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UV-VIS SPECTROSCOPIC PROFILE AS TAXONOMIC CRITERIA TO DISTINGUISH THE TREE FERNS (*CYATHEA*)

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ABSTRACT

UV-Vis spectroscopic analysis is applied to develop a rapid and effective analytical method for studying the main constituents in medicinal plant extracts, discriminating the extracts from different extraction process, comparing the categories of chemical constituents in the different extracts and monitoring the qualities of medicinal materials. In the present study, UV-Vis spectroscopic analysis was performed for different extracts (petroleum ether, chloroform, acetone, ethanol and aqueous) of *Cyathea nilgirensis* Holttum, *Cyathea gigantea* (Wall. ex. Hook.) Holttum and *Cyathea crinita* (Hook.) Copel. The results of the analysis provided direct preliminary information on the fingerprint of functional groups with varied absorption. The findings of the present study indicated variations in the UV-Vis spectrum and chemical constituents. Use of macroscopical fingerprint characters of UV-Vis spectrum not only identifies the chemical constituents of different extracts, but also compares the differences in the components. This analytical method is highly rapid, effective, visual and accurate for pharmaceutical research. The results of the present study may be applied to test the authenticity of particular products and also provides a useful tool in preventing the fraudulent substitution of one type of plant for another.

KEYWORDS: *Cyathea,* Spectroscopy, UV-Vis, Fingerprint

INTRODUCTION

Phytochemicals are constitutive metabolites that enable plants to overcome temporary or continuous threats integral to their environment and controlling essential functions of growth and reproduction. They are generally advantageous to the producing organisms but the inherent biological activity of such constituents often causes adverse consequences in other organisms that may be exposed to them. Careful observations on cause and effect followed by a coordinated approach to identify the responsible entities have proved extremely fruitful in discovering roles for phytochemical constituents (Molyneux *et al.,* 2007). Phytochemical analysis requires an efficient and unbiased extraction of metabolites from plant tissues (Aparicio and Aparicio-Ruiz, 2000), identification of specific groups (fingerprint) of compounds / plants and other living organisms without separation and directly based on their absorption characteristics using UV-Vis, FT-IR or Raman spectroscopy (Baeten and Aparicio, 2000).

In recent years, the use of medicinal products from plant extracts becomes more extensive. However, some counterfeits or products with poor qualities emerge in the medicinal market along with the popularity of extract products of medicinal materials. At present, the main method for identifying the qualities of medicinal plant extracts is to use spectroscopic analysis by examining the content of certain chemical constituent in the tested sample. It is well known that medicinal plants comprise hundreds of components and produce their curative effects through mutual effects of many ingredients, so the limited numbers of specific components cannot reflect the real qualities of the herbal medicines. Hence, there is a need for quick and effective analytical method to entirely monitor and reflect the whole constituents of medicinal plants and their corresponding extract products.

UV-Vis spectrum obtained directly from an instrument is simply a plot of wavelength (frequency) of absorption versus the absorption intensity (absorbance or transmittance). It is generally applied to molecules or inorganic complexes in solution. The spectra have broad features that are of limited use for identification and it is very useful for quantitative measurements (Baeten and Aparicio, 2000). This qualitative application requires recording atleast a portion of the UV-Vis spectrum for characterization of the optical or electronic properties of materials. However, validation studies must be performed in order to assure the ability of any analytical method to generate reliable and interpretable information, as well as to confirm whether the features implemented in the method comply with the required standards for the analytic applications through consistently documented evidence (Gunzler, 1996). In

order to contribute to the growing knowledge of analytical chemistry, the present study intends to investigate the potential of UV-Vis spectroscopy as a probe for the chemical and functional characterization of *Cyathea nilgirensis* Holttum, *Cyathea gigantea* (Wall. ex. Hook.) Holttum and *Cyathea crinita* (Hook.) Copel.

MATERIALS AND METHODS

Collection of plant materials

Specimens for the present study were collected from different parts of Tamil Nadu, South India. *C. nilgirensis* were harvested in and around Kaakachi stream (1,725 m), Tirunelveli hills, *C. gigantea* from the road sides near Nadugani (2,637 m), Nilgiris hills and *C. crinita* from the Anglade Institute of Natural History, Shenbaganur, Kodaikanal (2,195 m), Palni hills, Western Ghats, South India. The specimens were identified based on the "Pteridophyte flora of the Western Ghats, South India" by Manickam and Irudayaraj (1992). Herbarium specimens were deposited in St. Xavier's College Herbarium (XCH), Palayamkottai, Tamil Nadu, India for further reference (*C. nilgirensis* - XCH 25423; *C. gigantea* - XCH 25422 and *C. crinita* - XCH 25424).

Preparation of extracts

The collected species of *Cyathea* were thoroughly washed with tap water followed by distilled water. They were blotted on the blotting paper and spread out at room temperature in shade to remove the excess water contents. The shade dried plant samples were ground to fine powder using mixer grinder. 30 g powdered sample was extracted successively with 180 ml of petroleum ether, chloroform, acetone and ethanol using the Soxhlet extractor for 8-12 h at a temperature not exceeding the boiling point of the solvent. The aqueous extracts were prepared directly by boiling the powder with distilled water for 3 h and filtered using Whatman No.1 filter paper.

UV-Vis spectroscopic analysis

The UV-Vis absorption spectrum for different extracts of selected *Cyathea* species were recorded in the Shimadzu UV-Vis spectrophotometer capable of producing monochromatic light for measuring the absorbance. The 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 was diluted to 1:10 with the same solvent. The filtered extracts of selected *Cyathea* species were scanned in the wavelength ranging from 190-1000 nm and the characteristic peaks were observed and recorded. The analysis was repeated twice to confirm the spectrum. To demonstrate the inter-specific variation among the selected *Cyathea* species, UPGMA cladogram was constructed using NTSYSspc-2.0 software. Phytochemical (UV-Vis profile) similarities were estimated according to Nei and Li (1979) algorithm. For the cladogram construction, the UV-Vis

spectroscopic profile was converted into "1" and "0" matrix to indicate the presence or absence of peak values (absorption).

RESULTS

The qualitative UV-Vis fingerprint profile of different extracts of *C. nilgirensis, C. gigantea* and *C. crinita* was selected at the wavelengths from 190 to 1000 nm due to sharpness of the peaks and proper baseline. The absorbance reveals the concentration of compound present in the specific nanometer. These spectra are useful to identify the specific bioactive classes of molecules found in various extracts.

Among the five different extracts of *C. nilgirensis*, petroleum ether and ethanolic extracts exhibited five peaks which denotes the presence of maximum number of compounds. The chloroform, acetone and aqueous extracts demonstrated only three peaks. Aqueous extract of *C. nilgirensis* showed the highest absorbance (3.373) at 204 nm and petroleum ether extract determined the lowest absorbance (0.025) at 254 and 284 nm in the UV region. In visible region, ethanolic extract represented the maximum absorbance 0.300 at 412 nm and petroleum ether extract exhibited minimum absorbance 0.014 at 668 nm (Fig. 1; Table 1).

Table 1: UV-Vis peak values for different extracts of *C. nilgirensis*

Petroleum ether		Chloroform		Acetone		Ethanol		Aqueous	
λ_{max} (nm)	Abs	λ_{max} (nm)	Abs	λ_{max} (nm)	Abs	λ_{max} (nm)	Abs	λ_{max} (nm)	Abs
216	0.066	415	0.233	339	0.372	223	1.180	200	3.287
254	0.025	458	0.175	410	0.234	326	0.675	204	3.373
284	0.025	665	0.141	662	0.163	412	0.300	287	1.123
408	0.017					468	0.232		
668	0.014					663	0.210		

In *C. gigantea*, ethanolic, chloroform and aqueous extracts expressed more number of peaks (4) followed by petroleum ether and acetone extracts (3). Aqueous extract represented highest absorbance 3.625 at 214 nm and petroleum ether extract depicted least absorbance 0.110 at 216 nm in the UV region. In visible region, acetone extract showed maximum absorbance 0.693 at 408 nm and petroleum ether extracts demonstrated minimum absorbance 0.043 at 668 nm (Fig. 2; Table 2).

UV-Vis spectra of ethanolic extracts of *C. crinita* displayed more number of peaks (6) followed by chloroform and acetone extracts (4). Aqueous extract exhibited the

highest absorbance 3.860 and petroleum ether extract showed the lowest absorbance 0.214 at 215 nm in the UV region. In visible region, ethanolic extract represented the maximum absorbance 1.157 at 407 nm and petroleum ether extract illustrated minimum absorbance 0.096 at 669 nm (Fig. 3; Table 3).

Table 2: UV-Vis peak values for various extracts of *C. gigantea*

Petroleum ether		Chloroform		Acetone		Ethanol		Aqueous	
λ_{max} (nm)	Abs	λ_{max} (nm)	Abs	λ_{max} (nm)	Abs	λ_{max} (nm)	Abs	λ_{max} (nm)	Abs
216	0.110	414	1.051	408	0.693	214	0.614	199	3.451
408	0.059	457	0.067	533	0.209	221	0.901	207	3.613
668	0.043	537	0.332	664	0.386	329	0.641	214	3.625
		666	0.578			663	0.149	323	1.679

Table 3: UV-Vis peak values for different extracts of *C. crinita*

Petroleum ether		Chloroform		Acetone		Ethanol		Aqueous	
λ_{max} (nm)	Abs	λ_{max} (nm)	Abs	λ_{max} (nm)	Abs	λ_{max} (nm)	Abs	λ_{max} (nm)	Abs
215	0.214	245	0.783	468	0.572	326	1.515	215	3.860
406	0.129	411	0.821	664	0.622	335	2.676	316	2.214
669	0.096	458	0.565	796	0.354	336	2.696		
		666	0.545	992	0.393	340	2.808		
						407	1.157		
						663	0.678		

The cladogram was constructed based on the results of UV-Vis spectroscopic profile of selected *Cyathea* species. The results showed two clades viz., C_1 and C_2. The clade C_1 was shared between monophyletic species *C. nilgirensis* and *C. gigantea* whereas clade C_2 showed the unique presence of paraphyletic taxon *C. crinita* (Fig. 4).

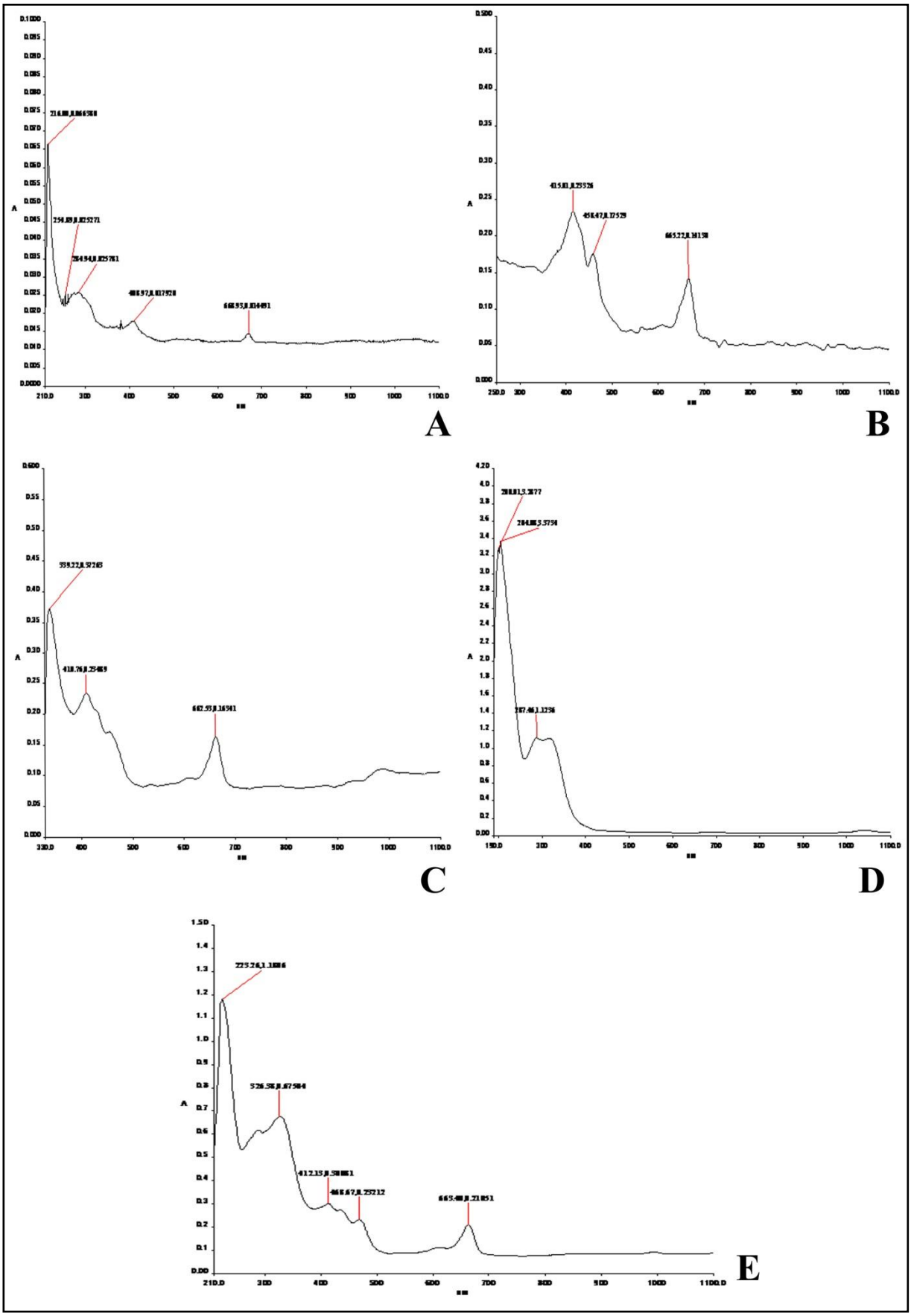

Fig. 1: UV-Vis spectrum of *C. nilgirensis* - A: Petroleum ether extract; B: Chloroform extract; C: Acetone extract; D: Aqueous extract; E: Ethanolic extract

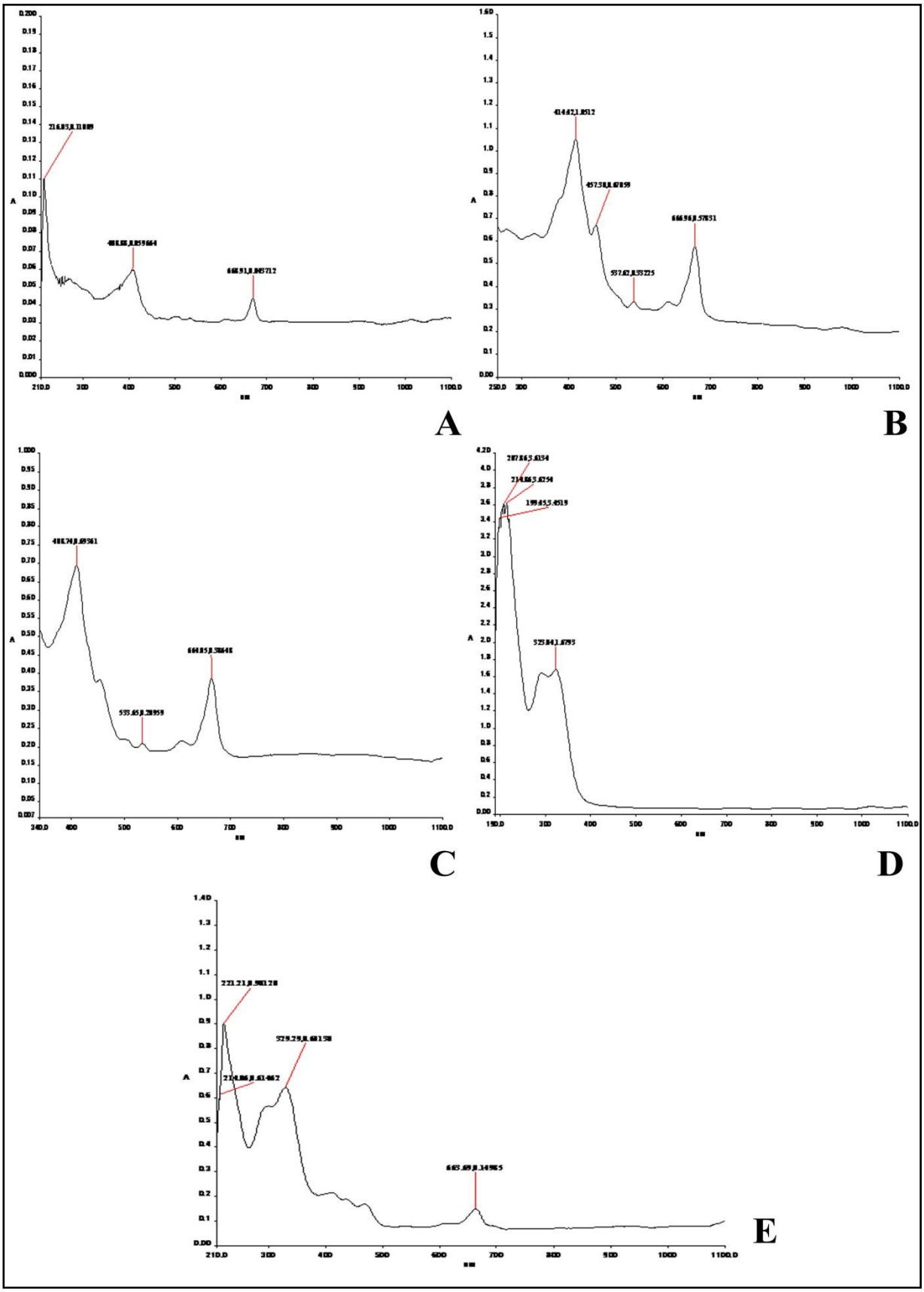

Fig. 2: UV-Vis spectrum of *C. gigantea* - A: Petroleum ether extract; B: Chloroform extract; C: Acetone extract; D: Aqueous extract; E: Ethanolic extract

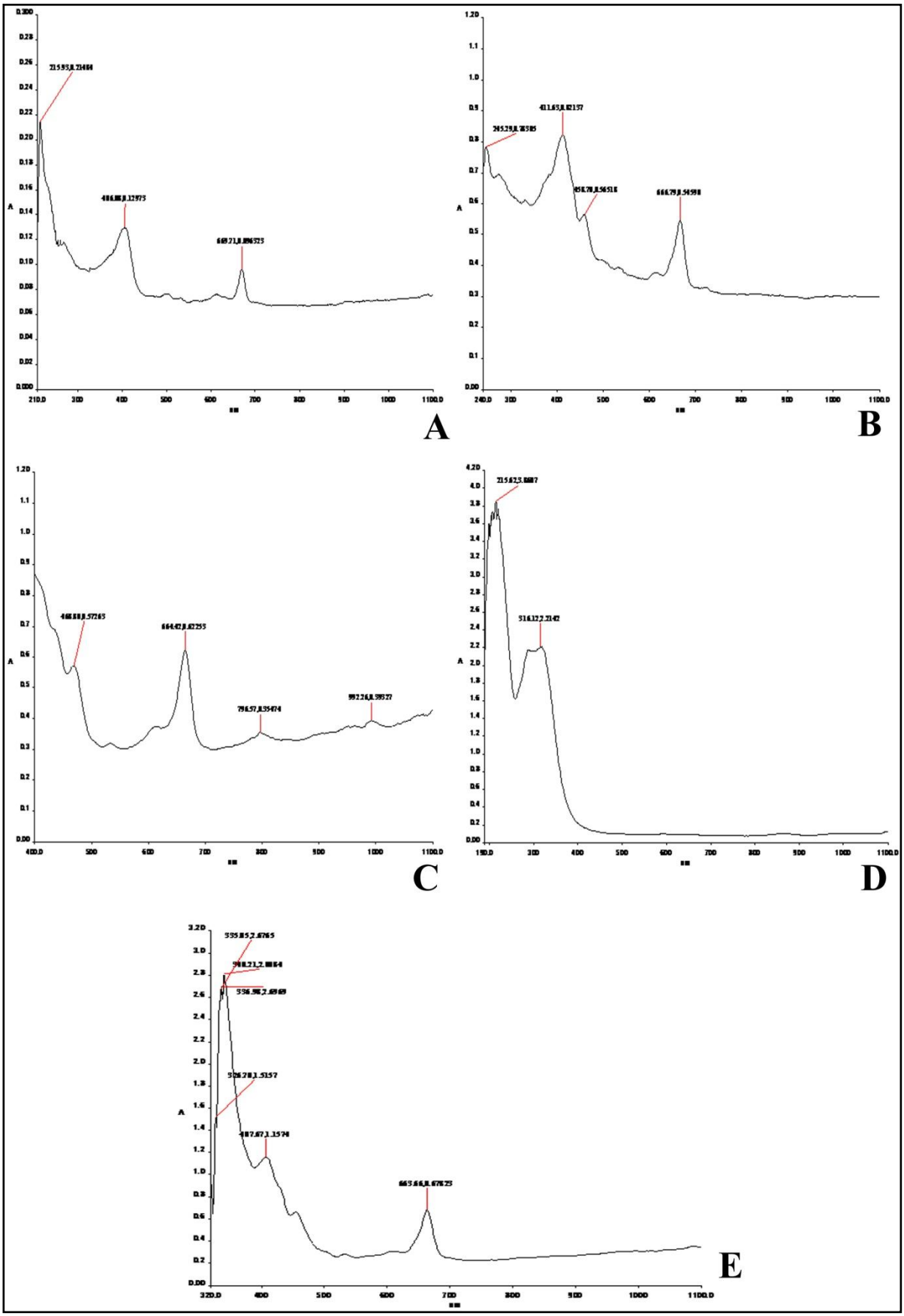

Fig. 3: UV-Vis spectrum of *C. crinita* - A: Petroleum ether extract; B: Chloroform extract; C: Acetone extract; D: Aqueous extract; E: Ethanolic extract

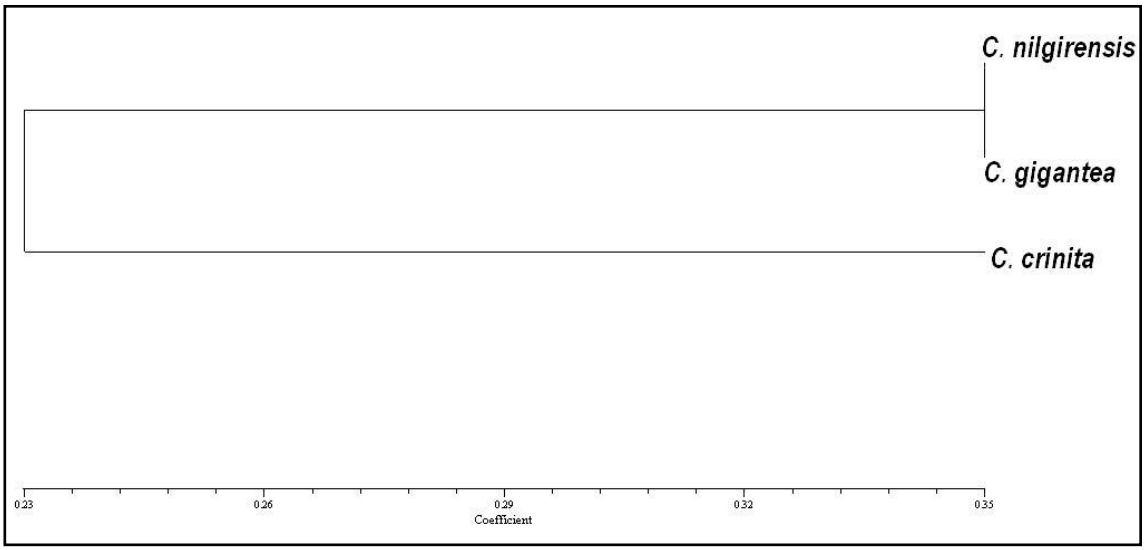

Fig. 4: Cladogram based on the UV-Vis Spectroscopic profile of *Cyathea* species

DISCUSSION

The UV-Vis spectra of *C. nilgirensis, C. gigantea* and *C. crinita* showed generally a decreased absorptivity or optical density as the wavelength increased. Although the spectra appeared to be broad and featureless showing no maxima or minima, the absorption intensity varied greatly among the selected five fractions. The presence of an absorbance spectrum at a particular wavelength is a good indicator for the presence of a chromophore. However, the position of the absorbance maximum is not fixed but it depends partially on the molecular environment of the chromophore and on the solvent in which the sample is dissolved.

Molecular absorption spectrophotometry in ultraviolet/visible light is an analytical method based on the property of an ion or molecular species to absorb at certain wavelengths of UV-Vis radiation. In the process of absorbing the radiation, the energy of the photons is transferred to the molecules of the medium under analysis to cause electron transitions associated with vibrational and rotational transitions (Leal *et al*., 2008). The changes that occur in the UV spectrum due to complex formation are generally identified by widening of peak areas. The displacement at maximum UV absorption by the effect of complex formation can be explained by the partial protection of excitable electrons and chromophores present in the sample. The development of less onerous methods, easier to execute and normally using less complex apparatus, requires validation studies of these techniques for each plant species, in order to assure the reliability of the results (Silva-Corazza *et al*., 2010).

UV-Vis spectra of selected *Cyathea* generally show only a few broad absorbance peaks. It provides a limited amount of

qualitative information compared with FT-IR which produces many narrow peaks. Although UV-Vis spectra do not enable absolute identification of an unknown, they are frequently used to confirm the identity of a substance through comparison of the measured spectrum with a reference spectrum. The number of peaks increases with higher orders of derivatives. This increase in complexity of the derivative spectra can be useful in qualitative analysis, either for characterizing materials or for identification purposes. The cladogram constructed based on the spectroscopic profile clearly distinguishes the inter-specific variation among the selected three *Cyathea* species.

CONCLUSION

The ethanolic extract of selected three *Cyathea* species produced more number of active constituents due to their stronger extraction capacity. The results of the present study paved a way to identify and isolate the active compounds from the selected taxa.

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