TOTAL PHENOLS AND FREE RADICAL SCAVENGING CAPACITY OF FIVE SELECTED HERBS IN CANTILAN, SURIGAO DEL SUR

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Abstract

Many plants possess antioxidant components that provide efficacy in lowering incidence and in lowering mortality rates of degenerative diseases in human. In this article, five selected herbs were evaluated for their total phenols using Folin-Ciocalteu (FC) reagent and gallic acid monohydrate as the standard. 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay was used to evaluate free radical scavenging capacity. Results showed that the phenolic content of each herbal plant extracts varied from 31.95 mg/g to 91.02 mg/g and their free radical scavenging capacity ranged from 32.82% to 93.48%. Correlation ($p<0.05$) between the phenolic content and the free scavenging capacity of the five selected herbal plants was observed.

Keywords: Phenols, antioxidant, herbs, radical scavenger, 1,1-diphenyl-2-picryl hydrazyl

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1.0 Introduction

Oxidative stress is widely known to contribute many illnesses including cardiovascular diseases, diabetes, inflammation, degenerative diseases, cancer, anemia, and ischemia due to a well-known reactive oxygen species (ROS) (Ravipati et al., 2012; Hazra et. al, 2012). According to Hazra et al, (2010), most living species have efficient defense systems to prevent themselves against oxidative stress induced by ROS. The reactive oxygen species and reactive nitrogen species are free radicals generated by our body by various endogenous reactions which include exposure to different physiochemical conditions or pathological states. Some free radicals are also generated from exogenous sources like environmental pollutants, radiation, chemicals, toxins, deep fried, spicy foods, and physical stress that cause depletion of the immune system (Pourmorad et. al, 2006).

Recently, modern medicine observed that despite its great advances, plants still make important contributions to health care. According to Ghasemzadeh et. al, (2012), over 250,000 higher plant species possess therapeutic properties—among which only 7000 to 7500 species are identified worldwide. Saeed et al., (2012) stated that a large number of these medicinal plants are investigated for their antioxidant properties. An easily rapid and sensitive method for the antioxidant screening of plant extracts is free radical scavenging assay using 1,1-diphenyl-2-picryl hydrazyl (DPPH) stable radical spectrophotometrically with the presence of an antioxidant (Pourmorad et al., 2006).

In recent investigations, antioxidant properties of plants have shown correlation with oxidative stress since they contain a wide variety of free radical scavenging molecules such as flavonoids, anthocyanins, carotenoids and other secondary metabolites (Otang et. al, 2012). As cited in Ravipati et. al, (2012) and Oladunmoye (2010), plant-based antioxidant compounds play a defensive role by preventing the generation of free radicals. One of the major groups of phytochemical found universally is polyphenol, a natural antioxidant which has many beneficial functions such as reducing agents (free radical terminators), metal chelator and singlet oxygen quenchers (Sasikumar et. al, 2010; Ozkok et al., 2010; Chew et al., 2012).

Natural antioxidants either in the form of raw extracts or in their chemical constituents are very effective in preventing the destructive processes caused by oxidative stress (Pourmorad et. al, 2006). Although many researches were done on some plants, there is still scarcity of data analysis on phenolic content and scavenging activity of plants particularly in Cantilan, Surigao del Sur. This region has a wide variety of plants that are considered therapeutic, and the geographical condition is different from the other places. Thus, herbal plants *Curcuma longa* (duyao), *Cymbopogon citratus* (tangyad), *Mentha arvensis* (herba buena), *Coleus aromaticus* (garabo), and *Capsicum frutescens* (sili) that were commonly used as medicinal plants in our place, were studied to determine their total phenolic contents and scavenging activities and correlate phenolic contents to its antioxidant activity.

2.0 Research Methodology

A. Plant Material

The samples of *Curcuma longa* leaf (duyao), *Cymbopogon citratus* leaf (tangyad), *Mentha arvensis* leaf (herba buena), *Coleus aromaticus* leaf (garabo), and *Capsicum frutescens* leaf (sili) were collected during the month of March, 2014 within Cantilan, Surigao del Sur. These plant materials were botanically authenticated and were deposited in the herbarium at Mindanao University of Science and Technology, Cagayan de Oro City.

B. Plant Preparation

The collected leaves of the plants were sorted and washed with tap water. These plant materials were air-dried thoroughly under shade for 1-2 weeks at room temperature. The dried materials were milled into a fine particle size, placed in airtight bottles and stored in the refrigerator at 4°C for succeeding analysis.

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C. Chemicals

All the chemicals used for the analysis were of analytical grade. Folin-Ciocalteau reagent, gallic acid, quercetin, sodium carbonate (Na_2CO_3), potassium persulfate, 1,1-diphenyl-2-picryl hydrazyl radical (DPPH), methanol, and ascorbic acid were purchased from Sigma Co. (St. Louis, MO, USA). Other reagents were obtained from Mindanao University of Science and Technology Chemistry Laboratory.

D. Plant Extract Preparation

Twenty (20) grams of each dried grounded plant samples were extracted twice (1500 ml for each sample) with 95% methanol at 20°C for 48h and concentrated to 100 ml using a rotary evaporator (Panchun Scientific Co., Kaohsiung, Taiwan). The extracts obtained were evaporated under pressure at 50°C to a constant weight. The weight of the filtrate was then transferred to the volumetric flask and raised to 250 ml volume. The extracts were then placed into the storage bottle and placed in the refrigerator at 4°C for succeeding activities.

E. Determination of Plant Extract Yield

Twenty (20) ml of extract solution was pipetted into an aluminum dish (pre-weighed). The dish was placed in an oven to evaporate the methanol at a temperature three degree (3°) higher than the boiling point of methanol. Then, it was oven dried for several hours until constant weight of the plant residues were achieved. The yield of each plant extract was calculated by dividing residue by volume of sample (20 ml).

F. Assaying Methods

1. Total Phenol Content(TPC) Determination

The TPC of each methanolic extracts samples were determined based on Chan et al., (2007) with slight modification according to the Folin-Ciocalteu method. In brief, 300 µL of extract was dispensed into test tube mixed with 1.5 ml of Folin-Ciocalteu's phenol reagent. After 5 min, 1.2 ml of 7.5% (w/v) Na_2CO_3 solution was added to the mixture followed by the addition of 15 ml of dionized distilled water and mixed thoroughly and allowed to stand for 30 min in the dark room at room temperature, after which the absorbance was read at 765 nm. The TPC was expressed as milligrams of gallic acid equivalents (GAE) per gram of dried sample and the values are presented as means of triplicate analysis.

2. DPPH Free Radical-Scavenging Capacity

The free radical scavenging capacity of *C. longa*, *C. citratus*, *M. arvensi*, *C. aromaticus*, and *C. frutescens* extracts were determined by 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) on the ability of the stability of free radicals to decolorize in the presence of antioxidants with slight modification as described by Sellappan et. al, (2002). The DPPH radical contains an odd electron that is responsible for the absorbance of 517 nm and also for the visible deep purple color. The decrease in absorption was taken as a measure of the extent of radical scavenging. All determinations were carried out at three trials.

G. Statistical Analysis

The data were expressed as the mean±standard deviation of triplicate separate observations. Significant correlation between total phenols and scavenging capacity were statistically analyzed using Statistical Package for Social Sciences (SPSS) version 17.

3.0 Results and Discussion

A. Total Phenol Content

Plant-derived phenolic compounds are well known to exhibit antioxidant activity (Shahidi et al., 1997) due to their hydroxyl groups that can eliminate free radicals (Hatano et al., 1989). The total phenol content of each plant extract is presented in table 1.

Table 1. Quantitative determination of total phenolic content (TPC) as milligram of gallic acid equivalents (GAE) in mg per gram dried sample

Plant Extracts	Gallic Acid Equivalents mg/g ± SD
Curcuma longa (duyao)	47.32 ± 2.47
Cymbopogon citratus (tangyad)	44.05 ± 0.147
Coleus aromaticus (garabo)	50.04 ± 2.29
Mentha arvensis (herba buena)	91.02 ± 5.09
Capsicum frutescens (sili)	31.95 ± 0.862

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

The values, as shown in table 1, were significantly different ($p<0.05$) in the order of increasing total phenol content: *C. frutescens* leaf<*C. citratus* leaf<*C. longa* leaf<*C. aromaticus* leaf<*M. arvensis* leaf. The total phenol content varied from 31.95 to 91.02 mg/g in the dried herbal plant. *M. arvensis* (91.02 mg GAE/gram of dried sample) had the highest amount of phenols among the herbal plants. In the study of Gruyal and del Rosario (2013) it was mentioned that *M. arvensis* and *C. frutescens* had the highest number of secondary metabolites and high flavonoids content. This depicts that the herbal plants may have antioxidant effect due to its flavanoidal and polyphenolic property which is to be further investigated. In fact, *M. arvensis* was endorsed by the Department of Health (DOH) that, it is one of the ten herbs used as an effective medicine for antiseptic, anti-cancer, anti-spasm and has anti-emetic activities.

On the other hand, *C. aromaticus* has 50.04 mg/g total phenol content. It was mentioned in Philippine Herbal Medicine (n.d.) that *C. aromaticus* was very rich in

anti-oxidant phytochemical flavonoids and phenolic acids which was also confirmed in the study of Gruyal & del Rosario (2013) that it has flavonoid content which is one of the secondary metabolites that is rich in antioxidant. Furthermore in this study, *C. longa* has 47.32 mg/g total phenolic content and *C. citratus* has 44.05 mg/g which is the least among the five herbal plants. Tosun et al., (2009) stated that phenols are very important plant constituents; they show high scavenging ability of free radicals due to their hydroxyl group. Therefore, the phenolic content of plants may contribute directly to their antioxidant action.

B. DPPH Free Radical-Scavenging Capacity

The antioxidant properties of samples were detected using DPPH radical scavenging activity. This method was based on the reduction of alcoholic DPPH solutions in the presence of a hydrogen donating antioxidant that shows a strong absorption band at 517 nm and appears as deep violet color (Cui et. al, 2011). Table 2 showed that all the five selected herbs in Cantilan, Surigao del Sur had the free radical scavenging capacity. *M. arvensis* scavenged the highest radicals at 93.48% followed by *C. citratus* 75.38%, *C. longa* 65.79%, *C. aromaticus* 55.62%, *C. frutescens* 32.82% while the standard ascorbic acid scavenged 96.45% radicals. There is correlation ($p<0.05$) between the phenolic content and the free radical scavenging activity of the five herbal plants studied.

Table 2. Free radical scavenging capacity of the different plant extracts

Plant Extracts	Gallic Acid Equivalents mg/g ± SD
Curcuma longa (duyao)	65.79 ± 0.0292
Cymbopogon citratus (tangyad)	75.38 ± 0.0149
Coleus aromaticus (garabo)	55.62 ± 0.0080
Mentha arvensis (herba buena)	93.48 ± 0.0006
Capsicum frutescens (sili)	32.82 ± 0.0323
Ascorbic acid	96.45 ± 0.0000

All analyses are of triplicate measurements ± standard deviation.

Tawaha et. al, (2007) estimated that a total phenolic content higher than 20 mg GAE/g dry weight could be considered as very high. This suggests that the methanol extracts of five selected herbs in Cantilan must be considered as good source of phenolic compounds since the TPC is higher than 20 mg GAE/g. However, a very high amount of phenolic content (>775 mg GAE/g extracts) was reported in *Acacia auriculiformis* (Singh et al., 2007). It was cited in the study of Goyal et. al, (2010) that polyphenolic compounds like flavonoids and phenolic acids have been reported to have multiple biological effects which includes antioxidant activity which act as free radical terminators. In addition, Razali et. al, (2008) stated that many of the phenolic have been shown to contain high levels of antioxidant activities.

M. arvensis exhibited highest free radical scavenging activity among the herbal plants. However, compared to ascorbic acid as standard reference, their activity is moderately lesser. It was also confirmed in the study of Raghavendra et. al, (2013) that the leaves of *M. arvensis* showed highest potential antioxidant and free radical scavenging activity. Moreover, the findings of the present study agreed with the findings of Gruyal et. al, (2013) study since it is rich in phytochemical constituent. Pidugu and Arun (2012) also mentioned in their study that the leaves of *M. arvensis* were used as contraceptive, carminative, antispasmodic, anti-peptic ulcer agent, skin diseases, cough and cold since it showed potent antioxidant activity due to its high contents of flavonoids and phenolic compounds. It was also stated in the study of Ghasemzadeh (2012) that many phenolic compounds were reported to possess effective antioxidant activity and anti-carcinogenic, anti-cancer, anti-inflammatory, anti-bacterial and anti-viral activities in a greater or lesser extent. The study of Ghasemzadeh (2012) is then in agreement with the findings of the study.

It has been known that ROS and RNS are both produced in a well-regulated manner to help maintain homeostasis at the cellular level in the normal healthy tissues. They play an important role as signaling molecules but if this production is not regulated it can become a free radical. According to Devasagayam et. al, (2004) free radicals have been implicated in the etiology of several human disorders like neurodegenerative diseases, cancer and aids. Several studies claimed that plants can help reduce these problems. As mentioned in the study of Sasikumar et. al, (2010) polyphenols in plant scavenge active oxygen species and effectively prevent oxidative cell damage. Oladunmoye also agreed (2012) that plants constitute chemicals that could act as antioxidants.

4.0 Conclusion

Analysis showed that these five herbal plants may prove to be beneficial to human beings health because of their high total phenol content and scavenging capacity. Among the five selected herbs *Mentha arvensis* showed the highest total phenol content (91.02 mg GAE/g dried sample) and the least is *Capsicum frutescens* with the amount of 31.95 mg GAE/g dried sample. The free radical scavenging capacity also showed that all the herbal plants has free radical scavenging capacity however, their capacity to scavenge varies from 32.82% to 93.48% depending on the herbal plants. There is correlation ($p<0.05$) between the phenolic content and the free radical scavenging activity of the five herbal plants studied.

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