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Assessment of Trace Element (Mg, Al, K and Mo) in 14 Types of Raw Herbs and Spices using SEM-EDX Analysis

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Herbs and spices are multi-purposes ingredients and essential in daily life such as in cooking as well as in traditional medicines. There was a lack of information on heavy metal composition of herbs and spices in Malaysia. Therefore, this study reveals the major and trace elements of elemental compounds in 14 types of herbs and spices using the Scanning Electron Microscopy Energy Dispersive X-Ray Spectroscopy (SEM-EDX) method. The pH of most herbs and spices values are found to be in the ranges of pH 5.70 until 7.60 for herbs, while pH for spices is found to be within pH 5.26 until 7.23. A statistically eloquent relationship ($r=0.91$, $P<0.05$) was found for Mg and its pH level in herbs by employing Pearson correlation. The SEM-EDX reveals the elements of carbon (C), aluminium (Al), magnesium (Mg), potassium (K) and molybdenum (Mo) are detected for the entire herbs and spices samples. C is the dominant element found at high concentration (>85%) in both herbs and spices. Basil and cardamom have the most elevated amount of K with atomic percentage of 1.82% and 2.14%. Basil and ginger samples consist of more than 1.00% atomic percentage. The values of Al found in the samples of oregano (0.11%) and black pepper (0.37%). Higher amount of Mo concentration was found in ginger with an atomic percentage of 0.99%. The values obtained in the assessment are within the safe level for both herbs and spices, according to WHO.

Keywords: herbs; spices; SEM-EDX; pH; trace element

I. INTRODUCTION

For almost a thousand years before, the Indians community in India had been using the herbs and spices such as black pepper, parsley, cinnamon, turmeric, and cardamom for culinary and general health purposes (Tapsell *et al.*, 2006). In Malaysia, herbs and spices specifically are added in traditional Malay, Chinese and Indian cuisine to ensure the unique taste of Malaysian food. Nowadays, Malaysia is famously known as one of the most important trading hubs for all kinds of herbs and spices (Ramli & Nik Abd. Rahman, 2009). With the long existing history related to the herbal and spice trading which took place in Peninsular Malaysia, still most of modern Malaysian society lacks the knowledge which can benefit their overall wellbeing by directly consuming herbs and spices in their food and others. Generally, the herb

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is a plant, or green leafy part of plants valued for its flavour, scent, or medicinal properties, which can sometimes be dried before use to give the taste and smell to the food as well as adding colour to the cuisine (Peter, 2001; Cruz-Zaragoza, Marcazzó & Chernov, 2012). Meanwhile, spices are from any dried part of a plant, other than the leaves, used for seasoning and flavouring food. In general, spices have a more pungent flavour than herbs (Mann, 2011).

Essentially, these herbs and spices are widely being used in the daily routine of the community, such as in cooking, medicine, and essential oils. Another purpose of herbs and spices were used as herbal medicines which to relieve and treat the various types of human diseases. Most of the herbs and spices may help to control the inflammation in the body and reduce blood sugar (Ghasemian, Owlia & Owlia, 2016). For example, antioxidants in cinnamon and turmeric can lower inflammation and reduce blood glucose concentrations in people with diabetes (Jurenka & Ascp, 2009; Nasri *et al.,* 2014). The benefits of using herbs and spices would help to protect against certain chronic conditions such as diabetes, cancer, and heart disease (Yagi *et al.,* 2013).

Moreover, the trace elements and heavy metals of herbs and spices can occur from the type of soil and the fertilisers used (Wuana & Okieimen, 2011). The herbs and spices also might be contaminated during processing products, packaging and storage (Łozak *et al.,* 2002). Otherwise, contaminated herbs and spices could have toxic reactions, allergic reactions and adverse effects on the human body (Ekor, 2014). Therefore, in order to protect consumers from contamination, there must be reasonable quality control on herbs and spices.

A study by Nordin and Selamat in 2013 has identified the heavy metals of As, Cd, Pb and Hg in common herbs and spices *via* the Atomic Absorption Spectroscopy (AAS) technique (Nordin & Selamat, 2013). Furthermore, a previous study investigated the common food additives such as turmeric, ginger, black pepper, cinnamon and red pepper using SEM-EDS and ICP-AES analysis (Hussain *et al.,* 2010). In this study, it has proven that potassium, K, was present in all spice samples followed by Ca, Si and Cl. Next, Al-Bataina (2003) stated that nutmeg, coriander, cardamom and black pepper contained Mg, Al, K, Si, P, Cl and Ca using the XRF technique (Al-Bataina, Maslat & Al-Kofahi, 2003).

Most of these researchers investigated the heavy metal and trace element using various techniques. However, there was no comprehensive study on trace elements in herbs and spices using SEM-EDX. Therefore, this study highlighted the trace element of herbs and spices obtained from the wholesale market in Malaysia using SEM-EDX analysis. SEM-EDX is a potential application in identifying the trace elements of herbs and spices (Ansari *et. al.,* 2020; Niemeyer, 2015). Apart from identifying and quantifying the trace elements in the sample, hopefully this investigation also will contribute to the recognition of analysing elemental composition using versatile and rapid analytical techniques of SEM-EDX especially for solid (powder) samples.

Moreover, this study also provides information on the pH level of herbs and spices. Previous study showed that most herbs and spices were classified as slightly acidic and alkaline (Samson & Girish, 2007). Therefore, it is crucial to investigate the correlation between pH and trace element of herbs and spices in order to strengthen the trace element analysis.

The main objective of this study is to assess the elemental compound in herbs and spices consumed in Malaysia. The significant objectives are to (1) determine the pH properties for all 14 samples of herbs and spices; (2) analyse the elemental composition for major and trace elements in herbs and spices using SEM-EDX. Hence, this research can contribute new knowledge to society regarding Malaysian herbs and spice elemental content. This study will provide essential information of trace elements in local products of herb and spices as well as increase the awareness to society of the main ingredients in the preparation of food and other applications.

II. MATERIALS AND METHOD

A. Sample Collection and Preparation

Herbs and spices that were commonly used in Malaysian cuisine have been identified and carefully selected. There are fourteen (14) samples of different types of herbs and spices purchased at the wholesale market in Larkin Wet Market, Johor Bahru, Malaysia was selected for this study; this includes seven (7) different types of herbs and seven (7) different types of spices, as shown in Table 1. All the samples for both herbs and spices were in dried and ground states, respectively. The samples were stored in a desiccator at room temperature (27 ˚C) to prevent humidification. The ground samples were packed in an airtight container in order to prevent it from exposure to moisture content and this ensured the samples remain to be dry for a longer time of period (Bunning, Woo & Kendall, 2014).

Table 1. The list of the sample used for herbs and spices

Types of samples		Scientific Name
Herbs	Oregano	*Origanum vulgare*
	Fennel	*Foeniculum vulgare*
	Cumin	*Cuminum cyminum*
	Basil	*Ocimum basilicum*
	Parsley	*Petroselinum crispum*
	Coriander	*Coriandrum sativum*
	Thyme	*Thymus vulgaris*
Spices	Black Pepper	*Piper nigrum*
	White Pepper	*Piper nigrum*
	Red Pepper	*Capsicum annuum*
	Turmeric	*Curcuma longa*
	Ginger	*Zingiber officinale*
	Nutmeg	*Myristica fragrans*
	Cardamom	*Elettaria cardamomum*

B. Analytical Method

1. pH Measurement

The pH level measurement was carried out using Mettler Toledo Seven Compact pH meter S220 at room temperature (27˚C). The pH of the samples was determined by dissolving the sample in the demineralised water in the ratio of 1:1. The pH measurement for all herbs and spices have followed the ASTM D4318 standard method (ASTM D4318, ASTM D 4318-10 and D4318-05, 2005).

2. Elemental Compound Analysis using (SEM-EDX)

Several analytical methods can be used to determine the elemental composition in herbs and spices: inductively coupled plasma–mass spectrometry (ICP–MS), inductively coupled plasma–atomic emission spectrometry (ICP–AES), atomic absorption spectrometry (AAS) and energy dispersive x-ray fluorescence (EDXRF) (Dghaim *et. al.,* 2015; Filipiak-Szok *et. al.,* 2015; Khan *et. al.,* 2014; Krejpcio, E.Krol & S.Sionkowski, 2007; Łozak *et. al.,* 2002; Tokalıoğlu, 2012). Thus, in this study, the samples were analysed using SEM-EDX (JEOL JSM – 6380LA). Each of the samples was placed on double-sided-coated carbon stubs. Then, the sample was allowed to dry at room temperature. The sputter coating was used to coat the samples with thin film 10nm of gold to acquire high-resolution imaging and improved the accuracy of identifying the composition of the element. Besides, this also avoids a charging process that reacts between electrons and the samples which influences the image information. The trace element is observed using SEM-EDX and it operates with high resolution (0.6 nm) at a voltage of 15kV at x100 and x500 magnifications. Both SEM and EDX analyses were carried out on the same instrument (JEOL JSM – 6380LA). This SEM was also facilitated with EDX, which allows elemental composition measurement for qualitative analysis.

3. Statistical Analysis

The output results appeared in the bar chart including mean ± standard deviation of three replicates using Microsoft Excel 2010 and Origin 9. Pearson correlation was used to show the relationship between the parameters under investigation in the herbs and spices. Significant levels were tested at $P < 0.05$.

III. RESULTS AND DISCUSSIONS

A. pH Meter Measurement

The pH value measurements for each type of herbs and spices were determined using a pH meter. According to Table 2, it is shown that the pH value for spices and herbs mainly were slightly acidic. However, two samples are found to be nearly neutral, which is basil and turmeric. This is because the ideal soil pH level for herbs and spices were in the range of 5.0 to 8.0 which is required to enhance healthy growth (Gentili *et al.,* 2018). In addition, the processing of the manufacture of herbs and spices can also interfere with the pH value of each sample (Adams *et al.,* 2004).

Table 2. The pH value measurements for herbs and spices

Types of samples		pH Value
Herbs	Oregano	6.15 ± 0.05
	Fennel	5.71 ± 0.05
	Cumin	5.87 ± 0.02
	Basil	7.60 ± 0.02
	Parsley	6.04 ± 0.02
	Coriander	6.17 ± 0.02
	Thyme	5.69 ± 0.02
Spices	Black Pepper	6.23 ± 0.03
	White Pepper	6.03 ± 0.03
	Red Pepper	5.27 ± 0.06
	Turmeric	7.23 ± 0.03
	Ginger	5.44 ± 0.02
	Nutmeg	5.82 ±0.03
	Cardamom	5.26 ± 0.03

B. Elemental Composition Analysis

1. Elemental Composition Analysis for Herbs

The elemental composition analysis was performed for the total of 7 herbs using EDX technique. Figure 1 shows the elemental composition of C, Al, Mg, K and Mo in all samples. The atomic percentage of each herb is then tabulated in Table 3. Based on Figure 1, it illustrates that C (>85%) is the dominant element in all herbs followed by Mg, Al, K and Mo. This result is as expected similar to previous research, due to carbon being widely spread in nature and may form many types of organic compound found on earth that enables it to function as a common chemical element in all living organisms (Prentice *et al.,* 2001). Therefore, this study has revealed carbon quantity is the highest found in most of the samples.

Table 3. Qualitative analysis of element in herb samples in atomic percentage*

Samples	Atomic Percentage (%)				
	C	Mg	Al	K	Mo
Oregano	99.11 ± 0.19	0.31 ± 0.09	0.11 ± 0.16	0.33 ± 0.12	0.14 ± 0.03
Fennel	99.38 ± 0.31	0.23 ± 0.25	0.02 ± 0.02	0.26 ± 0.02	0.11 ± 0.08
Cumin	99.41 ± 0.10	0.18 ± 0.13	0.01 ± 0.02	0.25 ± 0.02	0.15 ± 0.02
Basil	92.08 ± 0.52	1.77 ± 0.06	ND	1.82 ± 0.08	ND
Parsley	95.86 ± 0.62	0.31 ± 0.02	ND	1.39 ± 0.67	ND
Coriander	95.56 ± 1.13	0.50 ± 0.13	ND	1.53 ± 0.32	ND
Thyme	86.08 ± 19.46	ND	ND	0.80 ± 0.64	ND

*Means (n = 3) ± SD, ND: not detected, C: carbon, Mg: magnesium, Al: aluminium, K: potassium, Mo: molybdenum

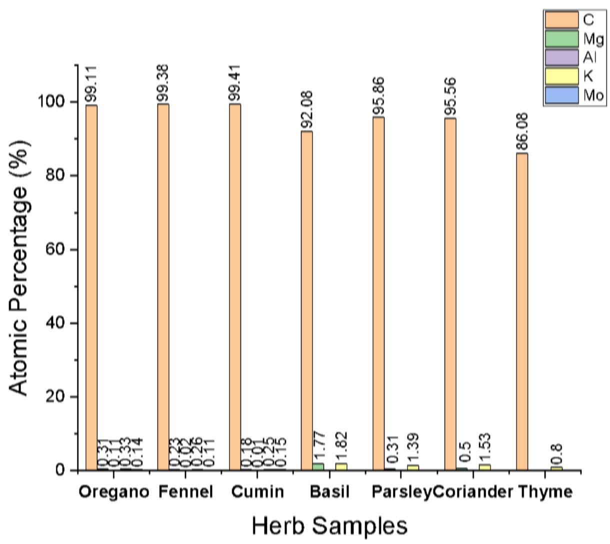

Figure 1. The distribution of the atomic percentage of C, Mg, Al, K, and Mo in herb samples

Figure 2 presents the detailed information on atomic percentage of Mg, Al, K and Mo in all of the herb samples: oregano, fennel, cumin, basil, parsley, coriander, and thyme. Basil has been found to be the highest value of Mg and K with atomic percentage of 1.77% and 1.82%. Followed by coriander, which contained the second highest value of Mg and K with atomic percentage of 0.5% and 1.53%. Next, Mg and K also presented in parsley with atomic percentage of 0.31% and 1.39%. Moreover, the highest value of Al exists in oregano followed by fennel and cumin with atomic percentage of 0.11%, 0.02% and 0.01%, respectively. On the other hand, cumin has the highest value of Mo with an atomic percentage of 0.15%. The atomic percentage of Mo in Oregano and fennel found to be slightly lower than cumin, with the amount of 0.14% and 0.11%. Hence, K is the only element that can be detected in thyme with the atomic percentage of 0.8%. Unfortunately, in basil, parsley and coriander there are no traces found for Al and Mo, while thyme only consists of K element.

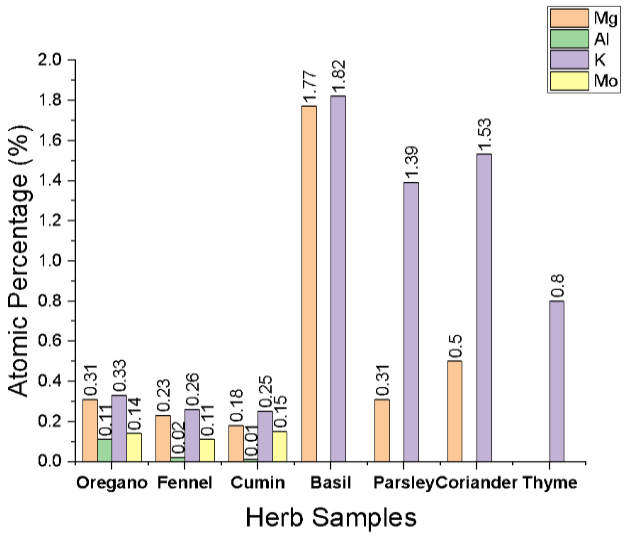

Figure 2. The distribution of the atomic percentage of Mg, Al, K, and Mo in herb samples

2. Elemental Composition Analysis for Spices

The elemental composition for each spice sample is tabulated on Table 4. As shown in Figure 3, the overall elemental composition is represented in a graphical chart. It shows that C is the dominant element in most of the spice samples. In this case, white pepper has been found to be the highest value of C with an atomic percentage of 99.47%.

Table 4. Qualitative analysis of element in spice samples in atomic percentage*

Samples	Atomic Percentage (%)				
	C	**Mg**	**Al**	**K**	**Mo**
Black Pepper	97.91 ± 0.77	0.62 ± 0.29	0.37 ± 0.05	0.84 ± 0.29	0.25 ± 0.19
Red Pepper	98.91 ± 0.21	0.35 ± 0.13	0.02 ± 0.01	0.62 ± 0.12	0.10 ± 0.03
White Pepper	99.47 ± 0.55	0.05 ± 0.01	0.02 ± 0.02	0.02 ± 0.02	0.12 ± 0.03
Turmeric	99.01 ± 0.17	0.47 ± 0.06	0.01 ± 0.01	0.39 ± 0.05	0.12 ± 0.06
Ginger	96.70 ± 3.75	1.19 ± 1.59	0.02 ± 0.02	1.10 ± 1.01	0.99 ± 1.12
Nutmeg	97.00 ± 0.29	0.74 ± 0.09	0.01 ± 0.01	0.24 ± 0.09	0.22 ± 0.10
Cardamom	99.23 ± 0.97	0.29 ± 0.39	0.01 ±0.01	2.14 ± 0.70	0.11 ± 0.06

*Means (n = 3) ± SD, C: carbon, Mg: magnesium, Al: aluminium, K: potassium, Mo: molybdenum

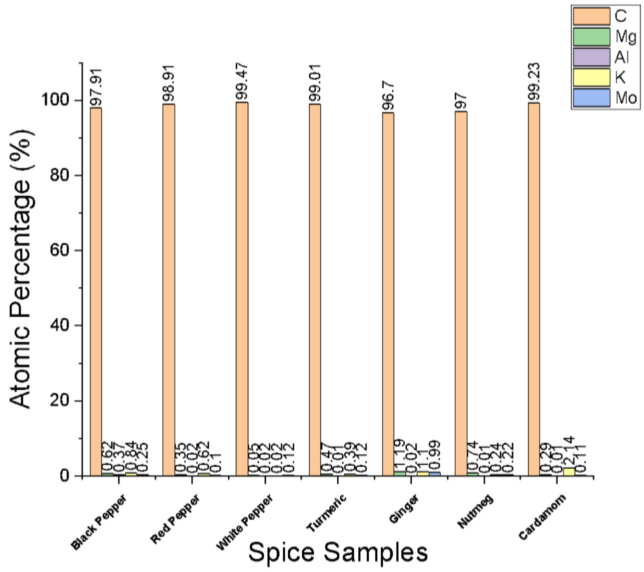

Figure 3. The distribution of the atomic percentage of C, Mg, Al, K, and Mo in spice samples

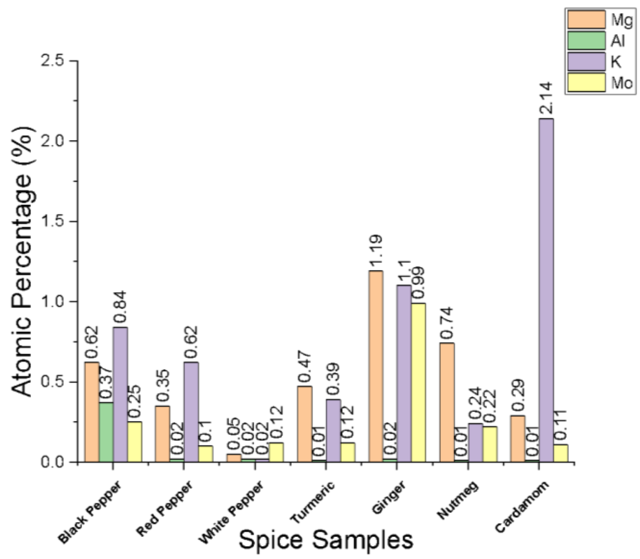

Figure 4. The distribution of the atomic percentage of Mg, Al, K, and Mo in spice samples

Moreover, the other elements, Mg, Al, K and Mo, are described in detail in Figure 4. Cardamom has the highest value of K with atomic percentage of 2.14%, but has low value of Mg (0.29%), Al (0.01%) and Mo (0.11%). In addition, ginger has the highest value of Mg, K and Mo with atomic percentage of 1.19%, 1.10% and 0.99%, respectively. Meanwhile, nutmeg contains the elements Mg, K and Mo with atomic percentages of 0.74%, 0.24%and 0.22%. Thus, the atomic percentage of Mg is greater than the atomic percentage of K in nutmeg. In contrast to red pepper, the atomic percentage of K (0.62%) is surpassing the atomic percentage of Mg. Turmeric, on the other hand, contains atomic percentages of Mg and K that are slightly lower than nutmeg which are at 0.47% and 0.39 %, respectively. Moreover, the highest value of Al is assigned to black pepper with an atomic percentage of 0.84%. On top of that, black pepper has a greater amount of K with an atomic percentage of 0.84%, followed by Mg with an atomic percentage of 0.62%. In contrast, white pepper has a low atomic percentage value in all elements, except C.

Plants are made of carbon just like every other living organism. Carbon, being one of the main ingredients in photosynthesis, existed in the form of carbon dioxide (CO_2) in the environment. This allows the plant to absorb the CO_2 to ensure it will grow properly. In this study, carbon (C) clearly appeared to be the most prevalent element in both herb and spice samples. According to the discussion in section 1 and 2 for Mg, Al, K and Mo are detected in all samples, except basil, parsley, coriander and thyme. In brief, basil has the highest value of Mg and K. This result has been agreed with Dzida (2010) where the basil has high value of Mg and K due to the soil types and fertiliser used for plant growth (Dzida, 2010). Moreover, the leafy green vegetables and herbs typically contain high amounts of K and Mg (Arasaretnam, Kiruthika & Mahendran, 2018). Generally, the soil composition also provided an essential role in determining the level of Mg content (Imelouane *et al.,* 2011).

Coriander, parsley and thyme have a high value of K compared to oregano, fennel and cumin. This is because the sample structure of coriander, parsley and thyme used in this study were in dried leafy parts of the plants. Therefore, it revealed that each sample of herbs contains a high concentration in K as K is natural minerals derived from the leafy part of plants (Kumar *et al.,* 2019). Moreover, dried herbs contained a higher source of K compared to fresh herbs (Zagula *et al.,* 2016). In this study, the amount of K and Mg in coriander were agreed with Bhat *et al.* (2014), where K and

Mg are the significant components in coriander (Bhat *et al.,* 2014). Meanwhile, cardamom also had the highest K value among the spice samples, followed by ginger, black pepper, red pepper, and turmeric. According to Khan *et al.* (2014), K and Mg are significant components in cardamom and turmeric (Khan *et al.,* 2014). As a result, the presence of K and Mg in the spice samples is undeniable.

Lopez and co-workers have reported that the presence of Al has been detected in basil, nutmeg, oregano, parsley, red pepper, black pepper, white pepper and thyme. However, oregano and red pepper had the most elevated Al concentrations (López *et al.,* 2000). For this study, black pepper has the highest value of Al followed by oregano and fennel. Other samples had a minimum value of Al (<0.02%). Therefore, it is proved that the presence of Al was identified in oregano, black pepper and other samples.

Furthermore, from the result found it has shown that Mo composition was deficient compared with other elements. In addition, a minimal amount of Mo is required for the growth of a plant. Hence, in this study, the amount of Mo does not exceed 1.00% for all samples. Moreover, the level of concentration of Mg, Al, K and Mo contained in the herbs and spices are still under the safe range of the WHO limit (Vázquez-Fresno *et al.,* 2019).

C. Correlation between pH Level and Elemental Composition

The correlation of pH and elemental composition of herbs and spices was conducted using Pearson correlation. The Pearson correlation matrix is tabulated in Tables 5(a) and 5(b). The pH values ranged from 5.26 – 7.60 as mentioned previously in Subsection A. Tables 5(a) and 5(b) show the r value significantly different ($P < 0.05$) among the samples. According to Table 5(a), the pH value derived positive significant correlation with Mg ($r=0.92$, $P<0.05$) and K ($r=0.72$, $P>0.05$) in herbs. Meanwhile, in Table 5(b), there was no significant correlation between pH and elemental composition in spices. In brief, there is a statistically positive significant correlation between the level of pH and elemental composition of Mg analysis using SEM-EDX for herbs. Therefore, it is recommended further analysis should be done in the future to analyse the relationship between the elemental composition with a pH value of herbs and spices.

Table 5. Pearson correlation of pH and elemental compound in (a) herbs and (b) spices in Malaysia

Elements	***r***	**p-value**
C	-0.256	0.580
Mg	0.918	0.004
Al	-0.084	0.858
K	0.723	0.067
Mo	-0.339	0.375

(a)

Elements	***r***	**p-value**
C	0.086	0.854
Mg	0.040	0.932
Al	0.196	0.673
K	-0.343	0.451
Mo	-0.266	0.564

(b)

IV. CONCLUSIONS

In this work, SEM-EDX has been successfully used as a rapid and versatile technique to determine the elemental composition measurement in atomic percentage for all herbs and spices powder samples. It was found that all 14 herbs and spices mainly contained a major concentration of C and some trace elements of Mg, Al, K and Mo. Furthermore, all herbs and spices are categorised into two categories of pH levels; weak acid and neutral with the range of 5.26 to 7.60. There is a statistically significant relationship ($r=0.91$, $P<0.05$) between Mg and pH level in herbs using Pearson correlation. This research shows that all elements in herbs and spices are essential especially for the Malaysian society, to know it is safe and suitable to be used in food preparation or to be applied as one of the main ingredients in traditional medicines and other products. Optimistically, the present study will provide information on the toxicological risk estimation of consumers in Malaysia.

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