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Compositional characterization of traditional medicinal plants: Chemo-metric approach

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ABSTRACT

Indian literatures mention the use of plants in the treatment of various human ailments. Leaf, flower, fruit, bark root or pltablant as whole been claimed to possess medicinal properties. Though ample literature on therapeutic application of medicinal plants is available but data on the proximate composition of medicinal plants is very scarce. The study was undertaken to investigate the compositions of some common medicinal plants. The medicinal plant samples (seeds/steams/leaves) were analyzed for crude fiber, fat, moisture, protein, acid soluble and total ash content. The energy content of the samples was obtained using Atwater factors. The obtained data were evaluated using multivariate methods: Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA).

Keywords: Multivariate analysis, Ayurvedic Medicinal Plants, Compositional analysis, Principal component, Chemometrics.

INTRODUCTION

Medicinal plants are natural resources, yielding valuable herbal products, which are often used in the treatment of various ailments. The uses of medicinal plants covered under investigation are widely reported in Ayurveda and are used since ancient times, which have therapeutic, preventive and curative properties. These herbs were selected from indigenous folk medicines, Ayurvedic, Unani and Siddha systems of medicines. In traditional methods, the medicinal plants being used, very often are in powder or paste forms of the crude herbs, which contain both the organic and inorganic constituents. Any attempt to incorporate these specific parts of herbal

plants as intakes of food components will be requiring a thorough analysis of energy and nutrient values, along with the specific knowledge of their metabolic actions and active biological components.
In present study, different macronutrient and proximate composition of the specific parts of the herbal plant samples were studied, for the classification and characterization on the basis of compositional characteristics. These medicinal plants were analyzed for the basic proximate composition to get the proximate composition profiles for the classification based on chemo-metric approach.

MATERIALS AND METHODS

Ten different types of medicinal herbs and plants portions were obtain from local market in dried form, which were cleaned, sorted and pulverized. The finely powdered dried parts of herbal medicinal plant were further subjected to proximate analysis.

The samples have been taken for estimation of nitrogen by using a Kjeldahl method. On an average protein contains 16% Nitrogen, therefore the nitrogen content is calculated by using factor 6.25 as in equation (1) to get approximate value of crude protein [1].

$$Crude\ Protein = \%Nitrogen \times 6.25 \quad (1)$$

Moisture analysis was performed using the oven-drying method 950.46 [2]. Fat was estimated using SOXTEC system. Samples were taken in pre-cleaned thimble and extracted in the unit, using hexane as solvent following the standard procedure prescribes in the manual. The final extract and solvent mixture obtain in the pre-weighed beaker is heated for an hour in hot air oven at 80˚ C to obtain the final extract content. This crude fat indicates a mixture of triglycerides, phospholipids, wax esters sterols and related compounds soluble in the solvent system [2]. The ash contents of samples were determined by the ash oven method 920.153 [2], which is the index of inorganic components and trace metals in the samples, where as the acid insoluble portion of the same indicate the silicates in the samples.

Crude fiber, values were obtained by Weinde method [2], which is the sum of plant, substances resistant to hydrolysis by acids and subsequently by alkali. This captures part of lignin, cellulose and hemicellulose. These values are substantially lower than for dietary fiber values which are generally used for food ingredients and components.

The calorific energy value of the medicinal plant samples has been calculated in kcal/joule with the help of Atwater general factors from the composition data [3], which indicates the net metabolizable energy available to the body from ingested foods. If precise energy values for experimental or therapeutic diets are not required, the Atwater general factors provide a good estimate of the energy content of the samples. The Atwater general factors are given as 4, 9 and 4 kcal per gram for dietary protein, lipid and carbohydrate respectively which are the rounded average net energy values to whole numbers of the same. The Available carbohydrate by difference is calculated by subtracting from total carbohydrate the dietary fiber value. However the total carbohydrate by difference is calculated as 100 g food minus the sum of grams of water, protein, fat, alcohol and ash.

Statistical Data Analysis

The statistical data processing was performed by using MINITAB v 13.2, SPSS v 11.0 and Microsoft Excel v 2000 software packages for proximate compositions data set. The Principal component and Hierarchical cluster analysis was applied to analyze proximate composition pattern in medicinal parts of different herbal plants and classify the samples on the obtain patterns of their proximate composition.

Algebraically, principal components are particular linear combinations of the p random variables $X_1, X_2 \ldots, X_p$. Geometrically, these linear combinations represent the selection of a new coordinate system obtained by rotating the original system with $X_1, X_2 \ldots, X_p$ as the coordinate axes. The new axes represent the directions with maximum variability and provide a simpler and more parsimonious description of covariance structure.

$$\begin{aligned} Y_1 &= a'_1 X = a_{11}X_1 + a_{12}X_2 + \ldots\ldots\ldots\ldots + a_{1p}X_p \\ Y_2 &= a'_2 X = a_{21}X_1 + a_{22}X_2 + \ldots\ldots\ldots\ldots + a_{2p}X_p \\ &\vdots \\ Y_p &= a'_p X = a_{p1}X_1 + a_{p2}X_2 + \ldots\ldots\ldots\ldots + a_{pp}X_p \end{aligned} \qquad (2)$$

Proximity between the samples is measured as squared Euclidean (straight-line) distance, which can be calculated for any two p-dimensional observations (samples) $x' = \left(x_1, \ldots, x_p\right)$ and $y' = \left(y_1, \ldots, y_p\right)$ using equation (3).

$$d\ (x, y) = \sum_{i=1}^{p} (x_i - y_i)^2 \qquad (3)$$

Clustering of the samples is done using Ward's method as an amalgamation rule which is based on minimizing the loss of information from joining two clusters [4]. This method is implemented with loss of information taken to be an increase in an error's sum of squares criterion, ESS. Let for given cluster *k*, ESS_k be the sum of squared deviations of every item in the cluster from the cluster mean (centroid). If there are currently *K* clusters, ESS will be defined as the sum of the ESS_k as showed in equation (1.3). At each step in the analysis, the union of every possible pair of clusters is considered and the two clusters whose combination results in the smallest increase in ESS (minimum loss of information) are joined. Initially, each cluster consists of a single item, and if there are *N* items, ESS_k = 0, *k* = 1.2……..*N*, so ESS =0, at the other extreme, when all the clusters are combined in a single group of *N* items, the value of ESS is given by equation (1.4) where X_i is the multivariate measurement associated with the *j*th item and $\bar{X}$ is the mean of all the items.

$$ESS = \sum_{i=1}^{k} ESS_i \qquad (4)$$

$$ESS = \sum_{i=1}^{N} (X_i - \bar{X})^2 \qquad (5)$$

RESULTS AND DISCUSSION

The biological effects of estimated proximate components (Protein, Moisture, Fat, Ash, Fiber, Acid soluble ash and Energy) in living system strongly depend on their concentration. Thus, should be carefully controlled when herbs & medicinal plants are used as food component. Energy and nutrient values of medicinal plant samples are mainly used to translate medicinal samples intakes as intakes of food components.

These data are important for nutritional assessment, researches linking diet and diseases, medicinal plant labeling and consumer education. In nutritional epidemiology, nutrient and energy intakes are used to calculate disease risk [5].

Table 1 Specific plant parts and therapeutic properties of the medicinal plants used under Investigation

Name	Scientific Name	Part	Therapeutic Properties
Jamun	*Syzygium jambolanum*	Seeds	Hypoglycemic, anti-bacterial and anti-fungal activity [8]
Aonla	*Emblica officinal*	Fruit	Hypolipidemic and hypoglycemic [9,10]
Tulsi	*Ocimum sanctum*	Seeds	Anti-inflammatory, anti-pyretic, analgesic and anti-arthritic activities [11, 12]
Kutki	*Picrorhiza kurroa*	Steam	Immunostimulant activity and hepatoprotective [13, 14]
Harda	*Terminalia chebula*	Fruit	Homeostatic, anti-tussive, laxative, diuretic and cardiotonic activities [15, 16]
Baheda	*Terminalia bellirica*	Fruit	Hepatoprotective and hypocholesterolemic [17, 18]
Gudmar	*Gymnema sylvestre*	Leaves	Against glycosuria and other urinary disorders [17, 19]
Babool	*Acacia arabica*	Pods	Anti-diabetic [20]
Amrita/Giloe	*Tinospora cordifolia*	Steam	Treatment of jaundice, diabetes, skin diseases and anaemia [21]
Kalmegh	*Andrographis paniculata*	Leaves	Anti-pyretic, detoxicant, anti-inflammatory, and analgesic agent for infections of the gastrointestinal tract, respiratory organs and urinary system [22, 23]

Table 2 Proximate composition and energy values of specific part of medicinal plants

Sample	Crude Protein (%)	Moisture (%)	Crude Fat (%)	Total Ash (%)	Crude Fiber (%)	Acid Insoluble Ash (%)	Energy (Kcal/g)
Jamun	2.42±0.44	9.34±1.99	0.92±0.52	2.93±0.82	6.08±1.11	0.53±0.06	3.31±0.16
Aonla	1.11±0.20	9.46±2.65	0.16±0.11	3.74±0.45	42.75±2.30	0.91±0.10	1.77±0.22
Tulsi	1.28±0.16	6.72±0.62	9.03±0.60	7.97±0.65	10.48±1.20	0.70±0.13	3.44±0.13
Kutki	0.56±0.12	10.81±2.66	1.40±0.42	3.73±0.51	9.10±0.75	0.05±0.01	3.18±0.12
Harda	0.62±0.12	8.74±0.83	1.16±0.39	2.39±0.54	33.79±1.47	0.38±0.08	2.12±0.06
Baheda	0.69±0.16	10.76±2.04	2.63±0.46	5.88±0.45	23.42±1.35	1.42±0.39	2.60±0.11
Gudmar	0.65±0.18	11.77±1.93	2.49±1.28	11.38±2.13	15.51±1.14	3.05±0.18	2.81±0.09
Babool	0.81±0.24	10.67±1.73	0.88±0.15	5.55±0.69	17.94±2.51	0.76±0.09	2.62±0.21
Amrita	1.43±0.23	9.51±0.91	1.32±0.57	6.87±1.18	23.45±1.19	1.51±0.20	2.39±0.13
Kalmegh	0.49±0.07	10.05±1.07	3.72±1.08	9.00±1.02	13.05±1.01	1.50±0.23	3.11±0.10

**Above values are wet percent with means ±SD*

The proximate concentration of ten medicinal samples (Table 1) are expressed on wet basis, are listed in Table 2. Each result is the mean value of at least three measurements. The relative standard deviations were in the range from 0.01 to 2.65, but majority of data have standard deviation below unity, confirming good reproducibility of the applied method. All medicinal plants sample showed higher content of fiber and very less protein and fat content (Table 2).

To study macronutrient patterns as well as different plant sample grouping, a Chemometrics approach was used. In first step of the statistic evaluation, using Shapiro-Wilk's test (significance level α was 0.05), it was found that the data set (Table 2) deviates from the normal distribution in contrast, the log-transformed data that are normally distributed [6]. The data matrix was also carefully observed by different descriptive statistics detecting no outliers.

The correlation matrix of seven proximate components is shown in (Table 3). More than 70 % of the correlation coefficients in the matrix are over 0.22.The energy content has very significant positive and negative correlation with fat and fiber content, while acid insoluble ash is positively correlated with total ash. The data set of the concentration measurements was subjected to principal component analysis (PCA), which removes the highly inter-correlated nature of variations in the macronutrient concentrations. The initial statistics of Eigen analysis is given in Table 4. It can be seen that three principal components (PCs) appeared to account for 87.41% of the total variance in the data. According to Kaiser Criterion only the first three PCs were retained because subsequent eigen values are all less than one [7]. Hence, reduced dimensionality of descriptor space is three.

Table 3 Correlation* Matrix of Proximate Composition Data

Variables	Protein	Moisture	Fat	Fiber	Total Ash	Acid Insoluble Ash	Energy
Protein	1.000						
Moisture	-0.402	1.000					
Fat	-0.039	-0.587	1.000				
Fiber	-0.236	-0.053	-0.409	1.000			
TAsh	-0.298	0.226	0.508	-0.329	1.000		
Acid Insoluble Ash	-0.210	0.476	0.072	0.006	0.832	1.000	
Energy	0.224	-0.131	0.588	-0.965	0.322	-0.073	1.000

**Pearson correlation*

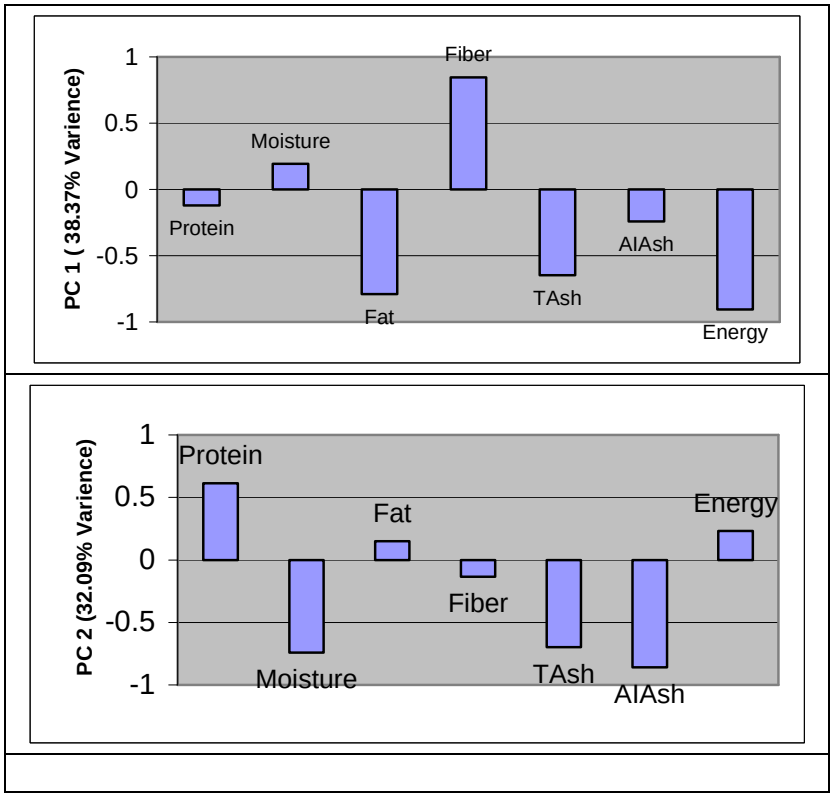
Table 4 Eigen analysis of principal components

Principal Component	Eigen values	Cumulative
PC1	2.686	38.370
PC2	2.246	32.090
PC3	1.187	16.960
PC4	0.808	11.540
PC5	0.046	0.650
PC6	0.021	0.300
PC7	0.006	0.090

One main objective of PCA is to identify factors that are substantively meaningful. The loading plots as shown in figure 1, for the three principal components. It can be seen that the first component explaining 38.37 % of variance is highly positively and negatively correlated with

fiber and energy respectively. Thus it classifies and distinguishes the score of medicinal plants on the basis of energy content. The second principal component explaining 32.09 % of total variance is highly correlated with acid insoluble ash. This component represents the average effect of both the ashes and fat content in medicinal plants, while the third component which explains only 16.96 % of total variance is positively correlated with moisture and negatively with fat and fiber thus explains contrast between the medicinal samples on the basis of fiber and moisture content.

Figure 2 illustrates the biplot of scores and loadings of the three principal components of specific parts of herbal medicinal plats, which further analyzed their positions according to their proximate composition with respected to the extracted principal components. The Biplot of PC1 and PC2 shows the separation of the medicinal parts of herbal plants according to their respective scores. First quadrant of the plot contains samples having positive PC1 and PC2 scores, where, less ash content clearly represented in Harda (Fig. 2). Second quadrant is expressing samples with negative PC1 & positive PC2 scores thus higher protein, fat and energy. Similarly the third quadrant contains samples with negative PC1 & PC2 scores, which comprises of Kalmegh and Gudmar samples, which are loaded heavily with ashes. Positive PC1 and negative PC2 scores sample are placed in fourth quadrant and having loaded with moisture and fiber. As PC1 and PC2 both contain about 70.46 % of the total variance and it can be seen that PC1 separates the samples on fiber contents and PC2 chiefly on moisture and ashes in composition. The PC2, PC3 and PC1, PC3 Biplots show that Kalmegh and Gudmar are loaded with acid insoluble ash, Baheda, Amrita, Harda and Aonla are loaded with fiber and Kalmegh and Tulsi are loaded with ashes and fat mainly.



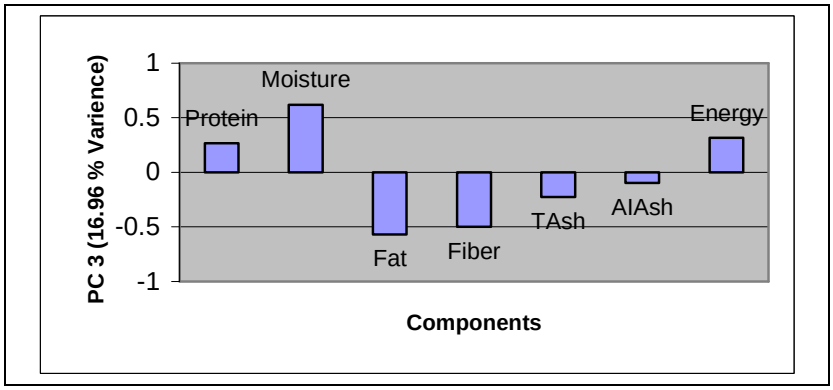
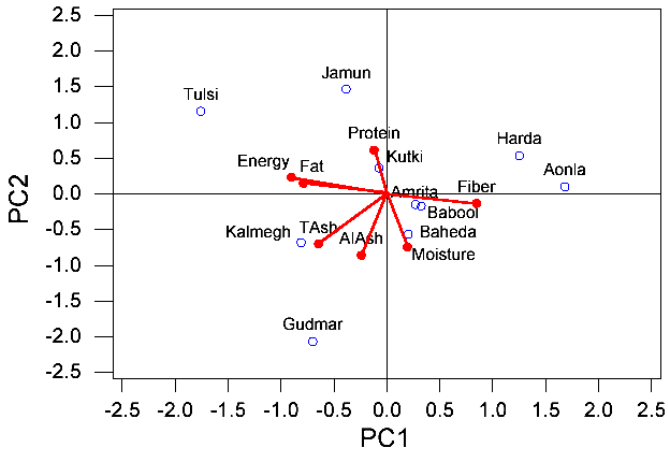
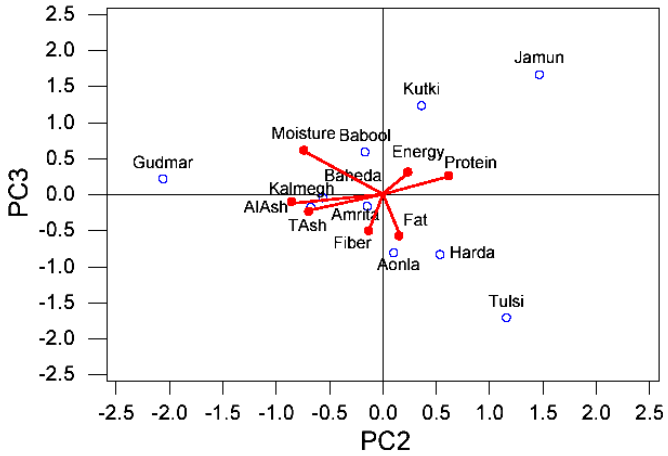


Fig. 1 Loadings of components on principal axes





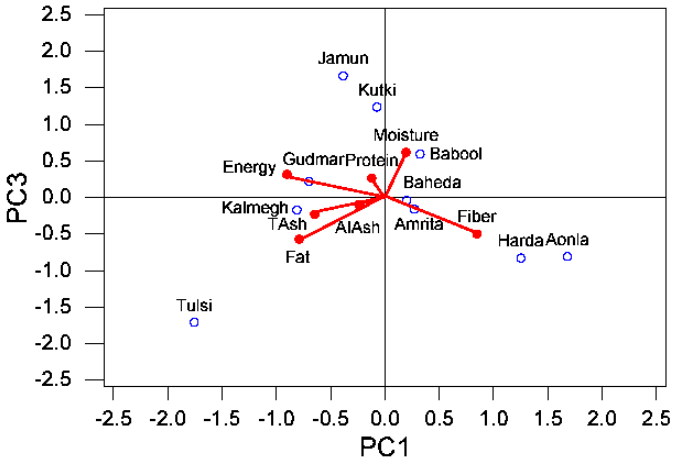


Fig. 2 Biplot of loadings and scores for specific parts of medicinal plants, projection on principal axes 1, 2 and 3

A graphical depiction of different parts of different herbal plant groupings was obtained by means of hierarchical cluster analysis (HCA) of standardized compositions using Ward's method as an amalgamation rule and squared Euclidean distances as the measure of proximity between samples [4]. A dendo-gram is shown in Fig. 3. As a result of applying HCA to the principle component score matrix, the parts of medicinal plants split to two main groups at very low similarity, but at considerable positive level of similarity they were classified into four main groups. Kutki was been isolated due to its exceptionally very less acid insoluble ash component and high energy, loading of moisture levels are low on PC1, PC2 & high PC3 scores.

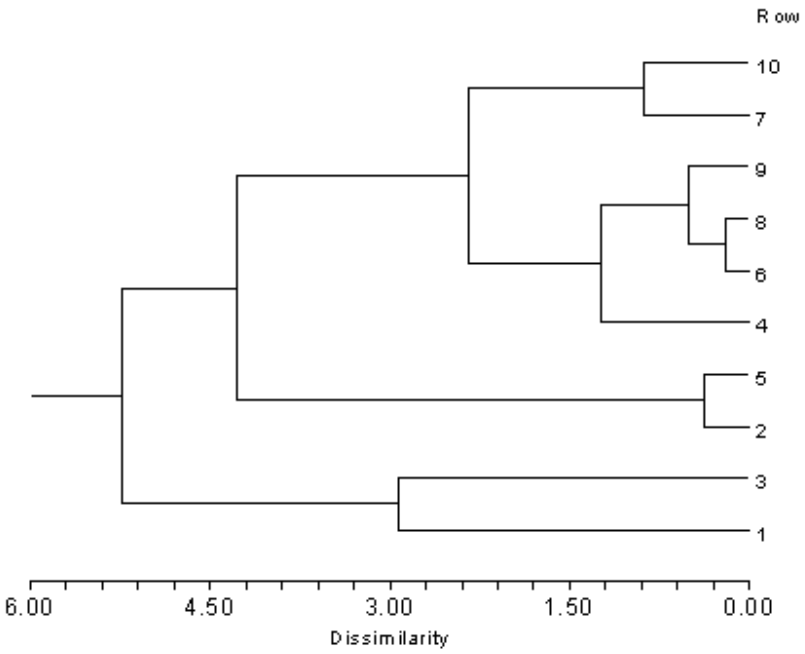


Fig. 3 Dendogram of Cluster Analysis for specific parts of medicinal plants

Jamun and Tulsi get clustered as having similar high energy, protein levels. Aonla and Harda having low energy and high fiber content provide them very high PC1 scores and separates from the rest of the parts of herbal medicinal plants. Finally the fourth group which further embedded two clusters of Gudmar, Kalmegh and Baheda , Babool with clear separation of Amrita form former cluster & its similarity towards later due to similar energy, fiber levels with former but different protein and moisture levels. While former cluster of Gudmar and Kalmegh is having same high amount of both ash (total and acid insoluble) components.

Thus the above characterizations of medicinal parts of herbal plants were obtained on the basis of dissimilarity in scores with respect to extracted principal components, which contain more than 87 % of total variance generated by the proximate composition data constituting moisture, fat, protein, total ash, acid insoluble ash, crude fiber and energy of these medicinal herbal parts of plant.

CONCLUSION

Different parts of medicinal plants were analyzed in order to get some useful information to be used in the preparation of therapeutic, sanative and nutraceutical foods. As there are no major reports in literature on detailed proximate composition and energy values of medicinal plants parts, this paper should be considered as a contribution to that course, being, far from the knowledge for the formation of active constituents from these medicinal parts of herbal plants.

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