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Photonic characterization of selected bioactive chemicals in Malaysian herbs

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Abstract

This work presents the characterization of selected bioactive compounds of local herbs through their photonic spectrum. A Shimadzu spectrophotometer was used to map bioactive compounds extracted from Andrographis paniculata, Ficus deltoidea, Orthosiphon stamineus and Centella asiatica. Dominant peaks and repeatability were the determinant parameters in this study. Absorbance peaks of bioactive compounds were calibrated against respective standardized chemicals obtained from reputable suppliers. The results obtained indicated that absorbance peaks from different bioactive compounds could be identified by photonic spectrum. Most bioactive markers were detected within the wavelength range of 200 nm to 350 nm, using a Deuterium (190 nm to 450 nm) light source and corresponding refraction grating. A mathematical relationship of concentration versus absorbance at different wavelengths for selected bioactive compounds were identified. The significance of the photonic characterization of these phytochemicals forms the basis for a mathematical model in a decision support system of a proposed mobile sensor prototype development.

Introduction

The herbal industry in Malaysia has been given a new life by the government when it embraced a policy recommendation to explore the country's rich biodiversity as a new resource for economic growth of the future. A number of promising herbal crops have been identified and selected as new commodity to be developed. The effectiveness of herbs as medicinal products or health foods can only be realized if the subsequent handling and processes retain most of its active compounds. Quantification of the active compounds in herbs and herbal products is considered an important issue in the herbal industry, especially to the processors and entrepreneurs. The bioactive compound of a herbal product might be lost due to industrial processing, packaging and storage procedure (Zafrilla *et al*., 2003; Noor Ismawaty *et al*., 2015). The storage duration of herbal products can be very long due to the logistics of the supply chain. The long storage duration of raw herbal drugs are prone to fungal contamination (Singh *et al*., 2008). The fungal deterioration adversely affects the chemical composition of the raw materials and thereby decreases the medicinal potency of herbal drugs (Dutta and Roy, 1987).

The current technology for evaluating the quality and authenticity of herbal medicines is based on extraction and wet chemistry quantification of bioactive compounds such as by high performance liquid chromatography (HPLC), gas chromatography and UV-VIS spectrophotometer (Yan *et al*., 2005; Han *et al*., 2011). These methods are expensive, require trained personnel, time consuming, and involve tedious sample cleaning (Ahmad *et al*., 2008; Iqbal *et al*., 2010).

Commercial application of the infra-red energy spectrum have been mainly for , N- , O-H and C-H based bond measurements (such as devices developed by National Instruments, Scientific Instrument, Foss-NIR Systems, DMA etc.) to quantify parameters such as moisture, protein, fat, sugar, acid and many other hydrocarbon compounds. They are also used for a wide range of food commodities, cosmetics, pharmaceutical products, fertilizers, minerals, fabrics, woods products, tobacco, etc. For instance, the FT-NIR analyzers developed by Yokogawa are used in gas analysis. A low cost portable NIR Spectrophotometer developed by getSpec.com is used for the detection of fat, protein and moisture.

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Other research works utilizes NIR spectroscopy to non-destructively detect internal defect and quality in agricultural produce (Ryu *et al*., 2002), while (Liu and Ying 2004) used the technique to measure sugar content in Fuji apples.

In view of the wide interest in herbs and herbal products and considering the herbal market size (health and pharmaceuticals market is worth US$ 42 billion), there is a demand for low cost, stable, portable and highly sensitive mobile bioactive sensors for the local herbs industry. This paper highlights the characterization of bioactive compounds using photonic spectrum, toward possibility of designing a dedicated sensor for field applications.

Materials and Method

Plant materials

Fresh leaves of A*ndrographis paniculata, Ficus deltoidea, Orthosiphon stamineus* and *Centella asiatica* were collected at commercial organic farms in Perlis, Tanjung Karang, Serdang and Melaka. The leaves were separated from stems and dried in an oven at 50°C for 24 hours. It was then ground by a hammer-mill (Cyun Tseh, Taiwan) and sieved using a 20 mesh screen. The powdered samples were stored at ambient temperature in obscurity and in a dry area.

Extraction process

For comparison purposes, three types of solvents (i.e. methanol, ethanol and water) were used for the extraction process. These solvents were chosen due to their different polarity characteristics. The extraction process for methanol and ethanol were the same, whereas extraction using water followed a different procedure.

Alcohol extraction

Two grams of dried powdered leaves were mixed with 200 ml of ethanol or methanol and the bioactive compounds were subsequently extracted by a Soxhlet for 8 hours. The alcoholic extract was then filtered using Whatman filter paper and concentrated in vacuum at 50°C using a rotary evaporator (Buchi rotovapor R-200) so that the dried bio-active compounds could be weighed. The dried crude extract was then dissolved in 10 ml of methanol or ethanol to be used for analysis. Each extraction was carried out in triplicate for ethanol and methanol.

Water extraction

Two grams of dried powdered leaves were mixed with 100 ml of distilled water, in a 150 ml amber bottle. The extraction was carried out at 50°C for 2 hours, with medium mode of sonic using ultrasonic cleaner (Jac 1505). The mixture was filtered through a Whatman filter paper to obtain a crude extract. The extract was frozen in a refrigerator at -80°C for 4 hours and then dried using a freeze-dryer until the extract becomes a powder. The dried crude extract was then dissolved in 10 ml of water for analysis. Each extraction was carried out in triplicate.

UV-VIS spectrophotometer analysis

In order to run the analysis, the extracts were diluted with their corresponding solvents (i.e. ethanol, methanol and water) respectively to obtain a 1:40 solution. The crude extracts containing the bioactive compounds were analysed using a Shimadzu UV-2450 model of UV-VIS spectrophotometer. Standard operating procedures of the spectrophotometer were followed according to its user manual (Shimadzu, 2012). The parameters observed for the spectrophotometer were light source, resolution, scan speed, slit width and scanned wavelength range. Apart from spectrophotometer settings, the type of sample holder material also influences the outcome of the analysis, namely the scanning efficiency. The absorption peaks of the bio-active compounds were calibrated against selected standardized chemicals obtained from reputable suppliers, namely andrographolide, sinensetin, vitexin and asiaticoside.

Results and Discussion

UV-VIS spectrophotometer setting

The optical resolution and throughput of a spectrometer is determined by the installed slit. Light entering the optical bench of a spectrometer is focused onto the pre-mounted and aligned slit. The slit then controls the angle of the light which enters the optical bench. The size of slit width directly affects output accuracy. Narrower slit widths produces a more crisp output compared to larger slit widths. The slit width of the spectrophotometer can be adjusted by installing a slit with a different sized width. They come in a number of different sizes and the most common are 0.5 nm, 1 nm, 2 nm and 4 nm. The slit width was set to the common values previously mentioned. Results indicated that slit setting of 1 nm gave the best output.

The spectrum range for the UV-VIS spectrophotometer was between 200 nm to 1100 nm. Initial scans done for the samples indicated that no significant data was detected above 400 nm. Therefore, the spectrum range above 400 nm was truncated for the experiment to reduce scanning time. The type of sample holder material also influenced the outcome of the analysis. A sample profile

captured using a plastic cuvette showed no peaks and only contained noise within the 190 nm to 400 nm range. Therefore, glass or crystal cuvettes were preferred over their plastic counterpart.

Repeatability

A known sample was repeatedly run for three cycles to check for repeatability of the spectrophotometer. The results indicated that the instrument has high repeatability where peaks were traced around the same wavelength at an absolute error of 0.032 nm as shown in Table 1.

Table 1. Observed peaks in repeated scans of the same sample on Shimadzu UV-2450 spectrophotometer

O. stamineus	Peak (nm)	Amplitude
Cycle 1	202.27	3.10
Cycle 2	205.81	3.07
Cycle 3	205.41	3.11
Average	205.49	3.09
Maximum absolute error	0.0032 (0.02%)	0.02 (0.65%)

Peaks of selected bioactive compounds

The peaks of the bioactive compounds studied, namely andrographolide, vitexin, sinensetin and asiaticoside appeared between 200 nm – 350 nm. These compounds either have a single peak, or a set of dominant and recessive peaks. For the sake of brevity, only the scanned result of sinensetin is shown in Figure 1. It can be seen from the figure that the absorbance falls to zero as the wavelength reaches 400 nm. This is true for the other studied bioactive compounds. Note that 400 nm is the upper boundary of the ultra violet spectrum. It can be concluded that the studied bioactive compounds reacts significantly to the ultra violet region of the light spectrum. The dominant peaks of andrographolide, vitexin, and asiaticoside as well as sinensetin are detailed in Table 2. There exist several dominant peaks for each studied compounds except for asiaticoside where only one dominant peaks was detected. It can be seen from Table 2 that some of the dominant peaks of the different compounds occur very close together, namely andrographolide (202 nm), vitexin (203 nm) and asiaticoside (201 nm). This result is important in the development of a mobile sensor prototype which is currently on going. The ambiguity of the detected bioactive compounds when only considering a single dominant peak has to be addressed. Therefore, the method of detection has to consider several peaks in order to confidently characterize and quantify the bioactive compound.

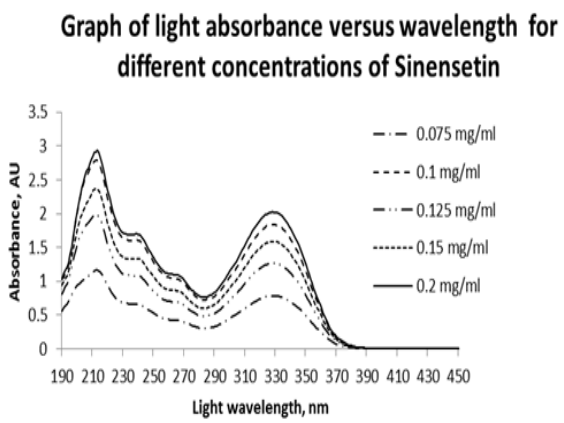

Figure 1. Scanned profile of standardized Sinensetin at concentrations of 0.075 mg/ml, 0.1 mg/ml, 0.125 mg/ml, 0.15 mg/ml and 0.2 mg/ml

Table 2 Peaks of studied bioactive compounds detected on Shimadzu UV-2450

Bioactive compound	Peaks
Andrographolide	202, 223
Vitexin	203, 271, 334
Asiaticoside	201
Sinensetin	214, 240, 268, 328

The absorbance at different concentrations of dominant peaks for bioactive chemicals were recorded and plotted. Again, for the sake of brevity, Figure 2 shows the plot for sinensetin. It can be seen from the figure that the amplitude of the peaks vary linearly with the concentration of the bioactive compounds. The same pattern was observed for all other bioactive compounds mentioned above. This result is significant in developing a mathematical model for a mobile sensor prototype.

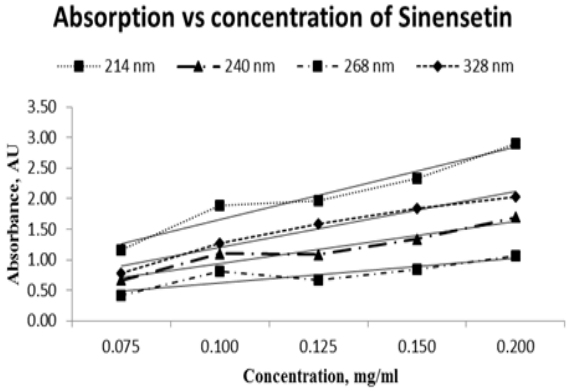

Figure 2. Graph of absorbance versus concentration of dominant peaks (214 nm, 240 nm, 268 nm and 328 nm) for Sinensetin and the fitted lines

The relationship between absorbance and concentration of each peak for all bioactive chemicals

studied were derived using simple linear regression in Microsoft Excel. The best fit linear regression line for each sinensetin peak is shown in Figure 2 as a solid line through the points. The mathematical representations for the best fit linear regression lines are listed in Table 3. It can be deduced that a very good correlation exist for all peaks of the studied compounds. The coefficient of determination, r^2 for all peaks exceed 0.95. This will simplify the model development for a mobile sensor prototype to quantify the detected bioactive compound in the future as each mathematical representation of the peaks can potentially be fused linearly. To the author's knowledge, the photonic characterization and the mathematical representation of the studied bioactive compounds have not been reported anywhere before.

Table 3 Mathematical representation of peaks for bioactive chemicals

Bioactive compound	Peak (nm)	Mathematical representation
Andrographolide	202	$y = 21.555x + 0.0735$, $r^2 = 0.9952$
	223	$y = 23.095x + 0.0521$, $r^2 = 0.9995$
Vitexin	215	$y = 32.95x + 0.6608$, $r^2 = 0.9639$
	271	$y = 21.01x + 0.3171$, $r^2 = 0.9797$
	334	$y = 21.745x + 0.3373$, $r^2 = 0.9773$
Sinensetin	214	$y = 98.9x – 0.166$, $r^2 = 0.9971$
	240	$y = 59.61x – 0.24$, $r^2 = 0.9998$
	268	$y = 38.21x – 0.15$, $r^2 = 0.9998$
	328	$y = 70.35x – 0.2478$, $r^2 = 0.9996$
Asiaticoside	201	$y = 7.015x – 0.0607$, $r^2 = 0.969$

Conclusion

The bioactive compounds of *Andrographis paniculata, Ficus deltoidea, Orthosiphon stamineus* and *Centella asiatica* were positively identified when extracted and scanned under a UV-VIS spectrophotometer. Dominant peaks for andrographolide, sinensetin, vitexin and asiaticoside were detected at 202 nm, 214 nm, 203 nm and 201 nm respectively. Due to the close occurrence of the peaks, it was concluded that the consideration of a single dominant peak was not sufficient in confidently characterizing and quantifying a bioactive compound when developing a mobile sensor prototype. The graphs of spectral absorbance versus concentration of the dominant peaks for all bioactive compounds studied were plotted, and the linear mathematical relationship recorded. These characterizations are significant and will form the basis for a mathematical model in a decision support system of a proposed mobile sensor prototype to detect and quantify herbs which is currently being developed.

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