PHYTOCHEMICAL PROFILES AND QUANTIFICATIONS OF FLAVONOID CONTENTS OF SELECTED HERBS IN CANTILAN, SURIGAO DEL SUR PHILIPPINES

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ABSTRACT

Phytochemical profiles and quantity of total flavonoid contents of methanolic extracts of selected herbs in Cantilan, Surigao del Sur, Philippines were studied. Results revealed that present among the crude extracts are secondary metabolites like carbohydrates, reducing sugars, saponins, flavonoids, steroids, alkaloids and glycosides. The total flavonoid content analysis showed that Capsicum frutescens had the highest total flavonoids with an equivalent value of 271.40mg quercetin per gram of crude extracts. This means that of the five extracts, Capsicum frutescens has the greatest free radical scavenging activity.

Keywords: *phytochemical profiles, flavonoids, scavenging activity herb extracts*

1.0 Introduction

Phytochemical or phytonutrients are compounds produced by plants which are considered nonessential nutrients but are required by the body for sustaining life, and protecting against certain diseases. Among these phytochemicals are alkaloids, tannins, flavonoids and phenols (Oloyede, 2009) compounds which are found to be the most beneficial to humans. There are more than thousand known phytochemicals. However, according to Ding (2012) only about 1,000 of these are identified and about a hundred are actually analyzed and tested for their medicinal value. Mostly, phytochemicals are found in fruits, vegetables, beans, grains, herbs and other plants.

Herbs like *Curcuma longa*, *Cymbopogon citratus*, *Mentha arvensis*, *Coleus aromaticus* and *Capsicum frutescens* are among those identified for their phytochemical content. They are commonly grown in home gardens. It is used widely for flavoring foods and beverages. It is also used as traditional folk medicine in the treatment of cough and colds, contraceptives (Pidugo et al., 2012), sprains, diabetes (Arutselvi et al., 2012), asthma, diarrhea (Rout et al., 2010), stimulant and carminative (Vinayaka et al., 2010).

In the last few years, a number of studies have been conducted on these herbs in different countries, however these studies focused only on the plant parts used, preparation, and route of administration for medicinal purposes. Research studies have shown that plants with potent bioactive compounds are often characterized as both poisonous and medicinal unless they are systematically evaluated and phytochemically profiled. The present study aims to screen phytochemical contents and quantify the total flavonoids.

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2.0 Materials and Methods

Chemicals

Aluminum chloride ($AlCl_3$), Sodium nitrite ($NaNO_2$), Sodium hydroxide (NaOH), Sulfuric acid (H_2SO_4), Ferric chloride($FeCl_3$), Ethanol (C_2H_6O), Magnesium ribbon, Hydrochloric acid (HCl), Chloroform, acetic anhydride, Ammonium hydroxide (NH_4OH), glacial acetic acid, Methanol (CH_4O), Napthanol, Iodine crystal (I_2), Potassium iodide (KI) and Distilled water were obtained from Mindanao University of Science and Technology, Chemistry Laboratory, Cagayan de Oro City. All chemicals and solvents are analytical grade.

Plant sample and sources

The plants used for the study are leaves of *Curcuma longa, Cymbopogon citratus, Coleus aromaticus, Mentha arvensis and Capsicum frutescens.* It was collected from various locations in Cantilan, Surigao del Sur, Philippines. It was confirmed in Biology Department, Mindanao University of Science and Technology, Cagayan de Oro City. The leaves of the plants were washed thoroughly and air-dried at Mindanao University of Science and Technology Laboratory. The dried leaves were then ground and stored separately in plastic containers and placed in the refrigerator at 4°C.

Preliminary Phytochemical Screening

The presence or absence of secondary metabolites of the grounded plant specimen was analyzed individually following to the method described by Goyal et al., 2012. The test were qualitatively expressed as negative (-) or positive (+). The tests of each plant specimen were done in triplicates.

Carbohydrates: 500 mg of each air-dried powdered leaves was boiled in 30 ml of distilled water. It was then filtered after boiling. One ml of the filtrate was (each sample) added with 1ml of Molisch's reagent and 1ml concentrated sulfuric acid (H_2SO_4). Formation of reddish ring indicates the presence of carbohydrates.

Reducing sugars: The above filtrates were used in this test. One ml of the filtrate (each sample) was added with 2 ml of Fehling's solution and boiled for 5 minutes. The presence of reducing sugar forms a brick red precipitate.

Tannins: Two milliliters from the above filtrates taken (each sample) and were added with Ferric chloride. To confirm the presence of tannins a blue-black or greenish-black precipitate was formed.

Saponins: Frothing test: From the above filtrate, 0.5 milliliter of each filtrate (sample) were added with 5 ml of distilled water and shaken for 30 seconds, a persistent frothing indicates the presence of saponins.

Flavonoids: Shinoda's Test: 200 mg of air-dried powdered plant (each sample) was extracted with 5 ml of ethanol. The extracts were then filtered separately. One ml of the filtrate was added with magnesium ribbon then added with concentrated HCl. Formation of pink or red color indicates the presence of flavonoids.

Steroids: Liebermann-Burchard's test: 200 mg of air-dried powdered plant sample (each) were subjected to 10 ml chloroform and was filtered. Two ml filtrate of each sample was added with 2 ml acetic anhydride and 1 ml of concentrated H_2SO_4. Blue-green ring formation is an indication that there is steroid present.

Alkaloids: 200 mg of (each) air-dried powdered plants sample were boiled to 20 ml methanol and was filtered. One ml of the filtrate was added with 3 drops of Wagner's reagent. Formation of brown or reddish brown precipitate indicates the presence of alkaloid.

Anthraquinones: Borntrager's test: 100 mg of (each) air-dried powdered plant sample was added with 5 ml of chloroform and was filtered. Two ml of the filtrate was added with 2 ml of 10% ammonium hydroxide (NH_4OH). Formation of bright pink color confirms the presence of anthraquinones.

Glycosides: Keller-Kiliani test: 2 ml (each sample) from the above filtrate was added with 1 ml of glacial acetic acid, 1 ml of ferric chloride ($FeCl_3$) and 1 ml concentrated sulfuric acid (H_2SO_4). A green-blue coloration indicates the presence of glycosides.

Determination of the Total Flavonoid Content

The flavonoid content of the plant samples was determined using aluminum chloride ($AlCl_3$) method with quercetin as standard.

A. Preparation of Standards

To quantify the total flavonoid content, quercetin was used as the reference, which was expressed as quercetin equivalent (QE). A standard curve of known concentrations of quercetin was generated by preparing and testing five concentrations of quercetin standard solution, which were 10,20,30,40 and 50 mg/L. Further dilution is applied to oregano, sili and yerba Buena since it exceeds to its flavonoid content. The UV-VIS Spectrophotometer took the absorbance readings at 510 nm.

B. Preparation of Samples

0.25 ml of plant extract (each plant extract) was placed in a vial and was added with 1.25 ml distilled water, subsequently added with 75 uL of 5% $NaNO_2$. The solution was allowed to stand for 5 min. at room temperature (RT). After 5 minutes, 0.15 ml of 10% $AlCl_3$ was added. It was further incubated for 6 min. at room temperature (RT). After 6 minutes, the mixture was treated with 0.5 ml of 1nM NaOH and was diluted with 275 uL of distilled water. The mixture was further subjected to incubation for 20 minutes at room temperature (RT). After 20 min., adsorbance readings were done using UV-VIS Spectrophotometer at 510 nm. The procedure were repeated to other plant extracts (duyaw, tanglad, oregano, yerba Buena, sili) and the tests were performed in triplicate. The following formula was used to calculate the total flavonoid content (Kiranmai et al., 2011)

$$TFC = \frac{R * D.F. * V * 100}{W}$$

where:

R = result obtained from standard curve

D.F. = dilution factor
V = volume of stock solution
100 = for 100g dried plant
W = weight of plant used in the experiment

Statistical Analysis

Results were expressed as mean ± standard deviation of triplicates. The groups were compared with a one-way ANOVA using portable minitab version 13.2

3.0 Results and Discussions

Phytochemical profiling and quantification of total flavonoid contents were conducted on the leaves of selected herbs in Cantilan, Surigao del Sur. The results obtained are presented in Table 1. Based on the selected secondary metabolites included in the study, *Mentha arvensis* was positive for the presence of the phytochemicals namely: carbohydrates, reducing sugars, saponins, flavonoids, steroids, alkaloids and glycosides. The results of the study revealed that *Mentha arvensis* is rich in potential secondary metabolites. As stated in Philippine Medicinal Plants (2012) *Mentha arvensis* is one of the ten herbs endorsed by the Department of Health (DOH) as an effective medicine for anti-septic, anti-cancer, anti-spasm and has anti-emetic activities. Moreover, the herbs also were found to function as stimulant and have restorative effect on the body system. Several researchers (Londonkar, 2009; Raja et al., 2010) also agreed that *Mentha arvensis* has secondary metabolites not only flavonoids but also terpenoids and phenols. Furthermore, on the study of Pidugu & Arun (2012) the *M. arvensis* leaves using different solvents showed results that in acetone extract, alkaloids, tannins, phenolic and carbohydrates were present but proteins, amino acids and flavonoids were absent. Isopropyl alcohol extract showed the presence of alkaloids, flavonoids, tannins, phenolic compounds and carbohydrates but absence of proteins, amino acids, anthraquinone, glycosides, saponins and phytosterol. In petroleum ether extract, it showed the presence

Table 1. Phytochemical profiles of the crude extracts of the five selected herbal plants in Cantilan

	PHYTOCHEMICAL HERBAL PLANTS				
Profiles	*C. longa*	*C. citratus*	*C. aromaticus*	*M. arvensis*	*C. frustescens*
Carbohydrates	+	+	-	+	+
Reducing sugars	+	+	+	+	+
Tannins	-	-	-	-	-
Saponins	+	+	-	+	+
Flavonoids	-	-	+	+	+
Steroids	-	+	+	+	+
Alkaloids	+	+	+	+	+
Anthraquinones	-	-	-	-	-
Glycosides	-	+	+	+	+

Note: (—) absence of secondary metabolite
(+) presence of secondary metabolite

of alkaloids and flavonoids, but proteins, amino acids, anthraquinone, glycosides, tannin, phenolic compounds and carbohydrates were absent.

On the other hand, the phytochemical analysis of the *Capsicum frutescens* extracts revealed the presence of secondary metabolites namely: carbohydrates, reducing sugar, saponins, flavonoids, steroids, alkaloids and glycosides. The result of the present study also confirmed with the finding of the study of Ghasemzadeh et al., (2011) that *Capsicum frutescens* had flavonoids content and had antioxidant activities. They also noted that it has high total flavonoids content compared to other tropical plants like cabbage, carrots, white radish, lemon grass and turmeric. Likewise, Vinayaka et al., (2010) did preliminary phytochemical analysis on the leaves of *C. frutescens* using methanol. The study revealed the presence of tannins, alkaloids, steroids and glycosides and has insecticidal potential.

Moreover, *Coleus aromaticus* leaf extracts were also positive for reducing sugars, flavonoids, steroids, alkaloids and glycosides. In India, Rout, et al., (2010) did preliminary phytochemical analysis on the leaves of *Coleus aromaticus* and the result of their study revealed the presence of alkaloids, carbohydrates, proteins, amino acids, flavonoids, tannins, phenolic compounds and terpenoids. It is also mentioned in Philippine Medicinal Plants (2012) that *Coleus aromaticus* is very rich in anti-oxidant phytochemical flavonoids and phenolic acids. However, in the present study some of the secondary metabolites of *Coleus aromaticus* were not present like carbohydrates, tannins and saponnins. Researchers in India, Ramya, Ganesh and Kumar (2012) assessed two important medicinal plants, *Coleus aromaticus* and *Leucas aspera,* of their phytochemical components and anti-microbial activity with an organic solvent methanol and ethanol for the extraction of plant materials. Results showed that the two plant extracts contained tannins, alkaloids and glycosides and both leaf extracts from methanol and ethanol exhibited antibacterial activity.

The phytochemical screening of *Curcuma longa* showed a positive result for carbohydrates, reducing sugars, saponins and alkaloids but other secondary metabolites were not detected like tannins, flavonoids, steroids, anthraquinones and glycosides. Arutselvi et al., (2012) conducted a study on phytochemical screening and antimicrobial activity of *Curcuma longa* leaves of different varieties; the result showed the presence of flavonoids, cardiac glycoside and phenols and has antimicrobial property. A parallel study was also done by Swadhini, Santhosh, Uma, Mythili, & Sathiavelu (2011). Results revealed that *Curcuma longa* has phytochemical constituents like flavonoids, cardiac glycosides and phenols but absence in alkaloids, tannins and saponins.

Cymbopogon citratus was negative for the presence of tannins, flavonoids and anthraquinones but was positive for carbohydrates, reducing sugars, saponins, steroids, alkaloids and glycosides. In the study of Oloyede (2009), aqueous extracts of *Cymbopogon citratus* leaves give a positive result for terpenoid phenol and cardiac glycosides. The in-vitro study of Nyananyo and Akada (2011) on methanolic extract of *Cymbopogon citratus* showed significant activity against *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Escherichia coli*. They also found that the extract was positive for alkaloids.

The presence of bioactive phytochemical components of these plants indicated that it has therapeutic properties. Steroids have biologic activities that include the development and control of the reproductive tract in man and contribute to a wide range of therapeutic application such as cardiotonics, anti-inflammatory agents and anabolic agents (Tyler et al., 1981). The five selected herbal plants were all positive for alkaloids. The alkaloids exhibited pharmacologic action (Madulid & Claustro, 2007) as analgesic, narcotics and central stimulants.

Saponins were present in *C. longa*, *C. citrates*, *M. arvensis* and *C.f rutescens* except for *C. aromaticus*. Plant saponins were classified into two types. One of these types is steroidal saponins which are of great importance because of their relationship to such compounds as sex hormones. It has also been found that saponins possess significant anti-cancer properties due to great variability of their structures (Man, et at., 2010).

The complete results of the phytochemical screening revealed that the five selected herbal plants in Cantilan are potential sources of bioactive components and can be further studied for the use in food, therapeutic agents and discovering the actual value of folkloric remedies.

Quantification of Total Flavonoid Contents of the Five Selected Herbs

The total flavonoids determination were done using methanolic leaf extracts of the herbal plant samples and was calculated from a quercetin calibration curve of 720ppm. Table 2 showed the total flavonoid content of the samples in mg quercetin per gram of crude extract.

The values, as shown, indicated that *Capsicum frutescens* had the highest total flavonoids with an equivalent value of 271.40 mg quercetin per gram of crude extract. *Mentha arvensis* ranked second, *Curcuma longa* ranked third, *Coleus aromaticus* ranked fourth and *Cymbopogon citratus* ranked fifth with

Table 2. Total flavonoid contents among the five selected herbal plants

Name of the Herbal Plants	Average total flavonoid contents $\frac{mg\ quercetin}{g\ crude\ extract}$		SD
Curcuma longa (Duyao)	141.09	±	5.94
Cymbopogon citratus (Tanglad)	86.26	±	12.93
Coleus aromaticus (Oregano)	107.00	±	18.8
Mentha arvensis (Helba Buena)	229.20	±	21.0
Capsicum frutescens (Sili)	271.40	±	18.3

Each value in the table was obtained by calculating the average of the three trials ± standard deviation.

the total flavonoid contents of 229.20, 141.09, 107.00 and 86.26 mg quercetin per gram crude extract respectively.

4.0 Conclusion

Based on the findings obtained, the following conclusions were drawn:

The phytochemical tests of five selected herbs showed different secondary metabolites present and absent. Among the five selected herbs two were predominantly rich in carbohydrates, reducing sugars, saponins, flavonoids, steroids, alkaloids and glycosides. This group of herbs was *Capsicum frutescens* and *Mentha arvensis*. The rest of the groups of herbs showed the presence of secondary plant metabolites but fewer numbers of bioactive constituents. Herbs *Cymbopogon citratus* rank second followed by *Coleus aromaticus* and the last one the *Curcuma longa.* The results implied that these herbs are capable of providing phytonutrients that could be used or synthesized for medical treatment or development of therapeutic drugs or agro-chemical products.

Results of the total flavonoid content analysis showed that *Capsicum frutescens* had the highest total flavonoids with an equivalent value of 271.40 mg quercetin per gram of crude extracts. *Mentha arvensis* ranked second (229.20 mg per gram), *Curcuma longa* ranked third (141.09 mg per gram), *Coleus aromaticus* ranked fourth (107.00 mg per gram) and *Cymbopogon citratus* ranked fifth (86.26 mg/gram). These five groups of herbs may prove to be beneficial to human health because of their high total flavonoid contents. According to the data obtained significant differences of total flavonoid contents has a p value < 0.05 which has been observed in plant extracts.

5.0 Recommendations

Based on the results obtained in the study, the following recommendations are drawn:

1. Quantitative analysis should be carried out of the five selected herbs for the presence of total phenolics and antioxidant activities.
2. Characterization and isolation of the bioactive compounds should be conducted in the five selected herbs.
3. Related research should be conducted on other herbs that are available in the locality.
4. Researchers should conduct scientific studies on the toxicity level of the five selected herbs.
5. Conduct scientific research on the synergistic effects of the five selected herb extracts on animal model to test for their biological activities.

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