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Research Article

Comparison of UV-Visible Spectrophotometric and FTIR Analysis of *Tinospora crispa* (L.) Hook. f. and Thomson Leaves and Stem

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Abstract

Herbals play an immense role in the primary health needs of local communities in developing countries. Nowadays, there is renewed interest in medicinal plants that have emerged because of their availability and less or nontoxic nature. The therapeutic potential of leaf and stem extracts of *Tinospora crispa* were analyzed by FTIR and UV-Vis spectroscopy. Major active molecules in the stem and leaf extract were interpreted using their functional groups. The peak (cm-1), intensity, corresponding intensity, base (H), base (L), area and corresponding area obtained from FTIR spectra data confirmed the presence of phenol, alkanes, aldehyde, secondary alcohol, amino acid, aromatic amines and halogens. The IR peak at 2800 to 2900 cm-1 refers to the presence of alkanes (C-H stretch), peak at 1700 to 1600 cm-1 corresponds the carboxylic acid group (C = O stretch), peak at 1500 cm-1 denotes the 1 ° amines (N-H bend), peak of 1300 to 1200 cm-1 showed the presence of aromatic amines (C-N stretch), peaks of 1200 to 1100 cm-1 indicate the alcohols, carboxylic acids, esters, ethers (C-O stretch). A peak of 600 -800 cm-1 revealed the alkenes (=C-H bend). Prominent UV-visible spectra peaks at 222, 350, 361, 317 nm, and 408 nm with the corresponding absorption reveal the presents of polyphenolic and alkaloids. Thus, the results of the present study provide a platform for using *T. crispa* leaves and stem as herbal alternative having multiple therapeutic potentials like anti-inflammatory, antioxidant, immunomodulatory, cytotoxic, antimalarial, cardioprotective, and anti-diabetic potentialities.

Keywords: *Tinospora crispa*; Secondary Metabolites; Medicinal Plants; FTIR Spectrum; Diseases

Introduction

Tinospora crispa (L.) Hook. f. and Thomson is a medicinal herbal of Menispermaceae. It is distributed along rainforests or mixed deciduous forests. It has been employed in traditional medicine to cure multiple ailments such as jaundice, rheumatism, urinary infections, fever reducing body temperature, malaria, diabetes, inflammatory disorders, fracture, scabies, blood pressure, reducing thirst, appetite and optimal health [1]. Deciduous glabrous climber, with long aerial roots, fleshy stem with conspicuous blunt tubercles, leaves were ovate to orbicular, margin entire, apex acuminate, 5- 7 veined, clustered flowers in an unbranched racemose inflorescence.

FTIR tool was applied for analyzing biomolecules fingerprinting among medicinal plants. FTIR spectroscopy can discriminate the various phytochemicals like proteins, sugars, phenols, tannins, alkaloids from plant materials. Pharmacopeia in many countries established the efficacy of the tool as a nondestructive analytical method gives structural data on molecular characteristics of range of molecules. It is considered as an affordable, sensitive technique for identifying the functional groups present in the herbal extracts with the help of IR region in the range of 400-4000cm^{-1}. Wavelength data from IR spectra reflect the chemical bond of the molecules like electrostatic, hydrogen, van der waals bonds, as well as effects related to electron-transfer and hydrophobic effects and thereby it is possible to predict their nature [2]. The knowledge gained may help to optimize processing strategies for extracting valuable phytochemical resources from herbal species. Research in drug metabolism, its biotransformation is important in toxicological, pharmacological and biomedical fields. Drug discovery from the herbals continues to yield new multiple pharmacological targets. In this scenario, the present study targeted to compare the FTIR and UV-VIS spectra of the leaf and stem of the medicinal plant *T. crispa* is attempted.

Material and Methods

Fresh leaves and stem of *T. crispa* were collected from the natural habitats of Nedumangad, Thiruvannathapuram district, Kerala. The plant was identified with flora and confirmed by referring to the herbarium of JNTBGRI, Palode.

Preparation of samples for FTIR analysis

Initially, the *T. crispa* leaf and stem powders were lyophilized to remove the water content. The powders were further ground using the mortar and pestle and were mixed with dried potassium bromide (ratio of 1/100), and the mixture was subjected to a pressure of 5 × 106 pa in an evacuated die to produce a Kbr pellet for application in FTIR spectrometer. The AR grade alcohol and Kbr was obtained from Sigma Aldrich. FTIR spectra were recorded with a FTIR 460 plus Jasco. The conjugated pellets were scanned at room temperature at 4000-400cm^{-1} spectral range. To improve the signal to noise ratio for each spectrum, 100 interferograms with a spectral resolution of ±4cm^{-1} were averaged. Background spectra, which were collected under identical conditions, were subtracted from the sample spectra. Each sample was scanned under the same conditions with six different pellets. Special care was taken to prepare the pellets at the same thickness by taking the same amount of sample and applying the same pressure. Therefore, in the present study it was possible to directly relate the intensities of the absorption bands to the concentration of the corresponding functional groups.

UV-visible spectrophotometric analysis

Leaf and stem extracts were analyzed by UV-visible spectrophotometer with a slit width of 2nm, using a 10-mm cell at room temperature. The extract was examined under visible and UV light in the wavelength ranging from 300-800nm for proximate analysis. For UV-VIS spectrophotometer analysis, the extract was centrifuged at 3000 rpm for 10 min and filtered through Whatman No. 1 filter paper. The sample is diluted to 1:10 with the same solvent.

Results and Discussion

FTIR analysis

Data obtained from the FTIR reflects the occurrence of many diverse functional groups in the leaves and stem of *T. crispa* (Figure 1A and B). The peak (cm^{-1}), intensity, corresponding intensity, base (H), base (L), area and corresponding area obtained from FTIR spectra were displayed in table 1 and 2.

In leaf, the peaks at 609.51, 680.87, 775.38 cm^{-1} denotes primary/secondary amines, halogen, alkyl halide and that at 956.69, 991.41, 1068.56 cm^{-1} refers benzene ring, four peaks, two medium, two strong. 1111 $^{-1}$ represents C-O, alcohol and ether strong, ester two bands or more; Benzene ring, three peaks, two medium, one strong. 6.33 was the corresponding intensity. 1325.1, 1396 $^{-1}$ (20.44 corresponding intensity) and 1598.99 cm-1 (26.74 corresponding intensity) displays with high intensity among the FTIR data analyzed interpreted as C-C, Aromatic medium-weak, series of sharp bands. 2856.58, 2927.94 cm^{-1} outlays C-H, alkane medium, sharp (stretch), while 3315.63 cm^{-1}H- bonded broad and strong, O-H, primary/secondary amine groups (Table 3).

Similarly in stem 615.29, 686.66 cm^{-1} denotes primary/secondary amines, halogen, alkyl halide, while 840.96, 908.47, 1062.78 cm^{-1} (13.694 corresponding intensity) benzene ring, four peaks, two medium, two strong; 1122.57 (15.95), 1259.52 cm^{-1} alcohol and ether strong, ester two bands or more; benzene ring, three peaks, two medium, one strong; 1388.75 cm-1 C-C, aromatic medium-weak, series of sharp bands; 1604.77 cm-1 showed the highest corresponding intensity 45.656 represents C-O, ester and carbonyl generally strong, conjugated lower; 2918.3 cm^{-1} C-H, Alkane medium, sharp (stretch) and 3213.41, 3383.14 cm-1 H- bonded broad and strong, O-H primary/secondary amines (Table 3).

Mariam., *et al*. [3] reported the phytochemical and biological activity studies of *T. crispa* stem using FTIR data substantiates with the present results. Kumar and Prasad [4] identified and compared biomolecules of *Tephrosia tinctoria* and *Atylosia albicans* by using FTIR i.e., the five unique peaks recorded were 1590 cm^{-1}, 1348 cm^{-1}, 1051, 3385 cm^{-1}, 1063 cm^{-1} and 456 cm^{-1}. Similarly, a marginal absorption peak at 453 cm^{-1} was attributed to the absorption of Y-O bonds. Significant shifts of 3086 and 1766 cm^{-1} reflects the role of -OH group (phenol, alcohol) and -C = O (carboxylic acid and its derivative) was previously reported by Apriandanu and Yulizar [5] of aqueous leaf extract of *T. crispa* as reducing and capping agents for synthesis of gold nanoparticles. The present findings are supported by previous studies; Rajiv., *et al*. [6] screened the phytochemicals via FTIR of *Myristica dactyloids* fruit extracts. Muruganantham., *et al*. [7] compared *Eclipta alba* and *E. prostrata* via FT-IR and SEM-EDS. Stem extract of *Bridelia montana* was analyzed by FT-IR and GC-MS and revealed the peaks at IR such as 3348.42 cm^{-1}, 2935.66 cm^{-1}, 1421.54 cm^{-1}, 1058.92 cm^{-1}and 690.52 cm^{-1} [8].

UV-VIS analysis

The UV-VIS analyses (solid and liquid mode) were carried to identify the compounds containing σ-bonds, π-bonds and lone pair of electrons, chromophores and aromatic rings. The UV-VIS profile was taken at the wavelength of 300 nm to 800 nm due to the sharpness of the peaks and proper baseline. The leaf profile

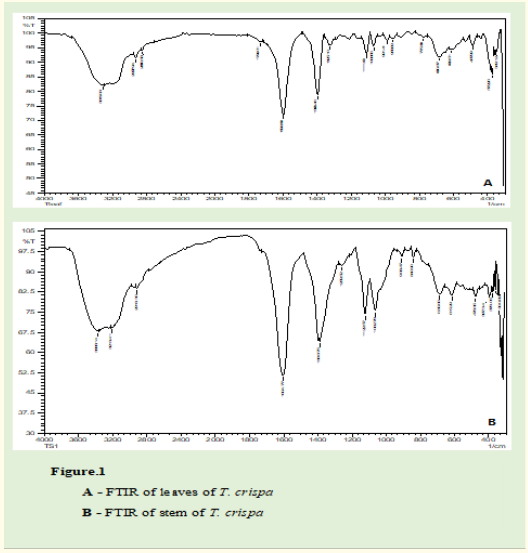

Figure 1: a: FTIR of leaves of *T. crispa.* B: FTIR of stem of *T. crispa.*

	Peak	Intensity	Corr. Intensity	Base(H)	Base (L)	Area	Corr. Area
1	349.12	93.57	1.1	351.04	335.61	0.3	0.11
2	372.26	85.94	5.08	378.05	364.55	0.71	0.21
3	487.99	95.74	3.35	509.21	459.06	0.55	0.35
4	609.51	94.38	1.41	630.72	545.85	1.6	0.33
5	680.87	91.76	4.98	750.31	630.72	2.92	1.34
6	775.38	98.77	0.78	823.6	752.24	0.22	0.15
7	956.69	98.32	0.87	974.05	935.48	0.22	0.09
8	991.41	97.61	1.6	1012.63	975.98	0.26	0.14
9	1068.56	95.78	3.89	1089.78	1047.35	0.44	0.38
10	1111	93.17	6.33	1143.79	1091.71	0.86	0.7
11	1325.1	95.82	2.7	1357.89	1296.16	0.75	0.36
12	1396.46	78.81	20.44	1487.12	1363.67	4.57	4.28
13	1598.99	71.95	26.74	1718.58	1489.05	10.4	9.03
14	1734.01	97.1	0.54	1811.16	1720.5	0.68	0.06
15	2856.58	94.08	0.35	2868.15	2713.84	2.72	0.3
16	2927.94	91.48	1.74	2953.02	2868.15	2.69	0.23
17	3315.63	82.2	0.64	3680.18	3102.13	17.88	0.8

Table 1: FTIR of leaves from *T. crispa* with peak, intensity, corresponding intensity, base(H), base (L), area and corresponding area.

	Peak	Intensity	Corr. Intensity	Base(H)	Base(L)	Area	Corr. Area
1	360.68	83.518	8.764	368.4	356.83	0.686	0.266
2	378.05	82.702	1.766	379.98	370.33	0.708	0.085
3	397.34	80.5	4.228	420.48	381.91	3.08	0.324
4	478.35	80.886	2.954	491.85	455.2	3.075	0.282
5	615.29	81.237	4.197	659.66	588.29	5.623	0.704
6	686.66	81.935	5.261	750.31	659.66	6.1	1.335
7	840.96	95.324	3.247	854.47	823.6	0.399	0.198
8	908.47	95.582	2.728	933.55	893.04	0.506	0.192
9	1062.78	75.576	13.694	1095.57	952.84	9.081	4.141
10	1122.57	74.269	15.95	1182.36	1097.5	5.584	2.661
11	1259.52	92.452	1.695	1278.81	1215.15	1.76	0.264
12	1388.75	64.147	1.532	1392.61	1278.81	10.98	0.107
13	1604.77	51.617	45.656	1728.22	1487.12	24.099	21.212
14	2918.3	83.88	1.566	2931.8	2777.5	9.124	0.482
15	3213.41	69.331	0.431	3219.19	2981.95	28.084	0.838
16	3383.14	67.976	1.212	3716.83	3371.57	31.42	1.468

Table 2: FTIR of stem from *T. crispa* with peak, intensity, corresponding intensity, base(H), base (L), area and corresponding area.

Sl. No.	Leaf (cm^{-1})	Characteristic group	Stem (cm^{-1})	Characteristic group
1.	609.51, 680.87, 775.38	Primary/secondary amines, Halogen, Alkyl halide	615.29, 686.66	Primary/secondary amines, Halogen, Alkyl halide, halogen
2.	956.69, 991.41, 1068.56	Benzene ring, four peaks, two medium, two strong	840.96, 908.47, 1062.78	Benzene ring, four peaks, two medium, two strong
3.	1111	C-O, Alcohol and ether strong, ester two bands or more; Benzene ring, three peaks, two medium, one strong	1122.57, 1259.52	C-O, Alcohol and ether strong, ester two bands or more; Benzene ring, three peaks, two medium, one strong
4.	1325.1, 1396, 1598.99	C-C, Aromatic medium-weak, series of sharp bands	1388.75	C-C, Aromatic medium-weak, series of sharp bands

5.	2856.58, 2927.94	C-H, Alkane medium, sharp (stretch)		
6.	3315.63	H- bonded broad and strongO-HPrimary/secondary amine	1604.77	C-O , Ester and carbonyl generally strong, conjugated lower
7.			2918.3	C-H, Alkane medium, sharp (stretch)
8.			3213.41, 3383.14	H- bonded broad and strong O-H Primary/secondary amine

Table 3: FTIR of leaves and stem from *T. crispa* showing characteristic groups.

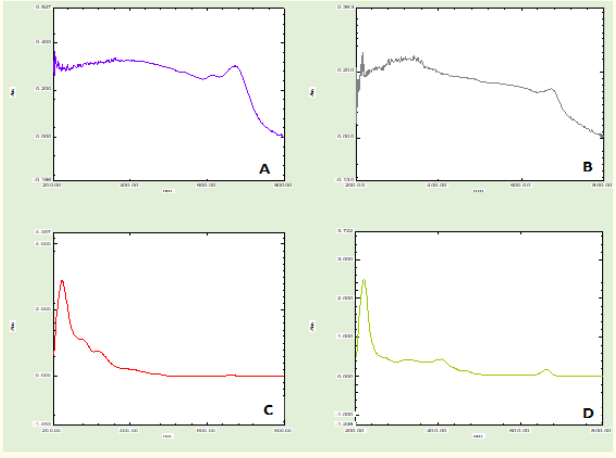

Figure 2: A: UV-VIS spectra (solid mode) of leaf from *T. crispa*, B: UV-VIS spectra (solid mode) of stem from *T. crispa*, C: UV-VIS spectra (Liqiud mode) of stem from *T. crispa*, D: UV-VIS spectra (Liquid mode) of leaves from *T. crispa.*

(liquid mode) showed the peaks at 664, 606, 535, 467, 408, 326, 221 and the corresponding absorptions were 0.182, 0.042, 0.038, 0.14, 0.437, 0.434 and 2.48 respectively. Similar data for stem were 717 (-0.021), 664 (0.03), 608 (-0.02), 317 (0.743) and 222 (2.894) (Table 4 and 5 and Figure 2. A and B). The leaf profile (solid mode) showed the peaks at 673, 609, 537, 361 and 288 the corresponding absorptions were 0.303, 0.263, 0.279, 0.338 and 0.32 respectively. Similar data for stem were 771 0.025), 673 (0.15), 537 (0.168), 350 (0.245), 286 (0.241) and 248 (0.217) (Table 4 and 5 and Figure 2. C and D). Occurrence of peaks between 200 and 400 nm in the UV-VIS spectra reflects the possibilities of unsaturated groups and heteroatoms like S, N, O [9]. Spectrum for *T. crispa* extract shows peaks at 326 nm and 408 nm confirms the presence of organic chromophores. However, the application of UV-visible spectrum analysis of extract is limited by the inherent difficulties in assigning the absorption peaks to any particular constituents in the system. So, UV-VIS may be corroborated with other analytical technique to enable proper characterization of the extract and constituent identification [10]. They analyzed the bioactive molecules in *Physalis minima* leaves using GC MS, HPLC and FTIR techniques.

Conclusion

In the present analyzes in *T. crispa* reveals by UV-VIS spectrum and FTIR analysis revealed the presence of polyphenolic molecules and alkaloids which provides the therapeutic features of the medicinal plant. Spectral area ranged between 3800–380 cm^{-1} could be considered as an important area for an easy and reliable discrimination between different plant species based on biomolecules, as provides a unique fingerprint for the biomolecules. Future studies are warranted to isolate, purify the key molecule and their various therapeutic potentialities.

	Solid mode		Liquid mode	
No.	**Wavelength nm.**	**Abs.**	**Wavelength nm.**	**Abs.**
1	673	0.303	664	0.182
2	609	0.263	606	0.042
3	537	0.279	535	0.038
4	361	0.338	467	0.14
5	288	0.32	408	0.437
6	-	-	326	0.434
7	-	-	221	2.48

Table 4: UV-VIS spectra (solid mode and liquid mode) *T. crispa* leaves.

	Solid mode		Liquid mode	
No.	**Wavelength nm.**	**Abs.**	**Wavelength nm.**	**Abs.**
1	771	0.025	717	-0.021
2	673	0.15	664	0.03
3	537	0.168	608	-0.02
4	350	0.245	317	0.743
5	286	0.241	222	2.894
6	248	0.217	-	-

Table 5: UV-VIS spectra (solid mode and liquid mode) of *T. crispa* stem.

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