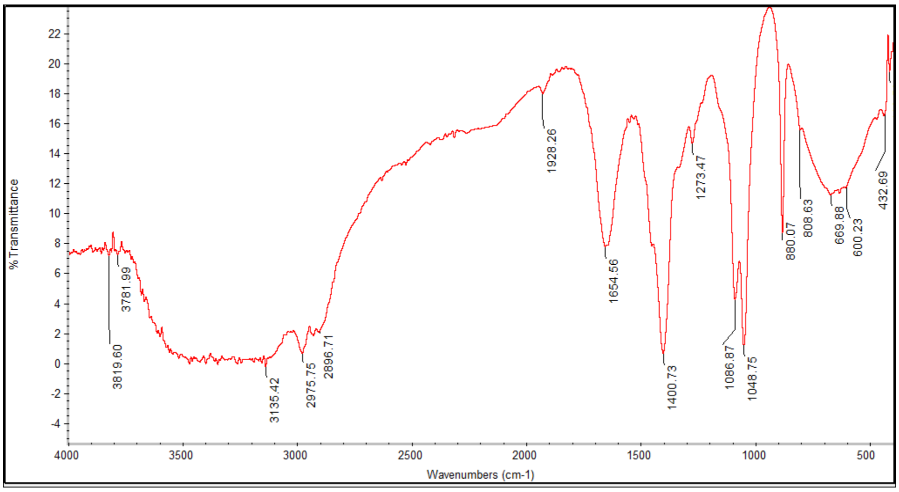

Fig. 9. FTIR spectra of *Cyathea gigantea* (Wall. Ex. Hook.) Holtt sporophyte ethanolic extract.

DISCUSSION

Alkene compounds were observed at 956.69 cm^{-1}, 964.41 cm^{-1}, 1658.78 cm^{-1}, 1651.07 cm^{-1} and 1658.78 cm^{-1} in *Cyathea* species [18] and at 827.25 cm^{-1} in *Ceratopteris thalictroides* [47]. In the present study also the alkene group was observed at 644.46 cm^{-1}, 669.88 cm^{-1} gametophyte of *A. schimperiana* subsp. *wightiana* and 804.54 cm^{-1} sporophyte of *C. gigantea* ethanolic extract.

Janakiraman and Johnson [18] identified O–H stretching with alcohol in *Cyathea* species by the presence of the peak at 3400 to 3450 cm^{-1}. The occurrence of O–H stretch with alcohol was observed at the peak of 3250 to 3450 cm^{-1} [7]. The observed results of the present study validated the observations of Janakiraman and Johnson [18] and D'Angelo and Zondrow [7] by showing the alcohol with O–H stretching in the gametophytes of two studied species ethanolic extract at the peak of 3135 to 3565 cm^{-1} and 3463 cm^{-1}, 3583.94 cm^{-1}.

Aliphatic compounds presence was observed in *H. glandulifera* (2925.81 cm^{-1}), *C. crinita* (1381.03 cm^{-1}, 1543.05 cm^{-1}, 1381.03 cm^{-1}, 1543.05 cm^{-1}, 1381.03 cm^{-1}), *C. gigantea* (1527.62 cm^{-1}, 1373 cm^{-1}, 1496.76 cm^{-1}, 1527.62 cm^{-1}, 1381.03 cm^{-1}), *C. nilgirensis* (1543.05 cm^{-1}, 1381.03 cm^{-1}, 1496.76 cm^{-1}, 1373.32 cm^{-1}, 1543.05 cm^{-1}) [18, 26]. Similar to the previous observation, the aliphatic compounds were observed in *A. schimperiana* subsp. *wightiana* sporophyte (1086.87 cm^{-1}) and *C. gigantea* gametophyte ethanolic extract (3377.18 cm^{-1}).

Presence of phenol was observed in the peak at 3332.36 cm^{-1} [31], 3364.58 cm^{-1} [7], 3270 – 3320 cm^{-1} [43], 3406. 43 cm^{-1} [3]. But in the present study the phenol was found in 1375.40 cm^{-1} in the gametophyte extract of *A. schimperiana* subsp. *wightiana.*

The aromatic compound was observed in *Hypolepsis glandulifera* at 2925.81 cm^{-1} [26]. Pradheesh *et al.* [38] found aromatic compound presence at 823 cm^{-1} in *C. nilgirensis*. Ramyajuliet *et al.* [40] validated the presence of aromatic compounds in *Marsilea quadrifolia* at 1441 cm^{-1}, 1559 cm^{-1} and 1541 cm^{-1}. Muthulakshmi and Anusya [31] observed the presence of aromatic compound at 1462 cm^{-1}, 1452 cm^{-1}, 1449 cm^{-1}, 1508 cm^{-1}. In this study, the aromatic compound presence was confirmed in the sporophyte and gametophyte of *A. schimperiana* subsp. *wightiana* at 1654.56 cm^{-1} and 1667.11 cm^{-1} and in the sporophyte of *C. gigantea* at 1928.26 cm^{-1}. The results of the present study supported the previous observation.

The halo compounds with the peak at 1046.80 cm^{-1}, 1043.56 cm^{-1}, 1013.41 cm^{-1} and 1041.43 cm^{-1} were observed [31]. Sahu and Saxena [43] observed the halo compound at 611.22 cm^{-1}. Likewise, Rani *et al.* [41] also studied the halo compounds at 1180.22 cm^{-1} and 1114.65 cm^{-1}. In the present study the halo compounds observed at 600.23 cm^{-1} and 808.63 cm^{-1} in the sporophyte of *A. schimperiana* subsp. w*ightiana and* 628.66 cm^{-1} (gametophyte), 669.44 cm^{-1} (sporophyte) of *C. gigantea* ethanolic extract.

Aliphatic amine compound was observed in *Sapindus mukrossi* at the peak of 1026 cm^{-1} [37]. Gnanasundaram and Balakrishnan [12] studied the aliphatic amine in *Cissus vitiginea* at 1030.73 cm^{-1}, 1017 cm^{-1} and 1054 cm^{-1}. Rani *et al.* [41] found the aliphatic amine at 1086.69 cm^{-1}. In this study the aliphatic amine presence was observed in the sporophyte of *C. gigantea* ethanolic extract.

Nazneen and Bhavani [35] studied the compounds of *Cissus quadrangularis* and found the presence of ester at 1745.64 cm^{-1}. In this study also the ester was observed at the peak of 1217.25 cm^{-1} in the gametophyte of *C. gigantea* ethanolic extract. Similarly, the presence of nitro compounds was confirmed at the peak of 1377.3 cm^{-1} and 1382.27 cm^{-1} [31, 43]. In this study also the nitro compound presence was observed in *A. schimperiana* subsp. w*ightiana* gametophyte (1400.73 cm^{-1}) and sporophyte (1434.4 cm^{-1}) ethanolic extract.

Plants are widely used for their medicinal values due to the occurrence of secondary metabolites like lignin, phenol, tannin, flavonoids, polyphenols and steroids [2, 21]. In UV Vis spectroscopy the peaks in between 400 to 450 nm indicated the presence of carotenoids and terpenoids [42]. The presence of phenolic and alkaloid compounds in *Cissus vitingnea* at the range of 234–676 nm was reported [12]. The existence of alkaloids, flavonoids and phenolic compounds were confirmed by the presence of peak between 237 – 700 nm and 200 to 676 nm [9] [36]. Muthulakshmi and Anusya [31] observed the flavonoid compounds at the range of 233, 274, 271 and 373 nm. Sujatha *et al.* [49] determined the phenol (125.27 µg/mg), tannin (115.22 µg/mg) and flavonoids (85.97 µg/mg) of *Anemia wightiana* leaves methanolic extracts. The results of UV-Vis analysis also confirmed the existence of phenols, tannins, flavonoids, carotenoids, and alkaloids in the gametophytes and sporophytes of *A. wightiana* by showing the peaks between 200 – 676 nm (Tables 1 and 2). Concerning available literature, the occurrence of peaks at 338, 411, 506, 537, 607, and 664 nm validated the presence of phenols, tannins, flavonoids, terpenoids, carotenoids, alkaloids in the gametophytes of *C. gigantea* ethanolic extract and peaks at 309, 409, 506, 537, 607, 665 nm confirmed the existence of phenols, tannins, flavonoids, carotenoids, alkaloids in the *C. gigantea* sporophyte ethanolic extract. The peaks at 664 and 665 nm confirmed the existence of chlorophyll pigments in the gametophytes and sporophytes ethanolic extract of *A. wightiana* and *C. gigantea.* Ferns possess alternation of generation and it offers a platform to assess the phytochemical changes in the content of bioactive compounds and qualitative profile in both sporophytic and gametophytic generation. The outcome of the study results revealed the similarities and variations between gametophytes and sporophytes of two pteridophytes by observing the different UV-Vis peaks values and the functional group and confirming the variation of the phytochemical constituents at different developmental stages. The gametophytes and sporophytes need varied metabolite profiles for their survival and protection against the biotic and abiotic components. The observed results also confirmed the varied amount of metabolites existence and chlorophyll pigments. The existence of metabolites and chlorophyll pigments in the sporophytes and gametophytes of *A. wightiana* and *C. gigantea* confirms the independent nature, self-defense mechanism and productivity.

CONCLUSION

The gametophyte and sporophyte stages of *A. schimperiana* subsp. *wightiana* and *C. gigantea* ethanolic extract expressed the existence of different functional groups and metabolites. The observed variations and similarities between the gametophytes and sporophytes confirm their independent nature of gametophytes and sporophytes. The observed results showed the variation in metabolite profile and quantities at gametophyte and sporophyte stages of studied ferns. The spectroscopic results confirmed the different secondary metabolites existence in the gametophytes and sporophytes of *A. schimperiana* subsp. *wightiana* and *C. gigantea* ethanolic extract with varied quantities. Secondary metabolites existence confirms the self-defense mechanism of the gametophytes and sporophytes of studied ferns. The existence of metabolites protects the plant (gametophytes and sporophytes) from the biotic and abiotic components of the ecosystem. Every plant requires different biochemical constituents at varied levels in the course of development. Further chromatographic and separation studies may bring out stage-specific bioactive principles from the gametophytes and sporophytes of studied ferns.

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