UV-VIS AND FTIR SPECTROSCOPIC ANALYSIS OF PHYTOCHEMICALS AND FUNCTIONAL GROUP IN *OCIMUM SANCTUM* AND A FEW MEDICINAL PLANTS

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Abstract. Simultaneous UV-VIS and FTIR spectroscopy is utilized to study and compare the concentration of the pigments-chlorophylls and carotenoids and to understand different functional groups of five medicinal plants, namely *Ocimum sanctum* L. (Tulsi), *Calotropis gigantean* L. (Akanda), *Paederia scandens* (Gadali), *Azadirachta indica* L. (Neem) and *Murraya koenigii* L. (Curry) available in India domestically. The UV-VIS spectra of five medicinal plants show the signature of pigments: chlorophyll a, b and carotenoids. The ratio of two types of chlorophyll is calculated in an uncommon way using the formulae of Lichtenhaler from the UV-VIS spectra and also directly by deconvoluting those spectra. The trend in both the cases corroborates each other, though the magnitude differs. The FTIR study of the samples shows the presence of keto, alcohol, amino groups in aromatic and aliphatic components in fingerprint-like spectra. However, significant presence of aromatic nitro group is found in *Paederia scandens* and *Ocimum sanctum* L. Thus UV-VIS and FTIR are cheap but highly powerful and easy-to-use tools to understand the phytochemical and polyphenolic compositions of medicinal plants for the betterment of herbal and alternative medicines.

Key words: Medicinal plants, *Ocimum sanctum*, UV-VIS, FTIR, chlorophyll, aromatic nitro group.

INTRODUCTION

In spite of the recent development in the field of pharmaceutical and chemical innovation herbal medicine has got its widespread acceptance among large population as herbal medicine is natural and therefore thought to have lesser side effects. These medicines have been produced from the leaves and stems of medicinal plants and herbs since ancient age. The traditional knowledge on herbal medicines has been passed from one generation to the next. However, chemical contents and composition of those medicinal plants are urgently needed to be

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explored for the betterment of an alternative form of natural medicines. The phytochemicals present are required to be understood in order to envisage the changes in these plants due to environmental degradation and pollution.

Fourier transform infrared spectroscopy (FTIR) is a physiochemical analytical technique which explores the chemical structure of the herbal plants along with its metabolites. It plays an important role in providing different band vibrations due to different functional groups of the herbal plants. A relative comparison of various bands provides the invaluable information of the relative concentration of existing functional groups.

Like FTIR, UV-Visible (UV-VIS) spectrophotometric analysis gives knowledge on the presence of various chlorophylls and carotenoids. The UV-VIS spectra, thus along with the FTIR spectra, provide information on the different pigments and the functional groups present in the plants.

Identification of chemical nature of different phytochemical compounds and different functional groups present in the medicinal plants is highly important in order to understand their medicinal properties. In this work we have investigated the pigments-chlorophyll a and b, carotenoids content in some medicinal plants in a simple way using the spectra of UV-VIS spectrophotometer. Simultaneously, using the dry leaves under the FTIR spectrophotometer we have tried to identify different functional groups. A survey of literature revealed that simultaneous FTIR and UV-VIS analysis have not been done on the medicinal plants *Calotropis gigantean* L., *Paederia scandens* and *Murraya koenigii* L. in the past. There is also no combined FTIR and UV-VIS data on them. Although, FTIR and UV-VIS analysis of *Azadirachta indica* L. and *Ocimum sanctum* L. exist in the old literature [2, 9], our effort to estimate the presence of pigments in a very simple way using the formulae of [8] is uncommon.

MATERIALS AND METHODS

In this work five medicinal plants, namely *Ocimum sanctum* L., *Calotropis gigantean* L., *Paederia scandens*, *Azadirachta indica* L. and *Murraya koenigii* L. have been selected and their respective medicinal properties [5, 10, 14] are listed in Table 1. The leaves of these herbal plants are collected and dried at room temperature so that the water content of the leaves becomes minimized to the maximum extent. A portion of the leaf is taken in an agate mortar and pulverized using a pestle to obtain a fine powdered form and subsequently mixed with acetone to make a solution. Importantly, the colour of all the solutions becomes green. Six drops of each of the solutions is mixed with acetone in a quartz tube to collect the UV-VIS spectra using a dual beam Perkin Elmer UV-Visible (model Lambda 25)

spectrophotometer having acetone as reference. Thereafter, the FTIR spectra of the samples are collected using a Perkin Elmer FTIR spectrophotometer (model Spectrum Two). The sample is mixed with KBr powder in an agate mortar and pelletized with a cold press at 5 ton pressure. The pellet is finally mounted in the FTIR to obtain the spectra.

RESULTS AND DISCUSSION

THE UV-VIS STUDY

Figure 1 shows the UV-VIS absorbance spectra of the extract of different leaves taken between wavelengths (λ) ranging from 380 nm to 750 nm. The spectra show the typical behavior of leaves composed of chlorophyll a and chlorophyll b along with carotenoids. The peaks near the red regions (*i.e.* λ ~ 620 to 750 nm) are due to chlorophyll a (at 661 nm) and chlorophyll b (at 644 nm) while in the blue region (λ ~ 450–490 nm) it is a compound spectrum composed of chlorophylls and carotenoids. However, contrary to the general expectation, the absorbance does not fall to zero in the regions where no chlorophyll peaks in nearby regions are present. This may be due to turbidity present in the extracts of the leaves. Turbidity and light scattering generally increase absorption between 400 to 800 nm wavelengths, especially towards the shorter wavelength side [8]. To check the turbidity for proper estimation of absorbance due to pigments, it is also reported [8] that the absorbance at 520 nm should be <10% of the maximum value of chlorophyll absorbance at 661 nm and that at 750 nm it should be zero. Keeping these in mind a base line (shown by red dashed straight line in Fig.2) has been drawn and accordingly all the spectral data are corrected by zeroing the base line data. The corrected spectra of the sample *Azadirachta indica* L. are shown in the same Fig. 2 by a green dashed curve from λ=550 to700 nm along with the spectra not corrected (shown by black curve). All the quantitative calculations are performed with these corrected spectra. A quantitative estimation of the chlorophylls has been done following the equations given in existing literature [8] in which the solvent was acetone similar to our present study. The concentrations of chlorophyll a (C_a), chlorophyll b (C_b) and total carotenoids (C_c) (expressed in μg/ml extract solution) are measured using the absorbance at the most prominent peak for the chlorophylls at 661.6 nm and 644.8 nm and 470 nm for the carotenoids in the spectra. The ratio Chlorophyll a/Chlorophyll b (= C_a/C_b) is also included following the equations,

$$C_a(\mu g/mL) = 11.24A_{661.6} - 2.04A_{644.8}$$

$$C_b(\mu g/mL) = 20.13A_{644.8} - 4.19A_{661.6}$$

$$C_c(\mu g/mL) = \left(1000A_{470} - 1.9C_a - 63.14C_b\right)/214 \quad (1)$$

where A_λ denotes the absorbance at the particular wavelength. The concentrations of all the pigments are given in Table 2.

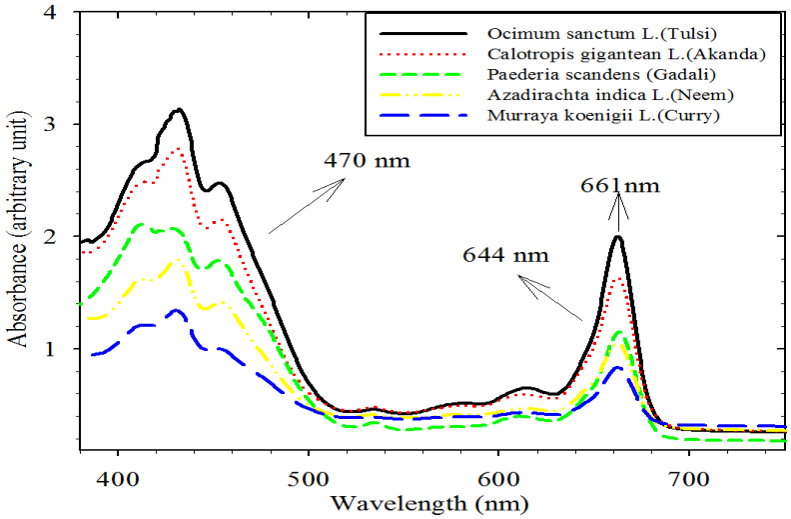

Fig. 1. (online colour) UV-VIS absorption spectra of five medicinal plants. Peaks at λ = 661 nm and 644 nm are due to chlorophyll a and b, respectively. The peak at λ = 470 nm originates due to carotenoids.

All the calculations are shown here after base line correction of the data. We have attempted to calculate the chlorophyll ratio by de-convoluting the two red region peaks. The de-convoluted Gaussian fits (shown by red dashed curves) along with the complete spectra (black continuous curve) are plotted in Fig. 3. In doing the de-convolution the two major peaks are selected as that at λ = 661 nm and another at 644 nm and thereafter obtaining the best fit data as shown by the chi square values. For all the cases studied here, the de-convoluted chlorophyll ratio is found to be higher (5th column of Table 2) than calculated as per the formulae shown in equation (1). However, the trend of the calculated relative ratio of chlorophylls amongst the different plant leaves conforms to the observed ratio obtained after de-convoluting the spectra. The ratio of C_a and C_b is found to be highest in *Paederia scandens* while the same is lowest in *Azadirachta indica* L. among the five herbal medicinal plants. The ratio of total chlorophyll and carotenoid is also calculated and listed in Table 3. It is seen that the greater the ratio of the chlorophylls (i.e C_a/C_b), the smaller is the ratio of total chlorophylls to

carotenoids (i.e $(C_a+C_b)/C_c$). Among all the plants studied here *Paederia scandens* leaf has the highest amount of carotenoids relative to its total chlorophyll contents.

FTIR STUDY

Fig. 4 shows the characteristics finger-print like FTIR spectra of all the samples under study. The major bands and peaks are pointed with arrow marks. They are also listed in Table 3 with their tentative assignment. All the spectra demonstrate some common features. They can be broadly divided into three regions, the mid region with wave number between 1700 cm^{-1} to 1000 cm^{-1} , the upper region around 3500 cm^{-1} and the lower region below 1000 cm^{-1}. The former has some unique features compared to the rest of the two regions. All the samples show similar type of FTIR characteristic bands as reported earlier for different plants [7, 13], however some literature also exist in which the spectra look quite different [3, 12].

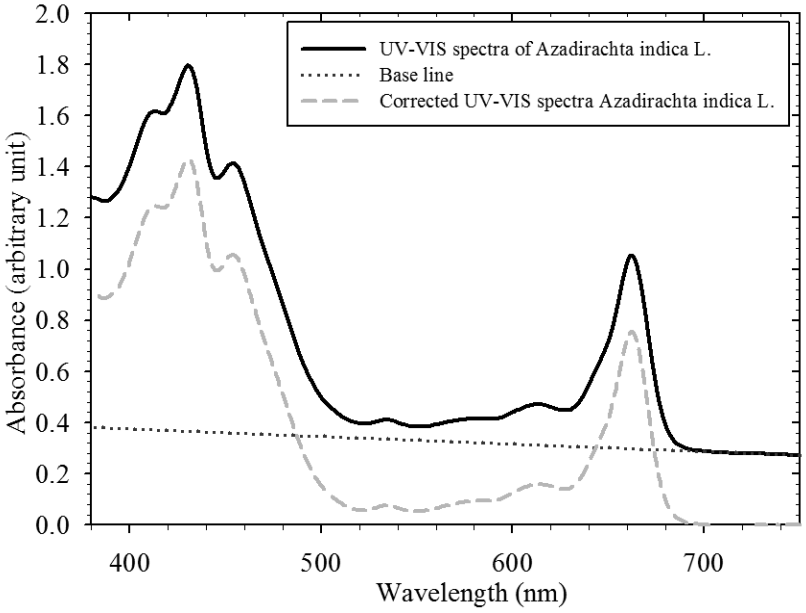

Fig. 2. The uncorrected UV VIS spectra (continuous curve) and base line corrected spectra (short dashed curve) of *Azadirachta indica* L.. The long dashed line represents the base line.

At around 3400 cm^{-1} there is presence of a strong and broad band for all the samples under study. The asymmetric nature of the band indicates that this is composed of more than one component at this region. This suggests that the broad natured band may be originated from the overlapping of O–H and N–H stretching

modes (confirmed later with other modes) of vibration of alcohol/water present in the carotenoid and amide present in all the plant leaves.

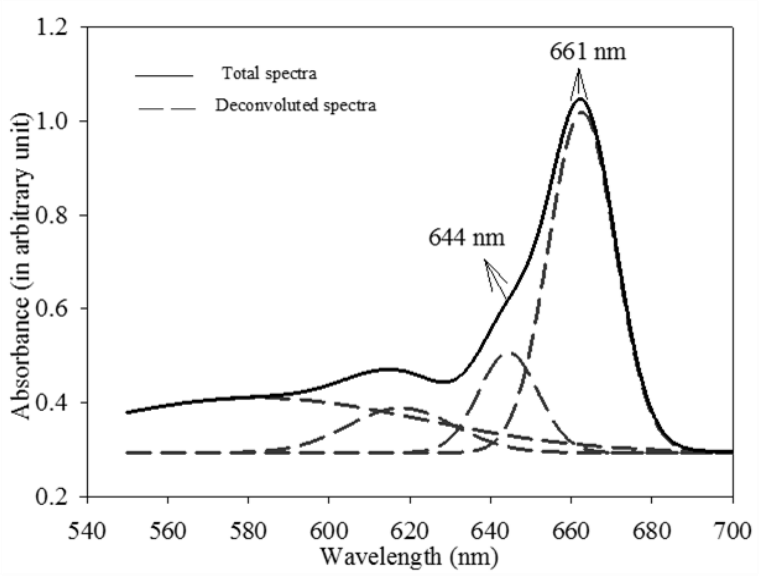

Fig. 3. The total UV VIS spectra (continuous curve) and deconvoluted Gaussian fits (dashed curves).

All the samples show absorption band at 2960 cm^{-1} region due to asymmetric C–H stretching of CH_3 of alkane group and at around 2925 cm^{-1} region due to asymmetric C–H stretching of CH_2 of alkane group. The band at around 2850 cm^{-1} is assigned due to the symmetric C–H stretching in CH_2 of the alkane group [1].

All the samples have a very weak and extended band at around 2130 cm^{-1} due to the asymmetrical stretching of C≡C of alkynes. The small band which is just resolved for all the samples at around 1730 cm^{-1} is assigned due to the C=O stretching of aromatic ester [4].

The strong band at around 1630 cm^{-1} for all the samples are assigned due to the C=O stretching vibration of derivative amide. The band around 1318 cm^{-1} is assigned due to C=N stretching vibration for all the samples. However, both the bands at around 1550 cm^{-1} and around 1320 cm^{-1}, found prominently in *Paederia scandens* and *Ocimum sanctum* L., are believed to be arising from the N–O asymmetric and symmetric stretching bands, respectively, of the aromatic NO_2 group which is not so prominent for the other samples [1].

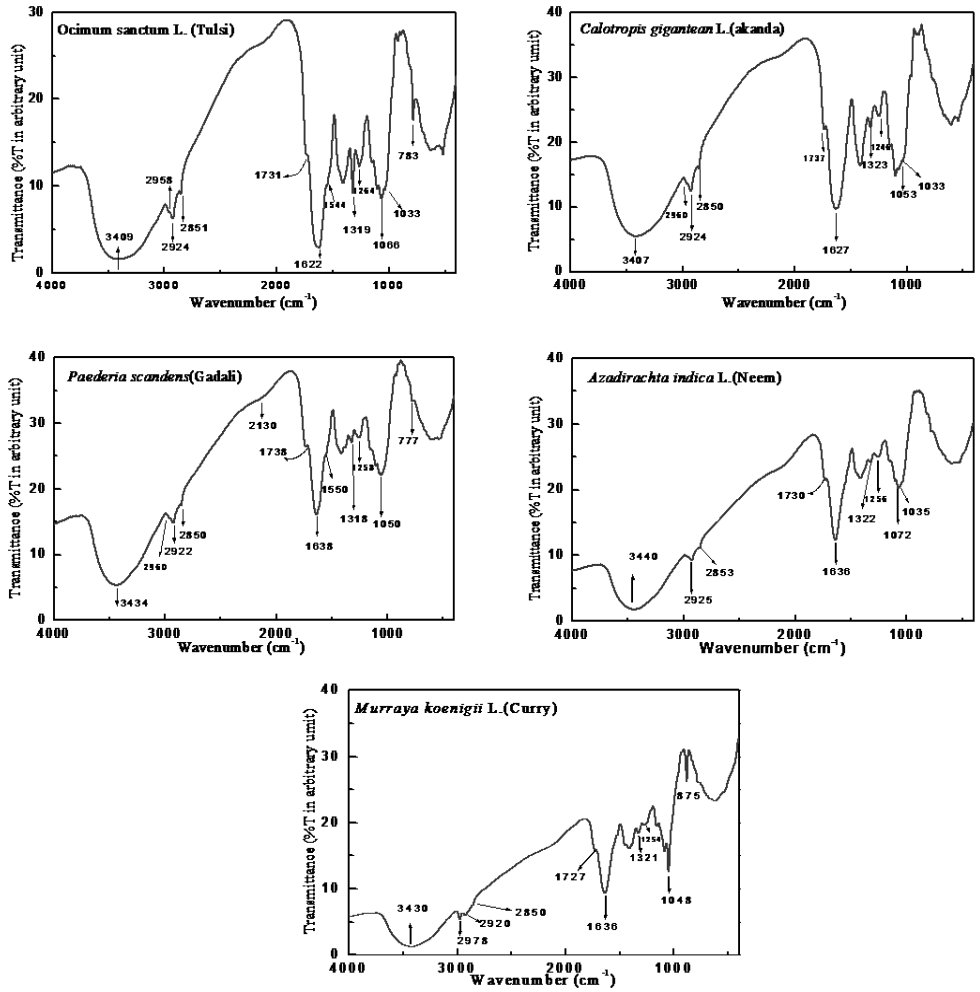

Fig. 4. The FTIR spectra of five medicinal plant samples.

A weak band at around 1250 cm^{-1} is observed for all the samples. This is assigned as C–O stretching mode in the aromatic acetate group [6, 15]. The relatively stronger band at around 1060 cm^{-1} and 1030 cm^{-1} is assigned as C–O stretching vibration of primary and secondary alcohols in cellulose [11]. In only two samples, namely *Paederia scandens* and *Ocimum sanctum* L., a notable absorption band at about 780 cm^{-1} is present which is probably due to the bending vibrational mode of O–N–O of the NO_2 group confirming the presence of nitro group in those samples. All the band assignments are summarized in Table 3.

CONCLUSION

Simultaneous measurement of UV-VIS and FTIR spectra of some herbal plants has been done to study their phytochemical composition and functional groups. UV-VIS spectra have shown typical absorption bands characteristics of chlorophyll a, chlorophil b and carotenoids. The ratios of concentration of two types of chlorophyll are estimated in a unique way as per the methods proposed by Lichtenthaler and Buschmann as well as applying mathematical techniques on the measured UV-VIS spectra. Although the trend of the ratio of the chlorophyll shows same type of variation between the two methods but their value differs significantly. The FTIR study shows presence of amine, keto, alcohol groups in the aromatic and aliphatic compounds. However, only in *Paederia scandens* and *Ocimum sanctum* L. the significant presence of aromatic nitro group is an exception. Such use of FTIR and UV-VIS should provide the opportunity to understand the polyphenolic structures and phytochemicals compositions of herbal samples in order to enrich natural medicines.

Table 1

Medicinal plants, scientific names and their medicinal uses

Popular name	**Scientific name**	**Medicinal uses**
Tulsi	*Ocimum sanctum* L.	Hypoglycaemic, hypolipidemic activities, antioxidant activity, anti-ulcer, anti-microbial.
Akanda	*Calotropis gigantean* L.	Latex is used for skin irritation, root decoction is used as analgesic.
Curry	*Murraya koenigii* L.	Used in diabetes, anti-bacterial and anti-fungal activities, used as spice in cooking.
Gadali	*Paederia scandens (Lour.)*	Used for treating sores in skin, stomach problems.
Neem	*Azadirachta indica* L.	Leprosy, skin problem, analgesic, cough and many more

Table 2

Medicinal plants and their pigments' concentration

Name of the plant	C_a **(μg/mL)**	C_b **(μg/mL)**	C_c **(μg/mL)**	C_a/C_b	C_a/C_b **(From deconvolution)**	$(C_a+C_b)/C_c$
Ocimum sanctum L.	16.51	6.12	5.08	2.7	3.59	4.45
Calotropis gigantean L.	13.28	7.31	3.57	1.82	3.47	5.76
*Paederia scandens (*Lour.*)*	7.71	1.19	4.35	6.45	6.83	2.05
Azadirachta indica L.	7.28	3.74	2.24	1.94	3.55	4.92
Murraya koenigii L.	5.73	1.69	1.65	3.4	4.67	4.50

Table 3

FTIR peaks and their tentative assignments for the samples

FTIR peak positions (cm^{-1})					**Tentative assignment**
Ocimum sanctum L.	*Calotropis gigantean* L.	*Paederia scandens*	*Azadirachta indica* L.	*Murraya koenigii* L.	
3409	3407	3434	3443	3430	O–H and N–H stretching
2958	2960	2959		2978	asymmetric C–H stretching of CH_3 of alkane group
2924	2924	2922	2925	2920	asymmetric C–H stretching of CH_2 of alkane group
2851	2850	2850	2853	2850	symmetric C–H stretching in CH_2 of the alkane group
1731	1737	1738	1730	1727	C=O stretching of aromatic ester
1622	1627	1638	1636	1636	C=O stretching vibration of derivative amide
1544		1550			N⟶O asymmetric stretching of NO_2 aromatic group

Table 3

(continued)

FTIR peak positions (cm^{-1})					**Tentative assignment**
Ocimum sanctum L.	*Calotropis gigantean* L.	*Paederia scandens*	*Azadirachta indica* L.	*Murraya koenigii* L.	
1319	1323	1318	1322	1321	N⋯O symmetric stretching of NO_2 aromatic group
1264	1246	1258	1256	1254	C–O stretching in the aromatic acetate
1066	1053	1050	1072	1048	C–O stretching vibration of primary alcohols
1032	1033		1035		C–O stretching vibration of secondary alcohols
783		777			bending vibration mode of O–N–O of the NO_2 group

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