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Quality control of herbal drugs through UV-Vis spectrophotometric analysis

Research Article

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Abstract

Quality control of herbal raw material is essential to maintain quality thus better efficacy as well as safety through *Ayurvedic* preparations. Among various quality control parameters UV-Vis spectrophotometric analysis provides both qualitative and quantitative standards. But markers are needed for quantitative analysis. An attempt is made to study UV-Vis Spectrometric analysis of some herbal raw materials for understanding qualitative and quantitative parameters without markers. *Pippali*, *Eranda*, Tea etc. extracts were prepared and were analyzed using UV-Vis Spectroscope with different concentrations. As control solvents used for extraction e.g. water or ethanol or methanol are tested. Double beam UV-Vis Spectrometer of Shimadzu model UV1800 along with UV Probe software was used for analysis. Analysis was done in Central Research Facility – (Dept. of AYUSH approved Drug testing Laboratory for ASU drugs), of KLEU's BMK Ayurveda Mahavidyalaya, Belagavi. Spectral data showed relationship between concentration and intensity of absorption. Thus by generating library of spectral data of genuine raw samples, it would be possible to test quality control using UV-Vis spectrometer even without costly markers.

Keywords: UV-Vis Spectrophotometric analysis, Herbal drugs, *Ayurveda*, quality control, *Pippali*, *Kankola*,Tea

Introduction:

Quality control of herbal raw material is essential to maintain quality thus better efficacy as well as safety through Ayurvedic preparations. TLC/ HPTLC has been used as one of the quality control parameter in The Ayurvedic Pharmacopoeia of India.(1) ICMR published Quality control methods of Indian Medicinal Plants also uses HPTLC, HPLC or GC as quality control parameter.(2) UV-Vis spectrophotometric analysis is one among various quality control parameters which would provide both qualitative and quantitative standards.(3) But both qualitative and quantitative analysis needs markers for confirmation. An attempt is made to study UV-Vis Spectrometric analysis of some herbal raw materials for understanding qualitative and quantitative parameters without markers.

Materials & Methods:

Raw materials procured from market are authenticated in Central Research Facility of KLEU's BMK Ayurveda Mahavidyalaya, Belagavi. Raw materials are pounded and coarse powder is made and used for extraction. Extracts are prepared using solvents of analytical grade viz. double distilled water, methanol, ethanol, chloroform, ether etc. For extraction based on sample either cold maceration method or Soxhlet extraction methods were used.(4,5)

UV-Vis Spectrophotometry was done using Shimadzu UV 1800 Double beam Spectrophotometer.(6) Sample analysis was conducted at room temperature. System calibration was automatically programmed in the Spectrophotometer. UV probe software provided by Shimadzu is used for analysis and interpretation of spectral data.

Raw materials' extracts are diluted randomly initially and tested for UV-VIS spectral analysis. Total spectrum analysis from 900 nm to 200 nm was done as it facilitates to understand various peaks arising due to multiple components present in the extract as well as to find out the 'ʎ max' ie wavelength at which maximum absorbance is observed. Total spectrum also done as 'ʎ max' of the extracts is not exactly known. Spectral analysis was done at different concentrations of extract after identifying representative spectra through trial and error method. Where possible, if isolation methods of said markers of the raw drugs are feasible in the laboratory, spectra were analysed for that isolated chemical also.

Analysis was done in Central Research Facility – (Dept. of AYUSH approved Drug testing Laboratory for ASU drugs), of KLEU's BMK Ayurveda Mahavidyalaya, Belagavi, Karnataka.

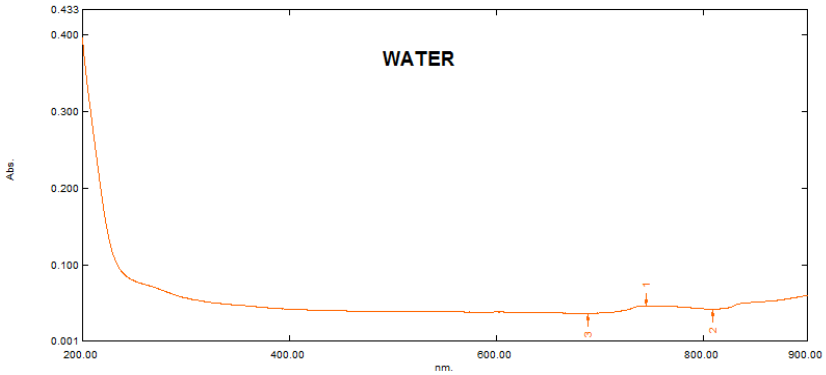
Observations & Results

During UV-Vis Spectrometric analysis initially was done with Blank cuvettes. And spectral analysis of solvents used for dissolving the extracts for analysis like distilled water, methanol, ethanol, chloroform etc. was done to understand the effects of solvent on spectra of drugs analysed. Instrument was made Autozero before analysis. Analysis was done with two cuvettes – one containing solvent and another containing extract dissolved in same solvent so that instrument nullifies the solvent effect on spectra.

Distilled Water spectral analysis:

UV-Vis Spectral analysis data is to observed to nullify its effect as solvent. (Image 1)

Image 1 (Distilled Water UV spectra)



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1. Pippali (*Piper longum* L)

Spectral data observed at different concentrations showed that ʎ max at 253-258 nm and another good peak at 338-341 nm and absorption showed increase with increasing concentration as represented in Table 1, 2 & 3 and Image 2.

Table 1 (*Pippali* at 25 µl concentration)

No.	Wavelength nm.	Abs.
1	663.00	0.098
2	606.00	0.025
3	535.00	0.031
4	505.00	0.035
5	408.00	0.291
6	**338.50**	**1.530**
7	307.50	1.287
8	**258.50**	**2.394**
9	206.50	4.000
10	626.50	0.016
11	581.00	0.011
12	524.00	0.027
13	491.00	0.030
14	401.00	0.285
15	314.50	1.252
16	300.50	1.264
17	233.00	1.910

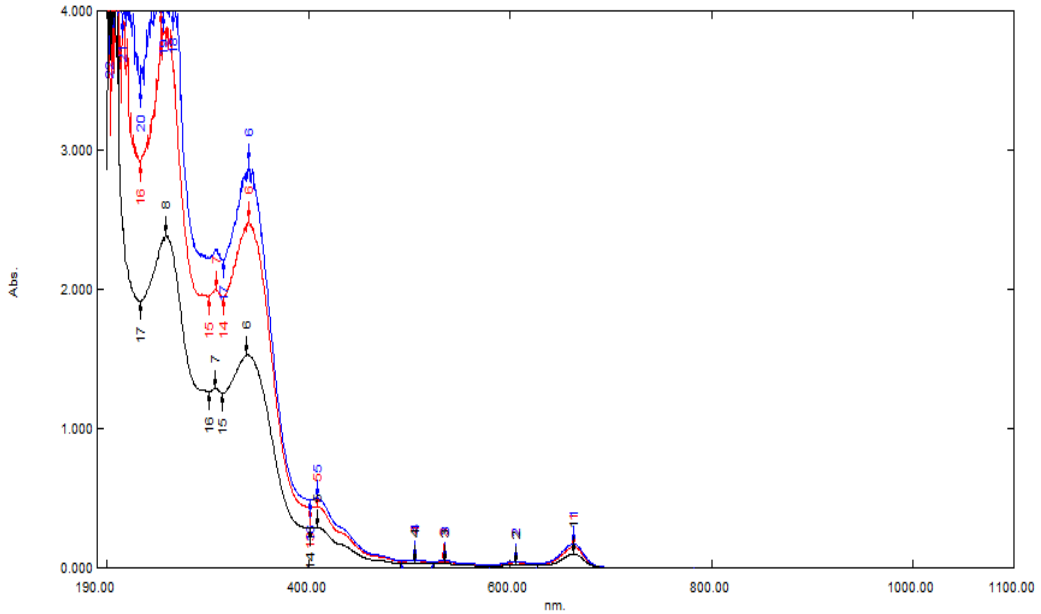
Table 2 (*Pippali* at 50 µl concentration)

No.	Wavelength nm.	Abs.
1	663.00	0.150
2	605.00	0.039
3	534.50	0.047
4	505.00	0.052
5	408.00	0.441
6	**341.00**	**2.501**
7	308.50	1.998
8	**253.50**	**3.896**
9	626.50	0.025
10	581.00	0.017
11	523.00	0.039
12	492.50	0.046
13	401.50	0.435
14	315.50	1.942
15	300.50	1.949
16	233.00	2.903

Table 3 (*Pippali* at 100 µl concentration):

No.	Wavelength nm.	Abs.
1	662.50	0.171
2	605.50	0.045
3	535.00	0.053
4	505.00	0.059
5	408.00	0.500
6	**340.00**	**2.913**
7	266.00	4.000
8	**256.50**	**4.000**
9	249.50	4.000
10	216.00	4.000
11	207.00	4.000
12	626.50	0.030
13	579.00	0.020
14	523.00	0.045
15	492.50	0.052
16	401.00	0.491
17	315.50	2.208
18	265.50	3.990
19	256.00	3.978
20	232.50	3.439
21	215.50	3.931
22	202.00	3.815

Image 2 (Overlay presentation of spectra of *Pippali* extract at different concentrations i.e 25 µl (Black), 50 µl (Red) & 100 µl (Blue))



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***2. Kankola* (*Piper cubeba* L.)**

Spectral data observed at different concentrations showed that ʎ max at 295-296.5 nm and another good peak at 352-359 nm and absorption showed increase with increasing concentration as represented in Table 4, 5 & 6 and Image 3.

Table 4 (At 10 µl dilution)

No.	Wavelength nm.	Abs.
1	**352.50**	**1.530**
2	**295.00**	**2.459**
3	207.00	4.000
4	332.50	1.478
5	253.50	1.368
6	203.00	3.877

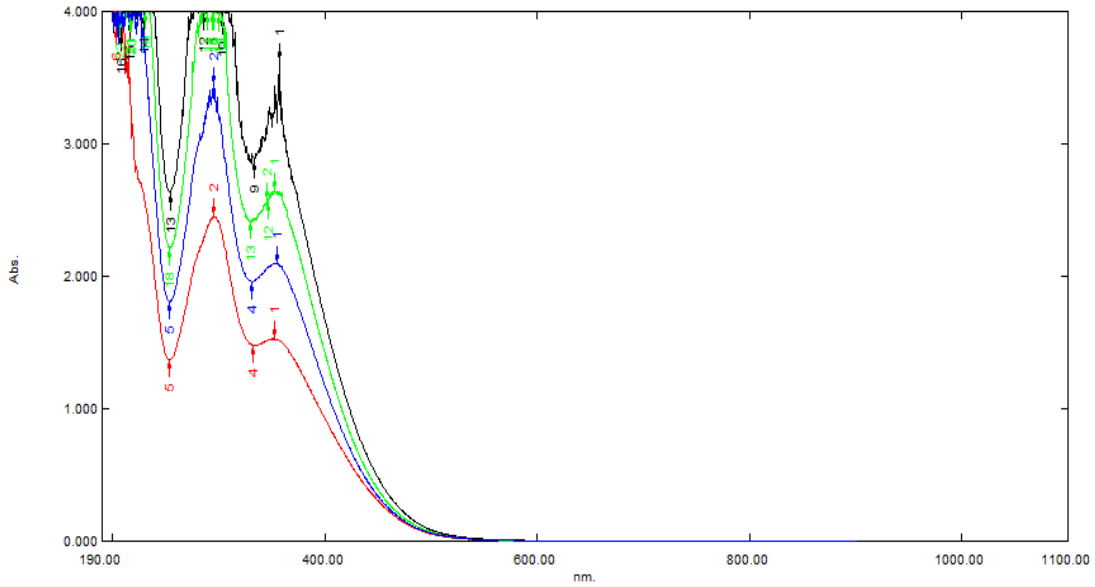
Table 5 (At 15 µl dilution)

No.	Wavelength nm.	Abs.
1	**354.50**	**2.101**
2	**295.50**	**3.447**
3	211.50	4.000
4	331.50	1.958
5	253.50	1.811

Table 6 (At 20 µl dilution)

No.	Wavelength nm.	Abs.
1	**359.50**	**3.048**
2	**296.50**	**4.000**
3	282.00	4.000
4	235.00	4.000
5	225.50	4.000
6	212.50	4.000
7	201.00	4.000
8	330.50	2.631
9	287.00	3.985
10	254.50	2.411
11	234.50	3.955
12	221.50	3.913
13	211.50	3.970

Image 3 (Overlay presentation of spectra shown at different concentrations i.e 10 µl (Red), 15 µl (Blue) & 20 µl (Green) and 50 µl (black))



3. Tea extract - *Camelia chinensis*

Spectral data observed at different concentrations showed that ʎ max at 272.5 nm and absorption showed increase with increasing concentration as represented in Table 7, 8 & 9 and Image 3

Table No.7 (at 50 µl)

No.	Wavelength nm.	Abs.
1	272.70	0.203
2	247.60	0.131

Table 8 (at 100 µl)

No.	Wavelength nm.	Abs.
1	748.50	0.047
2	272.50	0.385
3	247.00	0.212

Table 9 (at 200 µl)

No.	Wavelength nm.	Abs.
1	748.00	0.049
2	272.50	1.479
3	808.50	0.045
4	687.00	0.039
5	247.00	0.711

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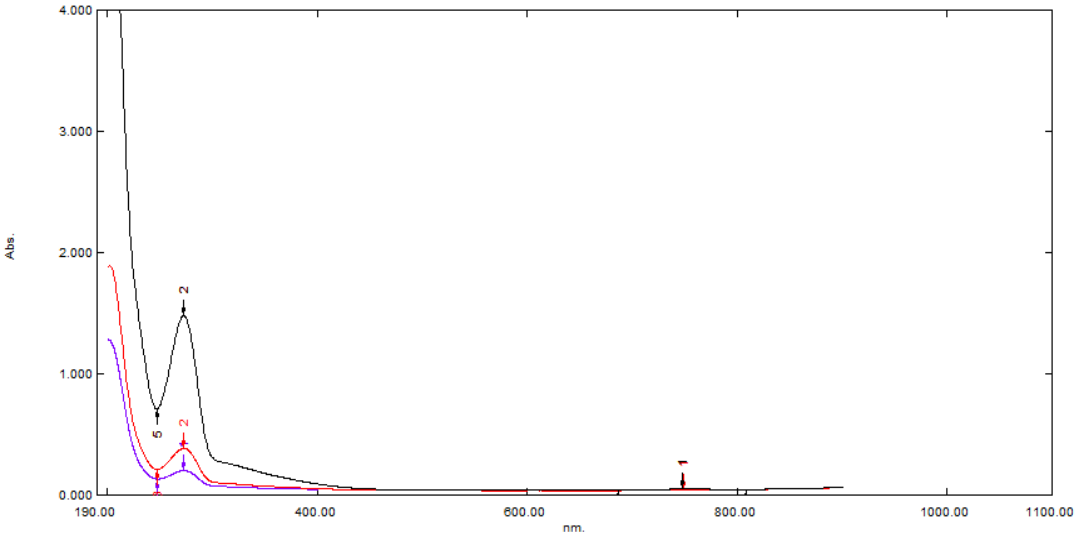
Image 4: (UV-Vis spectra at various concentrations (50 µl (Voilet), 100 µl (Red) & 200 µl (Black) as overlay presentation):



Discussion:

Studies with extracts of raw drugs used in *Ayurveda* showed that spectral analysis will help in quality control using UV-Vis spectroscopic analysis. Total spectral analysis showed many peaks in crude extracts indicating presence of multiple ingredients/ chemicals present in the tested extract. Fewer peaks are observed when extracts are purified for isolation of specific chemicals.

UV-Vis Spectroscopy is beneficial in qualitative analysis as we can get spectra with specific solvent extraction and dissolving in specific solvent. Spectra got can be used as fingerprint of the sample extract if it is obtained using an authenticated standard raw drug sample. And a library of spectra developed like this in the lab could be used to identify given specimen by comparing the spectra along with developed library. Adulterant can be found out by UV spectral analysis. But initial spectra should be obtained using authenticate specimen and tested multiple times with variations such as season of collection, place of collection, time of collection etc.

Quantitative analysis could also be done with the total spectra. But using specific markers will be better way. Total spectrum analysis will also show concentration of different chemicals through multiple peaks which cannot be identified if we use specific wavelength (Lamba Max) only. Even not totally scientific this study of UV-Vis spectrum form 200-900 nm of extracts will make us understand number peaks i.e. chemicals present in a sample as well as give us concentration through peak length which can be compared with known good/ standard authenticated sample spectra. Raw drugs which have been extracted, which lost their potency (*veerya*) due to more time after collection or infested etc. could be found out by comparing spectra with internal standard UV spectra developed in the laboratory even without marker.

Conclusion:

Spectral data showed relationship between concentration and intensity of absorption. Thus by generating library of spectral data of genuine raw samples, it would be possible to test quality control using UV-Vis spectrometer even without costly markers. Further studies are needed to come to conclusion that these data can

be used in quality control of herbal drugs. Drugs collected from various geographical regions and collected during said season and other seasons are to be used for analysis to understand the differences in spectral presentation. If spectra obtained in standard methods are developed by laboratories for many drugs and kept as an album, they could help in at least as internal quality control.

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