Laser Induced Breakdown Spectroscopy (LIBS) for Minerals Analysis and for Monitoring the Change in Elemental Compositions of the Mixtures of Herbal Medicines

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Abstract
In this research nutrient elements of ten Ethiopian herbal medicines promoting health advancing effect and practiced in local medicine were determined using LIBS. The LIBS analysis revealed the presence of many nutrient elements such as copper, zinc, carbon, manganese, phosphorous, calcium, magnesium, iron, sodium and potassium. In our study we have also tried to correlate the elemental compositions in the plants with their biological effects then, by using principal component analysis (PCA) we have characterized the herbal medicines with similar elemental compositions. Finally we have demonstrated the capability of LIBS to monitor the change in elemental compositions of the mixtures of herbal medicines.
Keywords: herbal medicines, mineral elements analysis, PCA, LIBS

1. Introduction.

Due to poverty and limited access to modern medicine, about four billion people, 80% of the world's population living in developing countries use herbal medicine as their source of primary health care (Bisset, **1994**; Farnsworth, **1985**; Mukherjee, **2002**; Bodeker, **2005**). In these communities, traditional medical practice is often viewed as an integral part of their culture.

In most countries herbal products are launched into the market without proper scientific evaluation, and without any mandatory safety and toxicological studies. There is no effective machinery to regulate manufacturing practices and quality standards. Consumers can buy herbal products without a prescription and one might not recognize the potential hazards in an inferior product. A well-defined and stable composition of the drug is therefore one of the most important prerequisites for the production of a quality drug (Bauer and Titte, **1996**; Baur, **1998**).

Most studies on such medicinal plants pertain to their organic contents like essential oils, glycosides, vitamins, alkaloids and other active components and their pharmacological and therapeutic effects. Besides several organic compounds, it is now well established that many trace elements play a vital role in general well-being as well as in the cure of diseases (Underwood, 1977; Prasad, 1993).

Many elements in trace amount in human body play an essential role in metabolic process (Dell and Sunde, 1997). Therefore, identification of elements in medicinal plant and food products are vital. Minerals are involved in structural components of human tissues, resources of acid-base balance and maintain the body fluids, transport of gases and muscle contractions (Omaye and Reddy, 1962).

Mineral deficiencies have manifested in forms of different disease conditions as goiter, rickets, and one form of metabolic dysfunction or the other. Minerals are divided into two groups: major minerals and trace minerals. The body needs larger amounts of major minerals than trace minerals, although trace minerals can be just as important for good health (Frank W. Cawood, 1997; Chaney, 2006; Crook, 2006).The major minerals include calcium, chloride, phosphorus, potassium, sodium, sulphur, and magnesium, while the trace minerals include iodine, iron, zinc, selenium, fluoride, chromium, copper, molybdenum, and manganese (Frank W. Cawood, 1997; Chaney, 2006; Crook, 2006).

Mineral contents of various medicinal plants were evaluated and correlated with their therapeutic action by numerous workers (Sahito, 2003; Pirzada, 2005; Januja, 1990; Sailey, 1994; Singh AK, 2011). Atomic absorption spectroscopy (AAS) (Herber and Stoeppler, 1994), energy dispersive X-ray fluorescence spectrometry (EDXRF) (Joseph, 1999), electro thermal atomic absorption spectrometry (ETAAS) (Scancar, 2000), inductively coupled plasma-atomic emission spectrometry (ICPAES), inductively coupled plasma mass spectroscopy(ICPMS) (Chan and Lo, 2003) are some of the techniques employed for the elemental analysis of medicinal plants.

In cases of ETAAS, ICPAES and ICPMS analytical methods medicinal plant samples require to be appropriately prepared before measurements by the digestion and the mineralization of their organic matrix.

Laser induced breakdown spectroscopy (LIBS) is an emerging spectroscopic technique that operates with high-power laser pulses focused onto a small spot of the sample material. The interaction of the pulsed laser beam with the target sample produces high temperature ionized plasma, containing excited elements that radiate the characteristic emission lines of the corresponding elements. LIBS has the capability of multi-elemental analysis of any type of material present in any phase (solid, liquid and gas) with minimal sample preparation, real-time, in situ, remote detection, and with the capability of non-destructive determination of elemental composition (De Lucia2003; Pandhija.S 2009).

Principal Component Analysis (PCA) has been called one of the most valuable results from applied linear algebra. It is a useful statistical technique that has found application in fields such as face recognition, image compression, neuroscience, computer graphics and is also a common technique for finding patterns in data of high dimension. It is a way of expressing the data in such a way as to highlight their similarities and differences. Since patterns in data can be hard to find in data of high dimension, where the luxury of graphical representation is not available, PCA is a powerful tool for analyzing data enabling effective visualization, regression and classification of multivariate data (I.T. Jolliffe 2002 22).

In PCA we arrange data in a matrix, for example, with samples arranged as a row and the spectra of the elements arranged as columns from which we will find the covariance matrix. Covariance provides a measure of the strength of the correlation between two or more sets of random variables. A large (small) value indicates high (low) redundancy. It captures the correlations between all possible pairs of measurements. The correlation values reflect the noise and redundancy in our measurements. The diagonal terms, by assumption, large (small) values correspond to interesting dynamics (or noise).The off-diagonal terms large (small) values correspond to high (low) redundancy. The eigenvectors and eigenvalues of the covariance matrix provide us with information about the patterns in the data. By this process of taking the eigenvectors of the covariance matrix, we have been able to extract the most important lines that characterize the sample.The eigenvector with the highest eigenvalue is the first principal component of the data set. We can decide to ignore the components of lesser significance, without losing much information of the original data. Here is where the notion of data compression and reduced dimensionality comes into PCA. Finally we project the input data onto the main principal components, which forms the representation of our data (Parinya Sanguansat 2012).

2. Experimental methods

2.1 LIBS set up

In the experimental configuration (shown in figure 1) we use a standard Nd:YAG laser (Brilliant, Quantel) to generate the plasma. It was running in its second harmonic at 532 nm, at a repetition rate of 2 Hz. Individual laser pulses had a pulse length of 4 ns. The pulse energy used was 30 mJ. A mirror is used to direct the laser beam onto the target surface. It is inclined at an angle of 45° to the direction of incident laser beam. The laser light was focused by a lens of 10 cm focal length. The distance between the plasma plume and the entrance slit of the monochromator (a JobinYvon THR 1500 monochromator equipped with a 1200 grooves mm^{-1} grating) was 40 cm. Light from the plasma was collected by a lens of 10 cm focal length placed at the same distance from the plasma plume and the entrance slit of the monochromator, under this condition the image magnification was 1:1. An ICCD (Intensified Charge Coupled Device) detector (Andor Technology, model DH520- 25F-03) was employed for spectral acquisition. Synchronization between the laser and the ICCD detector was ensured by microcomputer (Lab PC) via a pulse delay generator (Model DG 535, Stanford Research Systems, Inc). The microcomputer was equipped with software for data acquisition and plotting spectra. The selected spectrum was processed by averaging the signal over twenty successive laser shots. Due to this, we had a good signal-to-noise ratio. Then it was verified that the plasma was reproducible by recording the same average spectrum several times. The spectra were recorded with a delay of 1 µs after the laser pulse and a gate width of 10 µs.

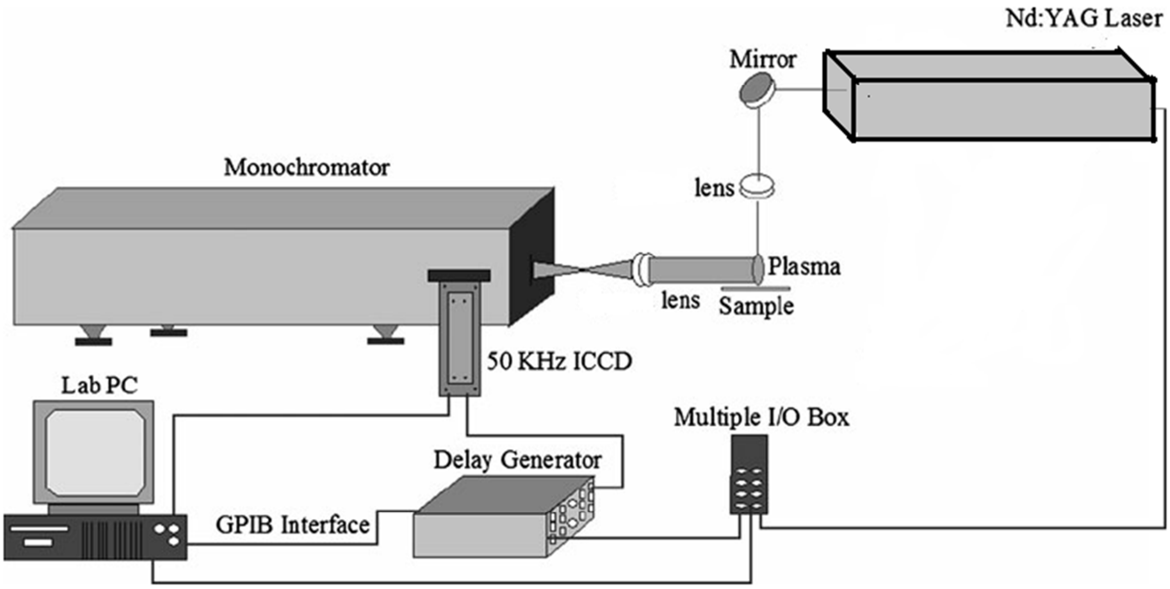

Figure 1: LIBS set up used at the LSAMA laboratory

2.2 Sample preparation and analysis

Ten herbal medicines purchased from local market places and authenticated by the Ethiopian bio-diversity institute were used as samples for analysis. The plant samples were made up of the leaves, stem and roots. The samples were gently and thoroughly washed with distilled water to avoid contamination. They were then dried at room temperatures in the range of 20°-26°C and then grounded into fine powder. About 300 mg of each sample were pelletized using a hydraulic press with a pressure around 2 tons/cm^2 to produce an intermediate thick pellet samples. We also prepared two samples by mixing two types of herbal medicines in equal amounts 150g each to see the capability of LIBS for monitoring the change in elemental composition of mixtures. For each sample three pellets were prepared to check the repeatability of the experiments. The preparation of the powders into pellets was necessary to have better ablation efficiency and higher repeatability of LIBS measurements. The names of the samples are shown in the table below.

No	scientific name	Local name
Sample 1	*Ocimum lamifolium*	Demakese
Sample 2	*Zingiber officinale*	Zingibil
Sample 3	*Moringa stenopetala packed*	Shiferaw packed
Sample 4	*Echinops kebericho*	Kebercho
Sample 5	*Foeniculum vulgare*	Ensilal
Sample 6	*Artemisia afra*	Ariti
Sample 7	*Hagenin abyssinica*	Fieto
Sample 8	*Moringa stenopetala natural*	Shiferaw natural
Sample 9	*Coriandrum sativum*	Dinbelal
Sample 10	*Ruta chalepensis*	Tenadam
Sample 11	Mixture of *Ocimum lamifolium* and *Foeniculum vulgare*	mixture of Demakese and Ensilal
Sample 12	Mixture of *coriandrum sativum* and *zingiber officinale*	Mixture of Dinbelal and Zingibil

Table 1: The different samples studied with the chosen nomenclature

3. Results and discussions

3.1 Comparison and correlation of mineral elements in herbal medicines

The LIBS spectrum of herbal medicines samples were collected in wavelength range 200-800 nm in air atmosphere. Spectra acquired by LIBS were processed using Igor software. A typical spectrum of sample 3 in the range of 424-436 nm is displayed in figure 2. The assignments of elements in the spectrum were done with consultation of national institute of standards and technology (NIST) atomic data.

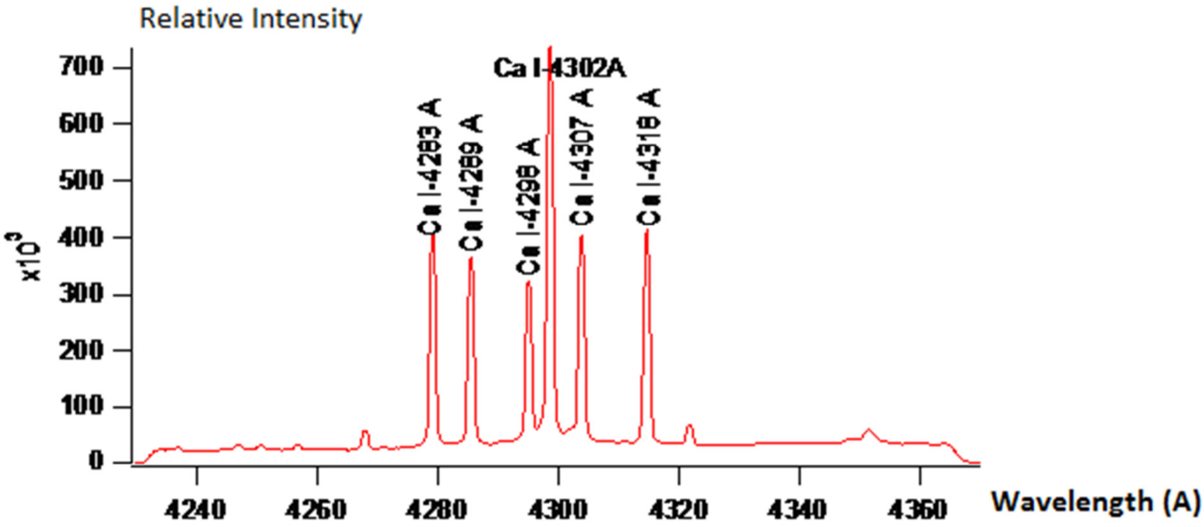

Figure 2: LIBS spectra of moringa sample in the spectral range of 424-436 nm.

The comparisons of mineral elements in terms of relative intensities of the emission lines are shown in figure 3 using histogram. From the histogram we can observe that the amounts of mineral elements were found to vary in different herbal medicines. It is also observed that in the herbal medicine samples the amount of calcium, magnesium and sodium are relatively higher than the amount of iron, potassium, manganese and phosphorus. Moreover in the samples we have identified zinc and copper but in small amount.

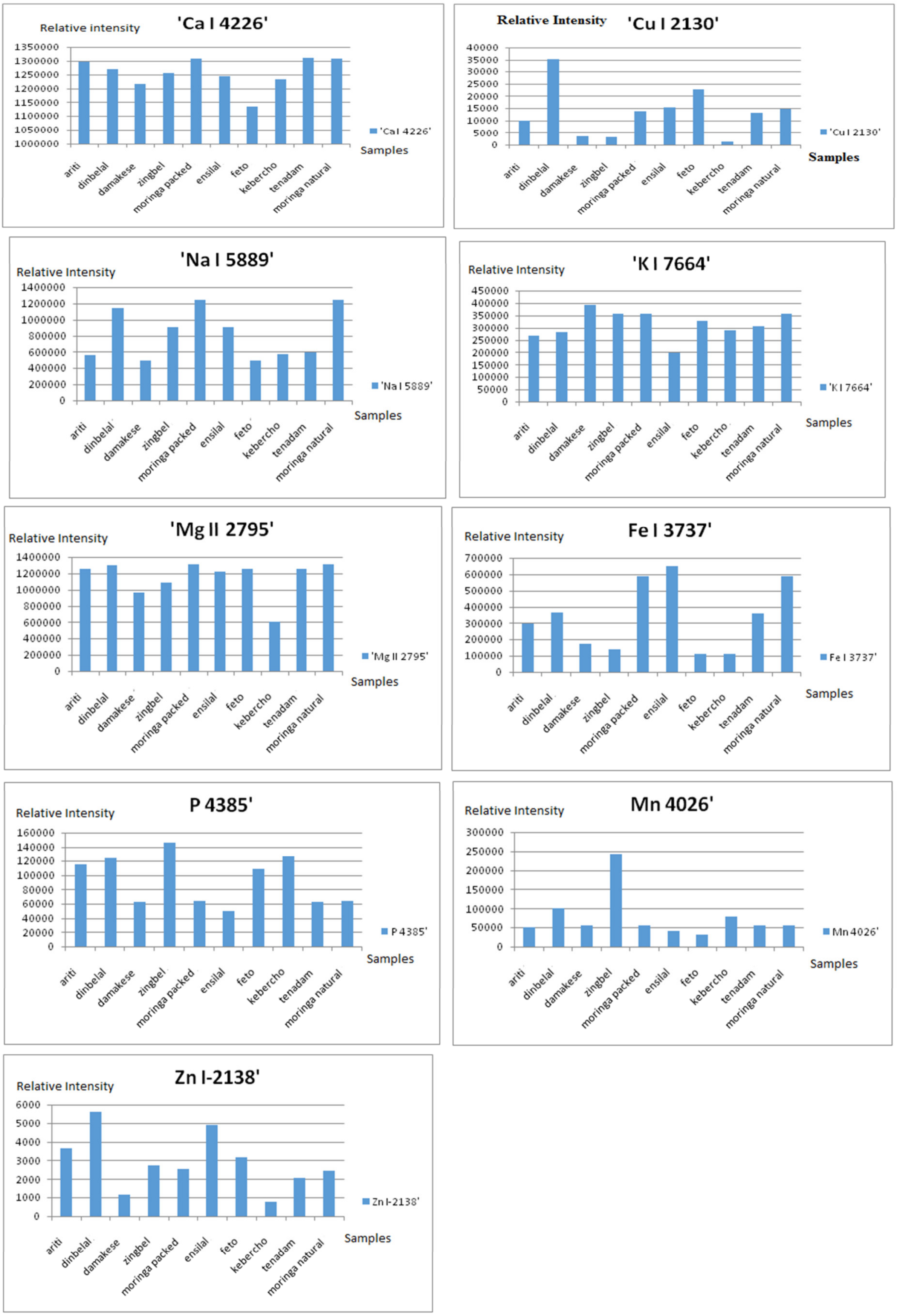

Figure 3 comparisons of mineral elements in term of relative intensity of the emission lines

Calcium is essential for the formation of strong bones and teeth and for the maintenance of healthy gums. It increases the rate of bone growth and prevents against bone loss associated with osteoporosis. Calcium is important in the maintenance of a regular heart beat and transmission of nerve impulses. It helps lower cholesterol levels and helps prevent against cardiovascular disease and certain forms of cancer including colorectal cancer. Calcium is important for normal blood clotting processes that aid in the early stages of wound healing. In addition, calcium also wards off the accumulation of an excess of acid or alkali in the blood. It is involved in the activation of several enzymes including lipase, which breaks down fats for utilization by the body (Balch, J.F. and P.A, 1997 Barney, 1998; Dunne, 1990).

Magnesium is an essential nutrient required for many biologic functions in the body, including more than 300 enzyme reactions. It also functions in the activation of amino acids, the syntheses of DNA, and is involved in neurotransmission and immune function. Numerous studies show that a magnesium deficiency may be an underlying cause of cardiovascular disease, hypertension, asthma, chronic fatigue and pain syndromes, depression, insomnia, irritable bowel syndrome, and many pulmonary disorders. Supplementing the diet with magnesium may also prevent depression, dizziness, muscle weakness, twitching, and premenstrual syndrome (PMS). (Fischer P., Kubena K. 2006; Balch, J.F. and P.A, 1997; Dunne, 1990).

Sodium is essential for maintaining blood pH and proper water balance. Together with potassium, it helps regulate the distribution of fluids on either side of the cell walls. Sodium and potassium are also intricately involved in muscle contraction and expansion as well as nerve stimulation. During intense exercise or extreme heat, sodium activates the thirst response. It keeps the other blood minerals soluble so that a buildup of other minerals will not accumulate in the blood stream. Sodium also acts with chlorine to improve blood and lymph health and aids in eliminating carbon dioxide from the body (Balch, J.F. and P.A, 1997).

Iron is involved in the production of hemoglobin and myoglobin. Hemoglobin carries oxygen from the lungs to the body. Iron is essential for many enzymes and is important for growth, proper cognitive function. Iron is vital in energy production and in maintaining an optimal immune system (Hunt, 2006).

Potassium is a key for a healthy nervous system, regular heart rhythm, and proper muscle function. It is necessary for chemical reactions within the cells and helps in maintaining normal blood pressure and in generating electrochemical impulses. In persons with hypertension, potassium can dramatically lower both systolic and diastolic pressure. It functions in cell metabolism, enzyme reactions and the synthesis of muscle protein from amino acids in the blood. It works with phosphorous to send oxygen to the brain and functions with calcium in regulating neuromuscular activity. Potassium will also stimulate the kidneys to eliminate poisonous wastes. It is also necessary for healthy skin (Balch, J.F. and P.A, 1997; Dunne, 1990).

Manganese is a component of several enzymes and, therefore, acts as a catalyst in the synthesis of cholesterol and fatty acids, and plays a role in protein, fat, and carbohydrate production. It activates a number of other enzymes including formation of cartilage in the bone and skin. Manganese is important for the production of milk, formation of urea, or part of the urine. It also maintains sex - hormone production, nourishes the nerves and brain, and is essential for the formation of thyroxin, an important component of the thyroid gland (Dunne, 1990; Montvale, 2001).

Phosphorous is needed for proper bone and tooth formation, cell growth and contraction of the heart muscle; it assists in the assimilation of vitamins and the conversion of food into energy. It also works with calcium to maintain the calcium - phosphorous balance in the bones. Deficiency of phosphorous can cause lack of appetite and weight loss (Dunne, 1990 Nielsen and Dunn, 2006).

Zinc has a variety of functions in the body. It is a component of at least 25 enzymes involved in digestion and metabolism, including carbohydrate digestion, and phosphorous metabolism. Zinc is essential for general growth and proper development of the reproductive organs and prostate gland function. It also may help prevent acne and control the activity of oil glands. It aids in the synthesis of protein and collagen formation, promotes a healthy immune system, aids in wound healing and allows for enhanced vision, taste and smell. Zinc is a component of insulin and many vital enzymes. It will fight and prevent against the formation of free radicals. Zinc also increases the absorption of vitamin A (Dunne, 1990; Balch, J.F. and P.A, 1997; Montvale, 2001; Cousins).

Copper aids in the formation of bone, hemoglobin, red blood cells. It aids in the conversion and transport of iron from the intestinal lumen into red blood cells. It works in balance with zinc and vitamin C to form elastin. It is involved in the healing process, energy production, hair and skin coloring, and taste sensitivity. It is involved in the development and maintenance of the cardiovascular system. It also helps maintain the myelin, which sheaths nerves and aids in the transmission signals from the brain to the body and vice versa (Balch, J.F. and P.A, 1997).

3.2 Clustering of the herbal medicines using PCA

Moringa leaves are known to have a high content of protein, minerals and vitamin, hence an ideal nutritional supplement, (Fletcher, 1998). Moringa leaves are also considered a rich source of minerals (Gupta et al, 1989). In this work we have tried to characterize the herbal medicine samples by using principal component analysis as shown in figure 4. From the figure it is clearly observed that *Foeniculum vulgare* (Ensilal), Ruta chalepensis

(Tenadam), *Coriandrum sativum* (Dinbelal), *Artemisia afra* (Ariti) and *Hagenin abyssinica* (Fieto) may have similar elemental composition as moringa because they are clustered together with moringa.

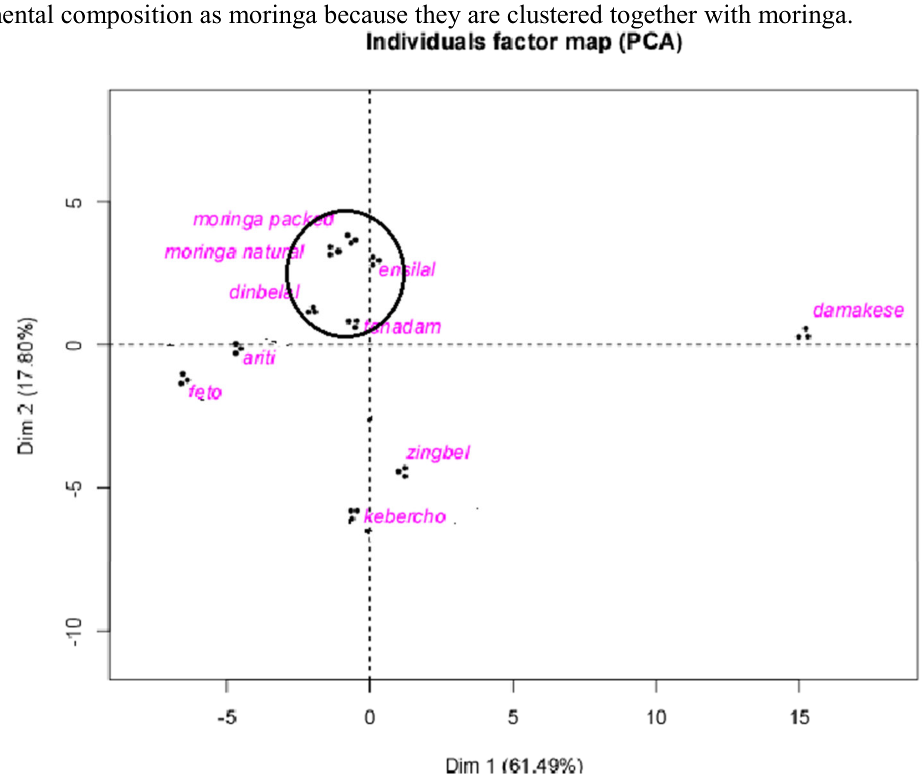

Figure 4: Clustering of the herbal medicines using PCA

3.3 LIBS used to monitor the change in elemental compositions of the mixed herbal medicines.

A well-defined and stable composition of the herbal medicines is most important prerequisites for the production of a mixture of herbal medicines. In our researches we have demonstrated that LIBS can be used to monitor the change in elemental compositions of the mixture of herbal medicines.

We prepared samples by mixing two types of herbal medicines in equal amount. Sample11 is the mixture of *Ocimum lamifolium* (Demakese) and *Foeniculum vulgare* (Ensilal). *Ocimum lamifolium* (Demakese) is relatively rich in potassium and foeniculum *vulgare* (Ensilal) is relatively rich in zinc. Sample 12 is the mixture of *coriandrum sativum* (Dinbelal) and *zingiber officinale* (Zingibil). *Coriandrum sativum* (Dinbelal) is relatively rich in copper and *zingiber officinale* (Zingibil) is relatively rich in manganese. We analyzed the mixtures by using LIBS and we have displayed the results by using histograms in figure 5 and figure 6

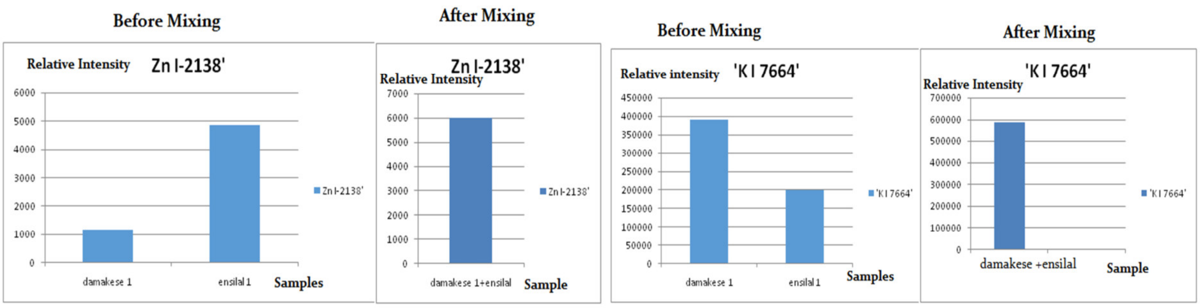

Figure 5: change of Zn and K of sample11 in terms of relative intensity of the emission lines

As we observe from figure 5 for sample 11 the increase in zinc (Zn) using the relative intensity unit is 22.84% compared to the amount of zinc in Ensilal before the mixing and the increase in potassium (K) using the relative intensity unit is 49.88% compared to the amount of potassium in Damakase before mixing.

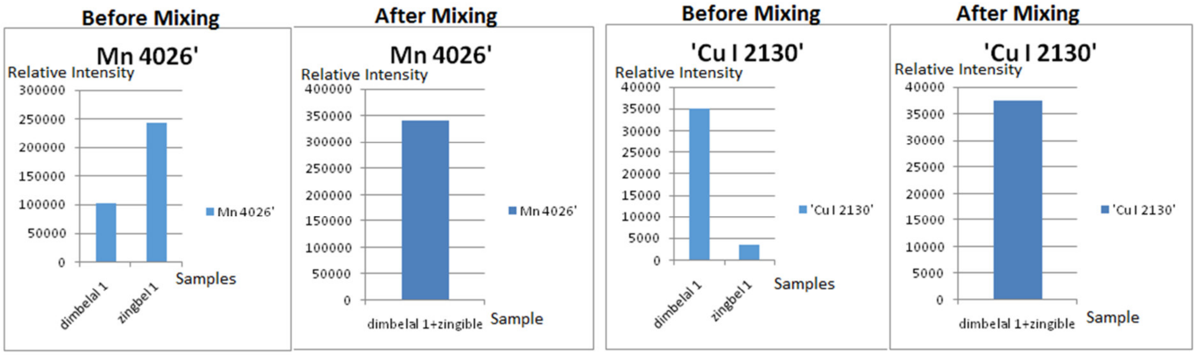

Figure 6: change of Mn and Cu of sample 12 in terms of relative intensity of the emission lines

Figure 6 shows result for sample 12, the increase in manganese (Mn) using the relative intensity unit is 40.31% compared to the amount of manganese in Zingbel before the mixing and the increase in copper (Cu) using the relative intensity unit is 6.5% compared to the amount of copper in dinbelal before mixing. This may show the capability of LIBS in monitoring the change in elemental composition of the mixture of herbal medicines.

Conclusion

In our researches we have demonstrated the potential of LIBS in elemental analysis, characterization and monitoring the change in elemental composition of herbal medicines. We have pointed out that the medicinal plants studied are a source of biologically important elements, which may play part in the observed therapeutic properties of these plants. The classification model obtained by PCA may be of great use for quality inspection of raw herbal material on a continuous basis as new product is produced. We have also shown the capability of LIBS to monitor the change in elemental compositions of the mixtures of herbal medicines. This study can be of help in deciding the quantity of various active constituents and also supervising the dose of a particular formulation since dosage has been a chief problem confronting traditional herbalists. The findings of this study can thus assist herbal medicine practitioners in their efforts to include these plants into a variety of formulations based on their mineral composition.

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