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RESEARCH ARTICLE

Quality control of herbal medicines by using spectroscopic techniques and multivariate statistical analysis

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Abstract

Herbal medicines play an important role in modern human life and have significant effects on treating diseases; however, the quality and safety of these herbal products has now become a serious issue due to increasing pollution in air, water, soil, etc. The present study proposes Fourier transform infrared spectroscopy (FTIR) along with the statistical method principal component analysis (PCA) to identify and discriminate herbal medicines for quality control. Herbal plants have been characterized using FTIR spectroscopy. Characteristic peaks (strong and weak) have been marked for each herbal sample in the fingerprint region (400–2000 cm⁻¹). The ratio of the areas of any two marked characteristic peaks was found to be nearly consistent for the same plant from different regions, and thus the present idea suggests an additional discrimination method for herbal medicines. PCA clusters herbal medicines into different groups, clearly showing that this method can adequately discriminate different herbal medicines using FTIR data. Toxic metal contents (Cd, Pb, Cr, and As) have been determined and the results compared with the higher permissible daily intake limit of heavy metals proposed by the World Health Organization (WHO).

Keywords: FPS; FTIR; herbal medicines; PCA; quality control

Introduction

Herbal products are being adopted increasingly by modern people worldwide for their therapeutic and cosmetic properties (Gogtay et al., 2002). Recently, the quality and safety of these herbal products has become a serious issue due to increasing pollution in air, water, soil, etc. The use of fake herbs to form drugs in some instances has also contributed to a negative opinion about herbal products (Yu et al., 1995). Several forensic reports and investigations have been published against herbal medicinal products from different parts of the world involving heavy metal toxicity (Steenkamp et al., 1999; Jung et al., 2006). Also, herbal medicines have multiple constituents including active compounds, inactive unknowns, and metallic elements (in macro and trace quantities). The quantitative composition of these

constituents depends on the soil and environmental conditions; therefore, it may vary from one place to another. The excess of these constituents over a specified limit may lead to several health problems (Au et al., 2000; Rai et al., 2001; Ernst, 2002; Saper et al., 2004). Hence, the discrimination and identification of herbal plants, their formulations (e.g. medicines made by extracts), and toxic heavy metal detection are essential for quality assurance (Martin, 1992; Woo et al., 1999; Cho et al., 2001; Garvey et al., 2001).

Traditionally, quality identification of herbal medicines was done by experienced herbalist doctors based on differences in the appearance of plants. Recently, several chromatographic techniques such as high performance liquid chromatography (HPLC), gas chromatography (GC), and thin layer chromatography (TLC), etc. have been employed for this purpose. These methods, though accurate, are time

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consuming and involve laborious sample preparation steps (Mao & Xu, 2006). It is therefore desirable to develop a rapid, simple, and accurate analytical method for herb identification and quality assessment.

The present article proposes a procedure for the identification of herbal products (plants or medicines) by combining molecular spectroscopy with multivariate statistical analysis. In the present work it was not attempted to identify the individual organic compounds present in the herbs, which is a time consuming, costly procedure, but several of the main bands present in the herbal sample in the mid-infrared (IR) region (the so-called fingerprint region of the spectra, 400–2000 cm^{-1}) were marked. These marked bands were used for the comparison of herbal plants and their medicinal formulations. The areas of the peaks of the marked bands were measured, and the ratio of the areas of two marked peaks of a specific sample from different parts of the country was calculated for comparison. The quantitative method of comparison proposed by Zou et al. (2005) was applied for the comparison of crude herbal samples and their formulations. Further, Fourier transform infrared (FTIR) spectral data for a large number of herbal samples were processed by principal component analysis (PCA) to cluster herbal medicines into different groups, to discriminate different herbal medicines even though they might be from different places. Finally, the toxic heavy metal (Pb, Cd, and Cr) content in these herbal drugs was also determined using atomic absorption spectroscopy (AAS), while the arsenic (As) content was determined using inductively coupled plasma atomic emission spectroscopy (ICP-AES). The concentrations of the various metals were compared with the maximum permissible daily intake limits for heavy metals prescribed by the World Health Organization (WHO).

Materials and methods

Plant materials

Herbal plants were collected from different parts of India (Varanasi, Ahmedabad, Jaipur, and Mumbai). These selected cities are in different climatic zones of India. Herbal plants Arjuna [*Terminalia arjuna* L. (Combretaceae)], Tulsi [*Ocimum sanctum* L. (Lamiaceae)], Aawala [*Emblic myrobalan* Burm. (Phyllanthaceae)], Aswagandha [*Withania somnifera* L. (Solanaceae)], Vaasa [*Adhatoda vasica* Nees. (Acanthaceae)], Meethi Neem [*Murraya koenigi* L. (Rutaceae)], and Bitter Neem [*Azadirachta indica* L. (Meliaceae)] were selected for this purpose, as these are some of the most common herbal medicines from India that are frequently used for herbal therapy (Khanna et al., 1986; Sharma 1993). Samples were collected in

the month of November and their identification was verified by Dr. Anand Chaudhary, an expert in herbal medicines at the Institute of Medical Sciences, Banaras Hindu University (BHU), Varanasi, India. Samples collected were processed (method given below) and used directly for the experimental work. Commercial samples (in the form of tablets, capsules, and powder) under the same vernacular names from various companies (Zandu Pharmaceuticals, Gyan Herbal Home, Himalaya Drug, and Nagarjuna Pharmaceuticals) were also collected from the local market of Varanasi, India.

Methods

Raw herbal samples were washed in fresh running water to eliminate dust, dirt, and possible parasites, and then they were washed again with deionized water. Commercial tablets were first cleaned in order to remove the outer coating. Both types of samples were dried and powdered using a mortar and pestle. For quantitative similarity, crude herbal samples (reference samples) were collected from the Ayurvedic Garden of BHU, Varanasi, India, and formulations (tablets and capsules) with the same vernacular names from the local market.

For FTIR analysis, a powder sample and KBr were mixed at an appropriate ratio (1:50) and pellets were prepared applying the same pressure to keep the thickness of the pellet constant. The utmost care was taken to obtain the same concentration of sample each time in every pellet. FTIR spectra were recorded in the 400–2000 cm^{-1} spectral region with a FTIR spectrophotometer (Spectrum RX-I; PerkinElmer). The spectra were collected at a resolution of 4 cm^{-1} . Each spectrum was the average of 10 scans.

PCA analysis was done by taking the FTIR spectrum at discrete wave number points over the 500–1800 cm^{-1} region of the spectrum at intervals of 100 cm^{-1} . In all, the spectra of five herbal medicinal plants (*Terminalia arjuna*, *Ocimum sanctum*, *Emblic myrobalan*, *Withania somnifera*, and *Adhatoda vasica*) and a total of 17 samples were considered. A 17 × 14 data matrix was formed by taking 17 samples along rows and 14 wave number points along columns. The different samples along the rows were arranged as follows: rows numbered 1–3 represented *Terminalia arjuna*, 4–7 represented *Withania somnifera*, 8–10 represented *Adhatoda vasica*, 11–13 represented *Emblic myrobalan*, and 14–17 represented *Ocimum sanctum*. The matrix elements in the data matrix were the % transmission values. The data were normalized by mean centering with respect to the column means.

For elemental analysis, first, digestion of the sample was done. All the chemicals (HCl (Qualigens, 36.5%), HNO_3 (Merck, 65%), and HClO_4 (Rankem, 73%)) used were of analytical grade. All the glassware was soaked

with aqua regia ($\text{HCl}:\text{HNO}_3$, 3:1) for 2 h (Levine et al., 2005). The completely dried sample (1 g) was digested in concentrated nitric acid and perchloric acid (3:1) until a clear solution with white fumes was obtained. After adequate cooling, the solution was reconstituted to the desired volume, i.e. 25 mL, with deionized water in a test tube. Quantitative estimation of heavy metals was done finally by using an atomic absorption spectrophotometer (Model no. 2380; PerkinElmer) and an inductively coupled plasma atomic emission spectrometer (PS-3000, UV-DRE; Leeman Labs Inc., USA).

Results and discussion

FTIR analysis for fingerprint spectra

FTIR spectra of the herbal samples and their formulations were recorded in the fingerprint region, $400\text{--}2000\text{ cm}^{-1}$, in

order to mark the characteristic peaks of these and check their presence in the formulations. The fingerprint spectra (FPS) of Arjuna (*Terminalia arjuna*), Aswagandha (*Withania somnifera*), Aawala (*Emblc myrobalan*), and Tulsi (*Ocimum sanctum*) obtained from different parts of the country are shown in Figure 1a–d, respectively. Under the same experimental conditions the FTIR spectra of different herbal samples showed obvious differences; however, they showed a good similarity for the same herbal samples obtained from different places. Some sharp, intense, and well-defined peaks were selected for each herbal (Table 1) to characterize their presence in a given sample. The intensity and the area of these characteristic peaks in the same sample obtained from different places showed significant changes; however, their peak positions remained unaltered. This clearly shows the effect of the environment and the soil on the samples. Second derivative spectra of the samples were also recorded (Figure 2 for Arjuna (*Terminalia arjuna*)).

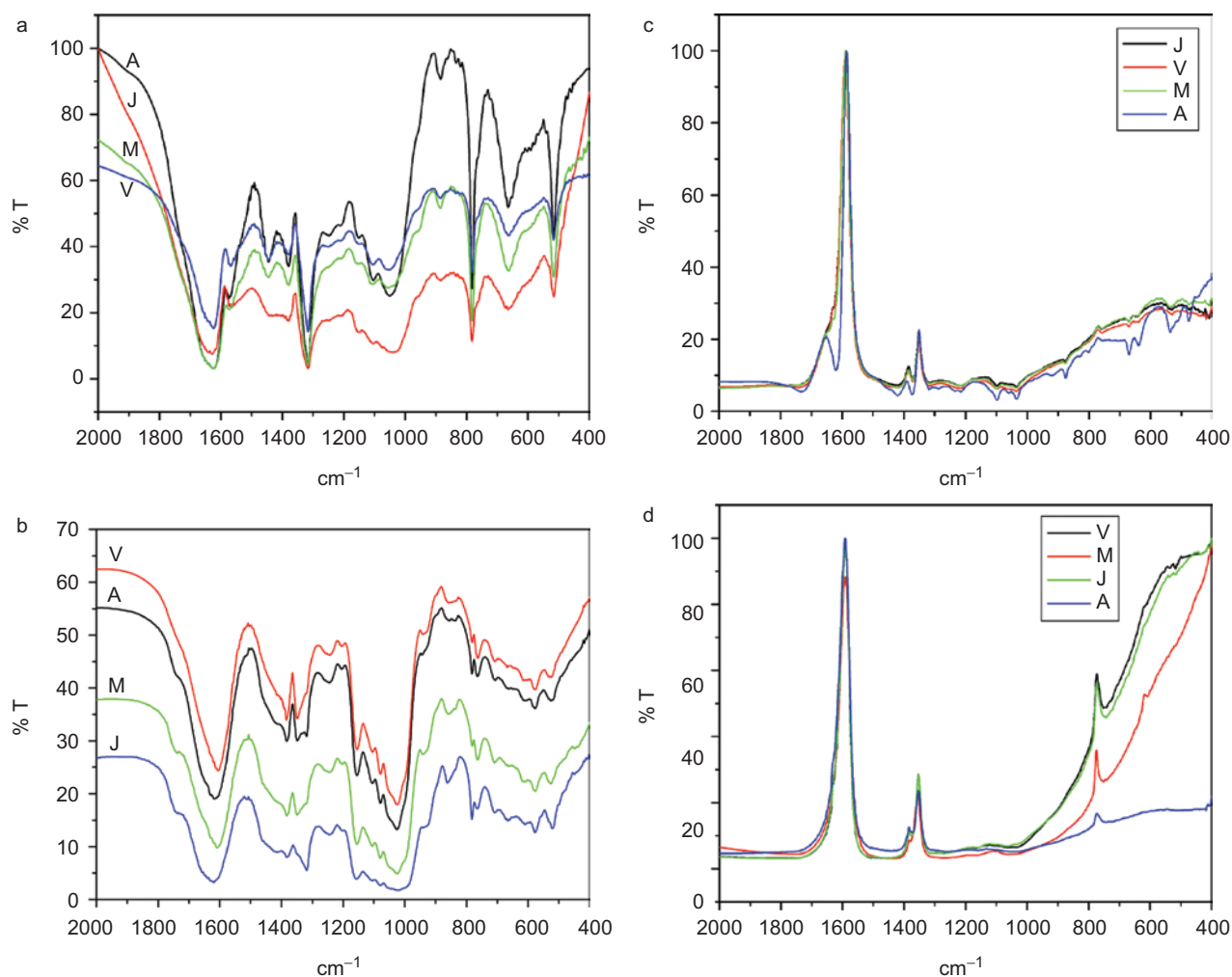


Figure 1. Fourier transform infrared (FTIR) spectra of (a) Arjuna (*Terminalia arjuna* L.), (b) Aswagandha (*Withania somnifera* L.), (c) Aawala (*Emblc myrobalan* L.), and (d) Tulsi (*Ocimum sanctum* L.) from different places showing similarity among them. J, Jaipur; M, Mumbai; V, Varanasi; A, Ahmedabad.

This helped to mark the peak positions in a better way, where the normal IR spectrum was slightly complex.

As mentioned above, the positions of the peaks in the same herbal samples obtained from different places were the same; however, their intensity and area differed from sample to sample. This was obviously due to a geographical effect. In order to check their similarity,

Table 1. Specific peaks (in cm^{-1}) in Fourier transform infrared-fingerprint spectra (FTIR-FPS) of herbal medicines for their characterization.

Herbal plant/medicine	Part used	Peak (cm^{-1})
Arjuna <i>Terminalia arjuna</i> L. (Combretaceae)	Bark	Strong 516, 780, 1316 Weak 884, 1445
Tulsi <i>Ocimum sanctum</i> L. (Lamiaceae)	Leaves	Strong 751, 1375, 1759 Weak 1060, 1029
Aawala <i>Emblis myrobalan</i> Burm. (Phyllanthaceae)	Fruits	Strong 876, 1035, 1097, 1371 Weak 472, 1216
Aswagandha <i>Withania somnifera</i> L. (Solanaceae)	Bark	Strong 520, 576, 1077, 1383 Weak 616, 1106
Vaasa <i>Adhatoda vasica</i> Nees. (Acanthaceae)	Leaves	Strong 430, 765, 1401, 1610 Weak 898, 1102, 1550
Bitter Neem <i>Azadirachta indica</i> L. (Meliaceae)	Leaves	Strong 1062, 1317, 1599 Weak 1155, 1255
Meethi Neem <i>Murraya koenigi</i> L. (Rutaceae)	Leaves	Strong 782, 1152, 1729 Weak 667, 959

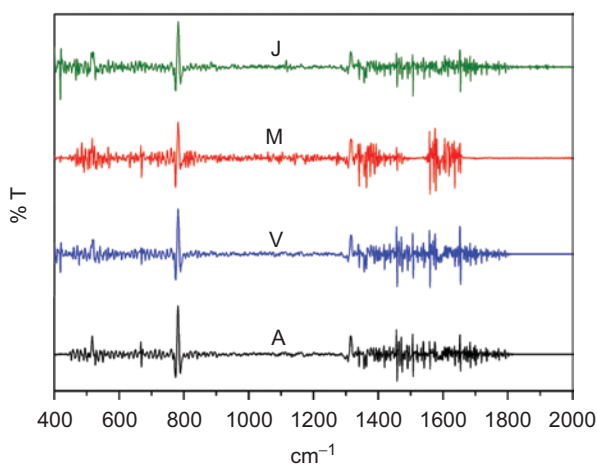


Figure 2. Second derivative Fourier transform infrared (FTIR) spectra of Arjuna (*Terminalia arjuna* L.) collected from different places.

ratios of the areas of two selected peaks for samples obtained from all four different places were calculated. Interestingly, this ratio was nearly consistent (Table 2) for a sample collected from four different places (Figure 3). Even though some remarkable differences between a raw herbal sample and its specific formulation might be observed, the characteristic peak positions were the same. Thus, peak position and ratio of the areas of the peaks could be used to judge the quantitative similarity of the drug.

Quantitative evaluation of similarity of two herbal medicines using FTIR spectrum

Zou et al. (2005) proposed the common peak ratio method to evaluate the similarity of any two (say, A and B) crude herbal medicines quantitatively. According to this method, the common peak ratio was defined as:

$$P = (N_g / N_d) \times 100\% \quad (1)$$

where P is the common peak ratio, N_g is the number of peaks that are common in the two IR FPS under consideration, and N_d is the total number of peaks that are different in the IR FPS compared. Hence:

$$N_d = N_g + n_a + n_b \quad (2)$$

where n_a is the number of variation peaks in IR FPS which belong only to IR FPS (A) and n_b is the number of variation peaks in IR FPS which belong only to IR FPS (B).

The samples can be arranged according to common peak ratio value from high to low, and the relationship between reference and other samples can be determined quantitatively. A higher value of the common peak ratio indicates a closer similarity between the reference sample and the formulation.

Here, we have utilized this method for the comparison of a crude herbal sample and its formulations. From this method, Aswagandha from Varanasi (reference sample) and its medicinal formulations Aswagandha capsule (contains viscous liquid) and tablets were compared, and the common variation peak ratio was found to be 52% and 95%, respectively. From these results it is clear

Table 2. Ratios of areas of assigned characteristic peaks.

Herbal plant/medicine	Peaks selected	Ratio of areas of peaks			
		Varanasi	Jaipur	Ahmedabad	Mumbai
Arjuna <i>Terminalia arjuna</i> L. (Combretaceae)	780/516	1.21	1.19	1.18	1.13
Tulsi <i>Ocimum sanctum</i> L. (Lamiaceae)	1759/1375	27.90	29.15	26.83	29.45
Aawala <i>Emblis myrobalan</i> Burm. (Phyllanthaceae)	1035/876	1.26	1.34	1.28	1.25
Aswagandha <i>Withania somnifera</i> L. (Solanaceae)	520/1077	3.14	3.35	3.26	3.45
Vaasa <i>Adhatoda vasica</i> Nees. (Acanthaceae)	1401/765	1.69	1.62	1.62	1.59
Bitter Neem <i>Azadirachta indica</i> L. (Meliaceae)	1317/1062	3.39	3.61	3.73	3.73
Meethi Neem <i>Murraya koenigi</i> L. (Rutaceae)	1152/782	2.54	2.42	2.68	2.65

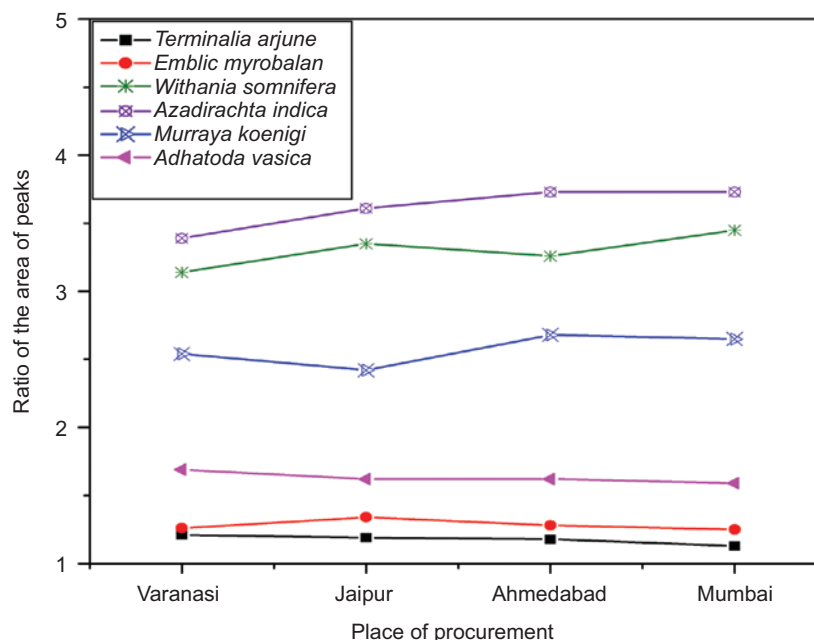


Figure 3. Graph showing consistent nature of ratio of areas of marked characteristic peaks for herbal samples collected from different places.

that the Aswagandha tablet shows a very good common variation peak ratio, and confirms that this sample is a formulation of Aswagandha. However, the Aswagandha capsule (extract form) shows a poor common variation peak ratio, even though most of the intensely marked peaks are present in the spectrum of the Aswagandha capsule. This confirms that although the prescription contains Aswagandha, the lower common variation peak ratio suggests that during the extraction process some other organic compounds were probably used and not removed completely from the final product, and were responsible for new peaks or a shift in peaks, giving rise to a poor common variation peak ratio.

Similarly, the common peak ratio for Arjuna (reference sample) and for the Arjuna capsule (contains powder) was found to be 95%, while for Aawala (reference sample) and Amlaki rasayan (powdered sample) it was 85%. Similar results were found for other herbal samples and their formulations, and it was predicted that if the formulation contained crude drug alone, the common variation peak ratio was around 90% or above, and if some additional organic impurity had been added during processing and was still present in the drug, then the common variation peak ratio was low. Thus, this method could be useful in the quality control of herbal medicines.

Principal component analysis of FTIR data

PCA transforms the data from a real measurement space to a virtual feature space defined by the principal axes. In the measurement space there is often a high level of

information degeneracy, and PCA removes or at least minimizes this degeneracy (or redundancy), and the data representations in the feature space become separated. Projections of the original measurement samples onto the principal axes are called the principal components (PCs). PCA is geometrically thought of as rotation of the axes of the original coordinate system to a new set of orthogonal axes, ordered in terms of the amount of variance of the original data (Andrew, 2002). The first PC finds the direction along which the variance is maximum; the second PC is defined by the direction orthogonal to the first with maximum residual variance; and so on, until the total variance is accounted. By plotting the PCs in different projection planes one can visualize grouping or clustering (a cluster is a collection of objects that are similar among themselves and are dissimilar to objects belonging to other clusters) of samples according to similarities or differences among them. PCA analysis is all about visualization and interpretation of these groupings.

Table 3 provides information about the percentage of variance in different PCs. The first three PCs account for 99.30% of the total variance. The remaining 11 principal components account for only 0.70% of the variance in the data representation, and therefore are ignored for further consideration. The first principal component PC-1 dominates the variance in data representation, containing 80.36% of the total variance; PC-2 has 18.11% of total variance; and PC-3 has only 0.83%. Figure 4 shows various PC plots as indicated. It can be seen that, in all plots, the five classes of medicinal herbs are discernibly

Table 3. Eigen values and percentages of total variance from principal component analysis (PCA) of Fourier transform infrared spectral data for each of first seven principal components.

Principal component	Eigen value	% Total variance	Cumulative % total variance
1	346,772	80.36	80.36
2	78,144	18.11	98.47
3	3568.17	0.83	99.30
4	1803.42	0.42	99.72
5	498.65	0.12	99.84
6	409.673	0.095	99.935
7	259.054	0.060	99.995

separated. The separation is, however, quite apparent in the PC-1 vs. PC-2 plot. In the PC-1 vs. PC-3 plot, the two samples of *Withania somnifera* overlap with the two samples of *Terminalia arjuna*. The remaining classes are again well clustered and separated. In brief, the PCA analysis of FTIR data leads to well-formed grouping in PC plots according to herbal type. This could be utilized for the identification of different species of medicinal herbs, and therefore the constituents of herbal medicine.

Thus, the FTIR/PCA combination may provide an effective method for the detection of spurious or fake content in herbal products.

Elemental detection for quality control

Table 4 shows the concentration of Cd, Pb, Cr, and As (in ppm) in crude herbal and commercial samples. From Table 4 it is clear that the heavy metal concentration varies significantly in a particular plant species collected from different regions. Cd is maximum in the Arjuna sample obtained from Ahmedabad (0.076 ppm), while its concentration is below the detection limit (<DL) in Arjuna gritha Ahmedabad. Pb is highest in Bitter Neem (0.752 ppm), while it is below the detection limit in Arjuna Jaipur. Cr is highest in Vijaysar Mumbai (1.006 ppm) and minimum in Bitter Neem Varanasi (0.30 ppm). As is maximum in Vaasa rishtha (0.34 ppm) and minimum in Vijaysar Jaipur (0.02 ppm). Thus, a large variation was observed, and it is clearly due to the water, soil, and environmental conditions of the location. Hence, monitoring of the heavy metal concentration in crude herbal material collected from a specific place seems quite essential.

It is clear from Table 5 that the concentration of Cd in some of the herbal preparations is slightly higher than the tolerance limit (in Arjuna). In order to compare the tolerance limit of herbal samples, the metal content of 1 g (or one tablet, one capsule, etc.) of sample was multiplied by the recommended dose. For example, the recommended intake of Arjuna preparation per day is 6 g, but that will contain Cd above the dangerous limit. In some other cases the abundance of

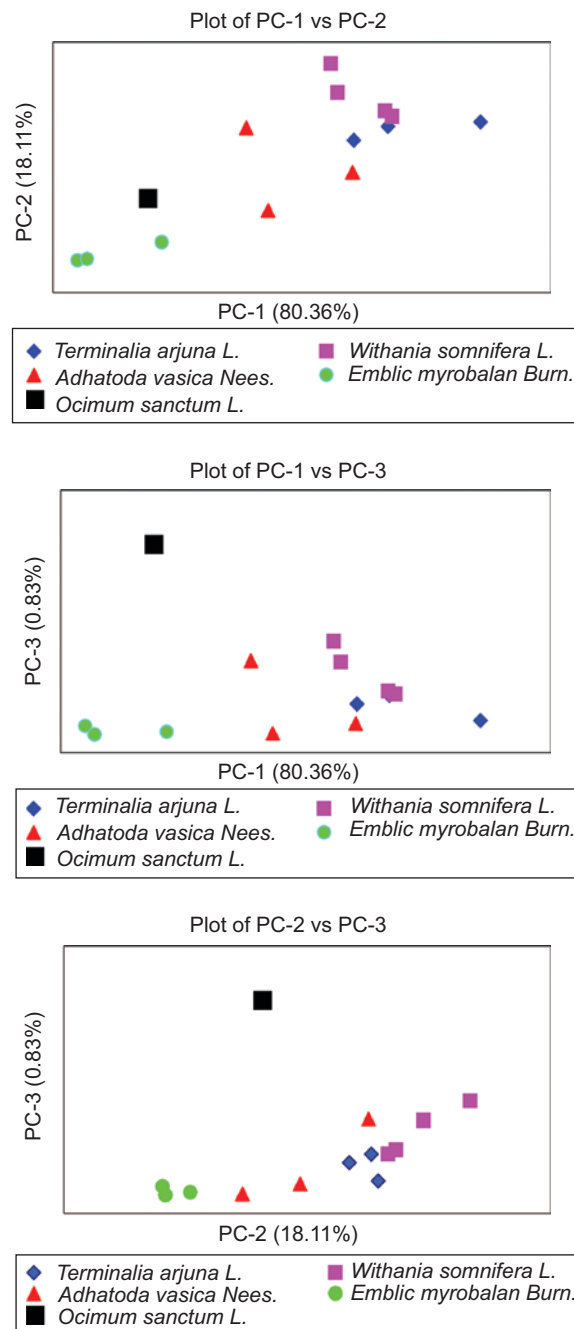


Figure 4. Principal component (PC) plots showing clustering of herbal medicines into different groups.

Cd is just at the tolerance limit (Table 5), which indicates that here also high doses or self-medication may lead to problems.

Further, in regard to other heavy metals whose presence was detected, no such difficulties were seen. It is a common belief that herbal products are safe; however, self-medication and high doses are common, and from the above observation it is clear that these may cause heavy metal toxicity.

Table 4. Heavy metal concentration (in ppm) in crude herbal/commercial Ayurvedic medicines.

Herbal medicine	Cd	Pb	Cr	As
Arjuna				
V	0.049 ± 0.012	0.425 ± 0.038	0.105 ± 0.012	0.09 ± 0.003
A	0.076 ± 0.117	0.500 ± 0.042	0.070 ± 0.008	0.03 ± 0.002
J	0.046 ± 0.012	<DL	0.072 ± 0.008	0.08 ± 0.015
M	0.012 ± 0.000	0.423 ± 0.048	0.098 ± 0.008	0.12 ± 0.018
Powder*	0.035 ± 0.027	0.496 ± 0.046	0.246 ± 0.021	0.20 ± 0.033
Gritha*	<DL	0.098 ± 0.008	0.195 ± 0.016	0.11 ± 0.008
Aawala				
V	0.024 ± 0.003	0.265 ± 0.024	0.056 ± 0.003	0.07 ± 0.010
A	0.030 ± 0.002	0.210 ± 0.0016	0.101 ± 0.014	0.16 ± 0.032
J	0.033 ± 0.006	0.140 ± 0.012	0.095 ± 0.008	0.13 ± 0.021
M	ND	ND	ND	ND
Aswagandha				
V	0.027 ± 0.002	0.030 ± 0.005	0.170 ± 0.013	0.15 ± 0.025
A	0.026 ± 0.003	0.110 ± 0.013	0.058 ± 0.006	0.24 ± 0.026
J	0.012 ± 0.002	0.230 ± 0.034	0.071 ± 0.007	0.06 ± 0.008
M	0.019 ± 0.004	0.260 ± 0.021	0.186 ± 0.01	0.27 ± 0.018
Tablet*	0.008 ± 0.001	0.010 ± 0.003	0.241 ± 0.042	0.28 ± 0.031
Powder*	0.032 ± 0.006	0.410 ± 0.033	.164 ± 0.009	0.09 ± 0.016
Capsule*	< DL	0.060 ± 0.013	0.085 ± 0.013	0.12 ± 0.018
Tulsi				
V	0.012 ± 0.002	0.286 ± 0.029	0.159 ± 0.009	ND
A	0.017 ± 0.003	0.330 ± 0.019	0.120 ± 0.011	0.03 ± 0.000
J	0.016 ± 0.002	0.396 ± 0.032	0.325 ± 0.038	ND
M	0.008 ± 0.001	0.460 ± 0.056	0.094 ± 0.010	ND
Vaasa				
V	0.028 ± 0.005	0.060 ± 0.005	0.045 ± 0.004	0.18 ± 0.035
A	0.021 ± 0.002	0.110 ± 0.001	0.152 ± 0.018	0.22 ± 0.032
J	0.024 ± 0.002	0.096 ± 0.008	0.086 ± 0.016	0.14 ± 0.021
M	ND	ND	ND	ND
Vaasa ristha*	0.008 ± 0.001	0.560 ± 0.042	0.125 ± 0.018	0.34 ± 0.041
Vrihat Vaasavaleh*	0.032 ± 0.004	0.390 ± 0.026	0.428 ± 0.062	0.23 ± 0.034
Bitter Neem				
V	0.001 ± 0.000	0.380 ± 0.000	0.030 ± 0.006	0.23 ± 0.041
A	0.033 ± 0.004	0.440 ± 0.033	0.091 ± 0.006	0.08 ± 0.000
J	0.022 ± 0.003	0.098 ± 0.009	0.050 ± 0.003	0.27 ± 0.035
M	0.006 ± 0.001	0.752 ± 0.082	0.056 ± 0.008	0.31 ± 0.048
Neem tablet*	0.009 ± 0.000	0.438 ± 0.037	0.248 ± 0.032	0.09 ± 0.006
Mithi Neem				
V	0.024 ± 0.003	0.390 ± 0.032	0.044 ± 0.002	ND
A	0.012 ± 0.002	0.540 ± 0.075	0.086 ± 0.003	ND
J	0.003 ± 0.000	0.350 ± 0.071	0.108 ± 0.009	ND
M	0.016 ± 0.002	0.429 ± 0.048	0.156 ± 0.012	ND
Vijaysar				
V	0.010 ± 0.002	0.098 ± 0.008	0.562 ± 0.073	ND
A	0.010 ± 0.002	0.684 ± 0.058	0.538 ± 0.054	0.08 ± 0.012
J	<DL	0.536 ± 0.102	0.502 ± 0.036	0.02 ± 0.007
M	0.060 ± 0.012	0.087 ± 0.007	1.006 ± 0.206	ND

Values are mean ± SD for $n=3$.

V, Varanasi; A, Ahmedabad; J, Jaipur; M, Mumbai; ND, not detected; DL, detection limit. *Commercial sample.

Conclusions

Herbal plants/medicines can be identified clearly by the characteristic peaks of their FTIR spectra. FTIR-FPS

along with PCA analysis was found to be an excellent technique to discriminate different herbal plants/medicines for quality control. The quantitative similarity method was found to be a mathematical, less

Table 5. Comparison of dose converted concentration of Cd in parts per million (ppm) with the recommended daily intake limit.

Herbal medicine	Concentration of Cd (ppm)	Dose converted concentration of Cd (ppm)	Remarks on permissible limit (0.30 ppm)
Arjuna			
V	0.049	0.294	Just equal
A	0.076	0.456	Above
J	0.046	0.276	Slightly below
M	0.012	0.072	Safe

time-consuming and valuable method to compare herbal formulations and crude herbal samples. Heavy metals in most of the examined herbal drugs were well within the permissible limit, except in a few samples of Arjuna. Differences in heavy metal concentration in plant samples from different regions are related to the site of collection, and therefore periodic monitoring of the heavy metal content in genuine as well as market samples seems essential.

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Declaration of interest: The authors report no conflicts of interest.

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