FT-IR SPECTROSCOPIC STUDIES ON THE GAMETOPHYTES AND SPOROPHYTES OF *PHLEBODIUM AUREUM* (L.) J. SMITH

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Abstract. In the present study, FT-IR analysis was used to study the functional groups present in the three different extracts of *in vitro* cultured gametophytes and sporophytes of *Phlebodium aureum* (L.) J. Smith. The powdered sample of each sample was loaded in FT-IR spectrophotometer (Shimadzu 8400S) in the range of 400 to 4000 cm^{-1}. The transmission percentage was verified against the wave number. The peak values of FT-IR were recorded, and the functional groups were predicted using Aldrich and Sigma IR chart table. The analysis revealed the presence of different types of compounds such as alkene, sulfoxide, esters, aromatic compounds, isothiocyanate etc. The observed results showed the similarities and variation between the gametophytes and sporophytes of *Phlebodium aureum*. The outcome of the study showed the unique profiles for gametophytes and sporophytes, and these may be employed to identify the gametophytes and sporophytes of *Phlebodium aureum.*

Key words: *Phlebodium aureum*, gametophyte, sporophyte, functional groups.

INTRODUCTION

To determine the functional groups in the unknown composition of the structure, the Fourier transform infrared spectrophotometer (FT-IR) is employed [2]. One of the rapidly expanding areas of chemosystematics is photochemistry, which utilizes chemical information to improve plant classification and identification. In Pharmacognosy, the identity of drugs confirmed using the morphological and microscopical characters was used. Identification based on morphological characters alone is questionable now. In addition to morphological and microscopical characters, phytochemical characters can be used to identify and distinguish drugs or species [4]. FT-IR has been used to reveal the functional constituents and the FT-IR profiles are employed as biochemical markers [1, 5–10]. Komal and Devi [6] used

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FT-IR to identify and compare the biomolecules in medicinal plants. Lethika *et al*. [7] studied few medicinal plants of Chhattisgarh using FT-IR. Maiten *et al*. [8] compared the features of the pteridosperm and host rock. Ragavendran *et al*. [9] studied the functional group analysis of *Aerva lanata* various extracts. Previously, UHPLC analysis was employed to identify the chemical variation between the gametophyte and sporophyte [11]. But there is no work related to determining functional groups existence in the gametophytes and sporophytes of Pteridophytes. Therefore, the present work is aimed to determine the functional groups present in the *in vitro* cultured gametophytes and sporophytes of *Phlebodium aureum* (L.) J. Smith.

MATERIALS AND METHODS

The in vitro spore-derived gametophytes and sporophytes of *Phlebodium aureum* (L.) J. Smith was used for the FT-IR analysis. 1 g of *in vitro* cultured gametophyte and sporophyte of *Phlebodium aureum* was taken with 60 mL of acetone, ethanol, and chloroform and kept at room temperature for 72 h. After 72 h the extracts are filtered and centrifuged at 5000 rpm. After centrifugation, the supernatant was collected and employed for FT-IR analysis.

FT-IR ANALYSIS

About 1.0 mL of the *Phlebodium aureum* gametophyte and sporophyte acetone, chloroform, and ethanol extracts were separately made into thin discs with 10–100 mg of potassium bromide using a mold and pressed under anhydrous conditions to prepare translucent sample disc. The powdered sample of each sample was loaded in an FT-IR spectrophotometer (Shimadzu 8400S) in the range of 400 to 4000 cm^{-1}. The transmission percentage was verified against the wave number. The peak values of FT-IR were recorded, and the functional groups were predicted using Aldrich and Sigma IR chart table.

RESULTS

The evaluation of the FT-IR spectra in terms of functional group corresponds to the absorption range of the frequencies of the *Phlebodium aureum* gametophyte (G) and sporophyte (S) sample for three different solvents were recorded in Tables 1–3 and Figs 1–3. Halo compounds with C–I stretching was observed in the sporophyte (536.93 cm^{-1}) of the acetone extract. C–Br stretching with Halo compounds was determined in the gametophytes (601.51 cm^{-1}, 673.78 cm^{-1}) and sporophyte (669.69 cm^{-1}). In the gametophyte (878.09 cm^{-1}) and sporophyte (910.78 cm^{-1}) C–H bending with 1.3 disubstituted compound was studied. Sulfoxide with S=O stretching; CO–O–CO with anhydride compound and aliphatic ether with C–O

stretching were unique to the sporophyte (1037.46 cm^{-1}); gametophyte (1046.15 cm^{-1}) and sporophyte (1092.72 cm^{-1}) of the acetone extract. C–N stretching of the amine compound was illustrated in the gametophyte (1231.01 cm^{-1}) and sporophyte (1233.6 cm^{-1}). In the sporophyte (1364.36 cm^{-1}), the uniqueness of S=O stretching with sulfonamide was observed. Flouro compound with C–F stretching was seen in the gametophyte (1400.49 cm^{-1}) and sporophyte (1400.6 cm^{-1}) of the extract. C=C stretching with alkene compound was determined in the gametophyte (1638.78 cm^{-1}) and sporophyte (1642.95 cm^{-1}). C=O stretching with conjugated acid and N=C=N stretching with carbodiimide compound were observed uniquely in the sporophyte extract at (1702.97 cm^{-1}) and (2144.52 cm^{-1}) respectively. C–H bending with aldehyde and C–H bending with alkane compound were seen in the gametophyte (2713.31 cm^{-1}, 2923.71 cm^{-1}) and sporophyte (2847.67 cm^{-1}). Alcohol compound with O–H stretching was determined in the gametophyte (3178.09 cm^{-1}) and sporophyte (3468.91 cm^{-1}) of the extract (Table 1; Fig. 1).

Table 1

FT-IR peak values with functional groups of gametophyte and sporophyte stages of *Phlebodium aureum* in acetone

Absorption range (cm^{-1})	Appearance	Group	Compound class	Acetone	
				G	S
420.43					+
436.78				+	
469.47				+	
536.93	strong	C–I stretching	Halo compound		+
601.51	weak	C–Br stretching	Halo compound	+	
669.69	strong	C–Br stretching	Halo compound		+
673.78	Weak	C–Br stretching	Halo compound	+	
767.7					+
878.09	strong	C–H bending	1,3 disubstituted	+	
910.78		C–H bending	1,3 disubstituted		+
1037.46	strong	S=O stretching	Sulfoxide		+
1046.15	Strong, broad	CO–O–CO	anhydride	+	
1086.49				+	
1092.72	strong	C–O stretching	Aliphatic ether		+
1231.01	medium	C–N stretching	Amine		+
1233.6	medium	C–N stretching	Amine	+	
1274.46	strong	C–O stretching	Alkyl aryl ether		+
1364.36	strong	S=O stretching	Sulfonamide		+

1400.49	strong	C–F stretching	Fluoro compound	+	
1400.6	Strong	C–F stretching	Fluoro compound		+
1638.78	strong	C=C stretching	Alkene	+	
1642.95	strong	C=C stretching	Alkene		+
1702.97	strong	C=O stretching	Conjugated acid		+
2144.52	strong	N=C=N stretching	carbodiimide		+
2713.31	medium	C–H stretching	Aldehyde	+	
2847.67	medium	C–H stretching	Alkane		+
2923.71	medium	C–H stretching	Alkane	+	
3178.09	Weak, broad	O–H stretching	Alcohol	+	
3468.91	Strong, broad	O–H stretching	Alcohol		+
3574.39					+
3753.37				+	
3795.21					+
3822.49				+	

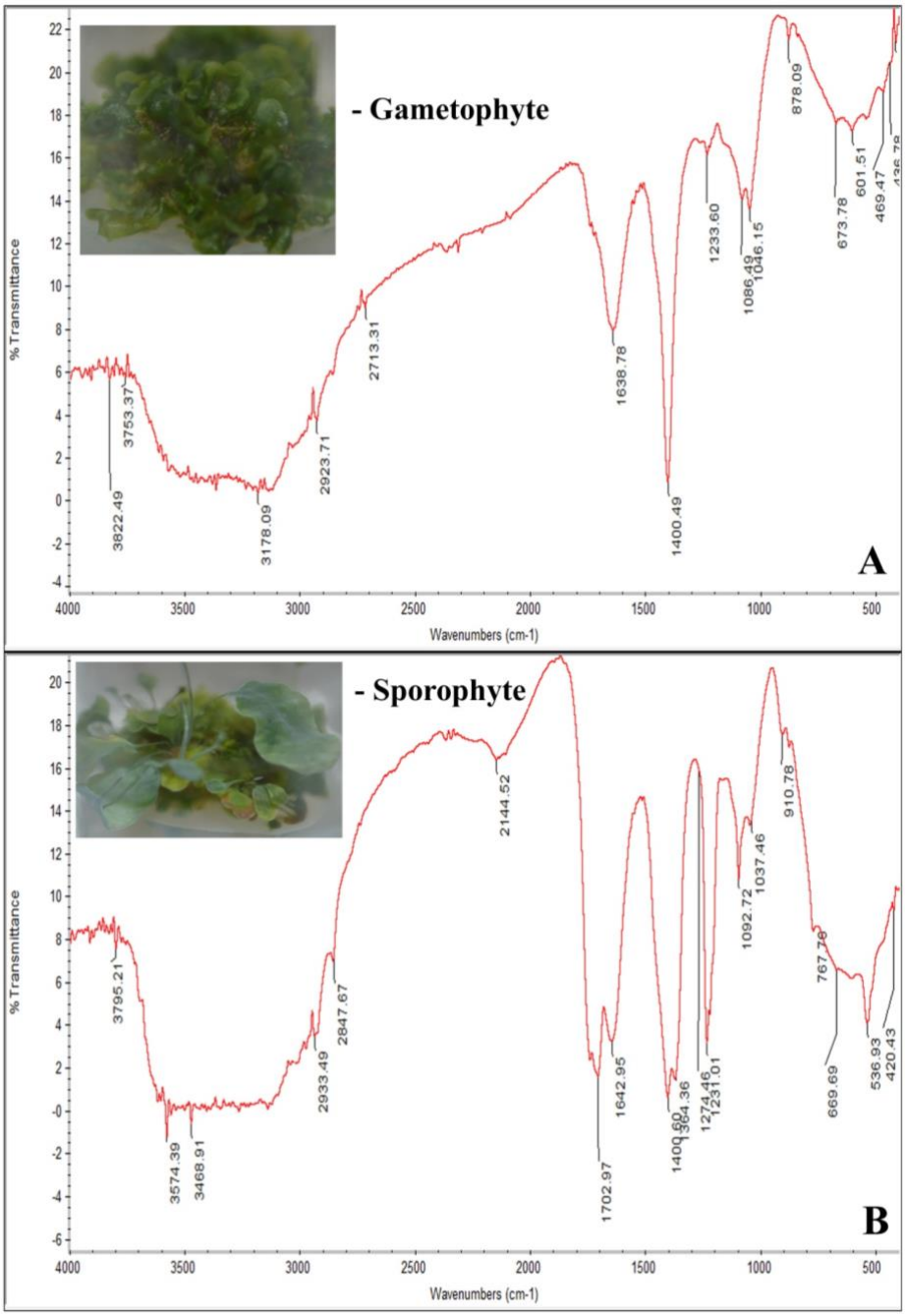

Fig. 1. FT-IR spectra of *Phlebodium aureum* acetone extracts.

C–Br stretching with halo compounds was seen in the gametophyte (604.31 cm^{-1}, 670.23 cm^{-1}) in the ethanol extracts. Alkene compound with C=C bending was observed in the gametophyte (808.03 cm^{-1}) and sporophyte (808.63 cm^{-1}) extracts. In the gametophyte (880.48 cm^{-1}) and sporophytes (880.42 cm^{-1}), C–H bending with 1,3 disubstituted compound was determined. Anhydride compounds were illustrated in the sporophyte (1044.85 cm^{-1}) and gametophyte (1049.52 cm^{-1}) extracts with CO–O–CO stretching group. C–O stretching with secondary alcohol was observed in the gametophyte (1088.09 cm^{-1}) and sporophyte (1088.92 cm^{-1}). Amine compound with C–N stretching showed uniqueness in the sporophyte (1229.51 cm^{-1}) extract. C–O stretching with alkyl aryl ether was determined in the sporophyte and gametophyte extracts. Aromatic amines (1323.5 cm^{-1}, 1331.67 cm^{-1}) were observed with C–N stretching in the gametophyte and sporophyte extracts. Flouro compounds with C–F stretching was illustrated in the 1400.57 cm^{-1} (gametophyte) and 1400.64 cm^{-1} (sporophyte) extracts. C=C stretching with alkene compound and C–H stretching with aromatic compounds were found in sporophyte (1652.54 cm^{-1}, 1924.49 cm^{-1}) and gametophyte (1652.91 cm^{-1}, 1924.9 cm^{-1}) extracts. Isothiocyanate with N=C=S stretching was seen in the gametophyte (2132.58 cm^{-1}) and sporophyte (2136.66 cm^{-1}) extracts. Nitrite compound with C≡C stretching and alkyne with C=C stretching was observed only in the sporophyte (2256.02 cm^{-1}) and gametophyte (2259.25 cm^{-1}) extracts. C–H stretching with alkane compounds was determined in the gametophytes (2928.93 cm^{-1}; 2973.61 cm^{-1}) and sporophyte (2971.93 cm^{-1}) extracts. Alcohol with O–H stretching and aliphatic primary amine with N–H stretching was observed in the sporophyte (3159.07 cm^{-1}) and gametophyte (3372.53 cm^{-1}) extracts (Table 2, Fig. 2).

Table 2

FT-IR peak values with functional groups of gametophyte and sporophyte stages of *Phlebodium aureum* in ethanol

Absorption range (cm^{-1})	Appearance	Group	Compound Class	Ethanol	
				G	S
435.32					+
435.58				+	
604.31	weak	C–Br stretching	Halo compound	+	
670.23	weak	C–Br stretching	Halo compound	+	
808.03	medium	C=C bending	Alkene	+	
808.63	medium	C=C bending	Alkene		+
880.48	strong	C–H bending	1,3 disubstituted	+	
880.82	strong	C–H bending	1,3 disubstituted		+
1044.85	Strong, broad	CO–O–CO stretching	Anhydride		+

1049.52	Strong, broad	CO–O–CO stretching	Anhydride	+	
1088.09	strong	C–O stretching	Secondary alcohol	+	
1088.92	strong	C–O stretching	Secondary alcohol		+
1229.51	medium	C–N stretching	Amine		+
1273.69	strong	C–O stretching	Alkyl aryl ether		+
1273.91	strong	C–O stretching	Alkyl aryl ether	+	
1323.5	strong	C–N stretching	Aromatic amine	+	
1331.67	strong	C–N stretching	Aromatic amine		+
1400.57	strong	C–F stretching	Fluoro compound	+	
1400.64	Strong	C–F stretching	Fluoro compound		+
1652.54	medium	C=C stretching	Alkene		+
1652.91	medium	C=C stretching	Alkene	+	
1924.49	weak	C–H bending	Aromatic compound		+
1924.9	weak	C–H bending	Aromatic compound	+	
2132.58	strong	N=C=S stretching	Isothiocyanate	+	
2136.66	strong	N=C=S stretching	Isothiocyanate		+
2256.02	weak	C≡C stretching	Nitrite		+
2259.25	weak	C=C stretching	Alkyne	+	
2928.93	medium	C–H stretching	Alkane	+	
2971.93	Medium	C–H stretching	Alkane		+
2973.61	Medium	C–H stretching	Alkane	+	
3159.07	Weak, broad	O–H stretching	Alcohol		+
3372.53	medium	N–H stretching	Aliphatic primary amine	+	
3580.76					+
3770.24				+	
3815.75				+	

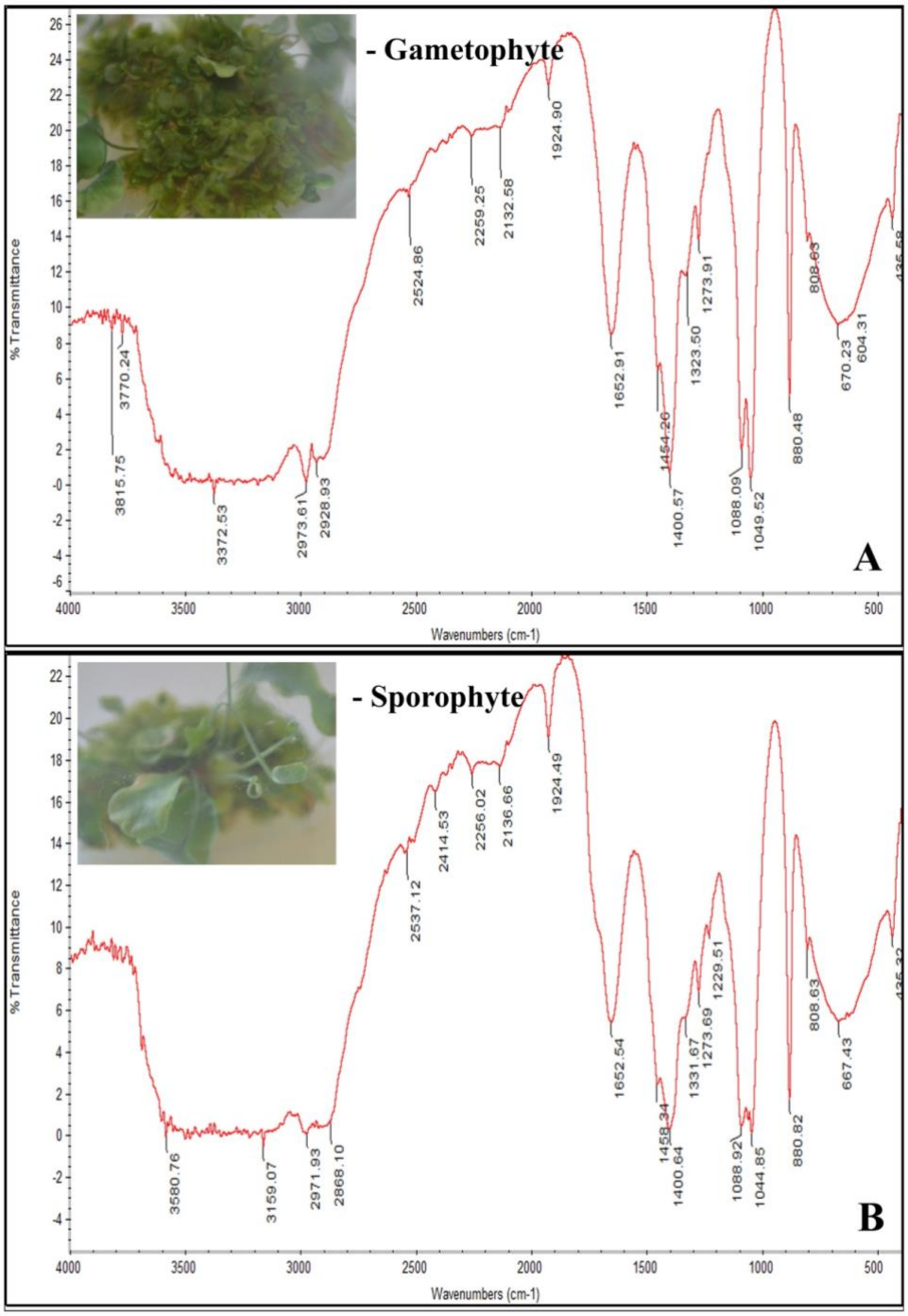

Fig. 2. FT-IR spectra of *Phlebodium aureum* ethanol extracts.

Halo compound with C–Br stretching was determined in the gametophyte (601.24 cm^{-1}, 670.75 cm^{-1}) and sporophyte (671.71 cm^{-1}) extracts. C–Cl stretching with Halo compound was illustrated in the gametophyte (772.35 cm^{-1}) and sporophyte (772.42 cm^{-1}) extracts. Alkene compound was unique to the gametophyte (840.13 cm^{-1}) extracts with C=C bending. 1, 3 disubstituted with C–H bending was found unique to the sporophyte (878.75 cm^{-1}) extract. In the sporophyte (1046.28 cm^{-1}) and gametophyte (1043.64 cm^{-1}), anhydride with CO–O–CO stretching was determined. Primary alcohol with C–O stretching was observed in the sporophyte extract only. C–O stretching with vinyl ether was observed in the gametophyte (1219.73 cm^{-1}) and sporophyte (1219.73 cm^{-1}) extracts. Aromatic ester with C–O stretching is unique to the sporophyte (1278.55 cm^{-1}) extract. Flouro compound with C–F stretching in gametophyte (1400.52 cm^{-1}); cyclic alkene with C=C stretching in gametophyte (1639.31 cm^{-1}); alkene with C=C stretching in sporophyte (1642.22 cm^{-1}); esters with C=O stretching in gametophyte (1744.38 cm^{-1}) and isothiocyanate with N=C=S stretching in sporophyte (2107.73 cm^{-1}) was only observed in the chloroform extracts. C–H stretching with alkane was determined in the gametophyte (2851.76 cm^{-1}; 2921.23 cm^{-1}) and sporophyte (2917.14 cm^{-1}; 2970.26 cm^{-1}) extracts. Alcohol with O–H stretching was illustrated in the gametophyte (3152.03 cm^{-1}; 3467.47 cm^{-1}) extracts. aliphatic primary amine with N–H stretching was unique to the sporophyte (3394.78 cm^{-1}) of the chloroform extracts (Table 3; Fig. 3).

Table 3

FT-IR peak values with functional groups of gametophyte and sporophyte stages of *Phlebodium aureum* in chloroform

Absorption range (cm^{-1})	Appearance	Group	Compound Class	Chloroform	
				G	S
473.55				+	
601.24	weak	C–Br stretching	Halo compound	+	
670.75	weak	C–Br stretching	Halo compound	+	
671.71	strong	C–Br stretching	Halo compound		+
772.35	strong	C–Cl stretching	Halo compound	+	
772.42	strong	C–Cl stretching	Halo compound		+
840.13	medium	C=C bending	Alkene	+	
878.75	strong	C–H bending	1,3 disubstituted		+
1043.64	Strong, broad	CO–O–CO stretching	Anhydride	+	
1046.28	Strong, broad	CO–O–CO stretching	Anhydride		+
1080.24	strong	C–O stretching	Primary alcohol		+
1086.49				+	

1219.12	strong	C–O stretching	Vinyl ether	+	
1219.73	strong	C–O stretching	Vinyl ether		+
1278.55	strong	C–O stretching	Aromatic ester		+
1400.32					+
1400.52	strong	C–F stretching	Fluoro compound	+	
1639.31	medium	C=C stretching	Cyclic alkene	+	
1642.22	strong	C=C stretching	Alkene		+
1744.38	strong	C=O stretching	Esters	+	
2107.73	strong	N=C=S stretching	Isothiocyanate		+
2851.76	medium	C–H stretching	Alkane	+	
2917.14	medium	C–H stretching	Alkane		+
2921.23	medium	C–H stretching	Alkane	+	
2970.26	Medium	C–H stretching	Alkane		+
3152.03	Weak, broad	O–H stretching	Alcohol	+	
3394.78	medium	N–H stretching	Aliphatic primary amine		+
3467.47	Strong, broad	O–H stretching	Alcohol	+	

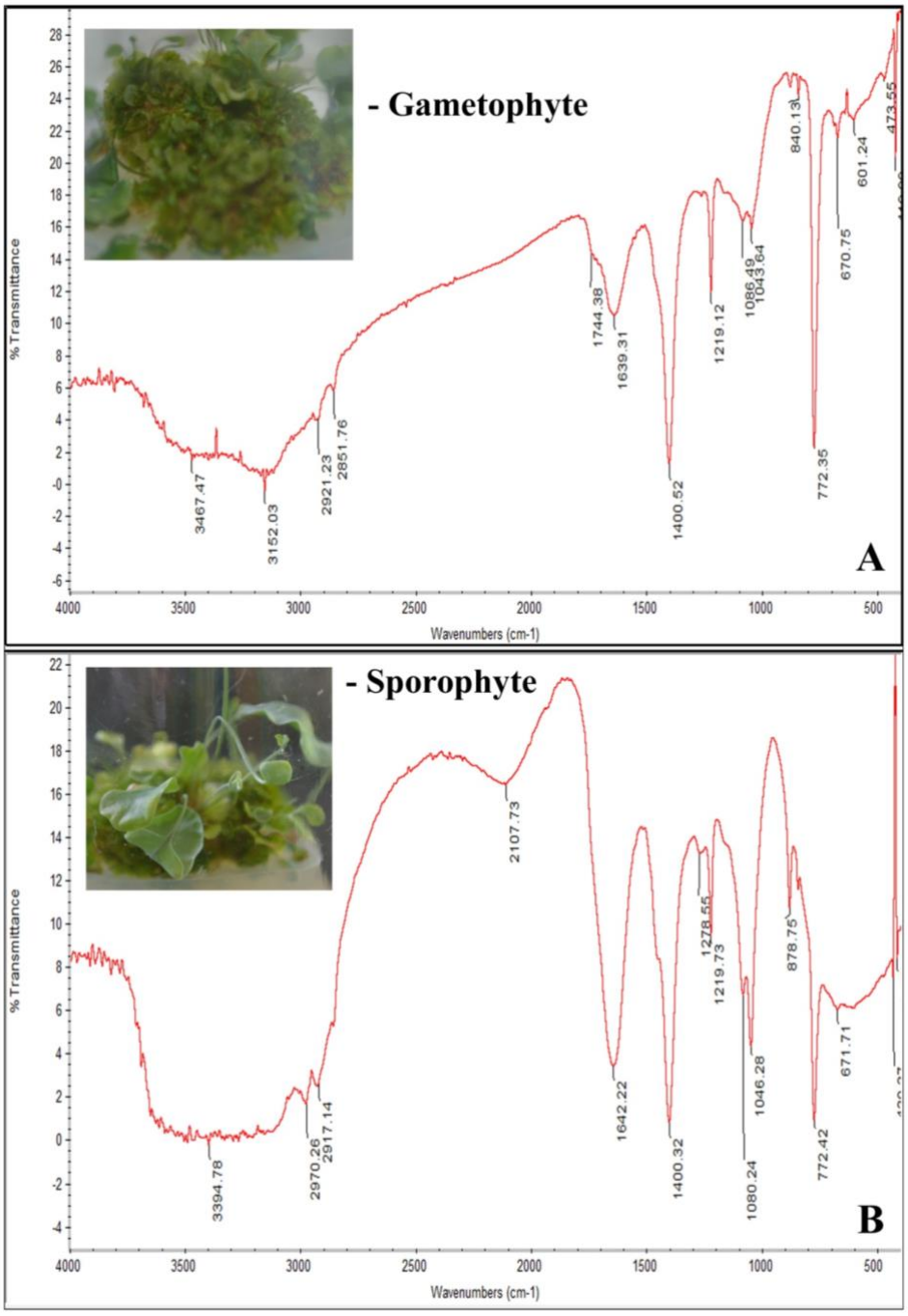

Fig. 3. FT-IR spectra of *Phlebodium aureum* chloroform extracts.

DISCUSSION

For the characterization and identification, FT-IR has become important for an unknown plant extract. Dalavi and Patil [2] screened the phytochemicals for Dashmula formulation using FT-IR. Janakiraman and Johnson [4] provided a chemical fingerprint for three species. They revealed differences in absorbances and band position. There was a relative intensity change of the bands. Maiten *et al.* [8] studied functional groups of different parts of *Ruflorinia orlandoi* and compare them with its host rock.

Lethika *et al.* [7] attempted to create a platform to treat various diseases by finding the compounds of various plants using FT-IR and to make research an interesting field. And it helps in chemical constituent identification, chemical structure elucidation and recognize the significance of the bio active constituents for various disease treatment. Sathish *et al.* [10] studied to observe the various chemical constituents in the fern *Vittaria elongata*. The compound such as alcohol, nitro groups, alkenes, alkynes, carboxylic acids, aromatic and alkyl halides were present. Deepashree *et al.* [3] made a comparative analysis of *Cleome gynandra* before and after the extraction process. The biochemical of different types were present in both extracts. Certain compounds were rich in the samples before extraction compared with after extraction. Hydroxyl compounds, amines, carbonyl, and halogens were present in both samples.

CONCLUSION

In the present study, an attempt is made to differentiate the gametophytes and sporophytes based on functional groups using FT-IR. The observed results showed the similarities and variations between the gametophytes and sporophytes of *Phlebodium aureum*. The functional group variation in the gametophyte and sporophyte confirms the different metabolites existence between the gametophyte and sporophyte. The outcome of the study showed the unique profiles for gametophytes and sporophytes and these may be employed to identify the gametophytes and sporophytes of *Phlebodium aureum*.

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