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Free Radical Scavenging (DPPH) and Ferric Reducing Ability (FRAP) of Three Selected Herbs in Cantilan, Surigao del Sur, Philippines

Gemma A. Gruyal

Abstract

Herbal medicines are natural herbs that treat, prevent diseases, disorders and promote good health. In the present study the methanolic extracts of *Conyza cinerea, Trema orientalis and Laportea interrupta* were evaluated in their phenolic, carotenoid and vitamin C contents and also their antioxidant activity using DPPH and FRAP assay. Result showed that among the three herbs *Trema orientalis* has highest phenolic and Vitamin C content, 9.13 ± 0.269 mg gallic acid/g and 53.85 ±0.269 mg ascorbic acid/g respectively. On the other hand, *Conyza cinera* has the highest total carotenoid content, 645.47±18.1 µmol/g. In terms of antioxidant activity *Trema orientalis* showed the highest scavenging activity and had the highest ability to reduce ferric ions. Generally, herbs contained different amount of phenolics, vitamin C, carotenoids content and each of these compounds possessed different expanses of antioxidant activity. It disclosed that herbs are an effective potential source of natural antioxidants. Thus, supplementing a balanced diet with herbs may have beneficial health effects.

Keywords:

Herbs;
Antioxidant activity;
Phenolics;
Vitamin C;
Carotenoids.

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1. Introduction

Herbal medicines are natural herbs that treat, prevent diseases and disorders, and promote good health. Herbal remedies are indispensable part of humanity's oneness with nature and are believed to be one of the ways that Mother Earth, cares for humanity [3]. Many herbs contain dozens of active constituents that, together, give the plant its therapeutic value [14]. Herbal plants contain a wide variety of free radical scavenging molecules such as flavonoids, antocyanins, carotenoids, alkaloids, tannin, saponins, phenolic, steroids and terpenoids which are rich in antioxidant activities [16].

Many free radicals have been implicated in the causation of several diseases such as liver cirrhoris, atherosclerosis, cancer, diabetes, ageing and Alzheimer's disease [18]. Antioxidant constituents of the plant material act as radical scavengers and helps in converting the radicals to less reactive species [17].

Ph. D. in Science Education major Chemistry

Conyza cinerea Linn. or locally known as "albahaka" belong to family Asteraceae is used as a traditional medicine in the Philippines. Traditionally, an infusion of plant were taken internally for cough, cold and fever remedy [19]. Leaves used for skin diseases: Handful of leaves pound and boiled in coconut oil and oil extract applied three times daily to treat leprosy and scabies [4].

Trema orientalis Blume, locally known as "hanagdong", belong to family Ulmaceae. Traditionally, decoction of leaves mixed with leaves of *Bidens pilosa*, *Citrus aurantifolia* and peels of unripe pineapple is used for jaundice. Macerated leaves in lemon juice is used for cough. Leaf decoction is used as anthelmintic for roundworm and hookworm [1].

Laportea interrupta Chew, locally known as "handiamay", belong to family Myrtaceae. Folklore used leaves for carbuncles and decoction of roots used as a diuretic. The fresh, hairy leaves are rubbed on skin to produce a hot and itching feeling, to combat muscular pains and fatigue [15]. The aim of the present study was to determine the phenolic, carotenoid, and vitamin C contents and evaluate the antioxidant activity, reducing power and free radical scavenging activity of the methanolic extract of selected herbal plants.

2. Research Method

2.1 Collection of plant materials

The three plant herbs studied: *Conyza cinerea* Linn., *Trema orientalis* Blume and *Laportea interrupta* Chew were collected from July to August 2015 from various areas in Cantilan, Surigao del Sur, Philippines. The plant specimens were identified in Biology Department of Mindanao University of Science and Technology.

2.2 Preparation of extracts

The plant herb materials were washed thoroughly and dried in shade and powdered in a mechanical grinder. One hundred grams of each respective plant leaves were soaked to 300 ml methanol and keep at room temperature for 24 hours. The extracts were filtered using whattman filter paper (No.1). The extracts were concentrated using a rotary evaporator with the hot water bath set at 40°C. The percentage yield of extracts ranged from 5 - 20% (w/w).

2.3 Chemicals

All Chemicals used including the solvents were of analytical grade. Gallic acid, Folin-Ciocalteau phenol reagent, ascorbic acid, 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) were purchased from Sigma Chemical Co. Ltd (USA). All other chemicals and reagents used were of the highest commercially available purity. Potassium ferricyanide, trichloroacetic acid, sodium phosphate buffer, sodium carbonate, ammoniacal acetone were from Elmar (Iligan, Philippines). The solvent methanol and sulfuric acid were purchased from Merteflor (Cagayan de Oro, Phil.)

2.4 Determination of Total Phenolic Content

Total phenolic content were recorded by Folin Ciocalteu reagent [10]. A dilute extract of each plant extract (0.5 ml of 1:10 g/ml) or gallic acid used as standard was mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) and aqueous Na_2CO_3 (4 ml, 1M). The mixture was allowed to stand for 10 min and the absorbance was measured by spectrophotometric analysis at 730 nm. The standard curve was prepared using 0, 10, 20, 30, 40, 50 and 60 mg/l solutions of gallic acid in methanol. The standard curved can be seen in figure 1. Total phenolic contents were expressed in terms of gallic acid equivalent (mg/g of dry mass), which is used as a reference compound.

2.5 Determination of carotenoids

The total carotenoid content of samples was determined according the method of [13] with a slight modification. Briefly, about 0.200 g of plant dried material was placed into the vial. The extract was then added with 5 ml ammoniacal acetone and shaken for 5 minutes. The solution was then subjected to centrifuge for 3-5 minutes and the supernatant liquid was transferred into another vial. The extraction was done again with another 5 ml of ammoniacal acetone. The first supernatants were combined with the new one. The empirical supernatants were subjected to centrifuge to ensure clean layer of solvent solution for the use in absorbance measurement. The absorbance was read at 480,645,663 and 710 nm against solvent blank. The absorbance @710 nm is in isobestic point or theoretical base and is deducted from all other absorbance readings from the same solution. This is an important point, particularly when comparing pigment values in different tissues or species. The corrected absorbance can be computed using this formula:

Corrected Absorbance = Abs at 480,645,663 – Abs at 710nm

Carotenoid content can be computed using the formula:

Carotenoid as µmol/unit area or wt. sample

$$\frac{(A480 + 0.114^* A663 - 0.638^* A645)\ ^*V^*1000}{112.5^* \text{ unit area or wt. sample}}$$

Where:

A480 – corrected Absorbance @ 480 nm
A645 – corrected Absorbance @ 645 nm
A663 – corrected Absorbance @ 663 nm
V – Total volume of the plant extract
V= 10ml

2.6 Determination of vitamin C content.

The vitamin C content of the aqueous extract was determined using the method reported by [6]. Briefly, 75 µL DNPH (2 g dinitrophenyl hydrazine, 230 mg thiourea and 270 mg $CuSO_4.5H_2O$ in 100mL of 5M H_2SO_4) was added to 500 µL reaction mixture (300 µL appropriate dilution of hydrophilic extract with 100 µL of 13.3% trichloroacetic acid and distilled water). The reaction mixture was subsequently incubated for 3 hours at 37^0C, then 0.5 mL of 65% H_2SO_4 (v/v) was added to the medium, and the absorbance was measured at 520 nm and the Vitamin C content of the sample was subsequently calculated from the calibration curve prepared with ascorbic acid standard. The calibration equation for ascorbic acid is Y = 0.0092x + 0.1305 (R = 0.9449) which is showed in figure.

2.7 DPPH radical scavenging activity.

The DPPH solution (0.006% w/v) was prepared in 95% methanol. The methanol extract of the leaves from the three plant herbs were mixed with 95% methanol to prepare the stock solution (1 mg/mL). Freshly prepared DPPH solution was taken in the test tubes and extracts was added followed by serial dilutions (100-1000 ug) to every test tube so that the final volume is 2 mL and discoloration was measured at 517 nm after incubation for 30 minutes in the dark (Thermo UV1 spectrophotometer). A measurement was performed at least in three trials. The control sample was prepared and contained the same volume without any extract and 95% methanol. It was used as the blank. The percentage scavenging of the DPPH free radical was measured using the following equation:

$$\text{DPPH scavenging effect (\%)} = \frac{(A_0 - A_1)}{A_0} \times 100$$

where, A_0 is the absorbance of the control and A_1 is the absorbance in the presence of the sample (methanolic leaf extract of the herbal medicine).

2.8 Ferric reducing antioxidant power (FRAP).

The reducing property of the methanolic extracts was determined by assessing the ability of the extract to reduce $FeCl_3$ solution as described by [13]. A 2.5 mL aliquot was mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 5^0C for 20 min. and then 2.5 mL of 10% trichloroacetic acid was added. This mixture was centrifuged at 650 rpm for 10 minutes. Then 5 mL supernatant was mixed with an equal volume of water and 1 mL of 0.1% ferric chloride. The absorbance was measured at 700 nm. The ferric reducing antioxidant property was subsequently calculated.

3. Results and Analysis

3.1 Total Phenolic Contents of the herb extracts

Phenolic compounds are a class of antioxidant agents which act as free radical terminators [20]. They are secondary metabolite of low molecular weight that are found in most land plants [2]. It has been reported that phenolic compounds have anti-cancer or anti-carcinogenic, anti-bacterial, anti-viral or anti inflammatory activities its either in their greater or lesser extent [7],[11] and [21]. In the present study, the total phenolic contents of the herb extracts were measured using Folin Ciocalteu reagent in terms of gallic acid equivalent which can be seen in figure 1 (the standard curve equation: $y = 0.0192x - 0.0149$, $r^2 = 0.9882$) the results were between 3.30 ± 0.196 to 9.13 ± 0.269 (Table 1). Among the three herb extracts *Trema orientalis* has the higher phenolic content (9.13 ± 0.269 mg/g) followed by *Conyza cinerea* (3.39 ± 0.155 mg/g) and the least is *Laportea interrupta (*3.30 ± 0.196 mg/g). This result confers with the study of [25] that *Trema orientalis* has high phenolic content. However, the amount is different from the present maybe due to geographical location.

Table 1. Total phenolics, Carotenoids and Vitamin C of three Herbal Plants

Herbal Plants	Total phenolics Content mg gallic acid/ g crude extract	Total Carotenoid µmol/g dried material	Vitamin C Contents mg ascorbic acid/ g dried sample
Laportea interrupta (handiamay)	3.40 ± 0.196	481.0 ± 32.7	9.67 ± 0.509
Trema orientalis (Hanagdong)	9.13 ± 0.269	75.2 ±33.3	53.85 ± 0.269
Conyza cinerea Linn. (albahaka)	3.39 ± 0.155	645.4 ±18.1	3.46 ± 0.118

Results triplicates for mean ± standard.

3.2 Determination of carotenoids

Carotenoid compound are involved in the proper conduct of important biochemical processes such as growth, reproduction and vision (provitamin A) of animals and humans. They also exhibited anticancer effects, antimicrobial and antioxidant activity [9]. Furthermore, carotenoid pigments have shown positive benefits in slowing the growth of induced skin tumors, treating dermatological diseases and lowering overall risk of cancer in human beings. In the current study, table 1 showed the carotenoid contents of *Laportea interrupta*, *Trema orientalis and Conyza cinerea.* Among the herbs, *Conyza cinerea* (645.4 ±18.1 µmol/g) has the highest content of carotenoids followed by *Laportea interrupta* (481.0 ± 32.7 µmol/g) and the least is *Trema orientalis* (75.2 ± 33.3 µmol/g.). With this result, the claim of [4] that the leaves of *Conyza cinerea*

can treat skin diseases, leprosy and scabies has scientific basis due to its high carotenoid content. It also conforms in the study of [9].

3.3 Determination of vitamin C content.

Vitamin C also known as ascorbic acid is a water-soluble substance found in some foods. This metabolite is one of the most abundant substances found in green leaves. Much attention has focused on the antioxidant role of Vitamin C in both plants and animals. Besides, this vitamin is also important as a cofactor for a large number of key enzymes [5]. Furthermore, vitamin C improves the absorption of iron particularly from plant-based foods and helps the immune system work properly to protect the body from diseases. In the body it acts as antioxidant. It helps protect the cells from the damage that is caused by free radicals. Results showed that *Trema orientalis* has the highest vitamin C content followed by *Laportea interapta* and the least is *Conyza cinerea*, 53.85 ± 0.269 mg/g, 9.67 ± 0.509 mg/g and 3.46 ± 0.118 mg/g respectively. It is mention in the study of [1] maceration of *Trema orientalis* leaves can be used for treating cough which approves with the present study that it has high content of vitamin C compared to other herbal plants studied. In addition, Vitamin C is needed in the body to make collagen in helping wounds to heal faster. The best source of Vitamin C is in fruits and vegetables.

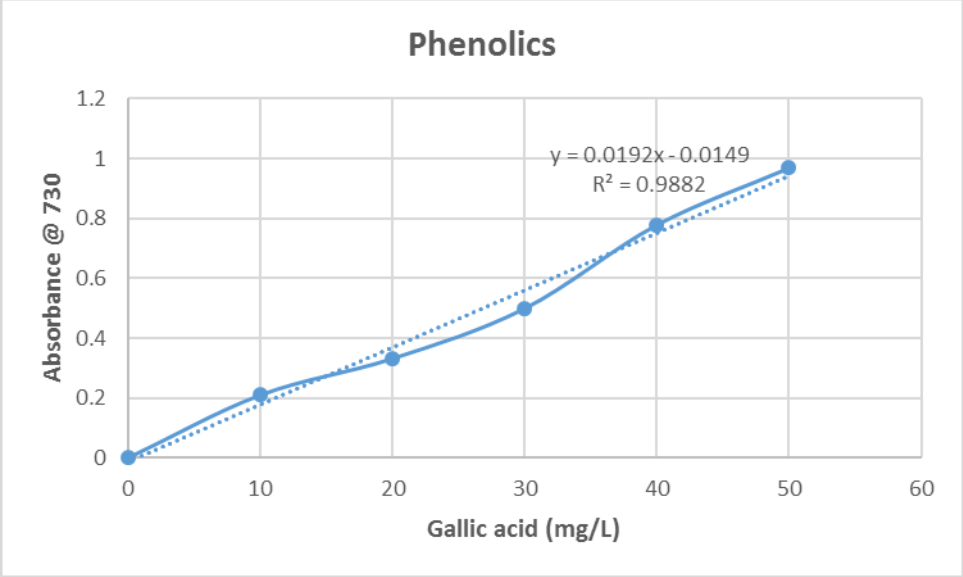
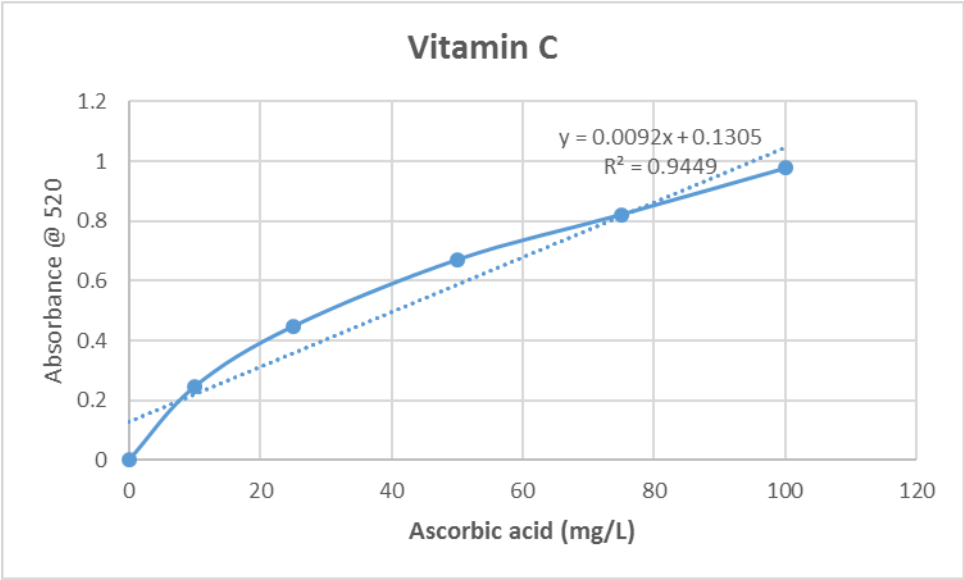


Figure 1. The standard curve using gallic acid in methanol.



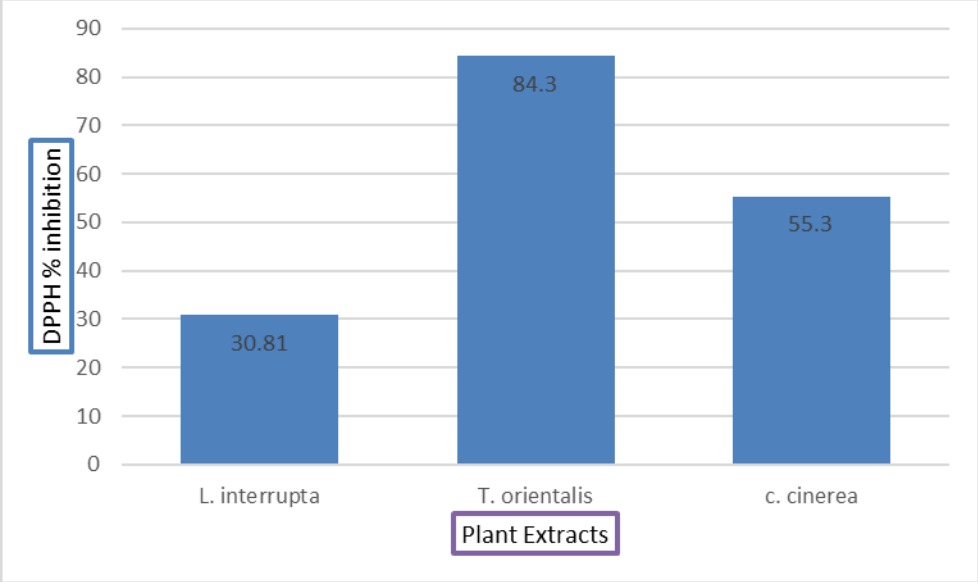


Figure 1: Free radical scavenging activity by DPPH radical in different methanolic plant extract

3.4 DPPH radical scavenging activity.

Antioxidants are the body's defense mechanism or internal army against free radicals [12]. These are specific compounds that protect human, animal and plant cells against the damaging effects of free radicals reactive oxygen species (ROS). They are molecules capable of inhibiting the oxidation of another molecule. They are also considered nutrients that generally act to cleanse the body of free radicals by giving up electrons or breaking the chain reactions of free radicals into stable substances

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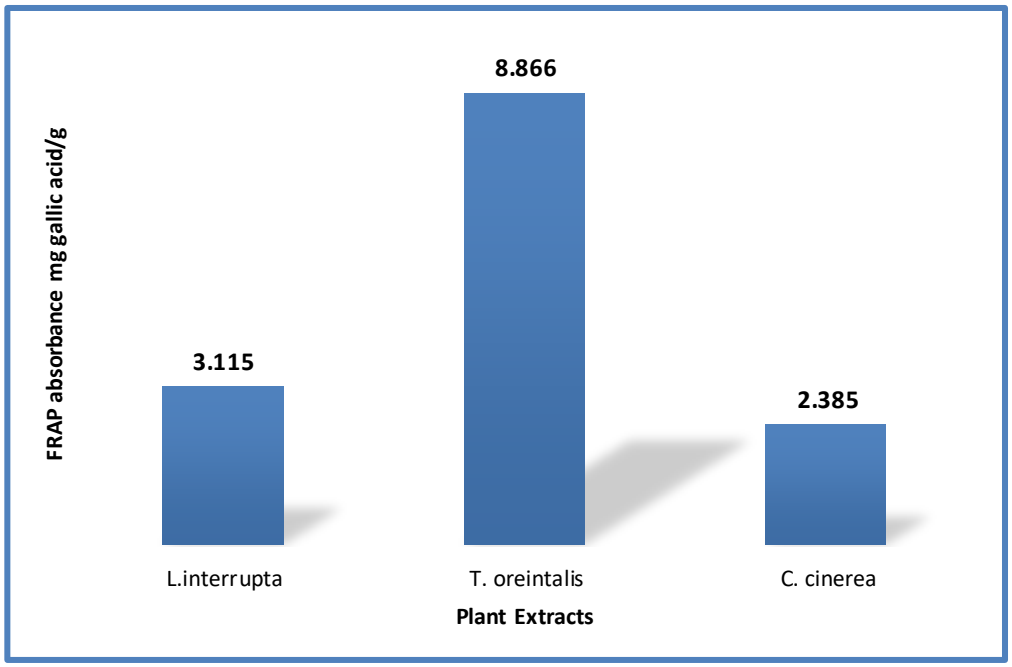

Figure 2. Ferric reducing antioxidant power in different plant extracts.

In the present study, several phytochemical constituents and free radical scavenging activities of three herbal plants were evaluated. Free radicals contribute to more than one hundred disorders in humans like atherosclerosis, arthritis, ischemia, gastritis, cancer and AIDS [20]. Antioxidants, due to their scavenging activity are useful for the management of those diseases. Figure 1 shows the amount of each extract required for inhibition of DPPH. The free radical scavenging action of methanol extracts of herbs are in the order as *T. orientalis* > *C. cinerea* > *L. interrupta*. Among the extracts, *T. oreintalis* showed the strongest DPPH radical scavenging activity while the others show moderate antioxidant properties. The present results confirme the study of [22] that methanol extracts of the leaves of *T. orientalis* showed potential free-radical scavenging activity. However, in aqueos extracts it showed very little free-radical scavenging activity.

3.5 Ferric reducing antioxidant power (FRAP).

FRAP assay measures the reducing ability of antioxidants against oxidative effects of reactive oxygen species. Electron donating anti-oxidants can be described as reductants and inactivation of oxidants by reductants can be described as redox reactions. Total antioxidant power may be referred analogously to total reducing power. The procedure of FRAP assay is relatively simple and easy to be standardized. However, this assay has been reported not to react fast enough with some antioxidants, such as glutathione [11]. In the present study, the FRAP values were expressed as gallic acid equivalent among the three plant herbs which can be showed in Figure 2. In the current study, *T. orientals* showed higher FRAP values (8.866 mg gallic acid/g) followed by *L. interrupta* (3.115 mg/g) and the least was *C. cinerea* (2.385 mg/g). This would further indicates that *T. orientalis* had the higher ability to reduce ferric ions. Although the trend for both DPPH and FRAP free radical scavenging activity appeared the same the absolute values obtained were higher in DPPH assay. According to [21], free radical scavenging activity may be due to its high total phenolic and vitamin C content.

4. Conclusion

In the present study, herbs generally contained different amount of phenolics, vitamin C, carotenoids content and each of these compounds possessed different expanses of antioxidant activity. This study revealed that herbs are an effective potential source of natural antioxidants. Hence, supplementing a balanced diet with herbs may have beneficial health effects.

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