Organoleptic Assessment of *Centella asiatica* by Taste Sensor

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Abstract

Medicinal plants and herbal preparations are usually evaluated utilizing methods such as physico-chemical, chemical, biological and pharmacognosical techniques. Organoleptic parameters such as taste, aroma and color are still useful to identify certain plants. The medicinal plants and their extracts possess characteristic odour and taste, which indicate their presence. Taste is based on the reactions of human senses when test materials come in contact with our tongue. We developed a multichannel taste sensor to identify and quantify taste. The sensor uses arrays of transducer (eight electrodes) composed of lipid polymer membranes to mimic the human tongue. It has the advantage of identification and quantification of tastes. The sensing principle in general, is based on the pattern of the electrical potentials of the eight electrodes for a sample. The potential data of the samples were analyzed using multivariate analysis of principal component analysis (PCA) to give characteristic chemical fingerprint. Different accessions of *Centella asiatica* extracts were successfully identified and assessed qualitatively and quantitatively by the taste sensor and is found to complement tastes experienced by human tongue.

Keywords: Organoleptic assessment; Medicinal Plants; Taste sensor; Chemical fingerprint.

Introduction

Plant materials have been known for their medicinal properties since long ago. Herbal medicine has been practiced by various ethnic groups long before the introduction of modern medicines in different countries. Currently the world is experiencing a wave of herbal revival and the issues related to quality and standards are being addressed as priorities.

A suitable standardization method is needed for the growing demand of herbal medicine. Typically, herbal standardization process involves microscopic, gravimetric, chromatographic and organoleptic assessment and uses of equipment such as GC, HPLC, TLC, HP-TLC to fingerprint and quantify marker compounds in the plant. The problem with this type of standardization is that there are hundreds of chemical compounds in the plant and only a few of them are identified. Yet, at the same time, more and more evidence is validating the usefulness of plant medicines, including the substantiation of synergism, where the clinical effect is more efficacious, less toxic or both than the isolated ingredients.

The taste of food and herbals are mainly evaluated by taste sensory organs, which provide objective scale of human expression. This method nevertheless, requires the training of human specialists. The evaluation differs from individuals and depends on the physical and mental condition of the person involved.

The Multichannel Taste Sensor is a device that mimics the human gustatory (taste) sense. This sensor has a "global selectivity". It has the ability to classify chemical substances to five basic taste qualities such as sourness, saltiness, sweetness, bitterness and umami (deliciousness). The sensor does not measure the amount of specific molecule but rather the taste quality and intensity as a whole. In the present paper, the taste sensor was used to obtain the characteristic fingerprint of *Centella asiatica* extracts and the compounds

isolated from the extract of the plant. The resulting fingerprint is useful for the identification and quantification of the herb.

Methods

Reagents: Poly(vinyl chloride) (PVC) used for membrane preparation was selectophore grade (Fluka Chemika, Switzerland). The lipids used were obtained from the following sources: oleic acid and decyl alcohol (~99.99%) from Fluka Chemika, Switzerland; trioctyl methylammonium chloride and dioctyl phosphate from Tokyo Chemical Industry, Japan, dioctyl phenylphosphate (plasticizer) (95%), oleyl amine from Aldrich Chemical. Chemicals and solvents used in the analytical procedure were all of analytical grade.

Samples - Ethanol extracts: *Centella asiatica* samples were supplied by the Forest Research Institute of Malaysia (FRIM). We studied different accessions of *Centella asiatica*. The herbs were dried at 50°C and grinded. Powdered plant (70gm) for each of the sample CA02, CA03, CA05, CA06 and CA11 were extracted in a Soxhlet extractor with ethanol for 48 hours and filtered. The extracts were evaporated to dryness under vacuum at 40°C in a rotary evaporator to yield dried extracts. From the crude dried extracts, 0.1%, 0.03%, 0.01%, 0.003% and 0.001% solutions were prepared.

Water and methanol extracts: Powdered *Centella asiatica* (2 g) for each of the sample CA02, CA03, CA05, CA06 and CA11 were refluxed with 40 ml water for 15 minutes and filtered. Methanol extractions of the samples were prepared by refluxing 2 g powdered samples for 15 minutes. The solutions were filtered and evaporated to dryness, and dissolved in distilled water to make 40 ml solution.

TLC separation: Crude ethanol extract (100 mg) was dissolved in 2 ml methanol and spotted on TLC plates. The TLC plates were developed in a solvent system chloroform/glacial acetic acid/ methanol/water as 100:40:16:8 ratios and were scraped at the areas having same hRf values as asiatic acid, madecassic acid, asiaticoside and madecassoside standards. Each fraction was extracted with 20 ml methanol for 3 times and the pooled extract was evaporated to dryness at 40°C. The dried fractions were made up to 25 ml with distilled water. Standard solutions of asiatic acid, madecassic acid, asiaticoside and madecassoside were prepared by dissolving 0.2 mg of standard in 25 ml distilled water in each case.

Taste sensor The detecting part, transducer of the sensor consists of eight lipid/polymer membrane electrodes. The lipid materials used and membran preparations are similar as reported by Wada with a additional channel No. 8 (DOP:TOMA=9:1). Th lipids were mixed with PVC and plasticizer in a tes tube, dissolved in tetrahydrofuran (THF) and cast int glass ring resting on the glass plate. The lipid membran thus prepared was a transparent colourless soft film abou 200mm thick. Each electrode was made of a silver wir whose surface was plated with Ag/AgCl, with an interna cavity filled with 3M KCl solution (Figure 1).

Negative charged electrodes (Channel 1,2,3 and 8) an positive charged electrodes (Channel 4,5,6 and 7) wer separated to prevent interferences. The potentia difference between the electrodes and referenc electrode (Ag/AgCl with saturated KCl) were measure using 8-channel high impedance Multi-Interface mete from Fylde Scientific U.K.

The electrodes were conditioned in 1mM KCl for 1 hou The electrical potential of the electrodes were measure for one hour to observe the performance of the electrod The measurements were taken from two separate beake and two reference electrodes were used. Eac measurement was taken for one minute. The sample w measured from lower to higher concentration and each tin electrodes were washed at least twice to avoid char carryover effect. The potential difference was taken as th difference between sample and 1mM KCl.

Principal component analysis: Principal compone analysis is a statistical technique that linearly transfor an original set of variables into a substantially small set of uncorrelated variables that represent most of t information in the original set of variables. Its goal is reduce the dimensionality of the original data s without losing information of the total variation of t original data set. Applying this method the respecti measurements are grouped into a database correspondi to the sensor array response matrix.

Result and discussion

Ethanol extracts: Figure2 shows the respon electrical potential pattern of different accessions a concentration of *Centella asiatica* ethanol extracts. T electrical potential of the electrodes change depends taste. The herbal's main ingredients cause the electri potential of the membranes to change. The original d were expressed on eight-dimensional spaces from ei kinds of membranes. The data were analyzed us principal component analysis, which is a kind multivariate analysis to reduce the dimension with losing information.

A plot of the PC1 and PC2 obtained from electrical potential data is shown in the Figure 3(a). The contribution rates of the original data to PC1 and PC2 are 74.13% and 12.44% respectively. If we look into PC1 vs. PC2 plot an observation can be made that the PC1 value decreases in proportion to the total extract concentration. The plot of PC1 vs. log concentration is shown in the figure 3(b). PC1 can be regarded as reflecting concentration and PC2 can be regarded as reflecting different accessions.

Methanol and water extracts: Figure 4(a) shows the plot of PC1 vs. PC2 of leaf, stem, stolon and whole plant extract at different concentrations. The variances of PC1 and PC2 were 58.67% and 27.53% respectively. PC2 can be regarded as reflecting different plant parts of the plant. It indicates different chemicals present in different parts. Furthermore, for different parts of the plant, PC1 decreases as the concentration increases as shown in figure 4(b). We can get an equivalent *Centella asiatica* extract concentration by calculating PC1 from the response electrical potential of unknown sample, and using the relation between PC1 and *Centella* extract concentration.

Methanol and water extracts and isolated compounds: The taste sensor was applied to water extracts, methanol extracts, isolated compounds, and pure compounds followed by TLC separation in order to classify different extracts. Data obtained from the samples were analyzed by principle component analysis. The plot of PC1 and PC2 of obtained data is shown in the Figure 5. The aim of principal component analysis is to get correlation between isolate compounds and crude extracts. In this principal component analysis methanol extracts are along the negative axis and water extracts are along the positive axis. PC2 along the negative direction can be regarded more triterpene compounds (asiaticoside, madecassoside, asiatic acid, madecassic acid) because methanol extract contain more triterpene. PC1 along the positive direction indicates the presence of triterpene compound. Each group of the sample separates well and distinguishable.

Conclusion

The taste sensor shows similar pattern for the chemical substances producing same taste qualities, while quite different patterns were obtained for substances with different taste qualities. The sensor has a concept of global selectivity. Discrimination of minute change is not important here but rather to transform molecular information obtained from taste receptors into several classes or groups of taste qualities and intensities.

The result obtained from the measurement of *Centella asiatica* samples with the sensor shows that it can identify and quantify different accessions and extracts of *Centella asiatica*. The sensor system can serve as a means to control and regulate raw herbs, extracts and finished product quality.

The sense of taste largely depends on subjective factors of human feelings. If we compare the standard index measured by means of the taste sensor against human sensory evaluation, we will be able to assess the taste objectively and could complement other methods in evaluating the quality of raw herbs, their extracts and finished products.

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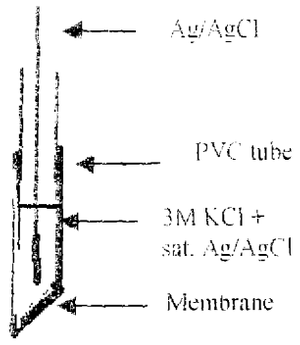

Fig. 1: Lipid polymer membrane electrode

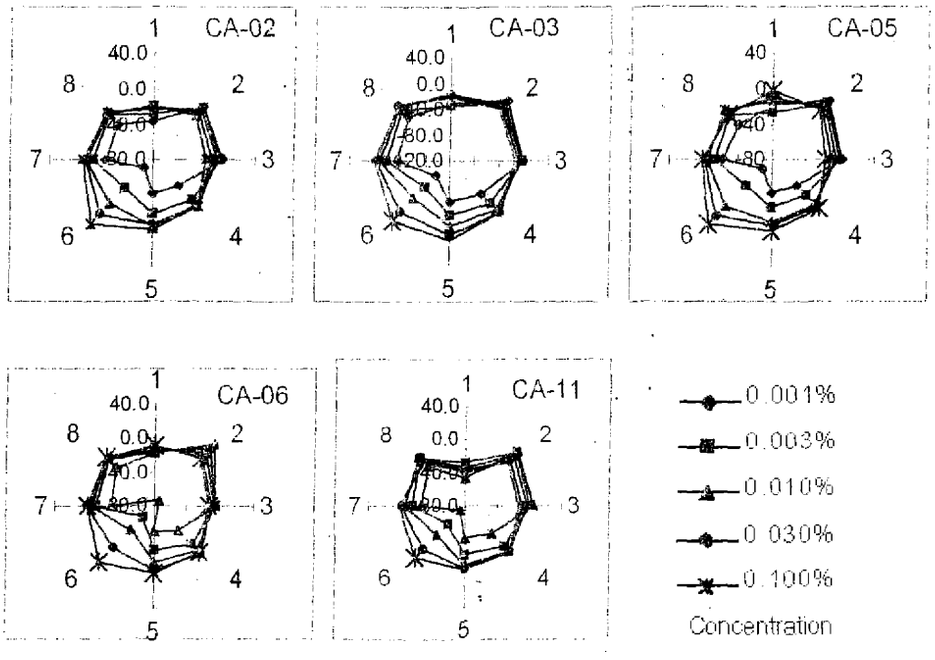

Figure 2: Response electrical potential fingerprint of Centella asiatica ethanol extracts by taste sen

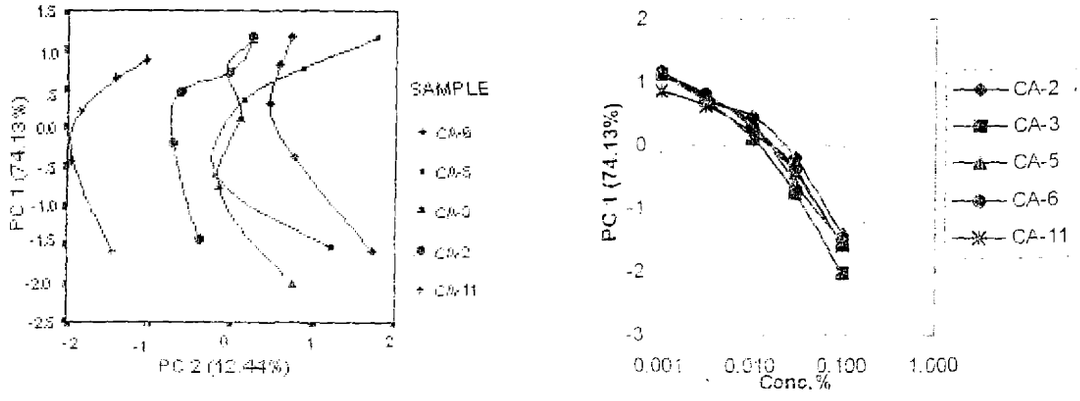

Figure 3 Plot of (a) PC1 vs. PC2 and (b) PC1 vs. concentration of crude ethanol extract of samples CA-03, CA-05, CA-06 and CA-11 at 0.1%, 0.03%, 0.01%, 0.003% and 0.001% concentrations.

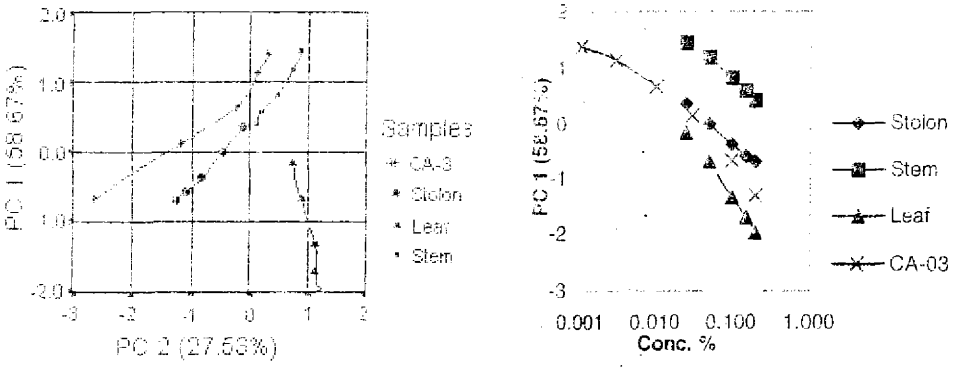

Figure 4: Plot of (a) PC1 vs. PC2 and (b) PC1 vs. concentration of methanol extract of CA-03 at CA-03 at 0.1%, 0.03%, 0.01%, 0.003 and 0.001%, and Water extracts of leaf, stem and stolon at 0.2%, 0.15%, 0.1%, 0.05% and 0.025% concentrations.

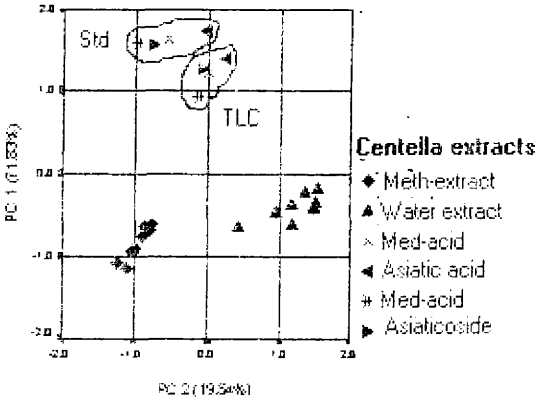

Figure 5: Asiaticoside, medicassoside, asiatic acid, medicasic acid (standard and TLC), Water extracts and methanol extracts,