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Research Article

Carotenoid content, phytochemical screening, and antioxidant potential of Kantutay (*Lantana camara* L.), Katuray (*Sesbania grandiflora* L.), and Blue Ternate (*Clitoria ternatea* L.) flowers in the Philippines

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Abstract: Natural product chemistry plays a pivotal role in drug discovery and development. This study investigated the phytochemical profiles and antioxidant capacities of three prominent Philippine plants: Blue ternate (*Clitoria ternatea*), Kantutay (*Lantana camara*), and Katuray (*Sesbania grandiflora*). The primary aim is to provide a comprehensive assessment of their bioactive constituents and evaluate their potential for pharmacological applications. Phytochemical screening identified a diverse array of compounds, including flavonoids, tannins, glycosides, terpenoids, and other major chemical constituent classes, highlighting their therapeutic potential. The pigment analysis revealed substantial variations, with Blue ternate exhibiting the highest concentration, suggesting it as a promising source of carotenoids. Thin Layer Chromatography (TLC) and chlorophyll analysis further revealed distinct compound profiles. Total Phenolic Content (TPC) analysis and the DPPH radical scavenging method marked Blue ternate to have the highest phenolic content and the most potent antioxidant activity among the plant samples. These findings collectively emphasize the significant therapeutic potential of these plants, warranting further exploration for pharmaceutical development.

1. INTRODUCTION

The significance of plants in the natural world extends beyond their well-known roles in oxygen production, and carbon dioxide absorption, and as a vital source of sustenance for both animals and humans (Fernando, 2012). They also play a pivotal role in mitigating the greenhouse effect and addressing climate change (Fernando, 2012). However, the true depth of their importance lies in the remarkable diversity of natural products they produce, each holding its unique significance.

For thousands of years, plants and their chemical compounds have been intertwined with various cultures, serving as the foundation for an array of remedies and tonics (Veeresham, 2012). Even today, these natural compounds continue to captivate researchers, forming the bedrock of modern drug development (Masinas *et al*., 2018). Extensive studies are dedicated

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to refining the safety profiles and exploring the efficacy of these compounds across various applications (Veeresham, 2012). Despite the introduction of groundbreaking technologies such as computer-based molecular modeling, combinatorial chemistry, and synthetic chemistry, the irreplaceable role of natural products in drug discovery and development remains (Fernando, 2012).

The advancements in technology, particularly in the realms of molecular biology and clinical observations, have ushered in a new era of understanding and application. However, the vast structural diversity found in many plant species leaves a wealth of untapped potential. These potential forms the basis of this study, which aims to give insight into three prominent plants found in the Philippines – Kantutay (*Lantana camara*), Katuray (*Sesbania grandiflora*), and Blue Ternate (*Clitoria ternatea*). This research focuses on determining carotenoid content, conducting a comprehensive phytochemical screening, and assessing the antioxidant capabilities of these plants.

Phytochemical screening is a method used to analyze the presence of phytoconstituents in a plant. This process starts with the preparation of aqueous and organic extracts (Srivastava *et al*., 2014). These extracts are obtained from plants that are rich in secondary metabolites (e.g., alkaloids, terpenes, flavonoids, etc.) and are commonly analyzed through thin-layer chromatography (TLC) (Purkait *et al*., 2023). The result of phytochemical screening depends on the morphology and structure of the plant. Moreover, analyzing the bioactive phytochemicals of a plant is substantial in determining its suitability for pharmacological development (Purkait *et al*., 2023).

Carotenoids are the pigments in many plants and vegetables that produce orange, red, and yellow hues (Healthline, 2017). Carotenoid contents of a plant mainly help in absorbing light and energy for photosynthesis. It has also an antioxidant function and hence acts as a type of antioxidant for humans which can fight certain types of cancer (Biology Dictionary, 2018; Szalay, 2015). Moreover, carotenoids are also viewed as safe chemicals for neutral chemical purposes and food supplementation due to their intense coloring abilities as well as their significance as precursors of Vitamin A (Hemalatha & Kailasam, 2022). It is composed of a long chain of alternating bonds and single C-C bonds which contain acid–functional groups, keto-hydroxyl-, and cyclic end groups (Hemalatha & Kailasam, 2022).

Plants are known as a rich source of antioxidants due to the many chemical components they contain, which may function alone or in concert to provide the body with defense against free radicals (Bhatt *et al*., 2013). These free radicals can cause oxidative stress which damages the cell and eventually results in some diseases like cancer, diabetes, eye disease, and age-related macular degeneration (Goodman *et al*., 2011). Given their significance, the three plants in this study will be analyzed to determine their bioactive components which will be of help in utilizing them for pharmacological developments in the Philippines and potentially in other countries.

Lantana camara, commonly known as Kantutay, belongs to the Verbenaceae family and is indigenous to the tropical and subtropical regions of the Americas (Kato-Noguchi & Kurniadie, 2021). This perennial herb or shrub can reach heights of 0.5-4.0 m and is characterized by its multicolored flowers, which can persist year-round in dense stands. While Kantutay's native habitat lies in the Americas, several of its taxa have been introduced and have become naturalized in Africa and tropical Asia (Negi *et al*., 2019). Kantutay encompasses an estimated 150 species, distributed across approximately 50 to 60 countries, with some being cultivated for their flowers (Munir, 1996).

Kantutay is classified as an invasive species in many regions, posing ecological challenges. Its rapid spread has disrupted terrestrial ecosystems and posed threats to agricultural areas by altering their structure (Kohli *et al*., 2006). Despite its invasive nature, Kantutay holds numerous medicinal applications. Experimental studies demonstrated its efficacy as an insecticidal, fungicidal, antimicrobial, and nematocidal agent (Begum *et al*., 2000). The bark of the Kantutay plant serves as an astringent and can be applied to leprous ulcers, while the leaves,

when they extracted, yield antimicrobial agents. Boiling the leaves and applying the solution alleviates body pain and swelling (Singh, 1996).

Furthermore, Kantutay leaves contain alkaloidal fractions that contribute to lowering blood pressure, stimulating intestinal movements, and enhancing respiration (Singh, 1996). Traditional uses for Kantutay extracts include addressing conditions like cancer, chicken pox, measles, rheumatism, tetanus, tumors, ulcers, high blood pressure, catarrhal infections, eczema, bilious fevers, and applying its oil for scabies and leprosy (Day *et al*., 2003; Ghisalberti, 2000; Sharma *et al*., 2007). A qualitative phytochemical screening conducted by Sardhara and Gopal (2013) in India revealed that Kantutay flowers contain a range of phytoconstituents including proteins, alkaloids, flavonoids, carbohydrates, sterols, tannins, terpenes, saponins, and glycosides. The ethanolic extract of Kantutay flowers was also analyzed, confirming the presence of flavonoids, terpenoids, ninhydrin, gelatin, phenols, and alkaloids. These identified phytochemicals underscore the pharmacological significance of Kantutay.

Sesbania grandiflora, commonly known as Katuray, belongs to the Leguminosae family and is endemic to Asia, including the Philippines, India, Indonesia, and Malaysia (Hasan *et al*., 2012). This perennial plant thrives in regions with an annual temperature range of 22-30°C and struggles in environments below 10°C. Katuray is known by various names, including Vegetable Hummingbird, West Indian Pea, Scarlet Wisteria, and Red Wisteria, but is predominantly referred to as Katuray in the Philippines. Katuray has a rich history of traditional use in folk medicine, particularly for its edible flowers (Kirtikar & Basu, 1995). The plant's flowers, roots, bark, leaves, and fruit or pod are all utilized for various medicinal applications. Katuray is described as a soft-wooded, short-lived, and quick-growing tree that can reach up to 12 meters in height (Wagh *et al*., 2009). Its flowers come in red and white varieties, and it produces elongated pods that hang vertically.

Unlike Kantutay, Katuray is not toxic and offers a wide range of medicinal benefits. In India, it has been traditionally used to address ailments like headaches, fevers, bronchitis, smallpox, rheumatism, inflammation, leprosy, gout, and anemia (Wagh *et al*., 2009). The plant also serves as a diuretic, emetic, laxative, and tonic in folk medicine. Notably, Katuray exhibits anxiolytic, anticonvulsive, and hepatoprotective properties (Wagh *et al*., 2009). The roots are powdered and mixed with water to create a poultice for body swelling, while the bark is employed for conditions like dysentery and sprue, acting as a laxative and emetic (Wagh *et al*., 2009). The leaves, when crushed or converted into juice, are used for various conditions including leprosy, fever, gout, and itchiness due to their anthelmintic and tonic properties. Additionally, Katuray flowers can be juiced and consumed to alleviate headaches, stuffy nose, and head congestion (Wagh *et al*., 2009).

Arun *et al*. (2014) conducted a phytochemical screening of Katuray leaves in India, identifying tannins, saponins, alkaloids, glycosides, cardiac glycosides, steroids, and flavonoids. Further studies confirmed the presence of valuable secondary metabolites, underscoring the medicinal potential of Katuray (Avalaskar *et al*., 2011; Gomase *et al*., 2012). Additionally, Katuray flowers were found to be rich in carotenoids, serving as a valuable source of Vitamin A and B9 (Bhokre *et al*., 2022).

Blue Ternate, scientifically known as *Clitoria ternatea*, belongs to the Fabaceae family and is recognized as a perennial herbaceous plant (Al-Snafi, 2016). Originating from the Ternate Island in Indonesia, it has spread to various Southeast Asian countries as well as other continents like Australia and America (National Nutrition Council, 2022). In the Philippines, it is commonly referred to as Blue Ternate but also goes by names such as Blue Bell Vine, Asian Pigeon Wings, Butterfly Pea, and Darwin Pea. Notably, the deep blue indigo color of its flowers adds to its ornamental value (NNC, 2022).

Blue Ternate holds both culinary and medicinal significance. In the Philippines, its flowers are frequently used to prepare herbal drinks and teas, and its natural pigment is employed as a food coloring agent for rice and bread, as well as a garnish for culinary. In addition, Blue

Ternate has a long history of traditional medicinal applications, particularly in Ayurveda. It has been utilized to address neurological disorders for centuries (Gollen *et al*., 2018). The plant's roots are used for sore throats, abdominal enlargement, skin diseases, and ascites, while seeds and leaves are employed for memory enhancement. The flowers and their juice serve as an antidote for snake bites, and the seeds are applied for joint swelling, colds, and urinary issues (Ragupathy & Newmaster, 2009). Furthermore, there have been several reports of the pharmacological characteristics of Blue Ternate including sedative, anti-inflammatory, analgesic, anxiolytic, and antipyretic activities (Gollen *et al*., 2018).

Phytochemical analyses have revealed the presence of a diverse array of compounds in Blue Ternate, including saponins, tannins, proteins, carbohydrates, triterpenoids, flavonoids, phenols, anthocyanins, flavanol glycosides, anthraquinone, volatile oils, cardiac glycosides, and Stigmast-4-ene-3,6-dione (Al-Snafi, 2016; Kelemu *et al*., 2004). Additionally, the flower contains flavonoids, phenols, and anthocyanin phytoconstituents (Chayaratanasin *et al*., 2015). These phytochemicals contribute to the plant's anti-inflammatory and analgesic properties (Srivastava *et al*., 2009; Malik *et al*., 2008). Notably, Blue Ternate flowers contain β-carotene, albeit in lower concentrations compared to some other plants, due to the prevalence of blue and violet hues associated with anthocyanins (Hemalatha & Kailasam, 2022).

In summary, the three plants hold immense botanical and medicinal significance in the Philippines and beyond. Their phytochemical compositions and biological activities offer a wealth of potential for various applications, ranging from traditional medicine to modern pharmacology. Further research and exploration of these plants' properties and compounds are essential for unlocking their full potential in the fields of health and medicine.

2. MATERIAL and METHODS

2.1. Sample Preparation

The three plant samples were collected and prepared at Pantabangan, Nueva Ecija, Philippines, 3124. The preparation of the samples for the extraction of phytochemicals and other natural products started with the air-drying at room temperature wherein the samples were placed in a room not directly hit by sunlight. The air-drying of samples lasted for 83 hours. Consequent to that, an osterizer was used to crush the plants into smaller pieces as shown in Figure 1. The crushed plant samples were extracted with ethanol (1 g plant material: 10 mL solvent) for 72 hours while being constantly stirred. By filtration, the extract and the residue were separated. With the use of a rotavapor drier (55-85℃), the extracts were dried and stored in glass bottles and centrifuge tubes coated with aluminum foil or paper and kept in the refrigerator until they were ready for analysis. Also, some grounded plant samples were kept in the fridge for pigment analysis.

Figure 1. Air dried (a) *Lantana camara*, (b) *Sesbania grandiflora*, and (c) *Clitoria ternatea*; (d) crushing the plant samples with osteorizer, (e) crushed plant sample subject for extraction, (f) and (g) are plant samples being extracted with ethanol subject for analysis.

2.2. Phytochemical Screening

In accordance with Pant *et al*. (2017) method, the extracts were subjected to qualitative phytochemical screening for the determination of the presence of the major chemical constituent classes (i.e., alkaloids, glycosides, carbohydrates, tannins, terpenoids, saponins, anthraquinones glycosides, cardiac glycosides, phenols, and flavonoids) through color reaction.

2.3. Pigment Analysis

2.3.1. *Total carotenoid analysis (TCA)*

The carotenoid analysis methodology was adapted from Natividad *et al*. (2014). After being placed in a 50 mL centrifuge tube, 500 mg of the plant powder was extracted with 10 mL of ethanol each using a vortex mixer for one minute. Measurements were taken of the supernatants. The carotenoid extract's ultimate volume was changed to 75 mL by adding 95% ethanol. The UV-Vis spectrophotometer was utilized to calculate the carotenoid extract's absorbance value at 450 nm. All plant samples underwent these procedures for three times each.

According to the following formula, the total carotenoid yield (dry weight) was determined:

$$\text{Total carotenoid yield } \left(\frac{\mu g}{g} \text{ dried weight}\right) = \frac{V(A - 0.0051)}{0.175W}$$

Where:

A – absorbance value of diluted extraction at 450 nm
V – final volume of the extract (mL)
0.175 – extinction coefficient of carotenoids
W – weight of the dried power (grams)

2.3.2. *Thin layer chromatographic (TLC) analysis*

With the use of a pencil, a baseline was drawn both at the bottom and top of a TLC distancing about 1 cm from the ends. Using a capillary tube, the sample extracts were applied to the TLC plate. The development chamber was a beaker with a watch glass lid and solvent within. The solvent mixture was poured into the development chamber after it had been lined with filter paper inside to ensure solvent saturation. Instead of using 5% methanol in toluene as the solvent, this experiment employed 5% methanol in xylene (Natividad & Rafael, 2014). Under the baseline of the TLC plates, more of the solvent mixture was injected into the chamber. Making sure that the baseline was above the solvent system, the TLC plate with spotted extracts was moved to the development chamber. It was noted how far the spot and solvent moved. An iodine chamber and an ultraviolet lamp were used to see the spots on the TLC plates. The spots were marked with a pencil (Velasco *et al*., 2018). Using the following formula, the retention factor (Rf) was calculated:

$$R_f = \frac{\text{distance travelled by the spot}}{\text{distance travelled by the solvent}}$$

To replicate the spots that were created, the TLC analysis was carried out three times.

2.3.3. *Chlorophyll analysis*

The method of Baluran and colleagues (2018a, 2018b) was used to determine the levels of chlorophyll in the samples of plants. Chlorophyll a and b concentrations of 2 mg/mL in methanolic plant extract solutions were measured at 666 and 653 nm using a UV-Vis spectrophotometer. The following formulas were used to determine the chlorophyll contents:

$$\text{Chlorophyll a } \left(\frac{mg}{L}\right) = 15.65\text{Abs}_{666} - 7.34\text{Abs}_{653}$$

$$\text{Chlorophyll b } \left(\frac{mg}{L}\right) = 27.05\text{Abs}_{653} - 11.21\text{Abs}_{666}$$

2.4. Total Phenolic Using Folin-Ciocalteau Reagent Determination

The Ortinero and colleagues' (2021) procedure was applied to analyze the total phenolic content (TPC) in the plant materials. 400 microliters of the extract, 1.0 mL of diluted Folin-Ciocalteau phenol reagent, and 800 µL of distilled water were added (1:10). 1.0 mL of 7.5% (w/v) sodium carbonate was mixed into the solution after waiting for five minutes. Several gallic acid standards were produced. With the use of a UV-Vis spectrophotometer, the absorbance of the extracts and the standard solutions were determined at 765 nm. The TPC of the plant extract was determined as milligrams of gallic acid equivalent per gram of the sample's dry weight (mg GAE/ g DW).

2.5. Determination of Antioxidant Activity Using DPPH Radical Scavenging Method

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assay) was carried out with a few minor adjustments using the procedure of Ortinero *et al*. (2021). The DPPH solution was made by mixing 6.0 mg of DPPH with 100 mL of methanol, whereas the extracts were produced in a range of solutions (0.01, 0.1, 1, 10, 100, and 1000 ppm). The test tube containing

2.5 mL of DPPH solution received precisely 1.5 mL of extract. BHA (beta hydroxy acids) served as the benchmark. After giving the mixture a vigorous shake, it was allowed to stand in the dark for 30 minutes. The UV-Vis Spectrophotometer 1500 was utilized to measure the solution's absorbance at 517 nm. The following equation was used to determine the DPPH radical scavenging activity of each extract:

$$\text{DPPH Scavenging activity} = \left(\frac{\text{Abs}_{\text{blank}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{blank}}}\right) \times 100$$

The IC_{50} values, which were determined by using nonlinear regression (sigmoidal dose-response) on the data, represented the extracts' scavenging activity.

2.6. Data Analysis

Three copies of each experiment were run throughout. Analysis of variance (ANOVA) was used to compare the total phenolic, carotenoid, and chlorophyll contents of the plant samples at the 5% level of significance. The statistical analysis was completed utilizing Statistical Tool for Agricultural Research (STAR) software version 2.0.1.

3. RESULTS

3.1. Phytochemical Screening

The phytochemical screening of the Kantutay, Katuray, and Blue Ternate is shown in Table 1. The flowers of Blue Ternate contain tannins, glycosides, carbohydrates, cardiac glycosides, terpenoids, and phenol while proteins and saponins were absent in the Blue Ternate. The test for alkaloids for Blue Ternate was found positive on Mayer's reagent but negative on Dragendroff's reagent, which is similar to Kantutay. Moreover, the Blue Ternate contained flavonoids as it was found positive on both the Shinoda and Alkaline reagent tests. The Kantutay and Katuray contained all the natural products aside from proteins and phenol. However, the test for alkaloids for Katuray was the opposite of the results for Blue Ternate and Kantutay, because it was found positive on Dragendroff's reagent and negative on Mayer's reagent.

Table 1. Phytochemical contents in the plant samples determined in various dissolving agents.

Phytochemical Screening	Blue Ternate	Kantutay	Katuray
Test for tannins	+	+	+
Test for alkaloids			
• Mayer's reagent	+	+	-
• Dragendroff's reagent	-	-	+
Test for glycoside	+	+	+
Test for Carbohydrates (Molisch's test)	+	+	+
Test for saponins	-	+	+
Test for cardiac glycosides	+	+	+
Test for flavonoids			
• Shinoda test	+	+	+
• Alkaline reagent test	+	+	+
Test for terpenoids (Salkowski's test)	+	+	+
Test for proteins	-	-	-
Test for phenol	+	-	-

(–) Absence of phytochemicals compounds
(+) Presence of phytochemicals compounds

Comparing the phytochemical screening results to other literature, the researcher found out that the Kantutay has almost similar results to that of the experiment of Sardhara and Gopal (2013) and Gul *et al*. (2020). From their experiment, the Kantutay also contained alkaloids, flavonoids, carbohydrates, tannins, terpenes, saponins, and glycosides. Sardhara and Gopal (2013) and Gul *et al*. (2020) also revealed that the test for protein had turned positive, which is in contrast to the negative result in this analysis. Further, Avalaskar and co-workers (2011) revealed that Katuray has tannin, polyphenols, saponins, and flavonoids. Their result is almost similar to the findings in this analysis, aside from the negative response for phenol. Moreover, Dethe and colleagues (2013) concluded that Katuray flowers have a negative response to terpenoids and glycosides, which have a positive response in this analysis. For Blue Ternate, the work of Al-Snafi (2016) with reference to Kelemu *et al*. (2004), stated that the Blue Ternate flower contained saponins, tannins, proteins, carbohydrates, flavonoids, phenols, and cardiac glycosides. The results were all the same with the analysis responses aside from the absence of saponins and proteins.

Tannins, being water-soluble phenolics found in all three plant samples, have applications in Asian medicine, particularly against diarrhea, and as astringents and diuretics (Chung *et al*., 2010; Khanbabaee & Ree, 2001; Sieniawska & Baj, 2017). They exhibit bioactivity in both absorbable and unabsorbable forms, influencing various organs systemically or targeting the gastrointestinal tract as antiviral, antimicrobial, and antioxidant agents (Navarro *et al*., 2018; Serrano *et al*., 2009).

Alkaloids, derived from amino acids, are detected in Blue Ternate and Kantutay with Mayer's reagent, and in Katuray with Dragendroff's reagent. Alkaloids are widely recognized in pharmacology for their antihypertensive, antiarrhythmic, antimalarial, and anticancer properties (Roberts & Wink, 1998).

Flavonoids, present in all three plant samples, have drawn interest in pharmacology due to their vitamin-like properties. They find applications in cosmetics, nutraceuticals, medicines, and pharmaceuticals, with demonstrated effects on cellular enzyme activity, as well as anti-carcinogenic, anti-mutagenic, anti-inflammatory, and antioxidative properties (Brandi, 1992; Panche *et al*., 2016).

Kantutay and Katuray contain saponins, which are increasingly valued for their diverse biological, pharmaceutical, and medicinal applications. Saponins exhibit anti-ulcer, adjuvant, anti-tumor, anti-inflammatory, hepatoprotective, and antibacterial properties (Moghimipour & Handali, 2014; El Aziz *et al*., 2019).

Terpenoids, present in all three samples, offer the potential for developing medications with fewer side effects. They are commonly found in essential oils and serve as flavoring and fragrance agents in the food industry. The pharmacological applications of these plants are extensive, as terpene-containing plants have been employed in traditional medicine for centuries, with various formulations available on the market (Ludwiczuk *et al*., 2017).

Among the three plant samples, only the Blue Ternate contained phenolics. Natural bioactive chemicals known as phenolic compounds are common in plant tissues. They have demonstrated fascinating bioactivities, including antibacterial and antiproliferative activities and antioxidant and anti-inflammatory properties, which has sparked a lot of interest in their utilization by many businesses (Albuquerque *et al*., 2021).

3.2. Pigment Analysis

3.2.1. *Total carotenoid analysis (TCA)*

The total carotenoid (TC) content expressed in µg/g for the three plant samples is shown in Table 2. From the TC analysis results, the values ranged from 213.03 to 538.36 µg/g in which the Kantutay has the lowest TC value, followed by Katuray, while Blue Ternate has the highest TC content. This result indicates that the Blue Ternate shows great potential as a source of carotenoids.

The TC content of the plant samples showed significant differences from the Analysis of Variance (ANOVA) at a 95% level of confidence. Moreover, from Duncan's Multiple Range Test (DMRT), the Blue Ternate has a significant amount of TC, followed by Katuray, and Kantutay has the least significant amount.

Table 2. Total carotenoid content of Blue Ternate, Kantutay, and Katuray.

Plant Samples	Total Carotenoid (TC) Content (μg/g)
Blue Ternate	538.36 ± 1.99^{a}
Kantutay	213.03 ± 0.55^{c}
Katuray	416.31 ± 0.75^{b}

Means with the same letter are not significantly different

The work of Meléndez-Martínez and colleagues (2021) revealed that the white, yellow, and red flowers of Kantutay have a TC content of 64.782±0.420, 2056.065±7.148, and 304.721±0.183 μg/g dry weight, respectively. These values were made up mostly of carotenoids like phytoene, 9-*Cis*-Violaxanthin, Lutein, Violaxanthin, 9-*Cis*-Anteraxanthin, α-carotene, and β-carotene. From the TC analysis in this experiment, the Kantutay has a TC of 213.03 μg/g, which is comparable to the white and red flowers' TC content from the previous studies.

Janarny and colleagues (2021) determined the TC of Blue Ternate and Katuray which are quantified as 18.2±0.1 and 82.1±0.5 mg β-carotene/g, respectively. Moreover, Weerasinghe and Gunathilake (2020) analyzed the β-carotene concentration of Katuray and revealed that it has 252.24±4.18 μg/g dry weight. Also, Bhokre and co-workers (2022) presented a carotenoid analysis of Katuray flowers and leaves and quantified it to be 420 μg/100g and 3,120 μg/100g, respectively.

The computed TC for Blue Ternate, Kantutay, and Katuray are close to the values written in the literature. Although there may be some obvious differences among the values, Dias and co-workers (2008) stated that the TC content could vary in terms of quantity and quality due to the variety, morphology, and maturity of the species involved. This statement is supported by the book of Rodriguez-Amaya (2001) about the protocol in food carotenoid analyses. It was stated in the book that factors like climate, maturity, geographic location, and various processes employed in the products affect the value and concentration of its carotenoid.

3.2.2. *Thin layer chromatography analysis*

TLC analysis was employed to separate the organic compounds, following the methods of Natividad and Rafael (2014). Instead of 5% methanol in toluene, 5% methanol in xylene served as the solvent system. The spots on the TLC plates were visualized under UV light and an iodine chamber, then marked with a pencil to record the distances traveled for *Rf* value calculation (Figure 2).

In total, 10 spots were identified in Blue Ternate, 11 in Kantutay, and 9 in Katuray. The *Rf* values ranged from 0.00 to 1.00 for Katuray and Kantutay, and from 0.00 to 0.99 for Blue Ternate. The ascending order of *Rf* values in Table 3 indicates differing polarities, with a value of 0.00 signifying a very polar compound. Similar *Rf* values suggest potential shared compounds in the plant extracts, with only two instances observed.

In particular, *Rf* values of 0.76 and 1.00 were observed in both the extracts of Kantutay and Katuray. It was also observed that there were values that did not have much differences. For instance, *Rf* values (in respective order) of 0.9 and 0.10 for Blue Ternate and Katuray, 0.15 and 0.14 for Blue Ternate and Kantutay, 0.36 and 0.35, 0.45 and 0.46, and 0.56 and 0.57 for Kantutay and Katuray, and 0.86, 0.87, and 0.88 for the three plant samples.

The polar compounds, which are very affine to the polar silica gel in the TLC plate, could be classified as oxygenated carotenoids i.e., lutein, echinenone, zeaxanthin, antheraxanthin, and

spirilloxanthin (Casuga & Natividad, 2023; Paiva & Russell, 1999; Velasco *et al*., 2018). The aforementioned oxygenated carotenoids may be present in the extract of the plant samples. On the other hand, those compounds that do not have a strong affinity for the silica gel in the TLC plate were considered to be very affine with mobile phase that has a great portion of xylene, a nonpolar solvent. In this case, high *Rf* values could be an indication of the presence of carotenoid hydrocarbon (i.e., β-carotene) (Natividad & Rafael, 2014).

Table 3. *Rf* values of the three plant samples separated by TLC.

	Blue Ternate	Kantutay	Katuray
Rf values	0.00	0.00	0.00
	0.09	0.05	0.10
	0.15	0.14	
	0.20	0.27	0.24
	0.30	0.36	0.35
	0.42	0.45	0.46
	0.48		
		0.56	0.57
	0.73	0.76	0.76
	0.86	0.88	0.87
		0.95	
	0.99	1.00	1.00
Total Spots	10	11	9

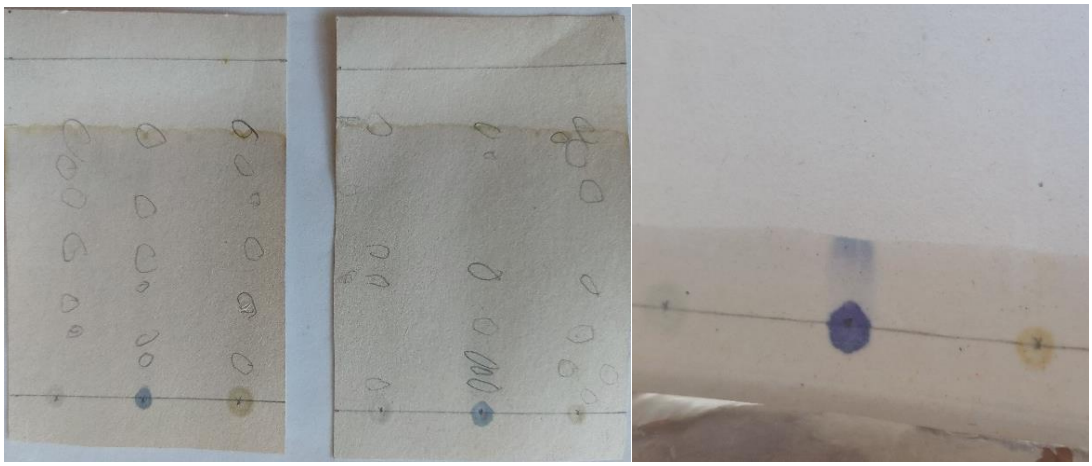

Figure 2. TLC analysis of Blue Ternate, Kantutay, and Katuray.

3.2.3. *Chlorophyll analysis*

The methods of Baluran et al. (2018a, 2018b) were adapted in this analysis for the determination of the chlorophyll concentrations. With the use of an ultraviolet-visible (UV-Vis) spectrophotometer, the methanolic solutions of the extracts of the sample plants for chlorophyll *a* and *b* were analyzed and the results are shown in Table 4.

Table 4. Chlorophyll *a* and *b* concentration in the three plant samples.

Plant Sample	Chlorophyll *a* (mg/L)	Chlorophyll *b* (mg/L)
Blue Ternate	0.81 ± 0.01^{c}	1.08 ± 0.02^{c}
Kantutay	1.12 ± 0.02^{b}	2.22 ± 0.03^{b}
Katuray	2.16 ± 0.01^{a}	3.13 ± 0.01^{a}

Means with the same letter are not significantly different

Chlorophyll *a* and *b* are known as two major types of chlorophyll that can be found in a plant where both are necessary for photosynthesis (Panawala, 2017). The key distinction between the two pigments is that chlorophyll *a* is regarded as a major or primary photosynthetic pigment, whereas chlorophyll *b* is an accessory pigment that gathers energy and transfers it to chlorophyll *a* (Panawala, 2017; Udayangani, 2011). The chlorophyll *a* concentration was recorded as 0.81, 1.12, and 2.16 for Blue Ternate, Kantutay, and Katuray, respectively. While chlorophyll *b* content was recorded as 1.08, 2.22, and 3.13 for Blue Ternate, Kantutay, and Katuray, respectively. Among the three plant samples, the Katuray has the highest chlorophyll *a* and *b* content, followed by Kantutay, while Blue Ternate has the lowest.

From the ANOVA (Analysis of Variance) with 5% level of significance, the concentration of chlorophyll *a* and *b* in the plant samples has significant differences. Moreover, from its DMRT, Katuray has a significant amount of chlorophyll *a* and *b*, followed by Kantutay, and Blue Ternate has the least amount of both concentrations.

3.3. Determination of Total Phenolic using Folin-Ciocalteau Reagent

The TPC in the extracts of the plant samples, expressed in mg of gallic acid equivalent (GAE) per gram dry weight of the sample was determined by the methods of Ortinero and colleagues (2021). The results of this analysis are shown in Table 5. Blue Ternate has the highest TPC amounting to 407.50 mg GAE/g dry weight, followed by Kantutay with 206.04 mg GAE/g dry weight, and Katuray has the lowest TPC of 107.07 mg GAE/g dry weight.

Table 5. Total phenolic content (TPC) determined in the plant samples.

Plant Sample	TPC (mg GAE/g dry weight)
Blue Ternate	407.50 ± 1.69^{a}
Kantutay	206.04 ± 4.39^{b}
Katuray	107.07 ± 2.52^{c}

Means with the same letter are not significantly different

Torres and co-workers (2021) determined the TPC of Blue Ternate and quantified it as 3.95 mg GAE/100 g (equivalent to 395 mg/GAE/g) which is comparable to the findings in this analysis of 407.50 mg GAE/g. Further, from the work of Baessa and colleagues (2019), different extracts were used in their experiment which are the infusion, decoction, and tincture. From those extracts, the TPC of Katuray was recorded as 163±1.00, 188±2.10, and 162±4.00 mg GAE/g DW, which values are close to the results in the TPC determined in this study. Moreover, Manzoor *et al*. (2013) employed different extraction techniques (i.e., Magnetic stirring, ultrasonic magnetic stirring, microwave-assisted magnetic stirring) and solvent systems (100% methanol, 100% ethanol, 80% methanol, and 80% ethanol) to determine the TPC of Kantutay. As a result, Kantutay contained 8.28±0.25 to 52.34±1.56 mg GAE/100 g DW.

From the ANOVA with a 5% level of significance, the TPC determined in the extracts of plant samples has significant differences. Moreover, from its DMRT, Blue Ternate has a significant TPC, followed by Kantutay, and Katuray has the lowest.

3.4 Antioxidant Potential using DPPH Radical Scavenging Activity

The antioxidant potential activity of the three plant samples was determined and different solutions of the plant sample extracts were prepared and tested to record the half-maximal inhibitory concentration (IC50) from the dose-response curve. IC50 is the concentration of the extract that indicates the amount of drug or test sample needed to inhibit a biological process or to produce the effect by half. Therefore, the lower the IC50 value, the more potent the substance for inhibiting a specific biological or biochemical function (Aykul & Martinez-Hackert, 2016).

The antioxidant potential activity of the plant samples is shown in Table 6. Blue ternate has the lowest value of 48.33 mg/mL, followed by Katuray with a value of 113.98 mg/mL, while Kantutay has the highest amount. Since this was evaluated with IC50, it means that the most potent among the plant samples is Blue ternate. Kantutay, on the other hand, is the least potent.

Table 6. Estimates of the half lethal concentration (IC_{50}) of the DPPH scavenging activity of the plant sample extracts

Plant Sample	IC_{50} (mg/mL)
Blue Ternate	48.33[c]
Kantutay	209.99[a]
Katuray	113.93[b]

Means with the same letter are not significantly different

4. CONCLUSION

This study provided a comprehensive insight into the bioactive components of Kantutay, Katuray, and Blue Ternate. Phytochemical screening revealed a diverse array of compounds including flavonoids, tannins, glycosides, carbohydrates, cardiac glycosides, terpenoids, and phenols in Blue Ternate. Interestingly, proteins and saponins were absent in this species. Kantutay and Katuray also exhibited a rich phytochemical profile, lacking only proteins and phenols. Additionally, the alkaloid tests displayed unique responses for each plant.

Carotenoid content analysis showed varying concentrations, with Blue Ternate displaying the highest levels, indicating its potential as a source of carotenoids. TLC analysis unveiled distinct spots, with only a few similarities between Kantutay and Katuray extracts, suggesting potential shared compounds. Furthermore, chlorophyll analysis demonstrated differential concentrations of chlorophyll *a* and *b* in the three plant samples. The antioxidant potential activity was determined using DPPH radical scavenging method and evaluated with half-maximal inhibitory concentration revealing that the Blue ternate is the most potent to inhibit a specific biological or biochemical function among the plant samples.

The results of this study provide valuable insights into the bioactive components and antioxidant potential of these three prominent Philippine plants. These findings hold significant implications for their potential utilization in pharmacological developments, contributing to the advancement of traditional and modern medicine in the country. Further research into the specific compounds and their potential applications is warranted to fully unlock the therapeutic potential of these plants.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors. **Ethics Committee Number**: Central Luzon State University ERC No. 2023-071.

Authorship Contribution Statement

Melanie D. Piedad: Investigation, Resources, Formal Analysis, and Writing Original Draft. **Lexter R. Natividad:** Visualization, Software, Methodology, Supervision, and Validation.

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