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Phytochemical profiles and concentrations of major antioxidative substances of selected medicinal plant leaves

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Abstract

Medicinal plants have been explored for the presence of phytochemicals and natural antioxidants for research and commercial utilization potentials. However, few studies were conducted on the selected medicinal plants' phytochemical profile and antioxidant contents. This study investigated the phytochemical profile, total phenolic, and total flavonoid contents of five methanolic leaf extracts of *C. igneus, A. bilimbi, M. charantia, A. esculentus, and B. glabra*. All five plants indicated the presence of carbohydrates, saponins, and alkaloids. *A. bilimbi, M. charantia, A. esculentus, and B. glabra* are potential sources of tannins. The total phenolic content of the extracts varied from 10.17±0.23 to 27.90±0.50 GAE/g. Whereas, the total flavonoid content of these plants ranges from 8.40±0.52 to 87.48±2.42 QE/g. The *M. charantia* leaves revealed the highest TPC while *A. bilimbi* yielded the highest TFC among the plants. The selected medicinal plants can be considered good sources of phytochemicals and natural antioxidants, phenolics, and flavonoids, which are the basis for the development and utilization of these plant leaves. The phytochemicals, phenolics, and flavonoids of these plants could be further isolated, purified, characterized, and utilized as antioxidants.

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Introduction

Humans have utilized medicinal plants since the start of human civilization. For thousands of years, plants have been considered the main source of medicines, nutrition, and therapy against diseases (Azam *et al.*, 2014). Plants contain phytochemicals that act as natural antioxidants that serve as their defense system which have the potential to treat various illnesses in humans (Aanouz *et al.*, 2021).

Diseases in humans are caused by oxidative stress brought about by environmental pollutants, radiation, unhealthy food, chemicals, and others. Oxidative stress plays a major role in cellular injury, aging, cancer, neurodegenerative disease, infectious disease, and other disorders. The reactive oxygen species produced during oxidative stress deplete antioxidants in the immune system. Plant- and animal-based antioxidants deter the oxidation of lipids and proteins. Thus, there is growing interest in natural sources of antioxidants due to the presence of phenolics and flavonoids that neutralize free radicals (Mihai *et al.*, 2023).

World Health Organization (WHO), posited that 80% of the world's population uses phytochemicals to treat diseases due to their bioactive compounds (Hussain *et al.*, 2018). Plants are considered rich sources of antioxidants (Mihai *et al.*, 2023). With this, various plants and their parts such as fruits, leaves, flowers, stems, and roots are evaluated for antioxidant concentrations and their antioxidant potentials.

The diverse phytochemicals play important roles in plants and have health benefits in humans. Carbohydrates exhibit potent antioxidant, antimicrobial, and antiviral properties, among others (Auwal *et al.*, 2023) whereas, saponins have expectorant, cardiotonic, hypolipidemic, and anticancer properties (Kumar *et al.*, 2023). Alkaloids have been found to have analgesic, anti-inflammatory, and anti-cancer properties (Kancherla *et al.*, 2019). Meanwhile, tannins, have antidiarrheal, anti-inflammatory, and anticancer properties (Kumar *et al.*, 2023).

For antioxidative substances in plants, phenolics are secondary metabolites that help reduce reactive oxygen species (ROS) generation and chelate metal ions. These compounds have potential health benefits such as antibacterial and anticancer properties and prevent chronic and oxidative stress-related disorders like cardiovascular and neurological diseases (Cosme *et al.*, 2020). On the other hand, flavonoids have strong biological activity and provide several health advantages in humans. These chemicals prevent the formation of reactive oxygen species (ROS) through antioxidant activity (Dias *et al.*, 2021) and have antibacterial, antifungal, anti-diabetic, antimalarial, neuroprotective, and anti-inflammatory which lowers the risk of cardiovascular disease and cognitive decline (Ullah *et al.*, 2020).

For the selected medicinal plants, most of the studies reported were focused on different parts of a single or two plant species from other countries and not from the Philippines with different environmental conditions. The studies on these selected plants have few or no studies extracted using methanol as a solvent or using various solvents such as water, n-hexane, acetone, and ethanol. Therefore, the study was conducted to compare the phytochemical profile and the total phenolics and total flavonoids contents of the methanolic leaf extract of the medicinal plants namely: *Costus igneus, Averrhoa bilimbi, Momordica charantia, Abelmoschus esculentus, and Bougainvillea glabra.*

Material and methods

Plant materials: collection and preparation

An ample amount of fresh, matured, pest-free leaves of selected medicinal plants were collected from different barangays of Valencia City, Lantapan, and Malaybalay City in the Province of Bukidnon, Philippines. The leaves were washed cleaned, air-dried, milled to particle size, labeled, and stored at room temperature in an airtight container.

Phytochemical profiling

The selected medicinal plant extracts were tested using standard methods for the presence of carbohydrates, reducing sugars, tannins, saponins, flavonoids, steroids, alkaloids, anthraquinones, and glycosides (Goyal *et al.*, 2010).

Determination of major antioxidative substances

Preparation of plant extracts

Twenty (20) grams of ground plant leaves were soaked in 300 mL of methanol for 12 hours with occasional shaking. The 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 of methanol and filtered. The filtrates were combined and concentrated to about 100 mL using the rotary evaporator. The concentrated extract was transferred to a 100 mL volumetric flask and diluted to volume. The methanolic extract was kept for analysis.

Determination of total phenolics contents

The total phenolics content was determined using the Folin-Ciocalteu (FC) reagent method described by Goyal *et al.* (2010) and Irondi *et al.* (2012) with slight modifications to the traditional FC assay by adding sodium carbonate instead of sodium molybdate to improve accuracy, precision, and reliability of results. The 0.5 mL methanolic extract was mixed with 0.5 mL of FC reagent (previously diluted 1:1 with distilled water) and incubated for 5 minutes at room temperature. Then 1 mL of 2% Na_2CO_3 solution was added. After incubation at room temperature for 10 minutes, the absorbance was read at 730 nm. All tests were performed in triplicates. Gallic acid monohydrate was used as the standard. The total phenolic content was expressed as milligram gallic acid equivalents (GAE) per gram dried sample.

Determination of total flavonoids contents

The total flavonoid content of the selected medicinal leaf samples was determined with the aluminum chloride, ($AlCl_3$) method described by Goyal *et al.* (2010) with slight modifications using quercetin as a standard. The 2 mL methanolic extract was added to 1.25 mL of distilled water followed by 75 µL of 5% $NaNO_2$. After five minutes at room temperature, 0.15 mL of 10% $AlCl_3$ was added. The mixture was incubated for six minutes at room temperature for the reaction to take place. Then, the reaction mixture was treated with 0.5 mL of 1 mM NaOH. The reaction mixture was diluted with 275 µL of distilled water and incubated further for 20 minutes at room temperature. The absorbance was measured at 510 nm using a UV-Visible spectrophotometer. This test was done in triplicates. The flavonoid content was calculated from the quercetin standard curve.

Statistical analysis

A series of analyses were performed in triplicates and the results are reported as the mean and standard deviation (SD). The data were analyzed using SPSS Statistics 13.0 (SPSS Inc, Chicago, USA) and the significant differences were determined using one-way analysis of variance (ANOVA) at a significance level of $p< 0.05$.

Results and discussion

Phytochemical profiling

The qualitative phytochemical screening of methanolic leaf extracts from the five medicinal plants revealed the presence of various secondary metabolites (Table 1). All plant extracts were found to contain carbohydrates, saponins, and alkaloids. Additionally, tannins were detected in *A. bilimbi*, *M. charantia*, *A. esculentus*, and *B. glabra* leaves, but not in *C. igneus* extracts. Reducing sugars, flavonoids, steroids, anthraquinones, and glycosides were not detected in any of the tested extracts using the employed methods.

The detected phytochemicals in these plants are known to have potential health benefits. Carbohydrates serve as a source of energy and may exhibit antioxidant, antimicrobial, and antidiarrheal properties (Auwal *et al.*, 2023). On the other hand, saponins, which are glycosides, possess expectorant, cardiotonic, and hypolipidemic activities, potentially reducing LDL cholesterol and hindering cancer cell proliferation (Kumar *et al.*, 2023).

Table 1. Phytochemicals present in the selected medicinal plants

Phytochemical	*Costus igneus* (Insulin plant)	*Averrhoa bilimbi* (Kamias)	*Momordica charantia* (Ampalaya)	*Abelmoschus esculentus* (Okra)	*Bougainvillea glabra* (Bogambilya)
Carbohydrates	+	+++	+	+	++
Reducing sugars	-	-	-	-	-
Tannins	-	+++	+++	+	+++
Saponins	++	++	+++	++	+++
Flavonoids	-	-	-	-	-
Steroids	-	-	-	-	-
Alkaloids	++	+	++	+	++
Anthraquinones	-	-	-	-	-
Glycosides	-	-	-	-	-

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.

Further, alkaloids are compounds that may have analgesic, anti-inflammatory, and anti-cancer properties (Kancherla *et al.*, 2019). Finally, tannins (present in *A. bilimbi, M. charantia, A. esculentus,* and *B. glabra*) are known to have antidiarrheal effects and may reduce inflammation and prevent cancer cell spread (Kumar *et al.*, 2023).

The absence of certain phytochemicals (reducing sugars, flavonoids, steroids, anthraquinones, and glycosides) does not necessarily indicate their complete absence. Certain factors may have led to negative results such as:

1. Interfering compounds: the presence of tannins might hinder the detection of other compounds (Kaur and Arora, 2009).
2. Extraction methods: suboptimal extraction methods may not yield sufficient quantities of certain phytochemicals (Sookying *et al.*, 2022).
3. Sample preparation: variations in source material, pre-treatment, and solvents can influence results (Sookying *et al.*, 2022).
4. Assay sensitivity: some assays might lack the sensitivity to detect low concentrations of specific compounds (Sookying *et al.*, 2022).
5. Uneven distribution: the uneven distribution of some compounds within the plant extract can affect detection (Yoo *et al.*, 2018).
6. Environmental factors: mineral composition, soil type, temperature, light, and water content can influence phytochemical profiles (Li *et al.*, 2012).
7. Plant-specific factors: genotype, maturity, soil conditions, fertilization practices, irrigation, pest control, and location can all impact phytochemical content (Solihah *et al.*, 2012).

It is always an option that in future studies more sophisticated analytical techniques can be employed, when accessible, for a more comprehensive profiling of phytochemicals in these plants.

Major antioxidative substances

Antioxidative substances: total phenolics

The total phenolics contents (TPC) of the methanolic extracts varied significantly ($p < 0.05$) among the five medicinal plants, ranging from 10.17 ± 0.23 mg GAE/g (*A. bilimbi*) to 27.90 ± 0.50 mg GAE/g (*M. charantia*) (Table 2). *M. charantia* exhibited the highest TPC, followed by *B. glabra* (17.47 ± 0.42 mg GAE/g), *C. igneus* (15.07 ± 0.25 mg GAE/g), *A. esculentus* (12.10 ± 0.20 mg GAE/g) and *A. bilimbi.*

Comparing with previous studies, the TPC of *M. charantia* leaves in this study (27.90 ± 0.50 mg GAE/g) fell within the range reported in previous studies (33.9-49.31 mg GAE/g) using methanol (Nagarani *et al.*, 2014). Similarly, the TPC of *C. igneus* (15.07 ± 0.25 mg GAE/g) was lower than the previous findings (25.30 – 78.89 mg GAE/mg) (Chacko *et al.*, 2018, Hajam *et al.*, 2022; Muthukumar *et al.*, 2019). For *A. esculentus* and *B. glabra*, the current TPC values (12.10 ± 0.20 mg

GAE/g and 17.47 ± 0.42 mg GAE/g, respectively) were comparable to some previous reports using methanol or ethanol (Liao *et al.*, 2012; Siddique *et al.*, 2022). However, substantial variations were observed in other studies, which may be attributed to factors like extraction conditions and plant maturity (Sookying *et al.*, 2022; Abdel-Razek *et al.*, 2023).

A. bilimbi presented the most significant discrepancy. While previous studies reported TPC values ranging from 50.23 to 103.79 mg GAE/g using various extraction methods (Niawanti *et al.*, 2019; Rahardhian *et al.*, 2019; Iwansyah *et al.*, 2021), the current study using methanol yielded a lower value (10.17 ± 0.23 mg GAE/g). This difference warrants further investigation into the optimal extraction conditions for TPC in *A. bilimbi* leaves.

Overall, the results suggest that all five plants are potential sources of phenolic antioxidants. *M. charantia* leaves exhibited the highest TPC among the studied plants.

Table 2. Total phenolic and flavonoid content of the selected medicinal plants

Medicinal plant	Total phenolics contents, mg GAE/g sample (Mean +SD)	Total flavonoids contents, mg quercetin/g sample (Mean + SD)
Costus igneus (Insulin plant)	15.97 ± 0.25	8.40 ± 0.52
Averrhoa bilimbi (Kamias)	10.17 ± 0.23	87.48 ± 2.42
Momordica charantia (Ampalaya)	27.90 ± 0.50	15.98 ± 0.50
Abelmoschus esculentus (Okra)	12.10 ± 0.20	13.02±1.38
Bougainvillea glabra (Bogambilya)	17.47 ± 0.42	72.73±3.80

Antioxidative substances: total phenolics

The total flavonoids contents (TFC) of the methanolic extracts varied significantly ($p < 0.05$) among the five medicinal plants, ranging from 8.40 ± 0.52 mg QE/g (*C. igneus*) to 87.48 ± 2.42 mg QE/g (*A. bilimbi*) (Table 2). *A. bilimbi* exhibited the highest TFC, followed by *B. glabra* (72.73 ± 3.80 mg QE/g), *M. charantia* (15.98 ± 0.50 mg QE/g), *A. esculentus* (13.02 ± 1.32 mg QE/g) and *C. igneus*.

The TFC of *A. bilimbi* in the current study (87.48±2.42mg QE/g) is higher than the reported values in the previous studies (32.80±0.37 and 29.71±4.66 mg QE/g) using methanol or ethanol, respectively (Arifin and Jumal, 2020; Iwansyah *et al.*, 2021). For the TFC of *B. glabra* leaves (72.73±3.80 mg QE/g) in the present study, no specific data were reported using the methanolic extraction on leaves, however, a related study on aerial parts with lower TFC value (41.51 mg QE/g) using methanol (Saleem *et al.*, 2019). Similarly, the current TFC of *M. charantia leaves* (15.98±0.50 mg QE/g) was greater than the previous reports (29.57±0.003 µg QE/mL or 0.02957±0.003 mg QE/mL) using methanol (Sharma *et al.*, 2020). On the contrary, the TPC of the recent study of *A. esculentus leaves and C. igneus* (13.02±1.32 and 8.40±0.52 mg QE/g) fell within the ranges in the previous studies (4.77- 49.30±2.20 mg QE/g and 3.02±0.04 - 58.30±0.2837 mg QE/g), respectively (Arifin and Jumal, 2020; Lin *et al.*, 2014). The significant disparities observed in other studies may be attributed to extraction conditions, part of the plant, and the species used (Iwansyah *et al.*, 2021; Sookying *et al.*, 2022; Saleem *et al.*, 2019; Lin *et al.*, 2014; Solihah *et al.*, 2012).

Generally, the results indicate that all five parts are potential sources of flavonoid antioxidants. *A. bilimbi* leaves exhibited the highest TFC among the selected plants.

Conclusion

In conclusion, the presence of phytochemicals in the selected medicinal plants confirms their traditional medicinal value and sheds light on their chemical composition and therapeutic properties. The identification of these diverse bioactive compounds underscores the potential use of these plants as sources of natural antioxidants. Furthermore, the variable concentrations of antioxidative substances-total phenolics and total flavonoids in the selected medicinal plants pave the way for future research into their antioxidants and health-promoting properties in pharmaceutical studies, the discovery of novel drugs, and treatments derived from plant-based sources.

Recommendation(s)

Future studies may utilize other plant parts and other solvents to determine which parts of these plants have the highest concentration of antioxidants and the greatest antioxidant activities for extraction, purification, isolation, and medicinal use of the selected medicinal plants. To minimize false negative results for phytochemical profiling, it is recommended to optimize extraction methods, choose appropriate assays, and consider the potential influence of interfering compounds, and other factors.

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