Applicability Of A Semi-Automated Clinical Chemistry Analyzer In Determining The Antioxidant Concentrations Of Selected Plants

Allan L. Hilario, Phylis C. Rio, Geraldine Susan C. Tengco, Danilo M. Menorca

Abstract: Plants are rich sources of antioxidants that are protective against diseases associated to oxidative stress. There is a need for high throughput screening method that should be useful in determining the antioxidant concentration in plants. Such screening method should significantly simplify and speed up most antioxidant assays. This paper aimed at comparing the applicability of a semi-automated clinical chemistry analyzer (Pointe Scientific, MI, USA) with the traditional standard curve method and using a Vis spectrophotometer in performing the DPPH assay for antioxidant screening. Samples of crude aqueous leaf extract of kulitis, Amaranthus viridis Linn, and chayote, Sechium edule Linn, were screened for the Total Antioxidant Concentration (TAC) using the two methods. Results presented in mean ± SD (µg/dl) were compared using unpaired Student's t-test ($P<0.05$). All runs were done in triplicates. The mean TAC of A. viridis was 646.0 ± 45.5 µg/dl using the clinical chemistry analyzer and 581.9 ± 19.4 µg/dl using the standard curve-spectrophotometer. On the other hand, the mean TAC of S. edule was 660.2 ± 35.9 µg/dl using the semi-automated clinical chemistry analyzer and 672.3 ± 20.9 µg/dl using the spectrophotometer. No significant differences were observed between the readings of the two methods for A. viridis ($P>0.05$) and S. edible ($P>0.05$). This implies that the clinical chemistry analyzer can be an alternative method in conducting the DPPH assay to determine the TAC in plants. This study presented the applicability of a semi-automated clinical chemistry analyzer in performing the DPPH assay. Further validation can be conducted by performing other antioxidant assays using this equipment.

Index Terms: Antioxidant Assay, Semi-automated Chemistry Analyzer, A. viridis and S. edule

1 Introduction

Several diseases and conditions including cancers, neurodegenerative and cardiovascular diseases had been found to be associated with the imbalance in the prooxidant and antioxidant levels in the human body [1-4]. Increased concentration of prooxidants promotes the generation of reactive oxygen species (ROS), putting the body under oxidative stress. When the level of antioxidant is insufficient to neutralize the detrimental effects of ROS, cellular damage, injury, or even death will eventually ensue. The normal physiology of the body will then be disrupted. Dietary intake of antioxidants had been found to be effective against oxidative stress [3]. Thus, various plants, particularly those in tropical countries, have been studied to determine their antioxidant concentration and activity. Amaranthus viridis Linn, locally known as kulitis, is an edible herb that has a high nutritional content [5,6]. It is one of those plants that had been subjected to antioxidant assays. Aside from being a good source of dietary antioxidant, this plant has a wide ethnobotanical utility as an alternative medicine for treating animal bites and stings, addressing digestive, respiratory, and skin diseases and conditions, alleviating pain during labor and stimulation of milk secretion [7,8]. Sechium edule Linn, locally known as chayote, is another edible herb that had been assayed for its antioxidant content [9].

- *Phylis C. Rio, MD, Geraldine Susan C. Tengco, MD and Danilo M. Menorca are full time-faculty members of the Department of Biochemistry and Nutrition, College of Medicine, Pamantasan ng Lungsod ng Maynila.*
- *Allan L. Hilario, MD, MHA, MSc is the main and corresponding author and presently a full-time Associate Professor at the Department of Biochemistry and Molecular Biology, College of Medicine, University of the Philippines-Manila. Email address: alhilario@up.edu.ph*

This plant possesses medicinal properties such as anti-ulcer, antiepileptic, CNS depressant properties and protective against hepatic injury [10-12]. In the Philippines, extensive numbers of edible and non-edible plants are possible sources of extracts that may be consumed; yet still remain understudied with regards to their functional properties including their antioxidant property, despite the wealth of botanical characterization and funtional studies available in the literature. In addition, health product development from natural substances is one of the current research priorities of the Philippine Council for Health Research and Development (PCHRD), while the Department of Agriculture (DAR) is propagating Philippine indigenous vegetables for their nutritive and functional properties. Thus, it is paramount that a high throughput screening method that would simplify and speed up most antioxidant assays be developed and used. Rendering a method as an indispensable hardware in enhancing the ethnobotanical profile of various plants in a developing country, it should be inexpensive and available, yet effective in screening the antioxidant property of various plant sources. This study aimed at assessing the applicability of programming the DPPH assay in a semi-automated clinical chemistry analyzer (Pointe Scientific, MI, USA) to determine the antioxidant concentrations of A. viridis Linn, kulitis, and S. edule Linn, chayote. Specifically, the objective was to compare the measurement of the semi-automated chemistry analyzer with that of the Vis spectrophotometer using the traditional method of creating a standard curve to determine the concentration of the Total Antioxidant Activity (TAC) of the test plants.

2 Materials and Methods

2.1 Research Review

The study was approved by the Pamantasan ng Lungsod ng Manila-College of Medicine Research Committee and the Pamantasan ng Lungsod ng Maynila-University Research Center. It was conducted at the PLM-Biochemistry Natural Products Laboratory. It was registered to the Research

Implementation and Development Office of the College of Medicine, University of the Philippines-Manila.

2.2 Plant Collection and Extraction

Leaf samples of Amaranthus viridis Linn, kulitis, and Sechium edule Linn, chayote, were obtained from the Bureau of Plant Industry. These plants were selected due to their availability during the collection period. Certificate of authentication were secured as a proof that the samples were identified and validated by a plant taxonomist from University of the Philippines-Los Banos. Fifty grams of macerated leaves of each plant were extracted with 100 mL of distilled water and were filtered and centrifuged at 10,000 rpm. The supernatant was subjected to antioxidant assay.

2.3 Antioxidant Assay

1,1-diphenyl-2-picrylhydrazyl (DPPH) assay was used to determine the antioxidant content of the aqueous leaf extract of A. viridis and S. edule. The procedure of the assay was programmed and calibrated in the semi-automated clinical chemistry analyzer according to the manufacturer's protocol (Pointe Scientific, MI, USA) using a single Trolox standard (112 µg/dl). For every 1 mL of the DPPH reagent, 500 µL of the leaf extract was used for each plant. The mixtures were allowed to react for ten minutes and read at a wavelength of 520 nm. In performing the assay using the Vis spectrophotometer, 2 mL of the DPPH reagent was used for every 1 mL of the leaf extract. The mixtures were read at the same wavelength as in the clinical chemistry analyzer. A standard curve was constructed by running five serial and different concentrations in an increasing concentration. The absorbance read using the Vis spectrophotometer was plotted in the standard curve to determine the sample's TAC. All runs of each test sample in the two methods were done in triplicates.

2.4 Data Analysis

Concentration readings obtained directly from the clinical chemistry analyzer and concentrations derived from the absorbance readings of the spectrophotometer using a standard curve were presented in mean ± SD (µg/dl) as the Total Antioxidant Concentration (TAC). Statistical analysis was done using unpaired Student's t test with significant result set at $P<0.05$ and a confidence level of 95%.

3 Results

The mean Total Antioxidant Concentration (TAC) of A. viridis were 646.0 ± 45.5 µg/dl as measured by the semi-automated clinical chemistry analyzer and 581.9 ± 19.4 µg/dl as measured by the spectrophotometer respectively (Figure 1). On the other hand, the mean TAC of S. edule was 660.2 ± 35.9 µg/dl as measured by the semi-automated clinical chemistry analyzer and 672.3 ± 20.9 µg/dl as measured by the spectrophotometer respectively (Figure 2).

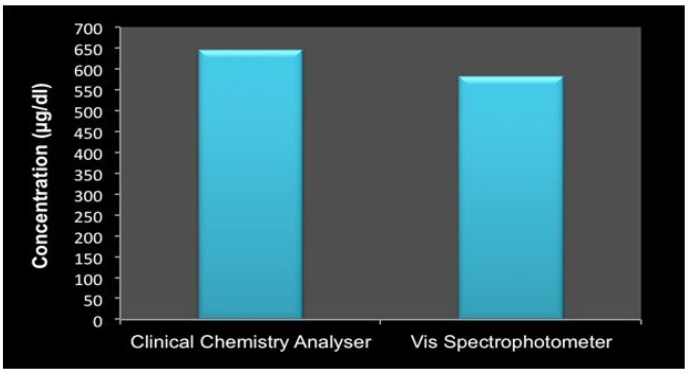

Figure 1. Mean Total Antioxidant Concentration of *Amaranthus viridis* measured using the Semi-automated Clinical Chemistry Analyser and Vis Spectrophotometer.

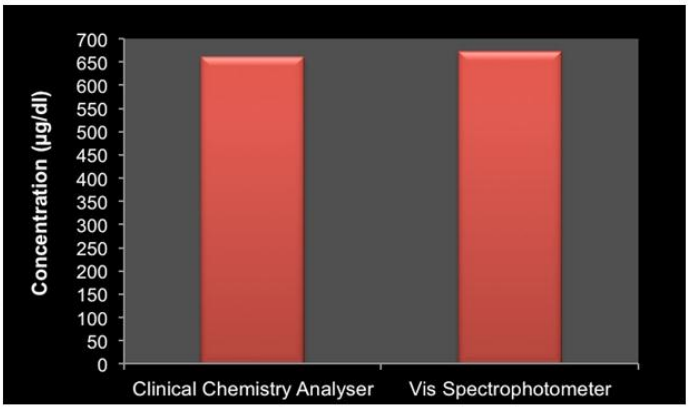

Figure 2. Mean Total Antioxidant Concentration of *Sechium edule* measured using the Semi-automated Clinical Chemistry Analyser and Vis Spectrophotometer.

5 Discussion

There were several validated assays that can be used to measure the antioxidant concentration of various plant sources. These assays include the 1,1-DPPH assay, which actually measures the activity of an analyte to reduce an oxidant by donating its proton. Thus, it is also called as free radical scavenging assay. 1,1-DPPH is a free radical that manifests a deep purple color in solution. Its reaction with an antioxidant results to a decrease in the intensity of color and its absorbance. The degree of discoloration, which is the absorbance, is directly proportional to the scavenging activity of the antioxidant and its proton content [13]. A spectrophotometer is routinely used instrument to measure the absorbance of any analyte in solution, regardless of the source, at a certain wavelength. In this study, its performance was compared with that of a semi-automated clinical chemistry analyzer, which is commonly used in clinical laboratories for measuring the amount of analytes in human specimens. The chemistry analyzer was considered for evaluation for its potential applicability in determining the antioxidant concentration in plants since it follows the same principle of the other equipment. In addition, the analyzer that was evaluated in this study has an open reagent system and is readily available. Statistical analysis of the mean TAC that had been obtained from the chemistry analyzer and the Vis spectrophotometer showed that there were no significant differences between the readings of the two methods for A. viridis and S. edule. This implies that the clinical chemistry analyzer can be used as alternative equipment in performing the DPPH assay to determine the TAC in plant sources. Since

the conception of the clinical chemistry analyzer in the 1950s, advances in technology had greatly improved its features. This applicability study had added another function to the analyzer's utility profile. This had also paved the way towards a high throughput method for antioxidant screening. DPPH assay programmed in a semi-automated clinical chemistry analyzer has a similar impact with the same assay performed in a Vis spectrophotometer in terms of eliminating the error that may occur in manual analysis and minimizing error due to interpersonal variation. But the former one seems to be more advantageous in terms of cost-reduction in reagents, since only half of the reagent required in the spectrophotometric measurement is required in the semi-automated chemistry analyzer's measurement, thus, miniaturizing the antioxidant assay. Another advantage is that more tests for phytochemical screening can be potentially done with semi-automated chemistry analyzer. The semi-automated chemistry analyzer is also a compact hardware, which combines the functions of water bath, spectrophotometer and computerized generation of results. This can be used in the field since it is lightweight and portable.

6 Conclusion

The applicability of programming the DPPH assay in a semi-automated clinical chemistry analyzer had been validated and established in this study. Further validation and reliability study is encouraged for the improvement of this potential screening method. Cost-effectiveness studies may also be done in the future to provide an exact assessment of the reductions in expenses and output of this when translated into service. Advanced and stand-alone models of clinical chemistry analyzers may also be evaluated as equipment in performing the DPPH assay. Such proposal may bring more benefit since these analyzers have a higher capacity in terms of batch testing and saving time. This applicability of this study may also be extended to other assays and procedures that involve the principle of spectrophotometry such as phytochemical screening.

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