

PJN

ISSN 1680-5194

PAKISTAN JOURNAL OF
NUTRITION

ANSI*net*

308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Phytochemical Profiles, Total Flavonoids, Total Phenolic Content and Antioxidant Activities Via Free Radical Scavenging Activities (FRSA) of Philippine Herbal Vines

Richard I. Licayan, Romeo M. Del Rosario, Nenita D. Palmes and Oliva P. Canencia
Mindanao University of Science and Technology, College of Arts and Sciences,
C.M. Recto Avenue, Lapasan Highway, Cagayan de Oro City, Philippines

Abstract: Philippines is bestowed with the abundance of plant biodiversity. Many of these plants have not been studied or thoroughly evaluated. Such plants could be a potential source of new molecules with impending therapeutic activities only waiting to be discovered. One of this is the group of vines that are widespread and fast growing including *Hiptage benghalensis*, *Antigonon leptopus*, *Macroptilium atropurpureum* and *Dioscorea bulbifera* L. These plants that have been used by traditional healers in rural communities in the Philippines as source of food, tea and medicine for a long time. In this study, the basic nutraceutical components of the crude extracted from the four herbal vines and their *in vitro* antioxidant properties was investigated to provide baseline data for the possible development of these plants as functional food and pharmaceutical products. Qualitative screening of the phytochemical constituents showed that alkaloids, tannins, saponins, steroids and flavonoids were present in their leaf extracts. All four showed varied free radical scavenging activities (FRSA). The greatest DPPH radical scavenging activity was observed in *H. benghalensis* (84.64%), followed by *A. leptopus* (68.21%), *M. atropurpureum* (26.62%) and *D. bulbifera* L. (19.04%). The FRAP assay revealed that *H. benghalensis* had the highest antioxidant activity (8.32 mg/g) while ABTS assay showed that *M. atropurpureum* had the strongest scavenging ability of free radicals (0.0842 mg Trolox/g). The total flavonoid content (TFC) analysis showed that *D. bulbifera* L. had the highest TFC (420.35 mg quercetin per gram-dried material). The total phenolic content (TPC) of the four herbal vines showed large variations, between 26.56 ± 0.160 and 55.91 ± 0.087 mg GAE/g dried material. The plant leaf extracts arranged in increasing values of TPC are *H. benghalensis* (26.565) < *A. leptopus* (37.29) < *D. bulbifera* L. (46.81) < *M. atropurpureum* (55.91). These plants appeared to be good sources of antioxidants for the development of functional food and biopharmaceuticals.

Key words: Antioxidant activities, herbal, total flavonoids, total phenolics, Philippine herbal vines

INTRODUCTION

Plants have been sources of most medicinal preparations to cure wide range of ailments and for isolation of bioactive compounds useful in biopharmaceutical industry. Many of medicinal plants contain bioactive compounds that can treat various infectious diseases and chronic conditions. A typical example is the discovery and isolation of artemisinin from *Artemisia annua* L., a Chinese medicinal plant used for 2000 years for fevers is now an effective drug to treat malaria.

In the advent of drug discovery and development predominantly bioactive lead compounds are natural products. Natural products are phytochemicals that are naturally occurring bioactive compounds in plants. Approximately 5000 phytochemicals have been discovered, isolated and named to date but a large percentage still remain unknown and need to be identified before their health benefits are fully understood (Liu, 2004). These bioactive non-nutrient

compounds found in plants impede important cellular pathways and play a vital role in maintaining homeostasis between health and disease. Among these phytochemicals, alkaloids, flavonoids, tannins and phenolic compounds are found to be the most beneficial to humans.

Philippines has bestowed with an enormous species of medicinal plants that are traditionally used and in most cases there are no scientific studies have been carried out to proved their efficacy. Taking stock of the abundance of Philippine biodiversity, medicinal plants remain to be critical in the development of common-day drug or medicine. Plant-based drugs continue to be an important source of alternative medicine because of the availability, relatively cheaper cost and non-toxic nature when compared to modern medicine (Narayanawamy and Balakrishnan, 2011). The National Integrated Research Program on Medicinal Plants (NIRPROMP) of the Philippines continues to develop plant-based drugs from indigenous plants/plant material in order to make available affordable yet safe and effective drugs.

Hiptage benghalensis commonly known as hiptage is naturally growing in the Philippines and Southeast Asia (Pagad, 2006). It belongs to family Malphigiaceae. It is a high-climbing woody vine with white or yellow hairs. The leaves and bark of this plant are used in treatment of cough, asthma, rheumatism, burning sensation, skin diseases and leprosy (Agharkar, 1991). The ethanolic extract of the stem have anti-diabetic property (Hridi *et al.*, 2013).

Antigonon leptopus locally known in the Philippines as cadena de amor is a climbing, somewhat woody, perennial vine, with stems attaining a length of 10 meters. It belongs to family Polygonaceae. It grows widespread in the Philippines. The leaves are used in Filipino folkloric medicine to reduce swelling, diabetes and flowers are used to treat hypertension. It is also used as herbal remedy for pain and gout-like symptoms in the Philippines (Apaya and Hernandez, 2014).

Macroptilium atropurpureum is commonly known as purple bush bean or siratro. It belongs to family Fabaceae, is a perennial herb with creeping and twining green stems and deep purple flowers. Decoction of the leaves of this plant is used as analgesic, treatment for fever and cough.

Dioscorea bulbifera L. is commonly known as air potato. It is a creeping perennial vine or yam used as a source of food and in folkloric medicine. It is native in the Philippines and other Asian countries. Research studies indicate that this plant has anti-inflammatory, estrogenic androgenic, antitumor, antifungal and cardiovascular activities (Sautour *et al.*, 2007).

Research studies showed that oxidative stress is among the major causes of many chronic and degenerative diseases including atherosclerosis, heart diseases, diabetes mellitus, cancer, neurodegenerative diseases and other prevalent diseases (Salihoglu *et al.*, 2013). Natural antioxidants help protect the human body from free radicals and impede the progress of many chronic diseases as well as hindering the lipid oxidative rancidity in foods (Pryor, 1991). Epidemiological studies have been investigated the potential of plant products as antioxidants against various diseases induced by free radicals (Hou *et al.*, 2003). Among these natural antioxidants, polyphenols such as flavonoids and phenolic acids are known in preventing oxidative damages (Silva *et al.*, 2000).

In the present study, the basic pharmacological components of the crude phytochemical components extracted from the four herbal vines and their *in vitro* antioxidant properties including DPPH radical scavenging activity, ferric reducing antioxidant power (FRAP) and ABTS radical assay was investigated to provide baseline data for the possible development of these metabolites in pharmaceutical products.

MATERIALS AND METHODS

Plant materials: The fresh leaves of the four species of herbal vines were collected from the localities of

Cagayan de Oro City, Philippines. The scientific names of this plant species were identified from the library of botanical plants in the Philippines and authenticated from Co's Digital Flora of the Philippines.

Phytochemistry of plant samples: Standard method of qualitative analysis of the phytochemistry of the plant samples was used with slight modifications adopted from Goyal *et al.* (2012).

Test for carbohydrates: Exactly 500 mg of the milled leaves of the plant samples were boiled in 30 mL distilled water and filtered. One mL of the filtrate was added with 1 mL of Molisch's reagent and 1 mL of concentrated sulphuric acid, H₂SO₄. The appearance of a reddish ring indicated the presence of carbohydrates.

Test for reducing sugars: One mL of the above filtrate was added to 2 mL of Fehling's solution and boiled for five minutes. The presence of a brick-red precipitate inferred the presence of reducing sugars.

Test for tannins: Exactly two mL of the above filtrate was added with 1 mL ferric chloride, FeCl₃. A blue-black or greenish-black precipitates indicated the presence of tannins.

Test for saponins: Frothing test was used for the screening of saponins. This was done by adding 5 mL distilled water to 0.50 mL of the filtrate in a test tube and was shaken for 30 sec. Persistent frothing confirmed the presence of saponins.

Test for flavonoids: The test for flavonoids was done using Shinoda's test. This test was done by extracting 200 mg of the plant material with 10 mL chloroform. This was filtered and 2 mL of the filtrate was added with magnesium ribbon and concentrated hydrochloric acid, HCl. A pink-red was noticed indicating the presence of flavonoids.

Test for steroids: Liebermann-Burchard's test was used for the screening of steroids. The plant samples were extracted by weighing 200 mg and soaking them with 10 mL chloroform. This was filtered and 2 mL of the filtrate was added to 2 mL acetic anhydride and 1 mL concentrated sulfuric acid, H₂SO₄. A blue-green ring was observed confirming the presence of steroids.

Test for glycosides: Screening for the glycosides was done using Keller-Killiani test. This was done by pipetting 2 ml of the crude extract and added with 1 mL of FeCl₃ and 1 mL of concentrated H₂SO₄. A green-blue color indicated the presence of glycosides.

Test for anthraquinones: Borstrager's test was used for the test for anthraquinones. This was done by extracting 100 mg of the milled leaves of the plant samples in 5 ml

of chloroform and filtered. Then, 2 mL of the filtrate was added with 2 mL of 10% NH₄OH. A bright pink color confirmed the presence of anthraquinones.

Test for alkaloids: This was done by extracting 200 mg of plant samples with 20 mL methanol. This was filtered and 1 mL of the filtrate was added with 2 or 3 drops of Wagner's reagent. A brown or reddish-brown precipitates indicated the presence of alkaloids.

Determination of the total flavonoid content: The determination of total flavonoid content was done by adopting the method used by Goyal *et al.* (2012) with slight modification.

Extraction of the plant materials: Twenty grams of pounded plant materials were soaked with 300 mL of methanol for 12 h with occasional shaking. This mixture was filtered and the filtrate was set aside. The residue was soaked with another 300 mL of methanol for one hour and the filtrate was collected. Then, the residue was soaked again with 300 mL methanol and filtered. The filtrates were then combined and concentrated to about 100 mL by using the rotary evaporator. This was then transferred and raised to volume in a volumetric flask and subjected further for the analysis of solid content via oven method.

UV-Vis spectrophotometric analysis for total flavonoid content: The total flavonoid content of the medicinal weed samples was determined with the aluminium chloride, AlCl₃, method using quercetin as the standard. The plant extract was piped out and added to 1.25 mL distilled water followed by 75 µl of 5% NaNO₂. After five minutes at room temperature, 0.15 mL of AlCl₃ was added. This was incubated for six minutes at room temperature to give time for the chemical reaction to take place. The reaction mixture was then treated with 0.5 mL of 1 mM NaOH. Then the reaction mixture was diluted with 275 µL of distilled water and incubated further for 20 min at room temperature. The absorbance was then read at 510 nm using UV-Vis spectrophotometer. This was done in three trials and the total flavonoid content was calculated from a quercetin standard curve.

Determination of the total phenolic content: The analysis for the total phenolic content of the four herbal vines was carried out using the Folin-Ciocalteu (FC) method adopted from Goyal *et al.* (2012).

Plant sample preparation: The collected samples were cleaned by removing the unwanted parts, washed thoroughly, sliced into smaller pieces and air-dried for a few days. The dried leaves were then pounded and stored in containers ready for extraction.

Extraction of the plant materials: Twenty grams of pounded plant materials were soaked with 300 mL of methanol for 12 h with occasional shaking. This mixture was filtered and the filtrate was set aside. The residue was soaked in another 300 mL of methanol for one hour and the filtrate was collected. Then, the residue was soaked again in 300 mL methanol and filtered. The filtrates were then combined and concentrated to about 100 mL by using a rotary evaporator. The concentrate was then transferred and raised to volume in a volumetric flask and subjected further for the analysis of solid content via oven method.

Solid content analysis: Twenty ml of extract solution was piped out into an aluminium dish (pre-weighed). This dish was placed in an oven to evaporate the methanol at a temperature three degrees (3°) higher than the boiling point of methanol. The resulting extract was oven dried for several hours until constant weight of the solids was achieved. The weight of the residue was calculated by dividing residue by volume of sample (20 mL).

UV-Vis spectrophotometric analysis for total phenolic content: The total phenolic content of the four herbal vines was determined using the Folin-Ciocalteu (FC) reagent method with slight modification. Concisely, the leaf extract (0.5 mL) was mixed with 0.5 mL of FC reagent (previously diluted 1:1 with distilled water) and incubated for 5 min at room temperature. Then, 1 mL of 2% Na₂CO₃ solution was added. After incubation at room temperature for 10 minutes, the absorbance was read at 730 nm. All tests were performed in three trials. Gallic acid monohydrate was used as the standard. The total phenolic content was expressed as gram of Gallic acid equivalents (GAE) per 100 g extract.

DPPH radical scavenging activity: The free radical scavenging capacity of the extracts was determined using DPPH. The DPPH solution (0.006% w/v) was prepared in 95% methanol. The methanol extract of the leaves from the four selected medicinal vines was mixed with 95% methanol to prepare the stock solution (1 mg/mL). Freshly prepared DPPH solution was taken in the test tubes and extracts were added followed by serial dilutions (100-1000 µg) to every test tube so that the final volume was 2 mL and discoloration was measured at 517 nm after incubation for 30 min in the dark (Themo UV1 spectrophotometer). The control sample was prepared, which contained the same volume without any extract and 95% methanol 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 was the absorbance of the control and A_1 was the absorbance in the presence of the sample (methanolic leaf extract of the medicinal vines).

ABTS radical-scavenging ability: The ABTS radical-scavenging ability of both extracts was determined according to the method described by Ironi *et al.* (2012). The ABTS radical was generated by incubating equal volume of a 7 mM ABTS aqueous solution with $K_2S_2O_8$ (2.45 mM) in the dark for 16 h at room temperature and adjusting the absorbance at 734 nm to 0.7 ± 0.02 with 95% ethanol. Then 0.2 mL appropriate dilution of the extract was added to 2.0 mL ABTS solution and the absorbance was measured at 734 nm after 15 min. The trolox equivalent antioxidant capacity (TEAC) was subsequently calculated.

Ferric reducing antioxidant power (FRAP): The reducing property of the methanolic extracts was determined by assessing the ability of the extract to reduce FeCl solution as described by Ironi *et al.* (2012). A 2.5 mL aliquot was mixed with 2.5 mL of 200mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. and then 2.5 mL of 10% trichloroacetic acid was added. This mixture was centrifuged at 650 rpm for 10 min. Then 5 mL supernatant was mixed with an equal volume of water and 1 mL of 0.1% ferric chloride. The absorbance was 1 measured at 700 nm. The ferric reducing antioxidant property was calculated accordingly.

Statistical method of data analysis: Descriptive-comparative method of analysis was used to evaluate the phytochemical constituents of the four herbal vines. The mean and standard deviation were used to distinguish which of these plants had the highest and the lowest total flavonoids, total phenolics and antioxidant activity.

RESULTS AND DISCUSSION

Phytochemical profiles of Philippine herbal vines: A standard method of phytochemical tests on the crude extracts was employed to evaluate the phytochemistry of the four selected species of herbal vines. This was done by ocular inspection on the physical changes observed on the crude extracts such as color change, formation of ring or precipitates and persistent frothing to confirm the presence or absence of the secondary plant metabolites.

Table 1 shows the results of the phytochemical screening of the crude extracts of the four herbal vines. Based on the results, *H. benghalensis* was positive for the presence of the phytochemicals namely; alkaloids, flavonoids, carbohydrates, reducing sugars, saponins and steroids. The results of the study demonstrate that *H. benghalensis* is rich in potential secondary metabolites.

On the other hand, the phytochemistry of the *A. leptopus* leaf extracts was positive for flavonoids, alkaloids, carbohydrates, glycosides, reducing sugars, tannins, saponins and steroids. *M. atropurpureum* leaf extracts were also positive for flavonoids, alkaloids, carbohydrates, glycosides, reducing sugars, tannins, saponins and steroids. The phytochemical tests also showed that *D. bulbifera* was positive for flavonoids, alkaloids, carbohydrates, reducing sugars, saponins and steroids. It was negative for tannins and anthraquinones.

The four medicinal weed samples were all positive for polyphenolic compounds known as flavonoids. These are polyphenolic compounds that are generally found in plants. These compounds are known for their potential beneficial effects on human health. They have been reported to have antiviral, anti allergic, antitumor and antioxidant properties (Ren *et al.*, 2003).

As a whole, the results of the phytochemical screening revealed that the four selected herbal vines are potential sources of bioactive compounds and can be further studied for use in food, pharmaceutical and the agro-petro chemical industry. This could lead to further investigation of this group of herbal vines, concentrating particularly on the synergistic effects of the bioactive compounds in their crude extracts for various biological activities or pharmacological targets.

Total flavonoid and total phenolic content: The total flavonoids determination was done using aluminum chloride method and was calculated from a quercetin calibration curve of 275 ppm. Table 2 shows the relative total flavonoid content of the samples in mg quercetin per gram of crude extract.

The values as shown indicate that *D. bulbifera* L. had the highest total flavonoids with an equivalent value of 420.35 mg quercetin per gram dried material followed by *A. leptopus* (111.47), *H. benghalensis* (93.29) and *M. atropurpureum* (84.69). Results of the total phenolic content analysis revealed that *M. atropurpureum* had the highest TPC (55.91) and the lowest was observed in *H. benghalensis* (26.56).

These groups of herbal vines may prove to be beneficial to human health because of their high total phenolic content. Research studies revealed that plants rich in phenolic compounds have been reported to have antiviral, antiallergic, antitumor and antioxidant properties. These compounds are considered as powerful antioxidants because they exhibited inhibitory properties against carcinogenesis in a number of *in vivo* studies (Ren *et al.*, 2003).

Antioxidant capacity: The antioxidant capacities using DPPH, ABTS and FRAP assay of the four herbal vines leaf extract are shown in Table 3. The highest antioxidant activity with respect to %inhibition of DPPH radical was observed in *H. benghalensis* with 84.64 % inhibition. *M. atropurpureum* showed the highest % inhibition of ABTS

Table 1: Phytochemical profiles of the crude extracts of the four herbal vines in cagayan de oro city Philippines

Phytochemistry	Plant extract			
	<i>H. benghalensis</i>	<i>A. leptopus</i>	<i>M. atropurpureum</i>	<i>D. bulbifera</i>
Alkaloids	+++	++	++	++
Anthraquinone	-	-	-	-
Carbohydrates	+++	+++	+++	+++
Flavonoids	++	+	++	+
Glycosides	-	++	++	-
Reducing sugars	+++	+++	+++	+++
Tannins	-	+	-	-
Saponins	+++	+++	+++	+++
Steroids	+	++	+	+

Legend: (-) absence of ring, precipitates, froth, or change in color, (+) slight appearance of ring, precipitates, froth, or change in color (++) definite appearance of ring, precipitates, froth, or change in color, (+++) heavy appearance of ring, precipitates, froth, or change in color

Table 2: Total flavonoids and total phenolic content of the four herbal vines of cagayan de oro city, Philippines

Sample	Total flavonoids mg Quercetin/g dried material	Total phenolics mg GAE/g dried material
<i>H. benghalensis</i>	93.29±4.215	25.56±0.160
<i>A. leptopus</i>	111.47±3.26	37.29±0.7939
<i>M. atropurpureum</i>	84.69±4.35	46.81±0.0624
<i>D. bulbifera</i> L.	420.35±4.35	55.91±0.087

Results are the mean of triplicate determinations ± SD

Table 3: Antioxidant activity of four herbal vines in cagayan de oro city, Philippines

Sample	DPPH	ABTS	FRAP
	% inhibition		
<i>H. benghalensis</i>	84.64±3.927	0.052±0.031	8.32±0.162
<i>A. leptopus</i>	68.21±4.56	0.0347±0.025	5.22±0.138
<i>M. atropurpureum</i>	26.62±1.77	0.0842±0.038	2.198±0.723
<i>D. bulbifera</i>	19.04±0.866	0.0512±0.0403	3.665±0.168

Results are the mean of triplicate determinations ± SD

radical (0.0842%). In FRAP assay, *H. benghalensis* obtained the highest antioxidant capacity (8.32%) while the lowest was observed in *M. atropurpureum* (2.198%). Based on these results, one more likely to conclude that methanolic extracts of the four herbal vines have different level of antioxidant activity. The results suggest that the phenolic compounds and the various phytochemical constituents might contribute significantly to the antioxidant capacity of these plant species.

Conclusion: The present study demonstrated the phytochemistry, antioxidant activity, total phenolic and flavonoid contents of four herbal vines of Philippine origin (*Hiptage benghalensis*, *Antigonon leptopus*, *Macrotillium atropurpureum* and *Dioscorea bulbifera* L). The obtained results provide support on their use in herbal medicine and alternative use against wide range of diseases and infections. This study holds to the significance in using medicinal plants as an alternative source for treating various diseases. Further studies should be done on the dietary intake of these medicinal plants and to evaluate the possible synergism among extract components for their antioxidant activity. The results provided evidence that the studied plant might indeed be potential sources of natural antioxidants.

REFERENCES

- Agharkar, S.P., 1991. Medicinal Plants. Bombay Presidency Scientific Publishers, Jodhpur, India, pp:88-89
- Apaya, M.K. and C. Hernandez, 2014. New steroidal saponin from *Antigonon leptopus* Hook. Pharmacognosy magazine.
- Goyal, A.K., S.K. Middha and A. Sen, 2012. Evaluation of the DPPH radical scavenging activity, total phenols and Antioxidant activities in Indian wild Bambusa vulgaris "Vittata". Retrieved from www.jnatpharm.org Accessed October 30, 2014.
- Hou, W.C., R.D. Lin, K.T. Cheng, Y.T. Hung and C.H. Cho *et al.*, 2003. Free radical scavenging activity of Taiwanese native plants. Phytomed., 10: 170-175.
- Ironji, A.E., G. Oboh and J.K. Akintunde, 2012. Comparative and Synergistic Antioxidant Properties of Carica Papaya and Azadarichta indica leaves. IJPSR, 3: 4773-4779.
- Liu, R.H., 2004. Potential Synergy of Phytochemicals in Cancer Prevention: Mechanism of Action. Retrieved from www.proquest.com.
- Narayanaswamy, N. and KP. Balakrishnan, 2011. Evaluation of some Medicinal Plants for their Antioxidant Properties. Int. J. Pharm. Tech. Res., 3: 381-385.
- Pagad, S., 2006. Hiptage benghalensis (vine, climber, shrub). Pacific Island Ecosystems at Risk (PIER). IUCN/SSC Invasive Species Specialist Group (ISSG).
- Pryor, W.A., 1991. The antioxidant nutrients and disease prevention-what do we know and what do we need to find out?. Am. J. Clin. Nutr., 53: 391S-393S.
- Ren, W., Z. Qiao, H. Wang and L. Zhang, 2003. Flavonoids: Promising Anticancer Agents. Retrieved From www.erbeofficinali.org/dati/nacci.pdf Accessed December 20, 2014.
- Salihoglu, E., G. Akaydin, E. Can and S.Y. Akaydin, 2013. Evaluation of Antioxidant Activity of Various Herbal Folk Medicines. J. Nutr. Food Sci., 3: 222.
- Sautour, M., A.C. Mitaine-Offer and M.A. Lacaille-Dubois, 2007. The Dioscorea genes: a review of bioactive steroid saponins. J. Nat. Med., 61: 91-101.

- Shehla Unaiza Hridi, Nafisa Ferdous, Fakhar Uddin Majumder and J.M.A. Hannan, 2013. Phytochemical screening and anti-diabetic efficacy of stem of *Hiptage benghalensis* (L) Kurz. *J. Sci. and Innovative Res.*, 2: 736-744.
- Silva, B.A., F. Ferreres, J.O. Malva and A.C.P. Dias, 2005. Phytochemical and antioxidant characterization of *Hypericum perforatum* alcoholic extracts. *Food Chem.*, 90: 157-167.