

Evaluation of DPPH Free Radical Scavenging Activity and Phytochemical Screening of Selected Folkloric Medicinal Plants in Tinoc, Ifugao, Cordillera Administrative Region, Philippines

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Abstract- Many folkloric medicinal plants are found in Tinoc, Ifugao, Cordillera Administrative Region, Philippines. These plants were used as traditional medicines by the local ethnic groups since time immemorial. This study aimed to evaluate the DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging activity and to determine the bioactive components present in the ethanolic leaf extracts with antioxidant activity. Ethanolic leaf extracts of *Medinilla pendula* Merr., *Rubus fraxinifolius* Hayata, *Melastoma polyanthum* Blume, *Desmodium sequax* Wall., *Astilbe rivularis* Buch.-Ham ex. D. Don, *Drymaria cordata* (L.) Willd. ex J.A. Schultes, *Allium odoratum* L., *Physalis minima* (L.), *Centella asiatica* (L.) Urban, and *Vaccinium myrtooides* (Bl.) Miq. were tested for *in vitro* antioxidant activity using the DPPH free radical scavenging assay with ascorbic acid as standard antioxidant. Different concentrations of the ethanolic leaf extracts and ascorbic acid were used in three replicates. Preliminary phytochemical screening was done using standard methods. The overall antioxidant activity of *Vaccinium myrtooides* (Bl.) Miq. was found to be the strongest, followed in descending order by *Melastoma polyanthum* Blume, *Astilbe rivularis* Buch.-Ham ex. D. Don, and *Rubus fraxinifolius* Hayata. As the concentration of the ethanolic leaf extract increased, antioxidant activity also increased. Phytochemical investigation revealed the presence of flavonoids and phenolic compounds in all four ethanolic leaf extracts with antioxidant activities. Only four out of ten plants showed potent inhibition of DPPH radical scavenging activity with *Vaccinium myrtooides* (Bl.) Miq. as the most potent. Results of these findings revealed that the free radical scavenging (antioxidant) activities are due to the phytochemicals present.

Index Terms- free radical; DPPH assay; IC₅₀; phytochemicals

I. INTRODUCTION

Plants are integral part of life in many indigenous communities. Being the sources of food, fodder, fuel, etc., they can be used as herbal medicines in curing several ailments that goes parallel to human civilization (Bhatia et al, 2014).

Medicinal plants are important sources of antioxidants (Rice, 2004). Antioxidants from plant materials terminate the action of free radicals thereby protecting the body from various diseases (Lai et al, 2001). Natural antioxidants increase the antioxidant capacity of the plasma and reduce the risk of diseases such as cancer, heart diseases and stroke (Prior & Cao, 2000).

The therapeutic effects of several medicinal plants are usually attributed to their antioxidant phytochemicals (Yildirim et al, 2001). The secondary metabolites like phenolics and flavonoids from plants were reported to be potent free radical scavengers. They are found in all parts of plants such as leaves, fruits, seeds, roots and barks (Mathew & Abraham, 2006).

Philippines is rich in biodiversities of plant species where only small portion of the species were investigated in detail. The plant samples of this study as shown in Table 1 are parts of the ethno-medicine and healthcare system of the Kalanguyas in Tinoc, Ifugao (Balangcod & Balangcod, 2011). These people still make use of plants as medicines despite the absence of scientific bases, hence this study.

Table 1. Plant samples and their traditional uses

| Plant Samples | Local Name/s | Traditional Use/s |
|---|------------------------------------|--|
| <i>Medinilla pendula</i> Merr. <i>Rubus fraxinifolius</i> Hayata | Ballangbang Pinit/ Buyot/ Habit | Decoction of leaves is given during cough. Crushed leaves are applied on sore eyes and wounds. Also, decoction of roots, stems, and leaves is a cure of diarrhea and urinary tract infection. |
| <i>Melastoma polyanthum</i> Blume | Bakhi/ Batgi | The stem is cooked with meat and consumed to treat high cholesterol level and hypertension. Also, the decoction of roots and leaves is given during dysentery and fever. |
| <i>Desmodium sequax</i> Wall. <i>Astilbe rivularis</i> Buch.-Ham ex. D. Don <i>Drymaria cordata</i> (L.) Willd. Ex J.A. | Pullet Kawan/ Cawad Hithit | Crushed leaves are applied on wounds. Decoction of the leaves or roots is given as a cure for diarrhea. It is used to treat boils. |

| | | |
|--|--------------------------------|---|
| <i>Schultes</i> <i>Allium odoratum</i> L. | Danggon hapon | The crushed leaves are used as poultice on blisters caused by measles and chickenpox. It also heals hematoma and sprains. |
| <i>Physalis minima</i> (L.) | Kamahit/ Batuwang/ Batiwang | Decoction of leaves is given during cough and diarrhea. |
| <i>Centella asiatica</i> (L.) Urban | Kannapa/ Canapa | Decoction of stems and leaves are taken orally to relieve cough. Also, crushed leaves and stems are applied on burns. |
| <i>Vaccinium myrtooides</i> (Bl.) Miq. | Gatmo/Ayohip/ Agohip | Decoction of the stem is used as wash or antiseptic during fever. |

II. MATERIALS AND METHODS

Sample collection and authentication

Fresh leaves of *Medinilla pendula* Merr., *Rubus fraxinifolius* Hayata, *Melastoma polyanthum* Blume, *Desmodium sequax* Wall., *Astilbe rivularis* Buch.-Ham ex. D. Don, *Drymaria cordata* (L.) Willd. ex J.A. Schultes, *Allium odoratum* L., *Physalis minima* L., *Centella asiatica* (L.) Urban, and *Vaccinium myrtooides* (Bl.) Miq. were collected from the different barangays of Tinoc, Ifugao. The plant samples were identified by Dr. Teodora D. Balangcod, a Botanist from the Department of Biology, University of the Philippines-Baguio, Baguio City, Cordillera Administrative Region (CAR), Philippines.

Sample preparation and extraction

Leaves of the ten (10) plant samples were removed from the stems and washed thoroughly with running tap water. Leaves of plant samples were air dried for a period of three weeks. These air-dried leaves were grinded and pulverized using a mechanical grinder. Two hundred grams of the ground samples were placed in a 3-L capacity bottle. Then 95 % ethyl alcohol was added until the plant samples were completely submerged. These amber bottles were covered and set aside and stored at room temperature for 48 hours. Then the plant samples were filtered using a Buchner funnel with gentle suction. Each filtrate was then concentrated in a rotary evaporator until approximately 20% of the filtrate was left.

DPPH scavenging assay

Radical scavenging activity by DPPH method was used following the different procedures (Molyneux, 2004; Bozin et al, 2006; Nya et al, 2009). Two hundred (200) milligrams of dried samples were extracted and mixed with five ml hot 70% ethanol in a 70°C water bath for about ten minutes with occasional vortex mixing. The mixtures were allowed to cool at a room temperature and then filtered using a Buchner funnel. The residues were further extracted and the filtrates were mixed. Cooled seventy percent (70%) methanol was added to the filtrates to make ten (10) ml solution to achieve an equivalent concentration of 20 milligrams dried sample per milliliter (ml) of solution. Aliquots were taken to prepare the different dilutions of 100 µg/ml, 200 µg/ml, 400 µg/ml, and 800 µg/ml.

Two microliter (ml) of the prepared concentrations of the leaf extracts such as 100 µg/ml, 200 µg/ml, 400 µg/ml, and 800 µg/ml were placed in microplates. About 2 ml of 30 µM solution were mixed by shaking gently. After 30 minutes of incubation at 37°C, the absorbance of each plate was read and recorded at 517 nanometer (nm) using a spectrophotometer. DPPH generated its yellow color if free radicals were scavenged. The degree of

discolorations of the solution indicates free radical scavenging activity.

Inhibition of the free radical, DPPH, by the sample in percent (%) was calculated using the formula:

$$\% \text{Free radical Inhibition} = \frac{\text{Abs}_{\text{blank}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{blank}}} * 100$$

where Abs_{blank} is the absorbance of the blank sample (containing all reagents except the test samples), and Abs_{sample} is the absorbance of the test samples.

Phytochemical Screening

Ethanol leaf extracts prepared were analyzed for the presence of alkaloids, glycosides, saponins, phytosterols, tannins, flavonoids, terpenoids and phenolic compounds (Himesh et al, 2011; Tiwari et al, 2011).

Detection of Alkaloids

Ethanol leaf extracts were dissolved individually using a dilute hydrochloric acid. These were filtered using a Buchner funnel in preparation for the detection of alkaloids.

A. Mayer's Test. Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow colored precipitate indicates the presence of alkaloids.

B. Dragendroff's Test. Filtrates were treated with Dragendroff's reagent (Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

C. Hager's Test. Filtrates were treated with Hager's reagent (saturated picric acid solution). Formation of a yellow colored precipitate indicated the presence of alkaloids.

D. Wagner's Test. Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicated the presence of alkaloids.

Detection of Glycosides

Ethanol leaf extracts were hydrolyzed with dilute HCl and then subjected to the different tests for the presence of glycosides. These include:

A. Modified Borntrager's Test. Ethanol leaf extract were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixtures were cooled and extracted with equal volumes of benzene. The benzene layer of each extract was separated and treated with ammonia solution. Formation of rose-pink color in the ammonical layer indicated the presence of anthranol glycosides.

B. Kedde Test. Five (5) ml of the ethanol leaf extract were dried using a water bath. The residue of the ethanol leaf extracts were dissolved in a 3 ml concentrated R-acetic acid. About one (1) drop of R-iron (III) chloride test solution was added to the liquid and was carefully transferred to the

concentrated R-concentrated sulfuric acid. A reddish brown ring was formed at the interface, and then the upper acetic layer soon turned bluish green to indicate the presence of glycosides.

Detection of Saponins

A. Froth Test. Ethanolic leaf extracts were diluted with twenty ml of distilled water. The diluted ethanolic leaf extracts were shaken in a graduated cylinder for about 15 minutes. Formation of one cm layer of foam indicates the presence of saponins.

B. Foam Test. About 0.5 gram of the ethanolic leaf extracts were placed in a test tube, diluted with 2 ml of water. Foam production that persisted for about ten (10) minutes after being shaken, indicated the presence of saponins.

Detection of Phytosterols

Libermann Burchard's Test. Ethanolic leaf extracts were treated with chloroform and then filtered using Whatman filter paper. The filtrates were treated with few drops of acetic anhydride, boiled and then cooled. Then concentrated sulfuric acid was added. Formation of brown ring at the junction indicated the presence of phytosterols.

Detection of Tannins

Gelatin Test. About 1% gelatin solution containing sodium chloride was added to the ethanolic leaf extract. Formation of white precipitate indicated the presence of tannins.

Detection of Flavonoids

A. Alkaline Reagent Test. Ethanolic leaf extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless with the addition of dilute acid, indicates the presence of flavonoids.

B. Lead Acetate Test. Ethanolic leaf extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicated the presence of flavonoids.

Detection of Terpenoids

A. Cupric Acetate Test. Ethanolic leaf extracts were dissolved in water and then treated with a few drops of copper acetate solution. Formation of emerald green color indicated the presence of diterpenes.

B. Salkowski's Test. Ethanolic leaf extracts were treated with chloroform and then filtered using Whatman filter paper. Filtrates were treated with few drops of concentrated sulfuric acid, shaken and then allowed to stand for five (5) minutes. Appearance of golden yellow color indicated the presence of triterpenes.

Detection of Phenolic Compounds

Ferric Chloride Test. Ethanolic leaf extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicated the presence of phenols.

III. RESULTS AND DISCUSSION

DPPH radical scavenging activity is one of the most widely used method for screening the antioxidant activity of plant extracts (Sahu et al, 2013). The DPPH test provides information on the reactivity of the test compounds with a stable free radical (Ayoola et al, 2008). Ascorbic acid was chosen as the reference antioxidant for this test. Table 2 shows the IC₅₀ values of the 10 different plant extracts. An IC₅₀ value is the concentration of the sample required to scavenge 50% of the free radicals present in the system (Sahu et al, 2013). The lower the IC₅₀ value, the higher will be the antioxidant activity of the extracts (Asadujjaman et al, 2013). Results revealed that the ethanolic leaf extracts of *Vaccinium myrtooides* (Bl.) Miq. (20.85 µg/ml) has the highest DPPH radical scavenging (antioxidant) activity which was even higher than that of ascorbic acid (21.56 µg/ml), followed by *Melastoma polyanthum* Blume (22.88 µg/ml), *Astilbe rivularis* Buch.-Ham ex. D. Don (24.90 µg/ml), and *Rubus fraxinifolius* Hayata (35.62 µg/ml). This assay suggest that only four out of ten plant samples are capable of scavenging free radicals via electron or hydrogen donating mechanisms and thus should be potent enough to prevent the initiation of deleterious free radical mediated chain reactions in susceptible matrices.

Table 2. The IC₅₀ of the ten different plant extracts

| Plant Samples | IC ₅₀ , µg/ml |
|---|--------------------------|
| <i>Medinilla pendula</i> Merr. | 255.363 |
| <i>Rubus fraxinifolius</i> Hayata | 35.642 |
| <i>Melastoma polyanthum</i> Blume | 22.875 |
| <i>Desmodium sequax</i> Wall. | 178.868 |
| <i>Astilbe rivularis</i> Buch.-Ham ex. D. Don | 24.904 |
| <i>Drymaria cordata</i> (L.) Willd. Ex J.A. | 899.273 |
| <i>Schultes</i> | 378.491 |
| <i>Allium odoratum</i> L. | 660.00 |
| <i>Physalis minima</i> (L.) | 607.790 |
| <i>Centella asiatica</i> (L.) Urban | 20.853 |
| <i>Vaccinium myrtooides</i> (Bl.) Miq. | 21.556 |
| Ascorbic Acid (Control) | |

The vital role of antioxidants is their interaction with oxidative free radicals (Amari et al, 2014). The efficacies of antioxidants are often associated with their ability to scavenge stable free radicals (Krishnaraju et al, 2009). Figure 1 shows the percentage inhibitions of the different concentrations of the ethanolic leaf extracts. The different concentrations of the extracts were 100, 200, 400, and 800 µg/ml respectively. The concentrations of ascorbic acid were 10, 20, 40 and 80 µg/ml respectively. Results revealed that there was a graded increase in percentage inhibition with the increasing concentration of the plant samples and ascorbic acid (Figure 1). This proves that the percentage scavenging effects on the DPPH radical increases sharply with the increasing concentration of the samples and the standard to a certain extent hence, they are said to be strongly dependent on the extent concentrations (Philip et al, 2012).

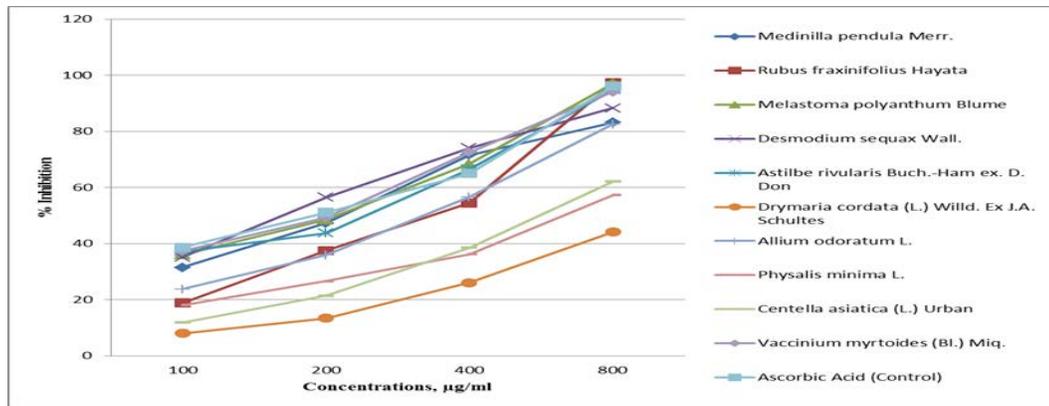


Figure 1. Percentage inhibitions of the different concentrations of the ethanolic leaf extracts

The antioxidant activity of the four plant extracts is due to their bioactive components present. Phytochemical investigation revealed the presence of flavonoids and phenolic compounds in all four plant extracts (Table 2). Also, tannins and phytosterols are present except in *Astilbe rivularis* Buch.-Ham ex. D. Don. Many studies confirmed that phenolic constituents, such as

flavonoids, phenolic acids and tannins are well known for their high antioxidant activities (Shahidi et al, 1990; Rice-Evans et al, 1996). Flavonoids and tannins are major group of compounds that act as primary antioxidants or free radical scavengers (Polterait, 1997).

Table 2. Phytochemical analysis of the four plant samples with antioxidant potentials

| Test | <i>Vaccinium myrtilloides</i> (Bl.) Miq. | <i>Melastoma polyanthum</i> Blume | <i>Astilbe rivularis</i> Buch.-Ham ex. D. Don | <i>Rubus fraxinifolius</i> Hayata |
|----------------------------|--|-----------------------------------|---|-----------------------------------|
| Alkaloids | | | | |
| Meyer's Test | - | - | - | - |
| Dragendroff's Test | - | - | - | - |
| Hager's Test | - | - | - | - |
| Wagner's Test | - | - | - | - |
| Glycosides | | | | |
| Modified Borntrager's Test | - | - | - | - |
| Kedde Test | - | - | - | - |
| Saponins | | | | |
| Froth Test | - | - | - | - |
| Foam Test | - | + | + | - |
| Phytosterols | | | | |
| | + | + | - | + |
| Flavonoids | | | | |
| Alkaline Reagent Test | + | + | + | + |
| Lead Acetate Test | + | + | - | + |
| Terpenoids | | | | |
| Cupric Acetate Test | - | - | - | - |
| Salkowski's Test | - | - | - | - |
| Phenolic Compound | | | | |
| | + | + | + | + |
| Tannins | | | | |
| | + | + | - | + |

Flavonoids linked were linked to reducing the risk of major chronic diseases including cancer because they have powerful antioxidant activities in vitro, being able to scavenge a wide range of reactive species (e.g., hydroxyl radicals, peroxy radicals, hypochlorous acid, and superoxide radicals) (Hollman & Kattan, 2000). Epidemiological studies also suggest that the consumption of flavonoid-rich foods protects man against diseases associated with oxidative stress, like coronary heart disease and cancer (Duthie et al, 2000; Lamber & Yang, 2003). The protective effect provided by fruits and vegetables against cancer, cardio and cerebrovascular diseases were attributed to their antioxidant compounds (Gey, 1990).

Phenols are very important plant constituents known for their free radical scavenging abilities due to their hydroxyl groups (Hatano et al, 1989). The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Hasan et al, 2008). Many phenolic compounds were reported to possess potent antioxidant activity and anticancer or anticarcinogenic/ antimutagenic, antiatherosclerotic, antibacterial, antiviral, and anti-inflammatory activities to a greater or lesser extent (Owen et al, 2000; Veeriah et al, 2006; Han et al, 2007; Baidez et al, 2007).

Tannins were observed to have remarkable activity in cancer prevention and anticancer (Li et al, 2008). In carcinogenesis, reactive oxygen species are responsible for initiating the multistage carcinogenesis process starting with DNA damage and accumulation of genetic events in one or few cell lines which leads to progressively dysplastic cellular appearance, deregulated cell growth, and finally carcinoma (Tsao et al, 2004).

IV. CONCLUSIONS

Only four extracts from *Vaccinium myrtilloides* (Bl.) Miq., *Melastoma polyanthum* Blume, *Astilbe rivularis* Buch.- Ham ex. D. Don and *Rubus fraxinifolius* Hayata showed varying antioxidant (free radical scavenging) activities when compared to ascorbic acid in the following order: *Vaccinium myrtilloides* (Bl.) Miq. > *Melastoma polyanthum* Blume > *Astilbe rivularis* Buch.-Ham ex. D. Don > *Rubus fraxinifolius* Hayata. The results suggest that the antioxidant activities of these plants are due to the bioactive components present.

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