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Phytochemical profiles and antioxidant activities of leaf extracts of euphorbia species

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Abstract

The study was conducted to identify the phytochemical constituents and to quantify the total phenolics, total flavonoids and free radical scavenging activity of leaf extracts from three plants belonging to Euphorbiaceae family namely *Euphorbia milii Des moul*, *Euphorbia trigona Mill* and *Euphorbia antiquorum L.* Preliminary phytochemical screening was also carried out. The total phenolics of the extracts was determined spectrophotometric ally according to the Folin-Ciocalteu procedure, while the totalflavonoids was determined by aluminum chloride colorimetric assay. DPPH assay was used for determining the free radical scavenging activity of the plant samples. Qualitative screening of the phytochemical constituents showed that alkaloids, carbohydrates, flavonoids, saponin and tannins were present in the metabolic leaf extracts. The total flavonoids analysis showed that *E. milii* had the highest (20.3 mg quercetin per gram-dried material) followed by *E. trigona* (19.5 mg quercetin per gram-dried material) and *E. antiquorum* (19.1 mg quercetin per gram-dried material). The total phenolics of the three samples showed a little variation with 3.61, 1.63, and 1.69 mg GAE/g dried material for *E. milii*, *E. trigona* and *E. antiquorum*, respectively. The highest DPPH radical scavenging activity was detected in the methanolic leaf extracts of *E. milii* (74.37%), followed by *E. trigona* (60.10%) and *E. antiquorum* (59.42%). The three plants appeared to be good sources of antioxidants and promising source of medicines against various types of chronic and degenerative diseases caused by oxidative stress.

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Introduction

Plants have a wide range of scientific and economic value. They are considerably valuable in agriculture, food, cosmetics, medical and pharmaceutical industries. Over the past few decades there have been much interest in plant and other natural materials as a source of new medicines. Plants are essential source of bioactive compounds which can be used to cure or treat diseases. Numerous plant species recorded to have active phytochemical compounds which are known as plant secondary metabolites (Abdallah, 2014). Secondary metabolites act as plant's natural system of defense and repair.

Plants produce a variety of secondary metabolites to survive the changing oxidative environment (Baba *et al.*, 2015). As such plant extracts are expected to contain a diversity of molecules with different structures and functions. This has led to development of a number of methods to evaluate the antioxidant activity of a plant extracts. Oxidative stress results from an imbalance between the body's production of free radicals and its ability to counteract their harmful effects through antioxidants. Antioxidants neutralize free radicals by either providing the extra electrons needed to complete them or by breaking them down to render them harmless (Zengin *et al.*, 2015). The natural antioxidants, polyphenols such as flavonoids and phenolics acids are known in preventing oxidative damages (Silva *et al.*, 2005). Antioxidants are highly capable agents to fight against free radicals (Tandog *et al.*, 2015). They promote growth of healthy cells, protect cells against diseases and most importantly, they provide excellent support for the body's immune system (Chakraborty *et al.*, 2009). Plants have long been a source of exogenous antioxidants (Kasote *et al.*, 2015). They produce a very impressive array of antioxidant compounds that includes carotenoids, flavonoids, and ascorbic acid among other stop event the oxidation of the susceptible substrate (Kaur *et al.*, 2014). It is believed that two-thirds of the world's plant species have medicinal importance, and almost all of these have excellent antioxidant potential (Kasote *et al.*, 2015).

Phytochemicals or plant secondary metabolites are not essential nutrients and are not required by the human body for sustaining life but have important properties to prevent or to fight some common diseases (Saxena *et al.*, 2013). Among the different secondary metabolites, alkaloids, flavonoids, tannins, carotenoids, and phenolic compounds are found to be most beneficial to humans (Licayan *et al.*, 2016).

In the Philippines, there are diverse varieties of plant species which are said to contain medicinal or herbal properties. Old folks, especially those living in far-flung places, know these medicinal plants well and are skillful in the preparation of these plants for the treatment of some common illnesses such as colds, fever, flu, etc. One of the traditionally used medicinal plant is the Euphorbia a genus from euphorbiaceae family. Some Euphorbia species such as *Euphorbia antiquorum L.* are used in Filipino folkloric medicine to reduce swelling and externally applied to sores, cysts, warts and calluses. Other Euphorbia species which has potential medicinal value are *Euphorbia milii Des moul* commonly known as corona de espina and *Euphorbia trigona Mill* also known as cathedral cactus. The study is designed to evaluate the phytochemical profiles and the antioxidant properties of the leaf extracts of *Euphorbia milii Des moul, Euphorbia trigona Mill. and Euphorbia antiquorum L.* The study employed the conventional method of extraction of the plant material to ensure that the bioactive components will not be degraded such that substantial data could be obtain during the phytochemical screening and in the quantitative analysis for the antioxidant properties.

Materials and methods

Plant materials and sample preparation

The fresh leaves samples of three different euphorbia species were collected from Tagoloan, Misamis Oriental and Balingasag, Misamis Oriental, Philippines. The scientific names of these plant species were identified and authenticated by a botanist from the Center for Biodiversity Research

and Extension in Mindanao, Central Mindanao University, Philippines.

The leaves of the plant sample were harvested during late afternoon considering the crassulacean acid metabolism (also known as CAM photosynthesis) where stomata of the leaves are open and higher possibility to get a good result for the test. Plant samples were then washed thoroughly and dried at room temperature in a well-ventilated room and ground into powder. The samples were placed into a zipper plastic bag and brought to Chemistry laboratories of University of Science and Technology of Southern Philippines, Cagayan De Oro City for chemical analyses.

Preparation of plant extracts

Powdered plant materials were soaked with 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 100m Lusing a rotary evaporator. The concentrated extract was then transferred in a volumetric flask and diluted with methanol.

Phytochemical screening

The concentrated extracts of selected plant were subjected to different chemical tests for the detection of different phytoconstituents using standard methods.

Test for alkaloids: About 200 mg of plant samples were extracted with 20 mL methanol. It was then filtered, and 1 mL of the filtrate was added with 2 or 3 drops of Wagner's reagent. Formation of brown or reddish-brown precipitates indicated the presence of alkaloids.

Test for anthraquinones: Screening for anthraquinones was done using Borntrager's test. This was done by extracting 100 mg of the milled leaves of the samples in 5 mL of chloroform and filtered. The 2 mL of the filtrate was added with 2 mL of 10% NH_4OH. Appearance of bright pink color confirmed the presence of anthraquinones.

Test for tannins: The ferric chloride test was used to test the presence of tannins. About two mL of plant extract was added with 1 mL ferric chloride, $FeCl_3$.The formation of blue-black or greenish-black precipitates indicates the presence of tannins.

Test for flavonoids: Shinoda's test was used for testing flavonoids. The 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. The appearance of pink-red color indicates the presence of flavonoids.

Test for carbohydrates: *About* 500 mg of the powdered leaves 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 sulfuric acid, H_2SO_4. The appearance of a reddish ring indicates the presence of carbohydrates.

Test forsaponins: *The* screening for saponins used frothing test. 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 seconds. Persistent frothing confirmed the presence of saponins.

Test for steroid: The Liebermann-Burchard's test was used for the screening of steroids. The analyte were extracted from the sample by soaking 200 mg plant samples with 10 mL chloroform. It was filtered and 2 mL of the filtrate was added to 2 mL acetic anhydride and 1 mL concentrated sulfuric acid, H_2SO_4. The formation of blue-green ring confirms the presence of steroids.

Test for glycosides: Keller-Killiani test was used for testing the presence of glycosides. This was done by

pipetting 2 mL of the crude extract and added with 1 mL of $FeCl_3$ and 1 mL of concentrated H_2SO_4. The appearance of green-blue color indicates the presence of glycosides.

Determination of the total flavonoids

The total flavonoid content of the plant samples was determined using the aluminum chloride ($AlCl_3$) method with quercetin as the standard. The1 mL of 1mg/mL plant extract was added to 5 mL distilled water followed by 0.300 mL of 5% $NaNO_2$.After standing for five minutes at room temperature, 0.600 mL of 10% $AlCl_3$ 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 0.2 mL of 1 m MNaOH. Then the reaction mixture was diluted with 1.1 mL of distilled water and incubated further for 20 minutes at room temperature. The absorbance was then read at 510 nm wavelength in a spectrophotometer. This was done in three replicates and the total flavonoids content was calculated from a quercetin standard calibration curve.

Determination of total phenolics

The total phenolic content of extracts was determined calorimetrically using folin-ciocalteu (FC) reagent method. The 0.5 mL leaf extract(at 1mg/mL solid content) was mixed with 4.5 mL distilled water. Then 0.50m Lfolin-ciocalteu reagent was added with constant shaking. It was followed by the addition of 5mL of 7% Na_2CO_3, then 2 mL distilled water.

After incubation at room temperature for 90 minutes, the absorbance at 730 nm was measured. All tests were performed in three replicates. Gallic acid monohydrate was used as the standard. The total phenolic content was expressed as milligram of Gallic acid equivalents (GAE) per 100g extract.

DPPH radical scavenging activity

The free radical scavenging activity of the extracts was determined using DPPH radical **(**1,1-Diphenyl-2-picrylhydrazyl radical)**.** The DPPH solution (0.006% w/v) was prepared from stock solution at concentration equal to 1 mg/mL with 95% methanol as solvent. The 10 mL of 1mg/mL plant extract was prepared using 95% methanol.

Then, 0.250 mL of 1mg/mL plant extract was placed in a clean vial, adding 1.75 mL of 95% methanol, mixed evenly. It was followed by the addition of 2 mL (0.006% w/v) DPPH solution, mixed evenly. It was incubated in the dark for 30 minutes. The absorbance was read at 510 nm using colorimeter. The free radical scavenging effect of the extract was determined using the following equation:

$$DPPH\ scavenging\ effect\ (\%) = \frac{(A_0 - A_1)}{A_0} x100$$

Where, A_0 was the absorbance of the control and A_1 was the absorbance of the sample. The control was prepared and analyzed in exactly the same way but without the extract.

Results

Phytochemical profile

The results shown in table 1 revealed that *Euphorbia milii Des moul*, *Euphorbia trigona Mill* and *Euphorbia antiquorum L.* were positive for the presence of the phytochemicals alkaloids, carbohydrates, flavonoids, saponins and tannins. Whereas, thethree plant extracts of Euphorbia species were negative for the presence anthraquinone, glycosides and steroids.

Antioxidant activity

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 *Euphorbia milii Des moul* had the highest total flavonoids with an equivalent value of 20.3 mg quercetin per gram dried material followed by *Euphorbia trigona Mill* (19.5) and *Euphorbia antiquorum L.* (19.1)Results of the analysis for total phenolics revealed that *E. milii* had the highest total phenolic content with an equivalent value of 3.61 mg GAE per gram of dried materials followed by *E. antiquorum* (1.69) and *E. trigona* (1.63).

Table 1. Phytochemical Profiles of the Crude Extracts of Three Euphorbia Species.

Phytochemicals	Plant Extracts		
	E. milli	*E. trigona*	*E. antiquorum*
Alkaloids	+ +	+ +	+
Anthraquinone	-	-	-
Carbohydrates	+	+	+
Flavonoids	+	+	+
Glycosides	-	-	-
Saponins	+ +	+ +	+ +
Steroids	-	-	-
Tannins	+ + +	+ + +	+ + +

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.

Discussion

Phytochemical Profile

Secondary metabolites contribute significantly towards the biological activities of medicinal plants such as hypoglycemic, antioxidant, antimicrobial etc. (Yadav *et al*., 2014). All the three selected Euphorbia plants were found to possess tannins. Tannins have amazing stringent properties. They are known to hasten the healing of wounds and inflamed mucous membranes (Yadav *et al*., 2014). Saponins were also present in the three plant samples. Numerous studies have confirmed that saponins possess the unique property of precipitating and coagulating red blood cells (Okwu, 2004).

Table 2. Total Flavonoids and Total Phenolics of the Three Euphorbia Species.

Sample	Total Flavonoids (mg quercetin/g dried material)	Total Phenolics (mg GAE/g dried material)
E. milli	20.3 ± 2.2	3.61 ± 0.81
E. trigona	19.5 ± 0.9	1.63 ± 0
E. antiquorum	19.1 ± 0.1	1.69 ± 0

Plants containing carbohydrates are known to exert a beneficial action on immune system by increasing body strength and hence are valuable as dietary supplements. Since all the plants sample were found present of carbohydrates this can be a good source of bioactive compounds. Likewise, alkaloids were also found present in all plant samples. Alkaloids represent a class which affects the central nervous system, reduces appetite and behaves as diuretic (Yadav *et al*., 2014). As a whole, the results of the phytochemical screening affirmed that all the three Euphorbia plant samples are potential sources of bioactive compounds and can be further studied for use in food, pharmaceutical and agro/petro--chemical industry.

Antioxidant activity

Plants with flavonoids have been reported to have antioxidant activity and are known to have health promoting effect like anti-cancer, anti-microbial, anti-inflammation and anti-viral properties (Ghasemzadeh *et al*., 2010). Quercetin is the most powerful flavonoids for protecting the body against reactive oxygen species produced during the normal oxygen metabolism or are induced by exogenous damage (Shah *et al*., 2016). Phenolic compounds have been reported to possess antioxidant activity which

allows them to scavenge both active oxygen species and electrophiles (Rice-Evans *et al.*, 1995).

Several studies have focused on the biological activities of phenolics, which are potent antioxidants and free radical scavengers. The antioxidant activity of phenolics including flavonoids is due to their redox properties which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers (Rice-Evans *et al.*, 1995). With the results obtained from this study, it indicates that those plant samples investigated have a potential to be used as an antioxidant. Furthermore, phenolic compounds have been reported to be beneficial in the treatment of chronic inflammatory diseases associated with overproduction of nitric oxide (NO) (Jiang *et al.*, 1995).

Table 3. Free Radical Scavenging Activity of Three Euphorbia Species.

Sample	Scavenging Effect on DPPH (%)
E. milli	74.37 ± 0.40
E. trigona	60.10 ± 0
E. antiquorum	59.42 ± 0.72

Radical scavenging activity

The DPPH radical had been used widely in the model system to investigate the scavenging activities of several natural compounds such as phenolic compounds, anthocyanins, or crude extracts of plants (Chang *et al.*, 2007). It is a stable nitrogen-centered free radical commonly used for testing radical scavenging activity of the compound or plant extracts (Pavithra *et al.*, 2014). DPPH radical was scavenged by antioxidants through the donation of hydrogen, forming the reduced DPPH-H^+. Substances which are able perform this reaction can be considered as antioxidants and therefore radical scavengers. With the result taken from the investigation, plant samples are a good source of antioxidant.

Overall, the study have shown that the methanolic leaf extract of selected Euphorbia species, *Euphorbia milii Des moul*, *Euphorbia trigona Mill* and *Euphorbia antiquorum L.* are sources of phytochemicals i.e, alkaloids, carbohydrates, flavonoids, saponins and tannins. Medicinal plants play a vital role in preventing various diseases, thus, the presence of these phytochemicals provide support on their use as an alternative medicine against certain diseases and infections. On the basis of total flavonoids, total phenolics, and radical scavenging activity, it is concluded that *Euphorbia milii Des moul*, *Euphorbia trigona Mill* and *Euphorbia antiquorum L.* possessed antioxidant activity. The findings on phytochemical analysis and antioxidant activity have significant use in both research institutes and pharmaceutical companies for the manufacture of the new drugs for treatment of various diseases.

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