Electroanalytical evaluation of antioxidant activity in monofloral honeys

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Abstract. The objective of this study was to develop an electroanalytical technique for the analysis of antioxidant compounds. Five different samples from five monofloral honeys were used. The electroanalysis experiments were conducted using two different techniques, namely cyclic voltammetry (CV) performed with a scan rate of 50 mV/s and differential pulse voltammetry (DPV) with a pulse amplitude of 50 mV and a scan rate of 10 mV/s. All samples were also analyzed by spectroscopy using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) microplate method. The electrochemical index determined using the DPV technique agreed with the spectrometric DPPH measurements. This result demonstrates that the electroanalysis method could potentially be utilized for antioxidant analysis of honey samples.

1. Introduction
Antioxidants are important compounds in living organisms as well as in food as they may delay or stop formation of free radicals by donating hydrogen atoms or scavenging them [1]. Free radicals are a major contributor to aging and to degenerative aging diseases such as cancer, cardiovascular disease, immune-system decline, brain dysfunction, and cataracts [2]. Antioxidants may be classified as natural or synthetic [3]. Due to safety concerning synthetic antioxidants, and increasing consumer preference for natural products, clean label and reduced usage of additives in food products, the use of natural antioxidants is widely preferred [4].

Honey is well known as a source of natural antioxidants. Honey typically contains more than 150 polyphenolic compounds, including flavonoids and cinnamic acid derivatives. These compounds have phenolic hydrogens as hydrogen-donating radical scavengers, which can predict their antioxidant properties [5]. Indonesia, as a country with a high diversity of flora, has many kinds of honey. Among them are: Ceiba pentandra, Nepheium lappaceum, Hevea brasiliensis, and Calliandra spp. honeys, and forest honey. That were analyzed for this study.

The antioxidants present in honey include both enzymatic substances—catalase, glucose oxidase, and peroxidase—and non-enzymatic substances—ascorbic acid, α-tocopherol, carotenoids, amino acids, proteins, organic acids, maillard reaction products, and more than 150 polyphenolic compounds including flavonoids, flavonols, phenolic acids, catechins, and cinnamic acid derivatives [6]. The most common technique for measuring antioxidant capacity is DPPH spectrophotometry as conducted by various researchers [1,6–10]. However, although it offers a simple alternative to spectrophotometric analysis, this method becomes unfeasibly time-consuming when applied manually to a large number of
samples [11]. As a response to this limitation, other researchers are examining alternative methods based on electrochemical approaches [5,12,13].

There are several reasons why electrochemistry has attracted increasing interest in this field. These reasons, among others, are as follows. (1) All termed natural or dietary antioxidants (which include mainly polyphenolic compounds and vitamins C and E) are believed to be effective dietary compounds that act as antioxidants, and all of them exhibit a moderate–marked native electroactivity. (2) In the particular case of controlled-potential techniques, oxidation potential is conceptually correlated with the antioxidant capacity. Indeed, one the one hand, low oxidation potentials found in food and biological samples point to their high antioxidant capacity. On the other hand, the amperometric current and/or charge measured under fixed oxidation conditions should give us an idea about the extension of their capacity as well as an estimation of their total content. (3) Owing to the complexity of the composition of food and biological samples, and also considering the possible synergistic interactions among the antioxidant compounds in the samples, separating each antioxidant compound and studying it individually is costly and inefficient. This dramatically opens up the analytical possibilities of electrochemical techniques that could be used in direct measurement when their inherent advantages of selectivity and sensitivity are accounted for [14].

In addition, to date there is only limited research in Indonesia on electrochemical evaluation of the antioxidant capacity of local honeys. This study aims to compare the determination of antioxidant capacity of five local monofloral honeys using spectrophotometry methods and electroanalytical methods. The spectrophotometry method used is the DPPH microplate method, while the electroanalytical methods used are cyclic voltammetry (CV) and differential pulse voltammetry (DPV).

2. Methods

2.1. Material

Five different types of monofloral honey obtained from local producer, namely *Nephelium lappaceum*, *Ceiba pentandra*, *Calliandra* spp., and *Hevea brasiliensis* honeys, and forest honey, were used. All honey samples were placed in glass bottles and stored at room temperature. 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma, and methanol was supplied by Merck.

2.2. Methods

2.2.1. Electrochemical method. Voltammetry analysis was performed by dissolving 2.5 grams of honey in PBS (Phosphate Buffer Solution) 0.1 M at pH 7. Measurements were made using a Potentiostat three-electrode system, with Ag/AgCl as the reference electrode, Pt wire as the counter electrode, and Pt plate as the working electrode. Electrode Ag/AgCl was rinsed using ethanol and Aqua Bidest alternately before use. The voltammetry analysis used were cyclic voltammetry (CV) and differential pulse voltammetry (DPV). CV was performed using the scan rate of 50 MV/s on the potential range of −1.0 to 1.0 V. While DPV was performed using a scan rate of 10 mV/s, a pulse amplitude 50 mV, and a pulse width of 50 ms.

2.2.2. Spectrophotometry. The spectrophotometry method used was the microplate AA method based on the 96-well plate assay described by Davidov-pardo [15] with few modifications. The sample solution was made by dissolving 2.5 grams of honey in 25 ml of methanol. A total of 20 µL of the diluted sample was added to 180 µL of DPPH solution (150 µmol/L) in methanol and shaken for 60 s in a 96-well microplate. After 40 min in the dark at room temperature, the absorbance was measured at 515 nm in the microplate reader of a Thermo Scientific Multiskan GO spectrophotometer (ThermoFisher Scientific). Trolox was used as a standard at 50–500 µmol/L to generate a calibration curve. The percent DPPH quenched was calculated using Equation 1, where $A_{\text{sample}}$ is the absorbance at 515 nm of 20 µL of extract or standard with 180 µL DPPH solution after 40 min; $A_{\text{blank}}$ is the absorbance at 515nm of 20
µL of water with 180 µL methanol after 40 min, and $A_{\text{control}}$ is the absorbance at 515nm of 20 µL of water with 180 µL DPPH solution after 40min.

Equation 1:

$$\%\text{DPPH quenched} = \left[1 - \left( \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{control}} - A_{\text{blank}}} \right) \right] \times 100$$  \hspace{1cm} (1)

3. Results and discussion

![Figure 1](image.png)

Figure 1. Cyclic voltammograms obtained from 2.5-gram honey samples in PBS 0.1 M at pH 7.

The electrochemical behaviors of the five local honey samples are shown in Figure 1. Three samples (forest honey, Calliandra spp., and Hevea brasiliensis) exhibited an anodic peak at 0.4–0.5 V, while the other samples did not exhibit significant peaks. Different phenomena were observed for the cathodic peaks, where all samples exhibited significant peaks. The occurrence of the peak is described redox reaction for specific compounds contained in honey. Some researchers have reported the electrochemical behavior of honey samples ranging from 0.4–0.5 V. For example, Ismail et al., reported the occurrence of hesperetin, syringic acid, caffeic acid, trans-ferulic acid [12]; Buratti et al., informed the anodic peaks of quercetin, isorhamnetin, caffeic acid, P-cumaric acid, kaempferol, and ferulic acid [5]. This range is also informed the level of antioxidant capacity from the five studied honeys increases in the following order: Nephelium lappaceum > Ceiba pentandra > Hevea brasiliensis > Calliandra spp. > forest honey. The absence of the corresponding reduction peak of Nephelium lappaceum and Ceiba pentandra honeys points to the irreversibility of oxidation of reaction products produced in this reaction according to the theory of Valek and Stipc [16].

Similar results were observed using the DPV method where the anodic peaks occurred in the range 0.475–0.570 V. It may be concluded that all honey samples have similar phenolic compounds. This DPV method was also performed by measuring the electrochemical index (EI) as described in Table 1. EI was highly correlated with total phenolics, indicating the suitability of the electrochemical approach as an alternative to measure total phenolics in the target samples where polyphenolics are minor constituents. Total phenolics indicates the antioxidant capacity of the target honeys [14].
Table 1 shows that *Calliandra* spp. honey has the highest EI value of 89.36 µA/V and *Ceiba pentandra* honey has the lowest EI value of 63.40 µA/V. The high value of EI for *Calliandra* spp. honey signifies that it has the highest antioxidant capacity compared to the other honeys. These electrochemical testing results were confirmed by conducting a DPPH test. In brief, this method is based on the reduction of the chromogenic DPPH radical by an antioxidant, which causes the radical to change color, and this change can be monitored and quantified using a spectrophotometer at 515–520 nm [17]. The result shows that *Calliandra* honey has the highest percent DPPH quenched, meaning that this honey has the highest antioxidant capacity. Different data was obtained using the CV method, where forest honey was observed to have the highest antioxidant capacity. However, as can be seen in Figure 2, the DPV and DPPH tests show similar trends of antioxidant capacity. Based on these results, we can assume that the electrochemical test can be used for determining the antioxidant capacity of honey samples. However, further investigation is still needed to evaluate the differences in the levels of antioxidant capacity of the samples obtained using CV and DPV testing.

**Table 1.** Electrochemical index (EI) of 2.5-gram honey samples in PBS 0.1 M at pH 7.

| Honey            | Ip (nA) | Ep (V) | EI (µA/V) |
|------------------|---------|--------|-----------|
| *Ceiba pentandra*| 0.34    | 0.53   | 63.40     |
| *Hevea brasiliensis* | 0.43    | 0.51   | 85.05     |
| Forest honey     | 0.44    | 0.57   | 76.94     |
| *Calliandra* spp.| 0.45    | 0.50   | 89.36     |
| *Nephelium lappaceum* | 0.41    | 0.47   | 86.63     |
Figure 3. Inhibition values and electrochemical index (EI) of the five different honey samples.

4. Conclusion
The results obtained were in agreement with previous findings in which polyphenols contained in honey were detected in the same range of potential oxidation. The electrochemical index determined using the DPV technique agreed with the spectrometric DPPH measurements. This demonstrates that the electroanalysis method could be potentially utilized for antioxidant analysis of honey samples. The presence of these phenolic compounds in Calliandra spp. honey and forest honey indicates that these honeys are rich in antioxidants and are therefore good sources of natural antioxidants.

Acknowledgements
This research was supported by Universitas Negeri Jakarta dan Kementrian Riset, Teknologi dan Pendidikan Tinggi Republik Indonesia through the research scheme Penelitian Unggulan 2020.

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