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Heavy Metal Detection in Medicinal Herbs Using Laser-Induced Breakdown Spectroscopy

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Abstract: Medicinal plants are the source of medication that have been widely used in Malaysia. The industrial revolution caused environmental pollution and contaminated the medicinal herbs with toxic metals. Healthy human body required essential elements to maintain diverse physiological and biochemical activities in the body. Laser-induced breakdown spectroscopy (LIBS) a powerful elemental analysis technique was used in this study. The single pulse, ungated LIBS system using Nd:YAG laser of 1064 nm and HR4000 spectrometer were used in this investigation to analyze the mineral composition in medicinal herbs spectroscopically. The herbs in the research are Peppermint (Menthapiperita L), and Aloe Vera (Aloe Barbadensis miller). Although these herbs are commercially utilized, the overuse of pesticides and herbicides cause heavy metals pollution in soil and gradually accumulated in plants. The spectral lines obtained from the herbs are used to identify the heavy metal elements. The LIBS elucidate the presence of essential elements metal like Al, C, Ca, Cu, Fe, K, Mg, Na, Si and Zn in herbs. The results obtained have detected the presence of toxic heavy metals such as Cd and Pb in peppermint, Co and Ni in aloe vera on overuse of pesticides and fertilizers as well as other contamination. The percentage of essential element K in peppermint is 6.93% significantly higher than in aloe vera with only 1.57%. LIBS is a helpful tool in analyzing the chemical composition of medicinal plants for quality control and monitoring purposes. Dispersive X-Ray

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Analysis (EDX) is performed to validate the presence of heavy metals identified in LIBS spectra of the samples.

Keywords: Energy Dispersive X-Ray Spectroscopy (EDX), Laser Induced Breakdown Spectroscopy (LIBS)

1. Introduction

Plants absorb the elements from soil, some of which are essential for biological function, and some are known to be toxic heavy metals at a trace amount. The study is based on detecting the presence of heavy element in medicinal herbs using LIBS technique. The herbs selected as samples are Peppermint and Aloe Vera. They are commercially utilized and easily obtained in Malaysia [8]. The LIBS method was employed to discover the heavy element contamination in medicinal plants locally and commonly used for treating various ailments, prevent and manage heart disease, cancer and diabetes. It may also help to regulate our body enzymatic and provide anti-inflammatory [12]. The environmental pollution caused the contamination in soil that leads to the bioaccumulation of heavy elements in plants [7]. Besides, the overuse of pesticides and herbicides in the plantation cause the heavy element to seep into the soil and be taken up by plants [7]. The overconsumption of heavy element contaminated herbs by patients lead to heart attack, high blood pressure, and liver damage. Hence, the monitoring of elemental content in herbs is very important with the use of LIBS technique which is rapid and easy to use for in-situ analysis. In this projects, spectral data from leaves of medicinal herbs by using LIBS will be recorded and analyzed with the aid of ASD-NIST.

2. Materials and Methods

The materials and methods section includes all the necessary details about the data collection strategies and experimental procedures performed in this research project.

2.1 Sample Preparation

Two types of medicinal leaves of herbs have been prepared as samples for the experiment in this study. The samples were bought from the supermarket in Pagoh where the crops were supplied by the farm from Bukit Gambir. There are:

i) Peppermint
ii) Aloe Vera

Firstly, the fresh leaves were plucked and collected from the farm, before it wash with distilled water and dry under the sun. Next, the samples were crushed using an electric rotor mill. The rough powder were grinded the with a mortar. Grinded sample powder were sieve with a 100 μm siever to obtain finer powder. Few grams of each sample powder were inserted into Pressing Die Sleeve with a spatula. The hydraulic press was used to compress the sample with pressure of 5000 Ib/in2 or 3.44 MPa and maintain this pressure for 5 minutes. Lastly, the resulting pellets have a thickness of approximately 2.0 mm for Aloe Vera and 3.0 mm for peppermint pellets.

2.2 LIBS Instrumentation

The plasma is formed on the sample in situ when the laser beam is focused on the sample piece. LIBS instrumentation consists of principal parts such as the source of laser, converging lens, Z-translation stage, fibre optic cable and a spectrometer. Firstly, the Nd: YAG laser acts as the source of the laser that emits light of a wavelength of nearly 1064 nm. The plano-Convex lens has a function to focus the collimated incident beam into a small spot. The Z-translation stage acts as the distance measuring mechanism in vertical. The radiation emitted from the plasma was collected by a fibre optic cable and delivered to a spectrometer. The spectrometer splits it into constituent wavelengths and projects over the detector array. Figure 1 shows the experimental set-up and instrumentation of LIBS.

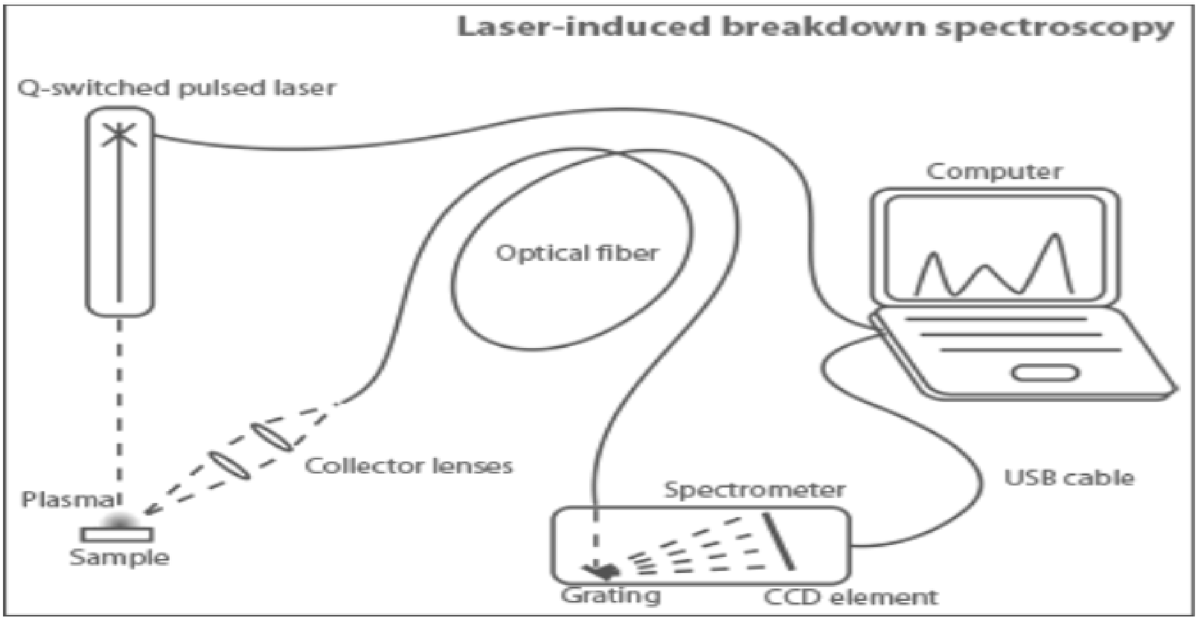


Figure 1: The experimental set-up and LIBS Instrumentation

2.3 Standard Operation of LIBS

Q Switched Nd:YAG Laser with the Laser pulse energy of 200 mJ, beam wavelength of 1064 nm, pulse width 10ns and repetition rate of 10 Hz are the setting for the experiment.

Firstly, adjust the height of the platform to the focal point. Next is the Alignment of the Z-translation stage. Mark the ‘X’ at the ablation spot on the sample stage. Position the pellet on the ‘X’ mark. Mount the fiber connector with the Ferrule Clamp towards the ablation spot. Connect the fiber optical cable to the Ocean Optics HR4000 spectrometer. Calibrate the settings on a software ‘SpectraSuite’, set the integration time to 100 ms and switch on the dark spectrum subtraction. Observe the spectra, repeat the alignment until more precise spectra are obtained. Experiments were repeated to collect the data for 10 different shots, on each spot 15 laser shots, out of these 5 are cleaning shots and 10 are measurement shots.

2.4 Experimental Procedure of EDX

Firstly, the sample pellets of peppermint and aloe vera were grinded to become powder after LIBS experiments were accomplished. The sample powders were slightly pour on a piece of white paper. Clean the SEM stubs by rubbing it with acetone. Removed the double-sided carbon tape, one side was pasted on the SEM metal stubs and stick the other side with sample powder for each stub per sample. The stubs were then blown with air pump to remove the not sticky residual powder. After that, the SEM stubs were placed on the stub holder in the sputter coating machine. During sputter coating, the processes involved were vacuuming, gas bleeding, sample coating and venting. After finish coating, the stubs were then inserted into SEM-EDX machine for analysis.

3. Results and Discussion

From the analysis of the recorded data, the presence of essential elements and toxic heavy metal were determined. Comparative differences were found in the composition of both samples.

3.1 Elemental Identification from spectral line

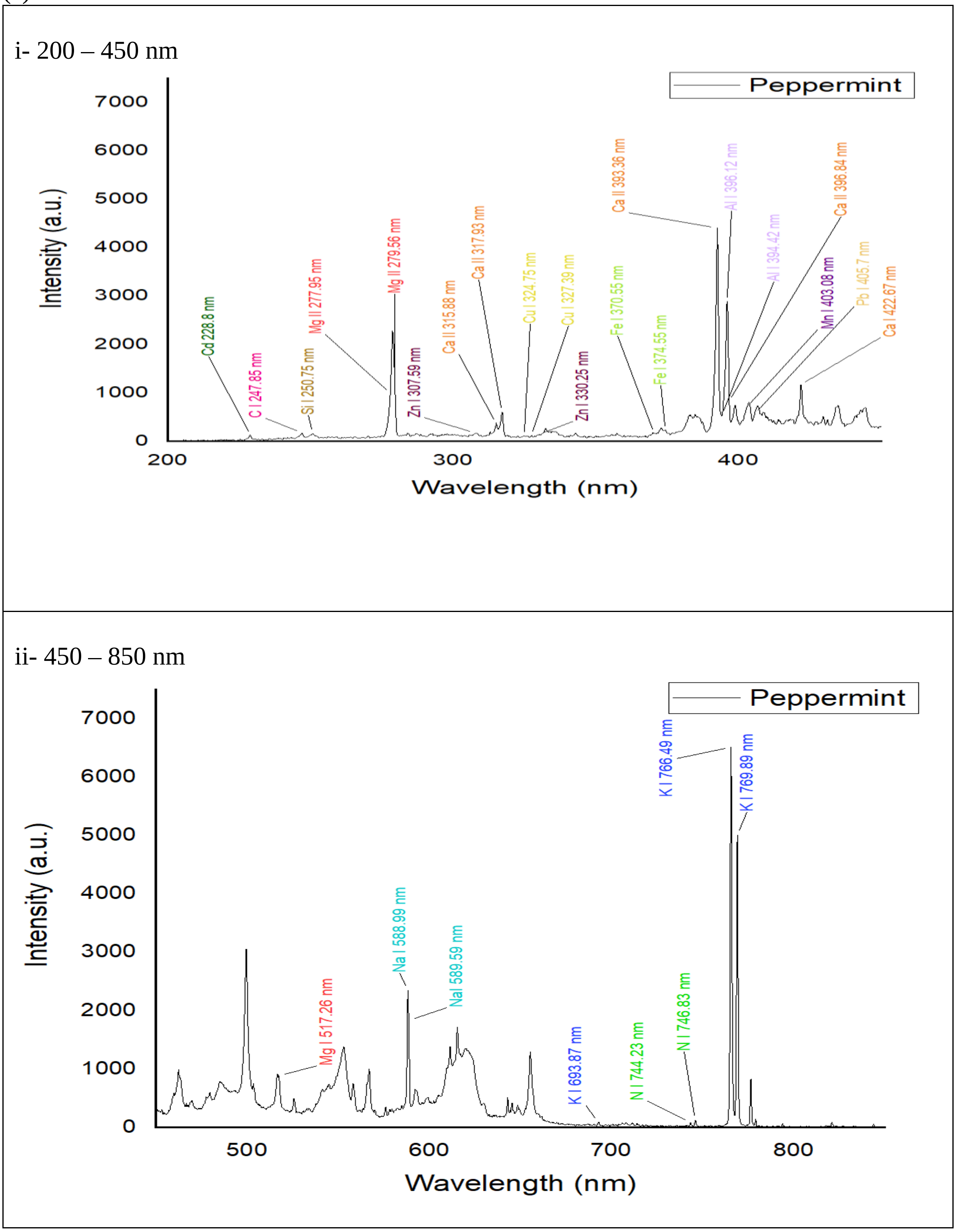
Figure 3 (a) and (b) shows the spectral of sample peppermint and aloe vera. Based on the spectral line identification of the sample herbs, LIBS spectrum consists of some useful spectral lines which gives information about the presence of essential elements and toxic heavy metals in the samples. The Y-Axis of intensity in the LIBS spectra represented arbitrary unit (a.u.). It is a relative unit of measurement to show the ratio of amount of substance, intensity or other quantities to a predetermined references measurement.

The carbon C-I of the 247.85 nm line and Si lines around 250.69 nm were observed. Carbon is an essential element while Si is a beneficial trace element for growth of crops [1]. The Mg lines at 277.95 nm and 279.56 for second ionisation state (Mg-II) were prominent which are essential elements for plants. The calcium lines were at 315.88 nm, 317.93 nm, 393.36 nm and 396.84 nm for second ionisation state (Ca-II) whereas 422.67 nm for first ionisation state (Ca-I). Copper lines were at 324.75 nm and 327.39 nm for the first ionisation state (Cu-I). The first ionisation state of iron (Fe-I) appeared with wavelengths at 370.55 nm and 374.55 nm with slightly low intensity. There were also aluminium lines (Al-I) at wavelengths of 394.43 nm and 396.12 nm. The zinc lines (Zn-I) with a less significant intensity can be barely seen at 307.59 nm and 330.25 nm.

Moreover, the magnesium line (Mg-I) was laying at 517.26 nm. The beneficial mineral element for plants such as sodium, Na and potassium, K were also detected. The potassium lines (K-I) can be found at 693.87 nm, 766.49 nm and 769.89 nm whereas sodium lines (Na-I) appear at 588.99 nm and 589.59 nm. It can be seen that the intensities of (K-I) in Peppermint were significantly higher than in Aloe Vera by comparing the height of the peak of intensity.

Other than that, there were also presents of a few toxic heavy metals from the spectral such as nickel and cobalt. Ni and Co are unhealthy for the human body and plant in high doses. The first ionisation state of cobalt (Co-I) can be found with the wavelength at 708.54 nm. The nickel lines (Ni-I) appeared at 341.28 nm. there were also presents of a few toxic heavy metals from the spectral such as lead and cadmium. Pb and Cd can build up disease and cancer cells in our body [2]. The first ionisation state of cobalt (Co-I) can be found with the wavelength at 708.54 nm.

(a)


i- 200 – 450 nm
Peppermint
Intensity (a.u.)
Wavelength (nm)
Cd 228.8 nm
C I 247.85 nm
Si I 250.75 nm
Mg II 277.95 nm
Mg II 279.56 nm
Zn I 307.59 nm
Ca II 315.88 nm
Ca II 317.93 nm
Cu I 324.75 nm
Cu I 327.39 nm
Zn I 330.25 nm
Fe I 370.55 nm
Fe I 374.55 nm
Ca II 393.36 nm
Al I 396.12 nm
Al I 394.42 nm
Ca II 396.84 nm
Mn I 403.08 nm
Pb I 405.7 nm
Ca I 422.67 nm
ii- 450 – 850 nm
Peppermint
Intensity (a.u.)
Wavelength (nm)
Mg I 517.26 nm
Na I 588.99 nm
NaI 589.59 nm
K I 693.87 nm
N I 744.23 nm
N I 746.83 nm
K I 766.49 nm
K I 769.89 nm

(b)

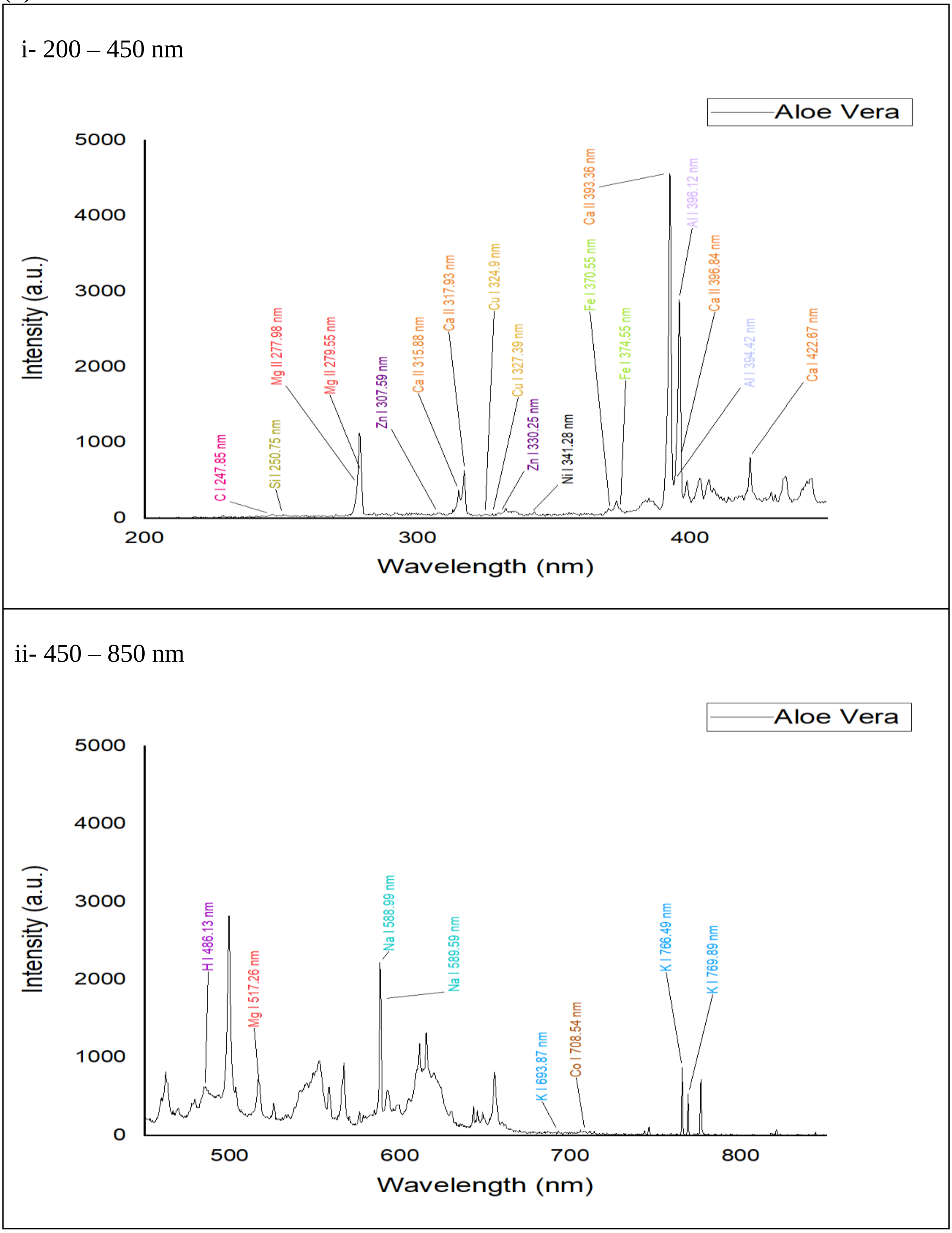


Figure 3: LIBS spectral emission obtained for samples (a) Peppermint ; (b) Aloe Vera

3.2 Elemental Analysis In Sample Herbs

The elemental analysis of sample medicinal herbs are classified into two categories. There are essential elements and toxic heavy metal. Based on the data obtained from the LIBS experiment, sample peppermint and aloe vera are prominent with few essential elements such as K, Na, Mg, Ca, Cu, Fe, Al and Zn. These samples are also slightly contaminated with toxic elements such as Pb, Ni, Co and Cd. Regarding the roles in biological systems, heavy metals are categorised as essential and nonessential. Essential heavy metals are necessary for living organisms and may be required in very low amounts in the body. Non-essential heavy metals have no recognised biological role and function in living organisms.

Table 1 shows the elemental classification of peppermint and aloe vera. The sample peppermint contains essential elements such as K, Na, Mg, Ca, Cu, Fe, Al, Si, Zn and Mn. Based on the spectral analysis with the validation of EDS, the peppermint was contaminated with toxic heavy metals like Pb and Cd which is caused by the soil pollution due to industrial activities, the impact of pesticides and inorganic fertilizers application [3].

The sample aloe vera contains essential elements such as K, Na, Mg, Ca, Cu, Fe, Al, Si and Zn. Aloe Vera was not detected to contain Mn elements. Based on the spectral analysis with the validation of EDS, the aloe vera was contaminated with toxic heavy metals like Ni and Co which is slightly different with peppermint. The presence of Ni and Co were due to the use of inorganic fertilizer such as copper sulphate or iron sulphate that contains high concentration of toxic heavy metals [4]. The presence of nickel could probably due to the high PH value of the soil from the quarry area [5].

Table 1: The Elemental Classification of Peppermint and Aloe Vera

	Peppermint	**Aloe Vera**
Essential Elements	*K, Na, Mg, Ca, Cu, Fe, Al, Si, Zn, Mn*	*K, Na, Mg, Ca, Cu, Fe, Al, Si, Zn*
Toxic Heavy Metals	*Pb, Cd*	*Co, Ni*

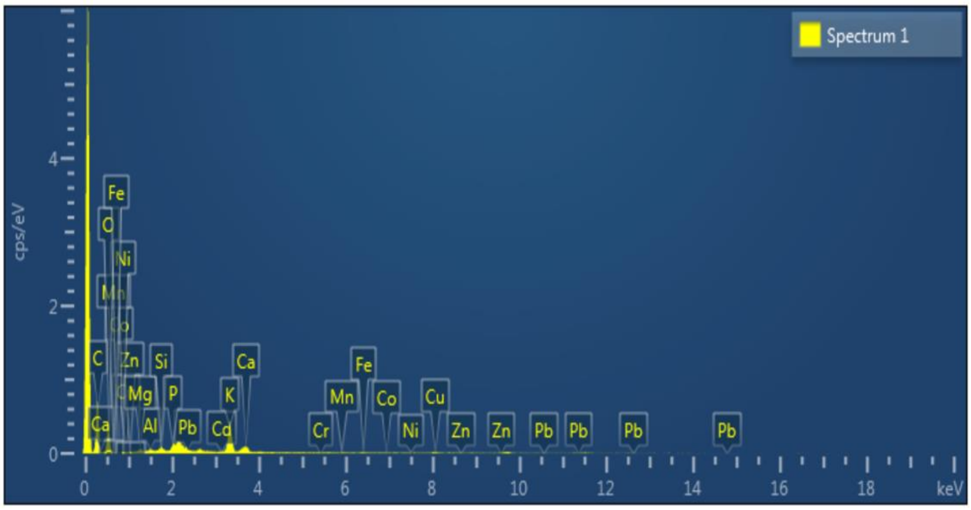
3.3 Energy Dispersive X-Ray Spectroscopy EDX Analysis

EDX is the Energy-Dispersive X-ray Spectroscopy. It is an analytical technique used for the elemental analysis or chemical characterization of a sample. It relies on an interaction of X-ray excitation with a sample. Its capability to characterize each particular element is because of the unique atomic structure that allows the unique set of peaks for emission of spectrum [6]. The selected samples were subjected to EDX to investigate the composition of elements of those samples. The highest peak at 0 KeV without labelling of element represent the electronic noise of the equipment.

Experiments were carried out to validate the presence of elements in the leaves of Peppermint and also to examine the composition of prepared samples. Peppermint is used as a medicinal plant. The spectra shown in figure 4 (a) validate the presence of the constituent essential elements such as K, Cu, Mg, Fe, Ca, Mn and Zn whereas toxic heavy metals like Pb and Cd in the sample peppermint. The rest of the peaks observed in the EDX spectrum such as C and O were non-metal elements. The weight percentage of K in peppermint of 6.93% which is higher than aloe vera of 1.57%.

Besides, another experiment was also carried out to validate the presence of elements in the leaves of Aloe Vera and also to examine the composition of prepared samples. Aloe Vera is used as a medicinal plant. The spectra shown in figure 4 (b) validate the presence of the constituent metal elements such as K, Cu, Mg, Ca, Fe and Zn whereas toxic heavy metals like Co and Ni in the sample aloe vera. The rest of the peaks observed in the EDX spectrum such as C and O were non-metal elements. The weight percentage of Ca in peppermint of 1.6% which is lower than aloe vera of 6.28%. Table 4.5 The Constituent Elements of (a) Peppermint and (b) Aloe Vera and their percentage values.

(a)



(b)

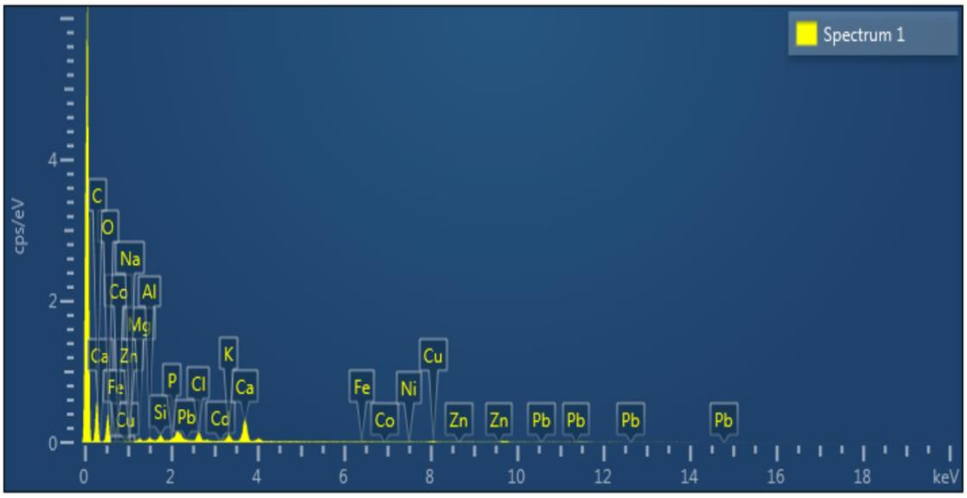


Figure 4: The EDX Spectra of Peppermint with the intensity (cps/eV) versus energy (KeV) (a) Peppermint (b) Aloe Vera

(a)

Peppermint

Element	Line Type	Weight %	Weight % Sigma	Atomic %
C	K series	46.68	2.34	57.56
O	K series	40.63	1.92	37.61
K	K series	6.93	0.35	2.62
Ca	K series	1.6	0.14	0.59
Cu	K series	1.34	0.26	0.31
Si	K series	0.64	0.09	0.34
Mg	K series	0.59	0.12	0.36
P	K series	0.54	0.12	0.26
Al	K series	0.39	0.09	0.21
Fe	K series	0.38	0.15	0.1
Cd	L series	0.13	0.24	0.02
Mn	K series	0.08	0.13	0.02
Pb	M series	0.05	0.4	0
Zn	K series	0.03	0.27	0.01
Co	K series	0	0.16	0
Cr	K series	0	0.12	0
Ni	K series	0	0.17	0
Na	K series	0	0.18	0
Total		100		100

(b)

Aloe Vera

Element	Line Type	Weight %	Weight % Sigma	Atomic %
C	K series	44.29	2.75	55.4
O	K series	41.38	2.14	38.85
Ca	K series	6.28	0.35	2.36
Cl	K series	1.74	0.13	0.74
Cu	K series	1.55	0.26	0.37
K	K series	1.57	0.13	0.6
Si	K series	1.01	0.1	0.54
Mg	K series	0.67	0.11	0.41
Al	K series	0.63	0.09	0.35
Na	K series	0.41	0.17	0.27
Ni	K series	0.23	0.17	0.06
Fe	K series	0.15	0.14	0.04
Co	K series	0.09	0.15	0.02
P	K series	0	0.09	0
Zn	K series	0	0.24	0
Cd	L series	0	0.18	0
Pb	M series	0	0.34	0
Total		100		100

Table 4.5: The Constituent Elements of (a) Peppermint and (b) Aloe Vera and their percentage values

3.4 Discussion on the Effect of Elements in Human Body

Silicon is the essential element that is necessary for the growth and bone calcification in human body [9]. It has beneficial effects on ageing of skin, hair and nails. Lack of Si will cause spinal deformity and reduce the amount of cartilage in human body [9]. Cadmium is a kind of heavy metal that is environmentally concerned. Cd is widely used in various industrial activities. Once absorbed, Cd will be retained in the human body and accumulate throughout life. Cd is toxic to the kidney and causes bone demineralization [10]. Besides, nickel and cobalt were toxic heavy metals that were found in aloe vera. Ni can be found mainly in nature. Ingestion of large amounts of nickel affects the stomach, liver, kidneys and body immune system. Ni in trace amounts does not harm the human body, but overdose will cause a variety of side effects such as allergy and nasal cancer [11].

4. Conclusion

LIBS is a highly capable and powerful elemental analysis technique that is rapid and easy to use for in-situ analysis. The spectral lines of peppermint and aloe vera were identified with the aid of ASD-NIST, analyzed by referring to other relevant literature reviews, and verified with EDX analysis. Based on the analyzed data, the essential element presence for both medicinal herbs is almost the same. The differences are the concentration of the element in the samples and the contamination of toxic heavy metals. Based on the weight percentage from EDX, it is significant that peppermint is richer in potassium with 6.93% whereas aloe vera is richer in calcium with 6.28%. The toxic elements like Pb and Cd were detected in peppermint with weight percentage of 0.05% and 0.13% whereas Co and Ni of 0.09% and 0.23% in aloe vera. There were in trace amount according to their respective weight percentage from EDX. The non-identical presence of toxic heavy metals are affected by the type of pesticides that may have been used, and the different pollution level of soil with respect to geographical location and the fertilizers used. EDX method has been used to validate the presence of heavy elements and calculate the weight percent composition of the elements in the sample. Lastly, the agriculture industry is advised to control the use of pesticides or fertilizers in order to control the pH value of the soil at a healthy level.

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