Correlation study between the electronic nose response and headspace volatiles of *Eurycoma longifolia* extracts

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Abstract

Most herb has its own characteristic smell due to the presence of volatile compounds. Traditionally, volatiles are analyzed using sophisticated and expensive gas chromatography (GC) in tandem with selective mass spectrometric (MS) detector. An alternative approach based on the use of electronic nose is described. The approach is much simpler than the traditional GC-MS counterparts, but providing key information of the samples analysed. Using lipids and gas chromatography stationary phase materials with different polarity as sensing membrane, a quartz crystal microbalance smell sensor array has been developed for the analysis of traditional medicinal plant. The headspace vapor of different types of Tongkat Ali (*Eurycoma longifolia*) extracts were analyzed by the smell sensor and GC-MS. Correlation between the sensors response and identified compounds were studied using principal component analysis. Some of the identified compounds exhibit good correlation with the QCM sensor array data.

Keywords: Electronic nose; lipids; medicinal plants; *Eurycoma longifolia*

1 Introduction

Herbal medicines have commanded special attention worldwide due to their neutraceutical and medicinal values. Therefore, the quality control of raw herbs and their products are essential to ensure consistent quality, safety and efficacy. Traditional methods used for odor analysis and regulation in the food industry involves GC-MS and human taste panels. These methods can give very detailed information about the contents of the odor. While (GC/MS) can separate, identify and quantify individual volatile chemicals, it is very difficult to correlate the data with sensory evaluation. Since odors are usually composed of complex mixtures of different volatiles, the technique is too cumbersome for practical everyday applications. Human sensory evaluation as conducted by human test panels is a powerful method for odor analysis. However, it has a number of limitations as human sensory panels are expensive and time consuming. Even the most highly trained human testers are subjective, and illness and other factors can influence their performance.

In contrast, the advantages of electronic nose include rapid, real-time detection of volatiles, lower costs, and the procedure can be easily automated. An alternative way to objectively analyze an odor is to design an instrument to mimic the human sense of smell. Application of quartz crystal microbalance array sensor for volatile and smell analysis has been reported [1-4]. Electronic nose consisting of chemical imaging [5] and multiparameter sensor systems [6] have been described. The sensing system can be an array of different sensing elements (chemical sensors), where each element measures a different property of the sensed chemical. Alternatively, a single sensing device (e.g., FT-IR, spectroscopy etc.) that produces a transient signal as a function of variables (e.g., absorbance vs. wavelength) for each chemical, or its combination can be used. Generally, an electronic nose is based on its evaluation of the sum of all the detected volatile species [7]. The volatile vapors present in the headspace interact with the array of non-selective sensor and produce a chemical fingerprint or pattern characteristic to the odor or volatile vapor.

Medicinal plants and their formulations may consist of hundreds of phytochemicals. As a result, it becomes very difficult or almost impossible in most cases to identify most of these components by means of common approaches [8,9]. Some of the volatile compounds, may be not sensitive to the human olfactory, could be responsible for the identity of a particular herb. In general, only a few marker or pharmacologically active components are employed for evaluating their quality and authenticity [10].

The volatile components contained in extracts are normally lower than the detection limits of the detector. Thus some forms of preconcentration become mandatory. Among these, simultaneous distillation-extraction (SDE) [11], dynamic headspace analysis or purge and trap method [12] using porous polymer and solid-phase micro-extraction (SPME) [13] are the most popular methods in aroma analysis [14]. The utilization of gas chromatography-mass

spectrometric (GC-MS) techniques for the discrimination of herbs with the same denomination has been described by several authors [14,15].

We have developed a smell sensor using gas chromatography stationary phase [16] and lipid [17] material to mimic the olfactory system adopted from nature. Gas chromatographic materials used were based on their different polarity. Lipid materials used were of amphiphilic character due to the hydrophilic head groups and hydrophobic tail [16]. The electronic nose gives an overall response to the complex mixture without identifying the single components [15].

In the present studies, we have analyzed *Eurycoma longifolia* Jack extracts with GC-MS using solid-state-micro-extraction (SPME) and correlated [14,18] twelve identified compounds with quartz crystal microbalance array sensor.

2 Experimental

2.1 Sample

Twelve *E. longifolia* Jack samples were studied. Samples 1-6 were freeze-dried and sample 7 and 12 were spray-dried. Sample 1 and 2 were water extracts spray dried from Nusantara. Sample 3 was petroleum ether extract spray dried. Details of the samples are shown in Table 1.

Table 1: Details of the *E. longifolia* Jack extract samples studied

Sl. No	Sample Name	Drying method
1	MeOH Extract	Freeze dried
2	Eu. Long Batch 1202-8002	Freeze dried
3	TA Stem(without bark)$(CH_3)_2CO$	Freeze dried
4	TA (Sabah) [(Ac)$_2$O Ext.]	Freeze dried
5	MMBPP206 (34)-84-TAFD	Freeze dried
6	MMBPP206 (34)-84-TAET	Freeze dried
7	MMBPP206 (34)-84-TASD	Spray dried
8	TA Powder (01) - Nusantara	Spray dried
9	TA Powder (02) - Nusantara	Spray dried
10	TA Ext. TAE2003/7-10 (1)	Spray dried
11	TA Ext. TAE2003/7-10 (2)	Spray dried
12	02A/TA-PE/06/02	Spray dried

2.2 Electronic nose

2.2.1 Instrumentation

The frequency changes (Δf) of the sensors were monitored by a Universal Sensor Array System (QTS-3) obtained from Quartz Technology Limited, U. K. The QTS-3 device was connected to a personal computer for data acquisition and processing.

2.2.2 QCM array sensors

The array sensor consists of eight AT-cut quartz crystals with gold electrodes on both sides. Eight

different types of gas chromatographic stationary phases and lipid materials were used to fabricate the array sensor. The sensing materials consist of polar, non-polar and amphiphilic materials (Table 2). The sensing materials were coated on the both side of the quartz crystal using spin and ultrasonic spray coating methods [19]. A simple in-house fabricated ultrasonic spray coating device using ultrasonic atomizer is used. The coated crystal (either method) with standard deviation less than 2 in terms of frequency changes is used in the array sensor.

Table 2: The materials used for the sensor fabrication

Sensor	Sensing material	Abbrev.
1.	Apiezon L	APZ-L
2.	Polypropylene glycol 1200	PPG 1200
3.	Polyethylene glycol 1000	PEG 1000
4.	Polyethylene glycol 4000	PEG 4000
5.	Ethyl cellulose	EC
6.	Dioctyl phosphate	DOP
7.	Trioctyl methylammonium chloride	TOMA
8.	Oleylamine	OAm

The samples (100 mg) were taken in 20 ml vials and heated at 60 ^{o}C for 15 minutes. The headspace vapors were supplied into the sensor chamber through a dried silica gel column. The sequences of the sample injection were: base line 60 sec, sample injection time 10 sec and recovery time 200 sec. The response of the sensor (ΔHz) is considered as the difference between baseline and their maximum frequency change when injected with sample.

2.3 GC-MS analysis

The samples were taken in 20 ml vials and heated at 60 ^{o}C for 15 minutes. The headspace volatiles were adsorbed on Supelco SPME fiber No. 57318 (Carboxen/Polydimethylsiloxane) for 15 minutes. The use of (PDMS/Wax) with addition of carbon sorbent led to more effective trapping of volatile compounds [13]. Inserting the SPME fiber in to the GC-MS injection port and holding for five minutes, the adsorbed components were effectively desorbed.

A Thermo Fennigan Trace GC-MS was used with a VB-WAX fused silica capillary column (60 m x 0.32 mm, film thickness: 0.32 μm, J&W Scientific Inc. Folsom, CA). The injection temperature was 210 ^{o}C. The initial oven temperature was 50 ^{o}C and held for 5 min, and rose to final temperature of 210 ^{o}C at 5 ^{o}C/min and hold for 5 min. The flow rate of He gas used was 1 ml/min. GC/MS was operated with an ionization voltage of 70 eV and ion source temperature of 180 ^{o}C. Peak areas of the individual compounds were used as the variables for the statistical analysis. Peak compounds were identified by matching their mass spectra with those in the Wiley Library of MS spectra and NIST Library and also based on retention indices. The relationship

between QCM array sensor response and headspace volatile compounds obtained by GC-MS was carried out using multiple factorial analyses using SPSS version 11.0.

3 Result and discussion

3.1 QCM data

The sensors are not compound specific but in general they are sensitive to chemical classes [20]. The volatile vapors are reversibly adsorbed when in contact with the sensing layer. This temporary and reversible mass change will lead to a "detuning" of the quartz resonator, resulting in a change of the vibrational frequency [21,22]. As aroma compounds are normally present at very low concentration [14], the array sensor responses to overall content and give characteristic chemical fingerprint of the headspace volatile vapor.

Principal component analysis was carried out on the sensor array data in order to find out the classification ability of the array sensor. The data were usually preprocessed before analysis. The preprocessing included standardization. This was done by expressing as deviations from means in units of standard deviation.

$$z = \frac{x - \hat{x}}{\sigma} \tag{1}$$

where x is the real value, $\hat{x}$ is the mean and σ the standard deviation. The principal component indicate that the total variance up to five principal components were 99.24 % (81.365 + 10.670 + 3.709 + 1.880 + 1.613). The plot of first and second principal component is shown in the Figure 1. The plot of first and second principal component was able to classify freeze and spray dried extracts.
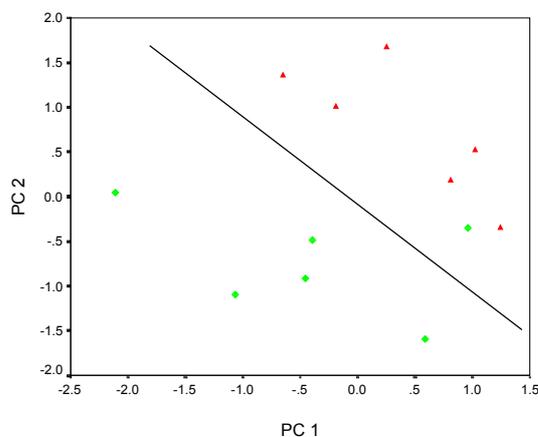

Figure 1: PC1 and PC2 plot of QCM analysis of spray dried and freeze dried extracts. (◆) Spray dried and (▲) freeze dried extracts.

3.2 GC-MS data

It is well known that herbs of the same variety share similar features such as taste, smell [23] etc. More than 65 different volatiles have been reported to be present in the *Eurycoma longifolia* extracts volatiles. Some of them are relatively higher concentration.

GC-MS profile of the headspace vapor of *Eurycoma longifolia* extracts using solid-state micro extraction method (SPME) is shown in the Figure 2. A total 133 volatiles were found. Freeze dried extract contained maximum 83 volatile whereas spray dried contained maximum 28 volatile compounds. Correlation between the sensor response and the volatiles were analyzed. A total of 9 major and volatiles that are common in all the samples were used as the predicted variables. Principal Component Analysis was applied to the peak area of the major compounds. The contribution rate (eigenvalue) of the PC1, PC2, PC3 PC4 and PC5 were 52.0, 23.3, 12.59, 9.47 and 1.5 respectively. With the exception of one freeze dried sample, PC1 and PC2 was able to classify freeze dry and spray dried extracts (Figure 2). This classification shows that in spray dried and freeze dried extracts the compounds are in different amounts.
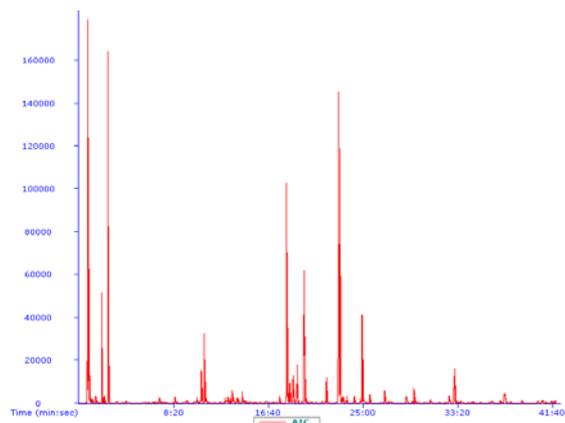

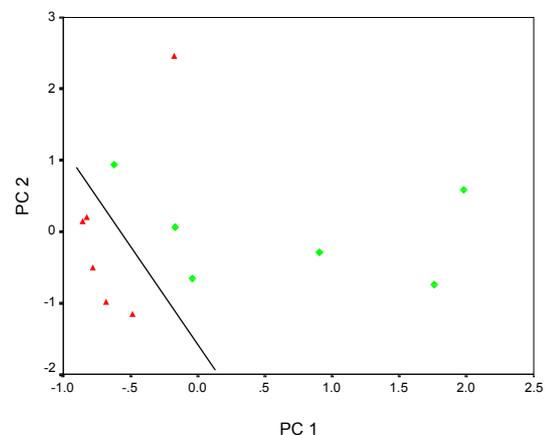**Figure 2**: SPME-GC-MS profiles of *Eurycoma longifolia* extracts. TA Sabah Acetone freeze dried

Figure 3: PC1 and PC2 plot of GC-MS data. (◆) Spray dried extracts and (▲) freeze dried extract.

3.3 Correlation between GC-MS and QCM

The correlation between QCM array sensors and 9 identified compounds using GC-MS was done using Varimox Rotated Principal Component Analysis. The plot of the first and second principal component was able to classify freeze dry spray dried extracts. The factor loading values above 0.3 of the identified compounds are shown in the Table 3. The identified compound, 3-phenoxy-1-propanol; 2-phenoxy-ethanol and benzoic acid has high loading value for PC1, Curcumene and, 4-ethynyl-4-hydroxy-3,5,5-trimetyl-2-cyclohex-1-enone has high loading to PC2, acetic acid and octanoic acid has high loading to PC3 and (R)-(-)- Massoilactone has high loading to PC4. The factor loading values above 0.3 of the sensors to the principal components are shown in Table 4. Sensor APZ-L, PEG4000, TOMA and oleyl amine has high loading for PC1, DOP have high loading for PC2, ethyl cellulose has high loading for PC3.

The correlation between QCM and GC-MS regression factor score are shown in the Table 5. The correlation between QCM and GC-MS regression factor score shows that QCM factor score 1 has high correlation with GC-MS factor score 3 (0.801). This result indicates that APZ-L, PEG-4000, TOMA and OAm sensors are more sensitive to acetic acid and octanoic acid. QCM factor score 2 has high correlation with GC-MS factor score 4 (0.591). This indicates that DOP sensor is more sensitive to (R)-(-)-Massoilactone. QCM factor score 3 has high correlation with GC-MS factor score 4 (0.628). This result indicates that ethyl cellulose sensor response to (R)-(-)- Massoilactone.

4 Conclusion

The *Eurycoma longifolia* extracts were classified based on the 9 common volatiles identified by SPME coupled with GC-MS present in all the samples. The headspace volatiles has been successfully used to classify according to freeze or spray dried extracts. Some correlation was found between the headspace volatiles and the quartz crystal sensors for the classification of plant extracts. Thus, high sensitivity of the e-nose to small changes in the smell-source offers an opportunity to evaluate herbal extracts. The method could be useful for the rapid evaluation of herbal volatiles.

Table 3: Factor loadings of the Varimax Rotated Principal Component Analysis of QCM data

Sensor Material	Principal Component							
	1	2	3	4	5	6	7	8
APZ-L	.767	.356	.432					
PPG 1200	.566	.530			.542			
PEG 1000	.538	.555		.564				
PEG 4000	.701	.529	.317					
EC			.971					
DOP	.453	.851						
TOMA	.844	.390						
Oleylamine	.829	.398	.340					

Table 4: Factor loadings of the Varimax Rotated Principal Component Analysis of GC-MS data

Volatiles	Principal Component							
	1	2	3	4	5	6	7	8
Acetic acid			.983					
Curcumene		.923						
(R)-(-)- Massoilactone	.325	.349		.878				
3-phenoxy-1-Propanol	.711	.626				.315		
Octanoic acid		.575	.712	-.367				
2-Phenoxy- ethanol	.942							
4-ethynyl-4-hydroxy-3,5,5-trimetyl-2-Cyclohex-1-enone		.836		.313	.304			
Benzoic acid	.977							

Table 5: Correlation between QCM REGR factor score and GC-MS REGR factor score. The parenthesis indicate the significance level

		QCM REGR factor score				
		1	2	3	4	5
GC-MS REGR factor score	1	-0.190 (0.553)	-0.406 (0.190)	-0.494 (0.103)	-0.154 (0.632)	-0.327 (0.300)
	2	-0.176 (0.583)	-0.101 (0.755)	0.332 (0.292)	0.382 (0.220)	0.020 (0.951)
	3	0.801** (0.002)	-0.058 (0.857)	-0.172 (0.593)	0.295 (0.352)	0.272 (0.392)
	4	-0.089 (0.783)	-0.591* (0.043)	0.628* (0.029)	-0.183 (0.570)	-0.225 (0.483)
	5	0.296 (0.351)	-0.437 (0.156)	-0.196 (0.542)	0.348 (0.268)	0.462 (0.131)

** Correlation is significant at the 0.01 level.
* Correlation is significant at the 0.05 level.

5 Acknowledgement

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