INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 25, No. 6, p. 1-9, 2024

RESEARCH PAPER OPEN ACCESS

A comparative study on the antioxidant activity of selected Philippine herbal teas

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Key words: Philippine herbal teas, Antioxidant activity, Phenolics

http://dx.doi.org/10.12692/ijb/25.6.1-9 Article published on December 03, 2024

Abstract

Tea ranks as the second most consumed beverage worldwide, surpassed only by water. With an annual production of approximately 2.5 million metric tons of dried tea, its popularity has surged in recent years, largely due to its recognized health benefits, particularly as a rich source of potent antioxidants. This study focused on evaluating the total phenolic content of seven commercial herbal tea samples from the Philippines using the Folin-Ciocalteu method. Additionally, antioxidant activity was measured using the DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assays, and the findings were correlated to the phenolic content. The results revealed that mango tea showed the highest phenolic content followed by pito-pito tea. In terms of antioxidant efficacy, the pito-pito tea showed the lowest IC_{50} value in the DPPH assay, while mango had the lowest IC_{50} value in the ABTS assay, followed closely by pito-pito. A correlation was found between the phenolic content and the antioxidant activities of the teas.

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Introduction

Tea, one of the world's most popular beverages, holds profound cultural and historical significance across various societies. With over 2 billion cups consumed daily, tea is produced in more than 60 countries, demonstrating its universal appeal. Traditionally, tea is made from the dried leaves of the *Camellia sinensis* plant, celebrated for its numerous health benefits. Green tea, for instance, is known to boost the immune system and improve cardiovascular health, while black tea has been linked to a reduced risk of chronic diseases such as heart disease and diabetes (Khan and Mukhtar, 2007). However, as awareness of the potential negative effects of caffeine has grown, many people have turned to herbal teas as a caffeine-free alternative that still offers a variety of health benefits.

Unlike traditional tea, herbal teas are not derived from the *Camellia sinensis* plant. Instead, they are made from a blend of ingredients known as tisanes, which include leaves, bark, nuts, fruits, and other botanical elements. These herbal blends are prized not only for their flavor but also for their medicinal properties. Often crafted with specific health outcomes in mind, herbal teas can help boost the immune system, provide antioxidants, or promote relaxation (Serafini *et al.*, 2011). For example, chamomile tea is well-regarded for its calming effects, while peppermint tea is commonly used to alleviate headaches and improve digestion (McKay and Blumberg, 2006).

The global rise in the popularity of herbal teas has coincided with a growing interest in natural antioxidants, which play a crucial role in maintaining the body's balance by neutralizing harmful reactive oxygen species (ROS) linked to degenerative diseases (Bocci and Valacchi, 2013). Recent studies have highlighted a preference for natural antioxidants like those found in herbal teas over synthetic ones as these have been associated with toxicity and carcinogenicity (Pokorny, 2007). This trend underscores the increasing demand for healthier, plant-based alternatives in both dietary and medicinal contexts.

In the Philippines, a country rich in biodiversity, herbal plants have long played a vital role in traditional medicine and are deeply embedded in the cultural fabric of the nation. Although many of these plants are not indigenous to the region, they have been used for centuries for their medicinal properties, contributing to a rich historical legacy. Philippine herbal teas, such as those made from guava, mango, guyabano, pito-pito, malunggay, and ampalaya, have gained popularity for both their health benefits and cultural significance. These teas are typically prepared by brewing or boiling plant materials in water, and are often enhanced with sweeteners or spices to improve their flavor. A growing body of literature emphasizes the importance of understanding the benefits of various Philippine herbal products to ensure their safe and effective use (Maramba-Lazarte, 2020), particularly in light of ongoing research into remedies for diseases like COVID-19, diabetes, and hypertension.

This study focuses on six popular Philippine-based herbal teas, examining their potential health benefits by analyzing their polyphenol content and antioxidant activity. The research aims to provide a deeper understanding of how these locally sourced herbal teas contribute to health and well-being, aligning with the global trend of turning to natural, plant-based remedies for maintaining health and preventing disease.

Materials and methods

Sample extraction

Tea bags from the samples (Fig. 1) were steeped in 200 mL of freshly boiled water for 5 minutes. The resulting tea infusion was then cooled and analyzed for phenolic content and antioxidant activity using the DPPH and ABTS assays.

Fig. 1. Herbal tea samples used in the study

Phenolic content

The phenolic content of the tea infusions was determined using the Folin-Ciocalteu assay. A 10% concentration of the Folin-Ciocalteu reagent and 1.0 M Na_2CO_3 solution were prepared. On a 96-well microplate, 20 µL of each tea infusion was mixed with 100 µL of the Folin-Ciocalteu reagent. After 4-minute incubation, 100 µL of 1 M Na_2CO_3 was added. The absorbance was measured at 645 nm after 2 hours of incubation in the dark. Distilled water served as the negative control, while gallic acid was used as the positive control and the standard for generating a calibration curve, which was used to express the total phenolic content (TPC) in µg gallic acid equivalents (GAE)/g tea.

Antioxidant activities

The antioxidant activities of each tea infusion extract were assessed using both the DPPH and ABTS assays. These assays were conducted using a microplate reader, where serial dilutions of the tea infusions were prepared and mixed with the respective reagents. Each extract (0.1 g/mL) was diluted three times in 10-fold increments, resulting in concentrations ranging from 0.01 to 1×10^{-4} g/mL. For the DPPH assay, 20 µL of the extract or standard was combined with 200 µL of 0.2 mM DPPH solution. After 30 minutes, the absorbance was measured at 519 nm. The antioxidant activity was calculated using the following formula, with EC_{50} values derived to express antioxidant potency:

% Scavenging activity = {(Blank absorbance – Sample absorbance)/(blank absorbance)} × 100

For the ABTS assay, the ABTS radical cation was prepared by mixing ABTS with potassium persulfate in a 1:1 ratio 24 hours prior to use. On a 96-well plate, 200 µL of the ABTS reagent was added to 20 µL of the extract at various concentrations with ethanol serving as the negative control. Samples were incubated in the dark at room temperature for 5 minutes. Absorbance was measured at 750 nm. The ABTS assay was performed in triplicate, and antioxidant activity was expressed as EC_{50} (mg/mL), the concentration at which 50% of the antioxidant activity was observed. The EC_{50} value was determined by plotting the absorbance of the serial dilutions against their concentrations, producing a sigmoidal curve from which the point of 50% effectiveness was derived using a logarithmic equation.

All absorbance readings were obtained using the Biotek Cytation 5 Image Reader. Both assays were replicated at least three times.

Statistical analysis

Statistical analysis using the student's t-test ($p < 0.05$) was performed on the experimental data (absorbance in specific wavelengths and assays) to compare if data obtained by different tea samples are significant. Correlation analysis of the phenolic content and IC_{50} from DPPH and ABTS assays was also determined using Microsoft Excel.

Results and discussion

Fig. 2 shows a microplate with the different assays performed in this study. These assays are commonly used to assess the health-promoting qualities of plant-based foods and beverages due to their ability to measure different aspects of antioxidant activity and total phenolics. The Folin-Ciocalteu assay on the other hand is a widely used method for measuring the total phenolic content in plant extracts. This assay involves the reduction of the Folin-Ciocalteu reagent by phenolic compounds in alkaline conditions, resulting in a blue color due to the formation of a molybdenum-tungsten complex (Singleton *et al.*, 1999). The intensity of this blue color correlates with the phenolic concentration, as phenolic compounds act as reducing agents. In the image, the first two columns show wells with varying intensities of blue, already indicating differences in the phenolic content among samples. Darker blue wells correspond to higher phenolic content, while lighter wells indicate lower phenolic concentrations.

On the other hand, DPPH assay measures free radical scavenging activity, an indicator of antioxidant capacity. DPPH is a stable free radical with a deep purple color in solution. When an antioxidant donates an electron to DPPH, the radical is neutralized, leading to a color change from purple to yellow or colorless (Blois, 1958). The degree of decolorization reflects the sample's ability to scavenge free radicals, with greater color change indicating higher antioxidant activity. In the image, the DPPH assay (columns 3-7) shows wells ranging from deep purple to yellow, signifying differences in antioxidant capacity among the samples. Yellow or pale wells represent samples with higher antioxidant activity, while darker purple wells indicate lower activity.

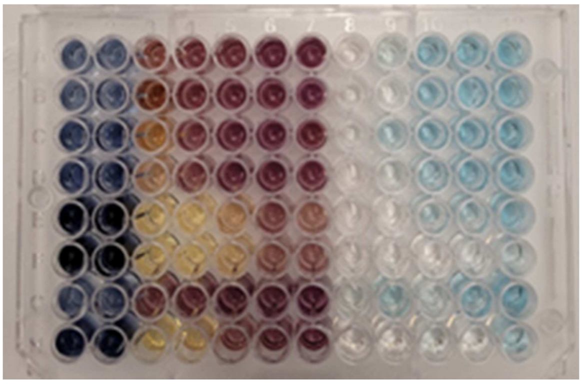

Fig. 2. Microplate well containing assay from Folin-Ciocalteu assay (first two columns) DPPH assay (next five columns) and ABTS assay (last five columns).

Lastly, the ABTS assay measures antioxidant capacity by generating the ABTS radical cation, a blue-green compound. When antioxidants are present, they reduce the ABTS radical cation, leading to a decrease in color intensity. The change from blue-green to colorless is measured spectrophotometrically, with more decolorization indicating higher antioxidant activity (Re *et al.*, 1999). The last five columns in the image represent the ABTS assay, with wells showing a gradient from dark blue to nearly colorless. Darker blue indicates lower antioxidant capacity, while lighter colors suggest higher antioxidant activity.

Fig. 3 displays the phenolic content of various herbal tea samples with error bars indicating the variability or standard deviation in the measurements. Among the samples, mango tea exhibits the highest phenolic content closely followed by the pito-pito tea. The phenolic content of these two herbal teas is not significantly different from one another, with both containing over 300 mg gallic acid equivalents (GAE) per gram of tea. Both samples displayed the darkest blue color in the Folin-Ciocalteu assay, occupying the 5th and 6th rows in the microplate.

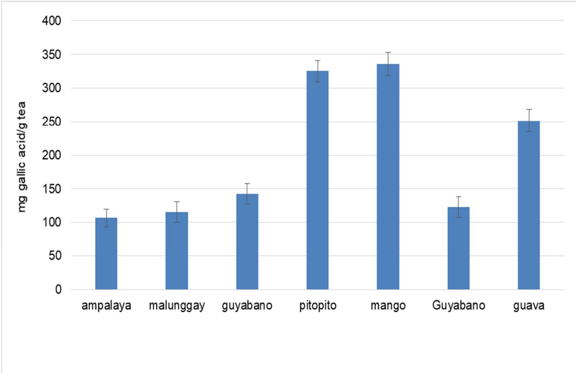

Fig. 3. Phenolic content of herbal teas used in the study

Following mango and pito-pito, guava tea (8th row in the microplate) shows the next highest phenolic content containing around 250 mg GAE/g tea sample. Its phenolic level is significantly different from that of mango and pito-pito, as well as from the lower-content samples (including two guyabano samples, malunggay and ampalaya). The two guyabano samples are close in phenolic content, slightly higher than the remaining samples but not significantly different from each other. Ampalaya has the lowest phenolic content, though it is not significantly different from malunggay and the two guyabano samples.

In terms of antioxidant activities, Table 1 summarizes the DPPH and ABTS assay results showing the IC_{50} values for each herbal tea. These IC_{50} values indicate the concentration required to achieve 50% free radical scavenging with lower values reflecting stronger antioxidant activity. In the DPPH assay, most samples exhibit significantly lower IC_{50} values except for mango that has a higher IC_{50} and ampalaya, which does not differ significantly in both assays. Pito-pito and mango teas show the lowest IC_{50} values in DPPH and ABTS assay, respectively, although there is no significant difference in ABTS between the two, unlike in DPPH. This trend is also apparent in the microplate results where both samples show lighter colors compared to the other samples, suggesting a strong antioxidant potential.

Table 1. Antioxidant activity (IC_{50}) of the different extracts

Samples	DPPH (mg/mL)	ABTS (mg/mL)
Ampalaya	2.30 ± 0.08	2.46 ± 0.15
Malunggay	1.31 ± 0.08	2.95 ± 0.12
Guyabano	1.23 ± 0.18	2.42 ± 0.21
Pito-pito	0.22 ± 0.05	0.42 ± 0.11
Mango	0.57 ± 0.05	0.40 ± 0.05
Guyabano	1.52 ± 0.04	2.23 ± 0.12
Guava	0.49 ± 0.11	0.99 ± 0.10

Guava tea has the next lowest IC_{50} value in DPPH, though not significantly different from mango and ranks third in ABTS, where it is significantly different from pito-pito and mango. The herbal tea with the highest IC_{50} value in DPPH assay is ampalaya while malunggay has the highest IC_{50} in the ABTS assay. Both samples are significantly different from the other samples that exhibit moderate IC_{50} values on both assays, including the two guyabano samples that show no significant difference between each other in either assay.

The phenolic content of herbal teas is a key indicator of their antioxidant potential, as phenolic compounds are well-documented for their ability to neutralize free radicals, and reduce oxidative stress, along with providing other various health benefits (Balasundram *et al.*, 2006). The results provide an overview of the phenolic content in seven different herbal tea samples: ampalaya, malunggay, guyabano (2 samples from different companies), pito-pito, mango, and guava. Each tea exhibits distinct levels of phenolic compounds, with pito-pito and mango showing the highest phenolic content, followed by moderate values in guava and guyabano, and the lowest content in ampalaya and malunggay. These differences highlight the variability in the antioxidant potential among these herbal teas, which can be influenced by factors such as the plant species, parts used to create the tea concoction, and their processing methods.

Pito-pito, a traditional Filipino herbal tea, is a blend that is made up of seven plant materials from the leaves of *Premna odorata* Blanco., *Lagerstroemia speciosa* Pers., *Psidium guajava* Linn., *Mangifera indica* Linn. and *Pandanus amaryllifolius* Roxb., and seeds of *Pimpinella anisum* Linn. and *Coriandrum sativum* Linn (Ramos *et al.*, 2021). These ingredients are commonly used for a variety of illnesses from cough, colds, fever, headache, asthma, migraine, and many more. The high phenolic content in pito-pito may reflect the synergistic effect of combining these different herbs, wherein they enhance each other's overall antioxidant capacity. This supports its traditional use for general wellness, especially as an anti-inflammatory and immune-boosting remedy (Rivera-Mancía *et al.*, 2011). High phenolic content in pito-pito aligns with findings in mixed herbal preparations, where multiple bioactive compounds interact to amplify antioxidant effects.

On the other hand, the mango (*Mangifera indica*) tea made from mango leaves, while less commonly consumed as a tea compared to the fruit, contain substantial amounts of phenolic compounds. One such compound is mangiferin, known for its antioxidant, anti-inflammatory, and antimicrobial activities (Garrido *et al.*, 2004; Yehia and Altwaim 2023). Mangiferin is also recognized for its ability to modulate cellular oxidative pathways (Rahmani *et al.*, 2023; Awny *et al.*, 2021) making mango leaf tea potentially beneficial for managing inflammation and supporting metabolic health. This high phenolic content suggests that mango tea could be marketed as a functional beverage with antioxidant properties, appealing to health-conscious consumers.

On the other hand, guava (*Psidium guajava*) results is consistent with literature that highlights its phenolic profile, including compounds like catechin, quercetin, and gallic acid, all of which are documented for their antioxidant effects (Jimenez-Escrig *et al.*, 2001). Guava is traditionally used in the Philippines and other tropical regions for its medicinal properties, particularly in managing diabetes, promoting good digestive health, and curing infections. The moderate phenolic content observed is a good explanation for its continued popularity in traditional medicine, as it provides antioxidant protection that contributes to its therapeutic applications (Luo *et al.*, 2019).

Similarly, guyabano (*Annona muricata*) tea also with its moderate phenolic content aligns with the plant's traditional use in folk medicine for immune support and general wellness. A study by Coria-Téllez *et al.* in 2018 has found that the phenolic content of guyabano teas depend on the extraction method, leaf maturity, or geographic origins, as phenolic content in plant materials can vary widely based on environmental and genetic factors. There is thus a need to establish processing protocols that are able to maximize the benefits from guyabano tea, if it is to be marketed as an important herbal tea in the future.

Lastly, despite their lower levels, the two other teas are valued for their unique medicinal benefits beyond phenolic compounds. Ampalaya for example is traditionally known for its hypoglycemic effects, attributed largely to its alkaloid and peptide compounds rather than its phenolic profile (Joseph and Jini, 2011). Nonetheless, it has also been reported to have phenolic compounds such as gallic acid, caffeic acid and catechin (Kubola and Siriamornpun, 2008).

Similarly, malunggay is rich in vitamins A and C, calcium, and essential amino acids, providing nutritional benefits that complement its mild antioxidant activity (Siddhuraju and Becker, 2003).

One study reported malunggay to be possessing high phenolic content and potent antioxidant properties with the presence of phenolics such as gallic, chlorogenic, ellagic and ferulic acid (Verma *et al.*, 2009).

The antioxidant properties of herbal teas are strongly associated with their phenolic content, as these compounds are known to contribute significantly to free radical scavenging. Results demonstrate variability in the antioxidant activity of different herbal teas, as assessed by DPPH and ABTS assays, where lower IC_{50} values denote stronger antioxidant capacity. In general, samples with higher phenolic content tend to show greater antioxidant potential, correlating well with the activity observed here. In terms of the correlation of the antioxidant activities with phenolic content, both are negatively correlated with DPPH having a higher correlation coefficient (-0.874) in comparison to ABTS (correlation coefficient = -0.760). The antioxidant activity obtained by DPPH is positively correlated to that of the ABTS assay with a correlation coefficient of 0.622.

With the lowest IC_{50} values in both DPPH (0.22 ± 0.05) and ABTS (0.42 ± 0.11), pito-pito tea demonstrates the strongest antioxidant activity among the tea samples analyzed. This is not surprising as this tea is a concoction of seven herbal components which also includes some of the herbal tea samples used in this study (mango, and guava). Mango also exhibited low IC_{50} values in both assays. As mentioned previously, it is possible that the herbal component in the pito-pito tea sample exhibits synergistic effects in antioxidant activity. This is confirmed in one study (Ramos *et al.*, 2021) where water and methanol extracts of pito-pito, as well as extracts from its individual components namely *Lagerstroemia speciosa*, *Psidium guajava*, and *Mangifera indica*, exhibited strong DPPH free radical scavenging activities, which correlate closely with high phenolic content. This result confirms the potential health benefits of pito-pito as a source of natural antioxidants, supporting its traditional use in treating various ailments and aligning with findings from other studies on the importance of phenolic compounds in antioxidative activity.

Mango tea had the lowest IC_{50} in ABTS but not significantly different as that of pito-pito. One study (Berardini *et al.*, 2005) reported that mango leaves contain notable amounts of phenolic acids, particularly mangiferin and quercetin, which are effective radical scavengers. Another study also showed the correlation of high phenolic content and stronger antioxidant property (Liu *et al.*, 2013). This aligns with the current findings, suggesting that mango tea's phenolic profile contributes significantly to its antioxidant capacity.

On the other hand, guava tea shows moderate antioxidant activity which aligns with prior findings on guava leaves' moderate phenolic content (Jimenez-Escrig *et al.*, 2001). Guava leaves have phenolic acids like gallic acid that contribute to its antioxidant activity, although less potently than the highly concentrated blends like pito-pito (Ruksiriwanich *et al.*, 2022). The lower IC_{50} value in ABTS compared to DPPH suggests that guava's antioxidants may be more selective in their reactivity, particularly with radical types assessed in the DPPH assay.

The antioxidant activity of malunggay tea on the other hand reflects the substantial phenolic content known in moringa leaves, which includes high levels of quercetin and kaempferol (Siddhuraju and Becker, 2003). This moderate antioxidant activity supports findings by Fahey (2005), who notes that while malunggay has impressive antioxidant properties, its efficacy may vary based on the phenolic concentration and type of extract used. This variability in antioxidant action between assays further suggests that malunggay's specific phenolic compounds may target particular types of radicals more effectively.

Despite ampalaya showing a lower antioxidant activity in comparison to the other tea samples in this study, it is also known for its antioxidant activity. One study (Kubola and Siriamornpun, 2008) found that ampalaya leaf and green fruit extracts showed the highest antioxidant activities in different assays, with the leaf extract excelling in DPPH radical-scavenging and ferric reducing power, while the fruit extract performed best in hydroxyl radical-scavenging, β-carotene-linoleate bleaching, and total antioxidant capacity. Additionally, the total phenolic content, dominated by gallic acid, showed a strong correlation with the FRAP assay (R^2 = 0.948), highlighting the importance of phenolics in the antioxidant potential of ampalaya extracts.

Lastly, even with their low antioxidant activity, the seeds and leaves of guyabano are reported to contain both enzymatic antioxidants, such as catalase and superoxide dismutase, and non-enzymatic antioxidants, including vitamins C and E (Vijayameena *et al.*, 2013). The antioxidant activity of *A. muricata* leaves was observed to be stronger than that of *A. squamosa* and *A. reticulata* species, as demonstrated through various *in vitro* models, including ABTS, nitric oxide, and hydroxyl radical assays (Baskar *et al.*, 2007).

These findings on phenolic content underscore the varied antioxidant potentials of different herbal teas. Teas with high phenolic content, such as pito-pito and mango, may appeal to consumers interested in maximizing their antioxidant intake to combat oxidative stress and support general health. On the other hand, teas like guava and guyabano, with moderate phenolic levels, could be promoted for their specific health benefits, such as immune support and digestive health, even if their overall antioxidant capacity is somewhat lower. Meanwhile, ampalaya and malunggay teas can be marketed for their unique health benefits, such as anti-diabetic and anti-inflammatory properties, which may appeal to consumers with specific health needs.

Conclusion

In this study, the antioxidant activity of various herbal teas was evaluated using DPPH and ABTS assays, and findings show a strong correlation between the antioxidant potential and the phenolic content determined using the Folin-Ciocalteu method. Pito-pito and mango teas, which demonstrated the lowest IC_{50} values and highest phenolic content, showed particularly potent antioxidant activity, likely enhanced by the presence of bioactive phenolic compounds such as mangiferin and quercetin. This suggests that pito-pito's blend of seven plants may provide a synergistic effect, aligning with its traditional use for wellness and immune support. In contrast, teas like guava and guyabano displayed moderate antioxidant activity, consistent with their phenolic profiles and traditional use in tropical medicine. Ampalaya and malunggay, though lower in antioxidant activity, still provide unique health benefits, such as anti-diabetic and anti-inflammatory effects, underscoring the diverse health-promoting potentials of these teas for specific consumer needs.

Recommendation(s)

To maximize the health benefits and market appeal of these herbal teas, it is recommended to promote phenolic-rich teas like pito-pito and mango for their strong antioxidant properties, targeting health-conscious consumers. Additionally, specific teas such as ampalaya and malunggay should be marketed for their unique health benefits, like hypoglycemic and anti-inflammatory effects, appealing to those with targeted health concerns. Further research into the synergistic effects of multi-herb blends, optimal extraction methods to enhance antioxidant content, and preparation guidelines for consumers could ensure the teas deliver maximum health benefits. These strategies highlight the teas' varied therapeutic potentials, supporting their role as natural wellness beverages.

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