

Full paper

## Development of Multichannel Artificial Lipid-Polymer Membrane Sensor for Phytomedicine Application

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**Abstract:** Quality control of herbal medicines remain a challenging issue towards integrating phytomedicine into the primary health care system. As medicinal plants is a complicated system of mixtures, a rapid and cost-effective evaluation method to characterize the chemical fingerprint of the plant without performing laborious sample preparation procedure is reported. A novel research methodology based on an in-house fabricated multichannel sensor incorporating an array of artificial lipid-polymer membrane as a fingerprinting device for quality evaluation of a highly sought after herbal medicine in the Asean Region namely *Eurycoma longifolia* (Tongkat Ali). The sensor array is based on the principle of the bioelectronic tongue that mimics the human gustatory system through the incorporation of artificial lipid material as sensing element. The eight non-specific sensors have partially overlapping selectivity and cross-sensitivity towards the targeted analyte. Hence, electrical potential response represented by radar plot is used to characterize extracts from different parts of plant, age, batch-to-batch variation and mode of extraction of *E. longifolia* through the obtained potentiometric fingerprint profile. Classification model was also developed classifying various *E. longifolia* extracts with the aid of chemometric pattern recognition tools namely hierarchical cluster analysis (HCA) and principal component analysis (PCA). The sensor seems to be a promising analytical device for quality control based on potentiometric fingerprint analysis of phytomedicine.

**Key words:** Lipid membrane sensor; Fingerprint profiling; Quality control; Phytomedicine; *Eurycoma longifolia*; Chemometric.

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## 1. Introduction

Quality control and standardization of phytomedicine poses many analytical challenges as herbal medicine is a complex system of mixture. Moreover, medicinal herb like other natural products exhibit significant variations in quality and quantity of phytochemicals due to species variation, different harvest time, growing conditions and processing. Standardization and quality control is the fundamental step towards modernization and development of herbal medicine into evidence-based medicines. Therefore, novel approaches and methods capable to ascertain consistency and repeatability of a particular herbal formulation are being sought after in recent years.

In medicinal plant research, chromatographic techniques for example thin layer chromatography (TLC), gas chromatography (GC) and high performance liquid chromatography (HPLC) are commonly used to obtain a characteristic fingerprint profile that represent the presence of a particular chemical constituents in a particular herbal sample [1]. However, the conventional separation-detection method are time consuming, expensive and depends largely on the polarity of the mobile phase (TLC and HPLC) and volatilities of the compounds (GC) to be separated.

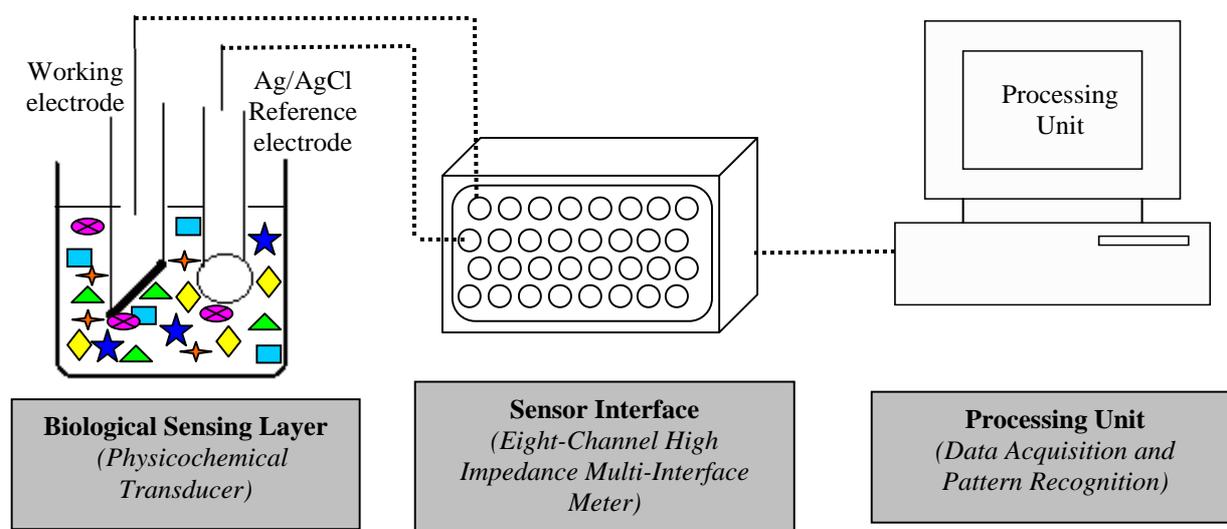
Considering the above mentioned analytical challenges, our research group in School of Material Engineering, Northern University College of Engineering Malaysia and School of Pharmaceutical Sciences, Universiti Sains Malaysia are currently embarking on an innovative, fast, economical and the most importantly accurate and repeatable analytical methodology. This is achieved through the integration of the state-of-the-art technology mainly for application in the phytomedicine industry. Recently, we reported our attempt of extending the areas of application of the electronic nose [2-3] and the electronic tongue [4-8] in phytomedicine applications using fingerprint analysis for quality control of phytomedicine.

As for the time being, the used of fingerprinting analysis to appraise the quality and to standardize phytomedicine formulation are mainly based on chromatographic separation techniques [9-12]. We report an innovative non-separation method utilizing an in-house developed sensor for performing qualitative profiling of a highly sought after herbal medicine in the Asean Region - *Eurycoma longifolia* or locally known as Tongkat Ali. The sensor is used for (1) potentiometric fingerprint profiling of the *E. longifolia* raw and extract samples, (2) identification variation of the chemical constituent from *E. longifolia* with different parts of plant, stage of maturity, batches and mode of extraction and (3) classification of *E. longifolia* extracts treated with different solvent system by chemometric analysis.

The multichannel artificial lipid-polymer membrane sensor are based on the principle of the bioelectronic tongue that mimicking human gustatory system through the incorporation of artificial lipid material as sensing element [13-14]. Figures 1 illustrate the system setup of the three main sensor components which consist of the biological sensing layer as the electrochemical transducer, the sensor interface and the processing unit. The sensor contains of eight non-specific sensor coated with different artificial lipid-polymer membranes with partially overlapping selectivity and cross sensitivity toward the complex mixture of liquid herbal sample.

Generally, the working principles of the sensor are as follow. Firstly, variations in the electrochemical properties of the chemical substances in herbal solution undergo different mechanism of response involving both electrostatic and hydrophobic interactions with the lipid membrane.

Secondly, the characteristic electrical response is acquired using a high impedance multi-interface meter. Thirdly, the generated potential electrical response was visualized using a radar plot in order to observe simultaneously the characteristic potentiometric fingerprint of a particular herbal sample. Lastly, multidimensional output of the sensor array was further analyzed using pattern recognition tools namely hierarchical cluster analysis (HCA) and principal component analysis (PCA). By using these two chemometric analysis algorithms, relevant information regarding the physicochemical properties of the analyzed liquid were extracted from the raw sensor readings thus help the analyst to correlate the observed pattern with the phytochemistry of the herbs.



**Figure 1.** Schematic diagram of the artificial lipid-polymer membrane based sensor system. Notes: 1) For simplicity, only single working electrode is show. 2) Different shape and color represents the characteristic chemical constituent present in the complex liquid herbal solution.

## 2. Experimental

### 2.1. Plant materials

All *E. longifolia* raw samples used in this study were supplied by the Forest Research Institute of Malaysia (FRIM). Different parts of plant (stem, leaves, branch and root) and plant harvested at different maturity stages (one year, two years and three years) were all represented with three different batches (batch 07, batch 09 and batch 12) except stem with batch 07 and batch 09 only. For preparing different solvent extract samples, the herbs were dried at 50°C and ground. The powdered plant of about 2 gm were refluxed for 5 min with 40 ml of solvent system (methanol, ethanol, chloroform and water) all of analytical grade and evaporated to dryness in a rotary evaporator to yield a dried extract. The dried extracts were reconstituted with 50 ml of deionized distilled water before potential measurements.

## 2.2. Artificial lipid-polymer membrane preparation

Eight kinds of lipid analogs (Table 1) were used for membrane preparation. The lipid materials and the sensor array arrangement are similar to those reported [15], with an addition of channel no. 8 (DOP:TOMA=9:1). Each lipid was mixed in a small beaker with 200 mg selectophore grade polyvinyl chloride (PVC) (Fluka Chemika, Switzerland) and 0.25 ml plasticizer dioctyl phenylphosphonate (DOPP) (Aldrich Chemical) and dissolved in 10 ml tetrahydrofuran (THF). The solution was then poured into the glass casting ring resting on the glass plate. A filter paper with weight was placed on top of the glass ring and left for a sufficient time (24 hours) to allow the solvent to evaporate. The lipid polymer membranes thus prepared are transparent, colorless as a soft film of 200  $\mu\text{m}$  thickness.

**Table 1.** Lipid materials used for membrane preparation.

Channel No.	Electrode Charge	Lipid Material
1	negative	Decyl alcohol (DA)
2	negative	Oleic acid (OA)
3	negative	Dioctyl phosphate (DOP)
4	neutral	DOP:TOMA=5:5 (D:T=5:5)
5	positive	DOP:TOMA=3:7 (D:T=3:7)
6	positive	Trioctyl methyl ammonium chloride (TOMA)
7	positive	Oleyl amine (OAm) (Decyl alcohol + Oleyl amine)
8	negative	DOP:TOMA=9:1 (D:T=9:1)

## 2.3. Multichannel sensor electrode fabrication

Stable Ag/AgCl coating was prepared by electrolytic method. The silver wires (Johnson Matthew, Switzerland) of 0.5 mm diameter and 2.5 cm length was thoroughly cleaned with detergent and then washed with multiple rinses of distilled water. The silver wires were then placed in concentrated  $\text{HNO}_3$  for one hour, following which were thoroughly washed with distilled water. Silver chloride was then electrically deposited on the silver wire. A solution of 0.1 N NaCl titrated to pH 11 to 12 with 6 M NaOH was used. A 5 to 10 mA current was then passed through the electrolytic cell for 30 minutes with the silver wire acting as positive electrode and a platinum wire serving as the negative electrode [16].

The lipid membranes were fitted on the bottom of the PVC tube. A glass tube is inserted through the other end of the PVC tube. The PVC tube was then filled with 3 M KCl solution. The other end of the glass tube was sealed with a stopper that held the Ag/AgCl electrode. The Ag/AgCl wire was immersed in the 3 M KCl solution. To prevent faster leaching of the lipid due to the opposite charge of

the lipid membrane, eight kinds of lipid membranes were separated into two groups according to their charges. Electrical potential measurements were taken from two separate beakers.

#### 2.4. Electrical potentiometric measurement

One Ag/AgCl reference electrode (Orion Research Inc., Switzerland) was used for each group of electrodes. The electrical potentials between the measuring electrodes and the Ag/AgCl reference electrodes were measured using Eight-Channel High Impedance Multi-Interface Meter (Fylde Scientific, U.K.). Prior to use, the electrodes were conditioned in 1mM KCl for one hour for better sensitivity and reproducibility [13] followed by washing with deionized distilled water. Then, the electrodes were left in the sample for one minute before taking reading for another minute. Each sample was measured three times. The electrodes were washed twice, each time for one minute and for each consecutive measurement with deionized distilled water to avoid charge carry over effect.

#### 2.5. Chemometric data analysis

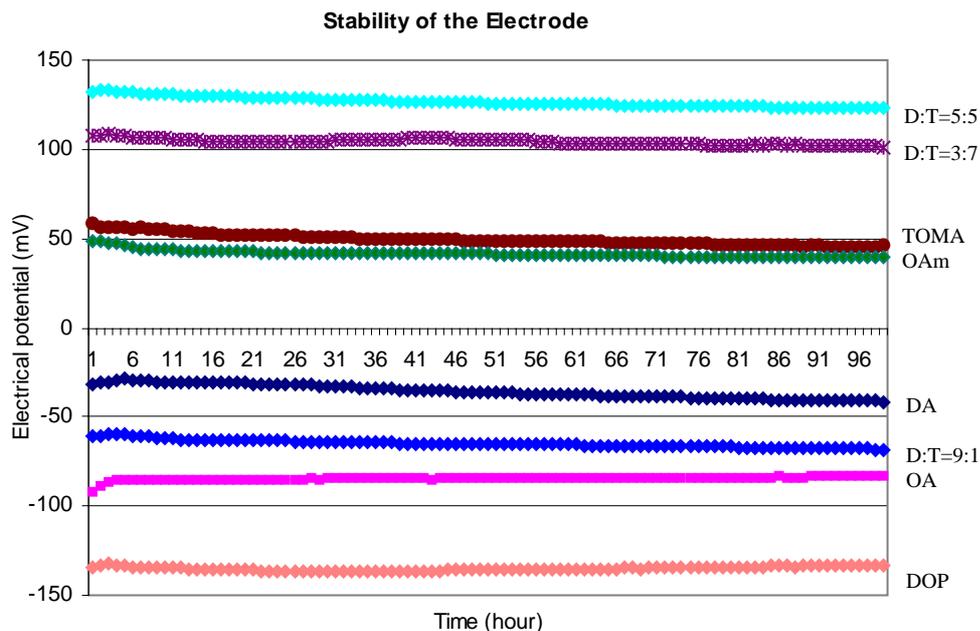
The electrical potential response of the sample was measured as the difference between the potential in the sample and in 1 mM KCl. The subtracted electrical potential data were then treated with suitable chemometric pattern recognition algorithms namely hierarchical cluster analysis (HCA) and principal component analysis (PCA) using statistical software package SPSS 11.5 for Windows.

### 3. Results and discussion

#### 3.1. Evaluation of the sensor array performance

In order to determine the stability of the eight lipid membrane over a period of time at room temperature ( $25 \pm 2^{\circ}\text{C}$ ), the electrode potential were recorded every 60 sec up to 98 hours or equivalent to four consecutive days in 1 mM KCl. The electrical potential of negative charge electrode DOP, DA and D:T=9:1 drift a little for the first one hour (Figure 2). The standard deviations of the electrode DA, OA, DOP, D:T=5:5, D:T=3:7, TOMA, OAm and D:T=9:1 electrical potentials were 0.42, 1.80, 0.97, 0.24, 0.32, 0.85, 0.50 and 0.52 respectively. After one hour, the electrode were almost stable and the standard deviation were 0.12, 0.05, 0.06, 0.13, 0.14, 0.24, 0.13 and 0.10 for DA, OA, DOP, D:T=5:5, D:T=3:7, TOMA, OAm and D:T=9:1 respectively. The standard deviation remains about constant till after 98 hours. Therefore, preconditioning for one hour in reference solution 1 mM KCl is a necessary step before sensor measurement enables repeatable results of sufficient accuracy to be obtained over an extended period of time. Beside this, sufficient 'settling-down' time or response time of about one minute to allow the system to come to equilibrium with the sample solution [17] before taking sensor reading is also an important factor that affecting the sensor performance.

Reproducibility of the electrode readings is limited by factors such as temperature fluctuations, drift and electrical noise [17]. Hence, the prepared samples and the reference solutions were kept at room temperature before and after the sensor measurement in order to prevent changes of the electrode potentials due to temperature effect. Consequently, all the sensor measurement was carried out under optimum control conditions by using the developed testing method.



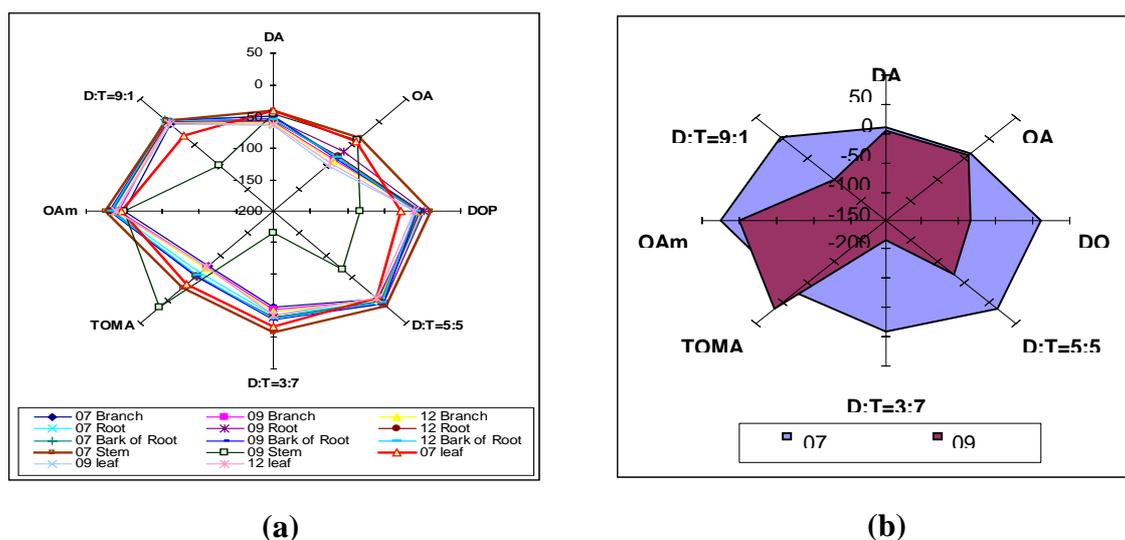
**Figure 2.** Time course of sensor array response electrical potentials in 1 mM KCl reference solution.

### 3.2. Characterization of *E. longifolia* by fingerprinting profiling analysis

The eight dimensional electrical response signals pattern generated from the sensor array provides a unique potentiometric fingerprint that might be a good representation of the complex chemical matrices presence in a particular herbal sample. The rationale behind the development of fingerprinting method towards standardization and quality control of phytomedicine are based on the observation that the pharmacological properties of a wide variety of herbal medicine is the results of the combined action of several active and non-active compounds that work synergistically to the targeted cells in our human body system.

#### 3.2.1. Potentiometric fingerprint profiling of *E. longifolia* from different parts of plant

The electrical potential patterns of *E. longifolia* from different parts of plant are shown in Figure 3. As can be seen, comparison using the relative electrical response signal intensity found that variability in the chemical constituent was found not significant with respect to different parts of plant [Figure 3(a)]. Although, the potentiometric fingerprint profiles of most of the plant parts are comparable with each others in the sensitivity range of the sensor, but stem from batch 09 show significant different fingerprint as illustrated in Figure 3(b). From the obtained fingerprint profile, we can implicit that inter-batch variation does occur in the stem samples investigated in this study. Batch-to-batch inconsistency of the raw herbal material may influence the quality of the final phytomedicine product as the concentration of the plant constituents can vary considerably. Hence, this section demonstrates the used of the developed potentiometric sensor for primary screening of the supplied raw herbal materials for batch-to-batch uniformity.



**Figure 3.** (a) Overall comparison of different plant parts by the relative electrical response signal intensity and (b) typical potentiometric profile of the stem of *E. longifolia* from batch 09.

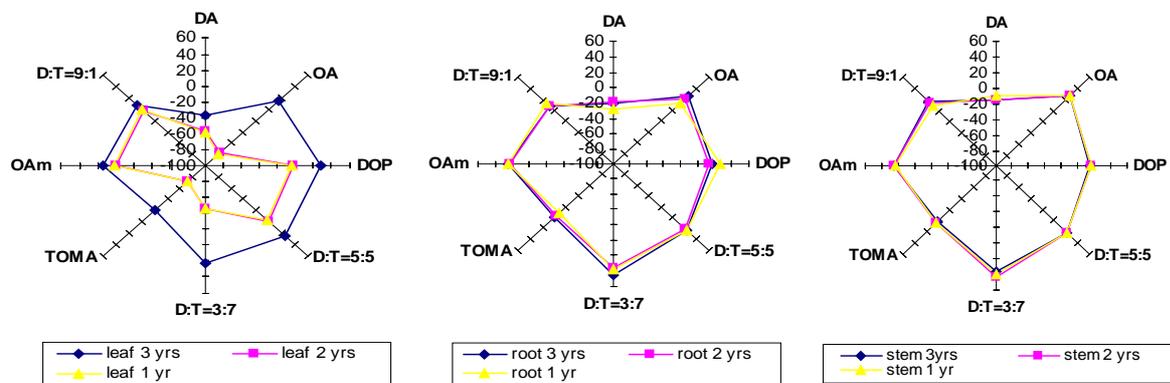
### 3.2.2. Potentiometric fingerprint profiling of *E. longifolia* harvested during different stage of maturity

It is well documented that the level of the plant bioactive constituent may vary substantially depending on environmental and genetic factor [18–19]. Among others, species variation, growing conditions, time of harvesting and not to mention the production and processing of the phytomedicinal product are the determining factors for herbal quality. Therefore, monitoring of the different stages of maturity of different parts of plant of *E. longifolia* was carried out in this study. The typical potentiometric fingerprint profile of *E. longifolia* leaf, root and stem during different stage of maturity at one, two and three years of cultivation are shown in Figure 4. For a reasonable qualitative comparison, the scalar effect on the fingerprint profile was eliminated by plotting the radar plot using the same scale factor. At sensitivity level of the sensor measurement, young leaf samples with one and two years of cultivation have significant differences in chemical constituent compare to others. On the contrary, the fingerprint profiles of the matured leaves under three years of cultivation are similar to the other parts of plant that are root and stem. This result is in agreement with those reported [20] as total quassinoid content was noticeably lower in leaves as compared to other plant part (i.e. root and stem). Furthermore, an active principle for aphrodisiac properties namely eurycomanone was high in both root and stem but was found absent in leaves of *E. longifolia* extract.

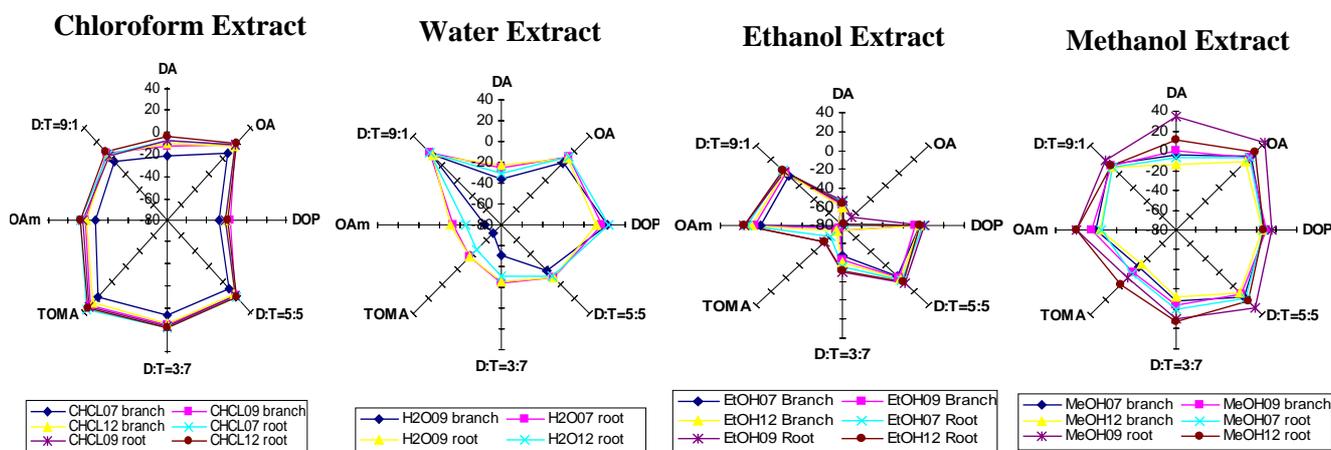
### 3.2.3. Potentiometric fingerprint profiling of *E. longifolia* extracted with different mode of extraction

The radar plot in Figure 5 give good characterization of the solvent system used to extract the *E. longifolia*. Overall, significant differences of the potentiometric fingerprint of different solvent extract especially water and ethanol extract were recorded. As solvent polarity and solubility of the plant constituent in the solvent might altered the absolute concentration of the certain class of compounds (i.e. lipid soluble and water soluble) extracted during the extraction process. Correspondingly, different part of plant only contributed slight differences in the signal intensity as the chemical

fingerprint are identical for both branch and root samples. Therefore, it can be concluded that solvent extract mainly contributed to variability of the phytomedicine chemical constituents, hence play an important role in extracting a certain biological active fractions present in a particular herbal medicinal plant. This study shows that the in-house developed potentiometric sensor is capable to be served as a fingerprinting device for qualitatively classifying the solvent extraction system.



**Figure 4.** Typical potentiometric fingerprint profile of *E. longifolia* leaf, root and stem during different stage of maturity at one, two and three years of cultivation.



**Figure 5.** Typical potentiometric fingerprint profile of *E. longifolia* under different mode of extraction.

### 3.3. Classification of *E. longifolia* using chemometric analysis

#### 3.3.1. Hierarchical Cluster Analysis (HCA)

In HCA, the geometrical distance which implies the degree of similarity among the studied samples (variables) in the multivariate data set is calculated and compared. A group of objects (samples) will be assign to its respective classes (cluster) so those similar objects are in the same classes [21]. This classification algorithm enables us to visualize grouping of the untreated data into cluster, thus provide additional insight of the sample attributes. Average Linkage (between groups) method and square

Euclidean distance as geometric distance measure between objects were used. The horizontal scale (0-25) gives pictures of similarity and dissimilarity among the study samples.

The dendrogram in Figure 6 clearly shows that four distinct clusters (A-D) were form at similarity distance of five. In general, most of the samples tend to cluster together according to its solvent system rather than according to the part of the plant. This classification results were in agreement with the above potentiometric fingerprint pattern. As the five representative *E. longifolia* extracts namely chloroform extract, methanol extract, water extract and ethanol extract form cluster A, B, C and D respectively. Therefore, once again we can conclude that the polarity of the solvent play an important role in determining the extraction of a certain bioactive fraction of the herbal extract. For example, water extract from the *E. longifolia* is use for its male aphrodisiac properties [22] whereas the methanol extract is intently used for antimalarial and antiplasmodial activity [23].

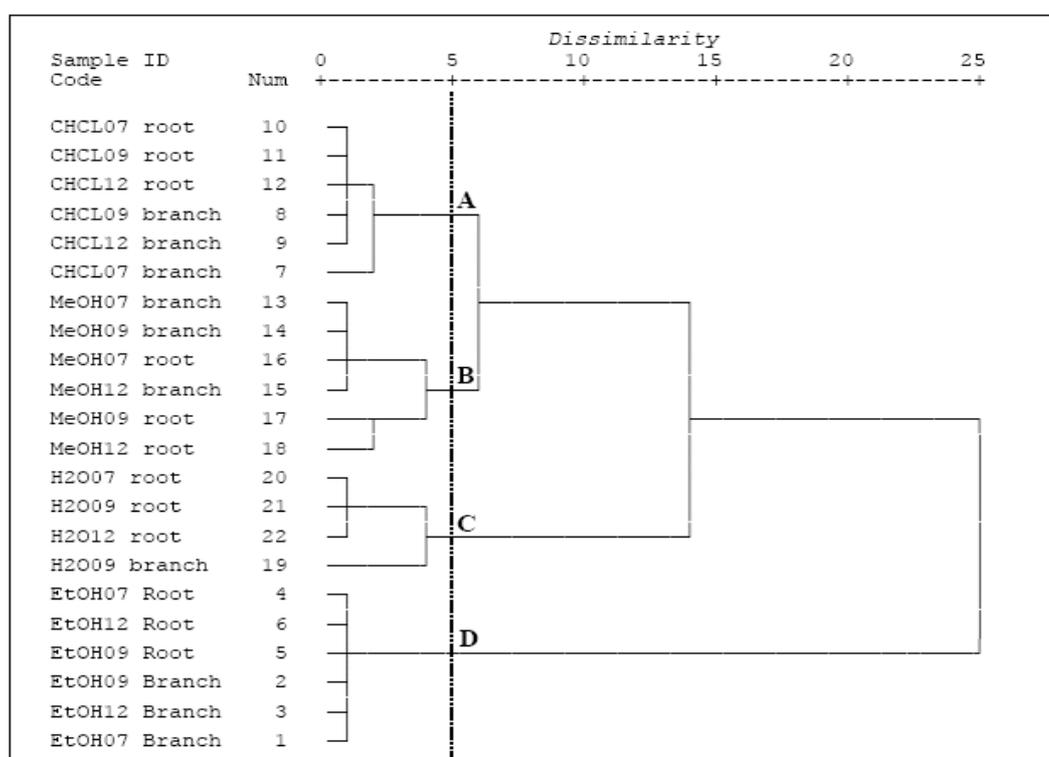


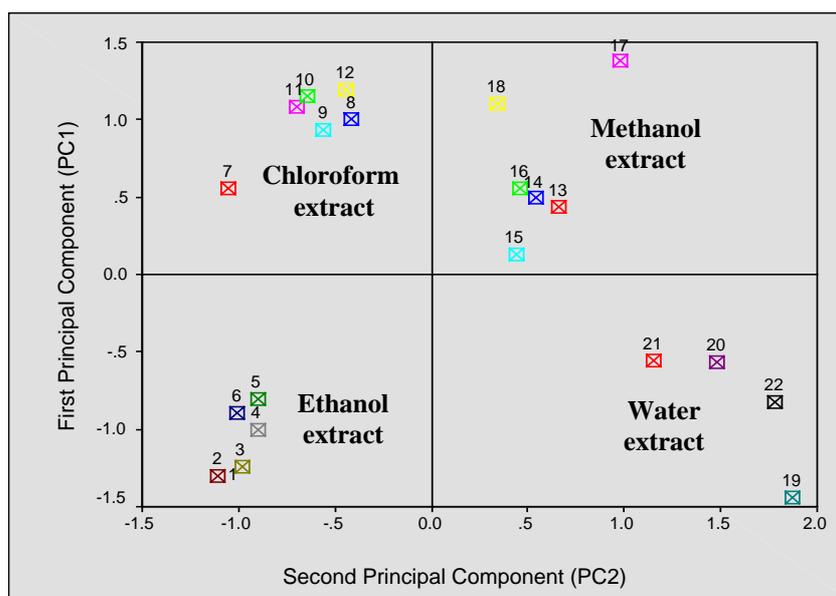
Figure 6. Dendrogram of cluster analysis on different mode of extraction *E. longifolia* samples.

### 3.3.2. Principal Component Analysis (PCA)

The original multidimensional sensor response were visualize using PCA with the purpose to reduce the dimensionality of the original variables to two or three dimensional scores plot that represent most of the chemical information contain in the original data set [21]. Natural grouping of various solvent extract *E. longifolia* samples were visualize in the two-dimensional score plot as shown in Figure 7. The identity of the sample extracts were label using numerical value, same as the above dendrogram (Figure 6). The first two principal components (PCs) represent 87.2% of the total variance with the first PCs consist 53.4% of the total variability followed by the second PCs with only 33.8%.

As can be seen, the *E. longifolia* sample extracts tend to cluster together based on its solvent system and clearly separated into four quadrants of the scores plot. The first PCs differentiate the chloroform

and methanol extract that having similar potentiometric fingerprint profile as indicate in Figure 5 from the ethanol and water extract samples. On the other hand, second PCs separated methanol and water extract on the positive region of the x-axis whereas chloroform and ethanol extract were clearly separated on the negative regions of the x-axis in the scores plot. Once again, we can conclude that considerable variability in the phytochemical content of the various solvent extract samples was found. The obtained classification model could be used to classify phytomedicine treated with different solvent system accordingly.



**Figure 7.** The scores plot of various solvent extract *E. longifolia* samples in two dimensional spaces.

#### 4. Conclusion

The in-house fabricated multichannel artificial lipid-polymer membrane sensor possesses the capability to qualitatively determining different parts of plant, maturity stage, batch-to-batch variation and mode of extraction of *E. longifolia* through the acquired potentiometric fingerprint and chemometric analysis. With the incorporation of the sensor, quality control of herbal medicine no longer a time consuming, complicated and expensive task to be accomplished as compared to conventional chromatographic separation techniques. However, further study that emphasized on quantification of the marker compounds in the complex herbal mixture is needed for fully demonstrating the analytical capability of the developed sensor in phytomedicine application.

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