Practical Report

Determining the Antioxidant Property of Plant Extracts: A Laboratory Exercise

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Vegetables and fruits are good sources of free radical scavengers or antioxidants. To evaluate the free radical scavenging activity of a plant extract, a few simple steps using the 1,1-diphenyl-2-picryl hydrazyl (DPPH) assay can be followed. Any plant part can be processed to produce crude extracts which can be used for testing. Using this technique, a small class of tertiary students determined the antioxidant property of ethanolic crude leaf extracts of some endemic and indigenous plants. The plants used were *Ardisia pyramidalis* Roth (Myrsinaceae), *Baccaurea tetrandra* (Baill.) Mull.Arg. (Phyllanthaceae), *Chisocheton pentandrus* (Blanco) Merr. (Meliaceae), *Ficus septica* Burm. (Moraceae), *Parameria laevigata* (Juss.) Moldenke (Apocynaceae), *Parartocarpus venenosus* (Zoll. & Moritzi) Becc. (Moraceae), *Streptocaulon baumii* Decne. (Asclepiadaceae), *Uncaria perrottetii* (A. Rich) Merr. (Rubiaceae), and *Voacanga globosa* (Blanco) Merr. (Apocynaceae). Crude leaf extracts of *U. perrottetti* and *B. tetrandra* were observed to possess a high free radical scavenging activity with values beyond 90% of that of gallic acid. These were fractionated further, and subsequent assays showed that ethyl acetate fractions for both plants had high free radical scavenging activity indicating that they contain potential chemopreventive agents against many diseases such as cancer, cardiovascular disorders and aging. Free radical scavenging activities demonstrated by leaf extracts of *A. pyramidalis* and *C. pentandrus* did not reach 70% of that of gallic acid. All the rest of the plant extracts showed very low or no free radical scavenging activity.

Keywords: antioxidant, chemopreventive agent, diphenyl picryl-hydrazyl, free radical scavenging activity

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Introduction

Production of free radicals takes place as a result of normal metabolic processes in the body especially that which happens during cellular respiration in the mitochondria. Reactive oxygen or nitrogen species are detrimental to biological molecules in the cells, hence destroying cell membranes, nucleic acids and proteins which in turn lead to aging and other diseases such as cardiovascular diseases and cancer.

The search for free radical scavengers is thus an important component in drug discovery. Most of the antioxidants are part of our diet including the polyphenolic variety such as flavonoids (Hanninen *et al*., 2000).

It is thus important to teach tertiary students

of biology and chemistry some techniques to determine the presence of free radical scavengers in any test samples.

This paper reports the results of undergraduate college students in a small laboratory class who collected test plants and processed them for determination of free radical scavenging activity. The class aimed to find antioxidant-rich plants from the forested mountains in Kanawan, Morong, Bataan, Philippines. As the area is a part of the ancestral domain of a community of indigenous Filipino people, a series of meetings was held between the community, the researchers and the students to come up with the Memorandum of Agreement (MOA) between the researchers and the community elders for this purpose. The preliminary investigations in the present study lead to a number of undergraduate theses.

Materials and Methods

Collection, Extraction and Purification

Students collected leaves from nine endemic and indigenous plants from the mountainous forests of Kanawan, Morong, Bataan, Philippines. Identification was authenticated by Mr. Leonard Co and Dr. Daniel Lagunzad, curators of the Jose Vera Santos Herbarium, Institute of Biology, University of the Philippines, Diliman. Voucher specimens were deposited also at Jose Vera Santos Herbarium, Institute of Biology, University of the Philippines, Diliman from 2007 to 2008.

Air dried leaves were homogenized, soaked in 95% ethanol for 48 hours, filtered and concentrated *in vacuo* to yield the crude ethanol extract. Extracts that were found to possess high free radical scavenging activity (as can be computed below) were partitioned in hexane and ethyl acetate. The hexane and ethyl acetate fractions were concentrated by rotary evaporation and air-dried overnight. Thereafter, the air dried samples were dissolved in dimethyl sulfoxide (DMSO) to a concentration of 4 mg/mL for use in the subsequent assays.

Assay for free radical scavenging activity using 1,1-diphenyl-2-picryl hydrazyl (DPPH)

Following the procedure of Hou *et al*. (2004), a 300 µM free-radical solution was prepared by dissolving 1,1-diphenyl -2-picrylhydrazyl (DPPH) in absolute ethanol. Then 95 µL of the DPPH solution was dispensed to 96-well plates. From the 4 mg/ml of extracts solutions, 5 µL was dispensed to each well to make final volumes of 100 µL. A 4 mg/mL gallic acid solution served as the positive control while DMSO served as the negative control for the assay. At least three replicates were made for the controls and extracts. The plate was then incubated at 37°C for 1 hour. After incubation, absorbance was read at 515 nm. From the absorbance, the free radical scavenging activity of each crude leaf extract relative to that of gallic acid was computed using the following formula with slight modification:

$$Percent\ Free\ Radical\ Scavenging\ Activity = \left(\frac{Abs_{DMSO} - Abs_{extract}}{Abs_{DMSO} - Abs_{gallic\ acid}}\right) \times 100$$

At least three trials were made for each extract..

Results and Discussion

As Hou *et al*. (2004) implied, the assay for free radical scavenging activity using the stable free radical DPPH was simple, rapid, and replicable. Similar exercises have been done by tertiary and secondary students using different plants for undergraduate thesis and science investigatory projects.

In this report, DPPH free radical scavenging activity with respect to gallic acid was calculated

for each of the ethanolic crude leaf extracts (Fig. 1). Two extracts were observed to have a high DPPH free radical scavenging activity (>90%): *B. tetrandra* and *U. perrottetii*. Hence, these were further partitioned in hexane and ethyl acetate fractions and were subsequently tested for the same DPPH free radical scavenging activity.

Results in Figure 2 showed that the ethyl acetate fraction had significantly higher DPPH free radical scavenging activity. This indicated that the active compounds were polar since ethyl acetate can extract more polar compounds compared to hexane.

All the other crude extracts yielded moderate

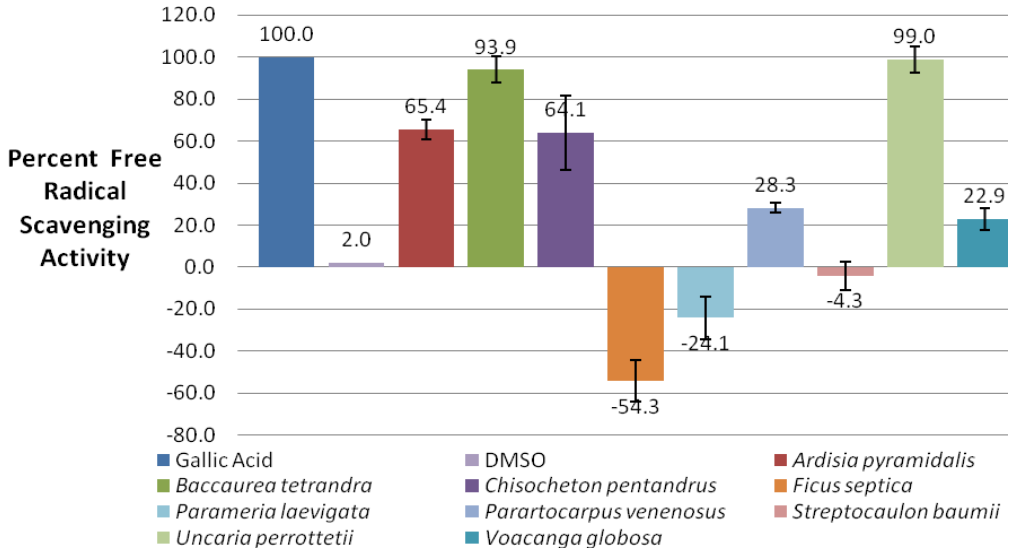

Figure 1 Free radical scavenging activities relative to gallic acid assayed using the 1,1-diphenyl -2-picryl hydrazyl in the crude ethanolic extracts of selected plants from Kanawan, Morong Bataan, Philippines. Each value represents the mean of three trials with three replicates per extract per trial. DMSO: dimethyl sulfoxide. Bars represent standard deviations.

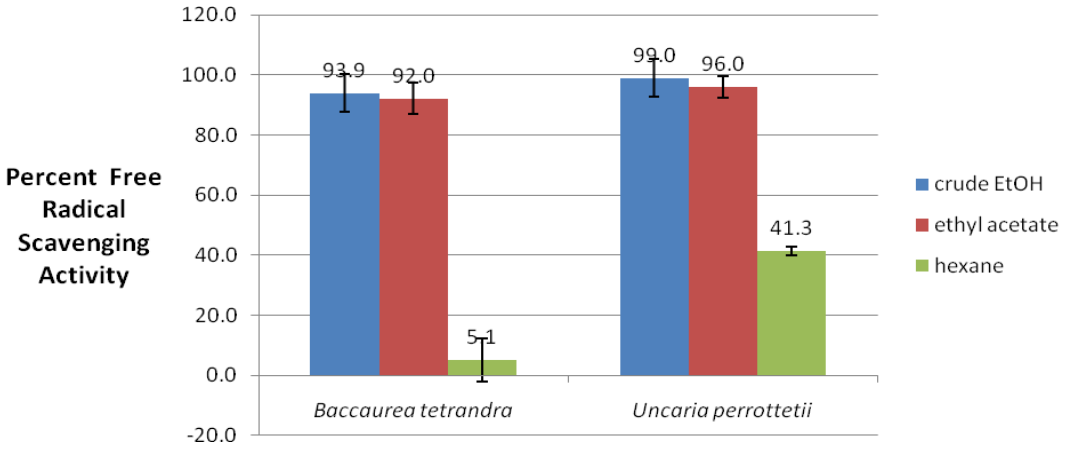

Figure 2 Free radical scavenging activities relative to gallic acid of the ethyl acetate and hexane fractions of the crude ethanolic leaf extracts of *Baccaurea tetrandra* and *Uncaria perrottetii* from Kanawan, Morong Bataan, Philippines using the 1,1-diphenyl -2-picryl hydrazyl assay. Each value represents the mean of three trials with three replicates per fraction per trial. Bars represent standard deviations.

activity, such as *A. pyramidalis* and *C. pentandrus*, or low activity, such as *P. venenosus* and *V. globosa*. *F. septica*, *P. laevigata* and *S. baumii* showed no activity (Fig. 1).

The high percent DPPH free radical scavenging activity indicates that these extracts contain antioxidants. The potent antioxidant nature of the active extracts makes them potential sources of chemopreventive compounds or compounds that can significantly prevent carcinogenesis and other diseases (Tseng *et al*., 2001; Albert-Baskar and Ignacimuthu, 2010). This suggests that further purification is needed to isolate the active compound or compounds that are antioxidants and are potentially chemopreventive.

It is interesting to note that the most potent free radical scavenging activity was observed from the extract of an endemic plant, *U. perrottetii*, which is used as food by the indigenous tribes of Aytas in Bataan. As *U. perrottetii* has not been studied at all in terms of its medicinal potential, this is the first report of bioactivity coming from this plant. *U. tomentosa*, a related plant, is used as tea by native Peruvians and is believed to possess the following properties: immunomodulation, antibacterial, antimutagenic, diuretic, depurative, hypotensive and vermifuge (http://www.herbalremedies.com/samstren6030.html). *U. perrottetii* may also confer the same dietary benefits because of its antioxidant property.

Development of tea from *U. perrottetii* is also next in the plans as part of the MOA between the indigenous group of Aytas and the University of the Philippines, Diliman, with the former as the primary beneficiaries of any nutraceuticals that may result from the research project.

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