International Journal of Medicinal Plants and Natural Products (IJMPNP)
Volume 11, Issue 1, 2025, PP 1-8
ISSN (Online) 2454-7999
https://doi.org/10.20431/2454-7999.1101001
www.arcjournals.org

Phytochemical Analysis and Antioxidant Capacity of Ulasimang Bato (*Peperomia pellucida*) Leaf Extracts

Juris Alexandra A. Pagasian[1*], Sebastian Miguel M. Abrajano [1], Jadee Kai L. Sereno[1], and Ian Jay P. Saldo[2]

[1]*Integrated Basic Education, San Isidro College, Malaybalay City, Bukidnon, 8700 Philippines*

[2]*School of Education, San Isidro College, Malaybalay City, Bukidnon, 8700 Philippines*

Corresponding Author:** ***Juris Alexandra A. Pagasian, *Integrated Basic Education, San Isidro College, Malaybalay City, Bukidnon, 8700 Philippines*

Abstract:

Aim/Background: *Plants with medicinal properties are the mainstay treatment among patients having difficulties acquiring medicines that need to be purchased at a high rate in local drug stores. It is a consumable plant and is often used as greens or condiments in various areas of the tropics, but in-depth examination is needed as Peperomia pellucida is being intake and is identified as a traditional treatment as a whole plant. The study focused on Ulasimang bato (P. pellucida) in Barangay 10, Impalambong, Malaybalay City, Bukidnon.* **Materials and Methods:** *The study aimed to examine the amount of phenols and flavonoids, to know the antioxidant capacity, and to examine which bioactive compounds of Ulasimang bato (P. pellucida) supports the anti-gout properties. The leaf samples were gathered at Battalion Camp Training, Impalambong, Malaybalay City, Bukidnon, Philippines.*

Results: *In this study, the Total Flavonoid Content (TFC) acquired the highest value among Total Phenolic Content (TPC) and Total Antioxidant Capacity (TAC) in the Ulasimang bato (P. pellucida) leaf extracts. Additionally, the TPC resulted in a mean of 0.767 mg and a standard deviation of 0.182. Meanwhile, TFC showed a mean of 4.477 mg and a standard deviation of 0.582. Finally, TAC revealed a mean of 1.149 mg and a standard deviation of 0.214.*

Conclusion: *The results of the phytochemical analysis were influenced by thermal treatment, quantity, plant part, and solvent. The bioactive compounds that support Ulasimang bato (P. pellucida) as anti-gout compounds are phenols and flavonoids. Furthermore, Ulasimang bato (P. pellucida) can be a natural alternative medication to anti-gout drug prescriptions.*

Keywords: *Antioxidant, Flavonoid, Phenol, Phytochemical, Ulasimang bato (P. pellucida)*

1. INTRODUCTION

Plants with medicinal properties are the mainstay treatment among patients having difficulties acquiring medicines that need to be purchased at a high rate in local drug stores. People utilize this to seek holistic modalities that can sustain their health. Despite the rapid advancement of technology in the medicinal world, there is still a wide range of consumers of herbal plant care. In the Philippines, proper precautions are being held and adequately studied to ensure safety and efficacy by the Philippines Institute of Traditional and Alternative Health Care (PITAHC); its objective is to adapt the medicinal way in the national health care administration [1]. However, using plants, their water-based extracts, essential exudates, resins, gums, oils, or other sophisticated outcomes from plant parts therapeutically to give proactive aid to many physiological systems is known as herbal medicine. In a more traditional sense, herbal treatment utilizes plants to aid, heal, or avoid diseases in humans or animals. Many people avoid the risk of using herbal medication due to allergic reactions [2].

However, information on the Ulasimang bato plant (*P. pellucida*) and detailed herb analysis is limited in Malaybalay City, Bukidnon. It may hinder an individual from attaining the needed results. The compounds entailed in the Ulasimang bato *(P. pellucida)* are unfamiliar to many. Some people may

disregard it which eventually ends up with plant waste. This assessment will address a significant problem in current herbal medicinal studies by identifying the total phenolic content, total flavonoid content, and total antioxidant capacity. With this, researchers may assess Ulasimang bato *(P. pellucida)* to gain insights into the content of herbal plant in aiding traditionally.

P. pellucida, an herbaceous plant belonging to the *Piperaceae* family, has been deemed a medicinal plant with therapeutic benefits for alleviating many various kinds of sicknesses by cultural traditions in several Southeast Asian nations, including Indonesia. The annual weed *P. pellucida (L.) Kunth* prefers damp environments with low sun radiation. The Neotropics, Africa, Southeast Asia, and Australia are the primary occurrence regions for this plant. It is widely used to treat a range of illnesses, especially issues with the kidneys, conjunctivitis, measles, skin sores, abdominal pain, and abscesses. Many studies have reported its cytotoxic, antidiabetic, antibacterial, and numerous other bioactivities [3].

The World Health Organization (WHO) defines traditional treatment as the entirety of intellect, capabilities, and habits derived from indigenous theories, perspectives, and lived experiences from many historical eras, may they be explained. It's application to both healthcare and the avoidance, prescription, development, or healing of physical and cognitive diseases. Vast amounts of written material and archives of theoretical ideas and valuable techniques underpin some traditional medical systems; other systems are passed down from generation to generation [4].

This study aimed to contribute to the medicinal field in the community. Emphasizing that selecting natural ways is more efficient than taking painkillers that may result in heart problems. To indicate that these herbs can be grown nearly anywhere like light shaded or damp areas. It is also a cheaper alternative to drugstore-based medication. Implying that this kind of plant does not require high maintenance but with consideration of the safety measures required.

2. MATERIALS AND METHODS

2.1. Research Design

This study utilized a quantitative method, specifically a descriptive design. It performed phytochemical screening of the Ulasimang bato (*P. pellucida*) leaves. Moreover, it ascertained the anti-gout compounds of the extracts through antioxidant capacity testing. The researchers quantified the amount of the present compounds on the leaves, which one possessed more depending on the test conducted, to thoroughly contrast and observe what activates them as anti-gout compounds.

2.2. Data Gathering Procedure

2.2.1. Identification of Plant Species

The researchers first gathered samples of Ulasimang bato (*P. pellucida)* at the study site. Then, they utilized various web-based research to pre-identify the samples. For final identification, the photographs of the samples were sent to the University Museum, Center for Biodiversity Research and Extension in Mindanao (CEBREM), situated in Musuan, Maramag, Bukidnon.

2.2.2. Collection and Preparation of the Samples

The identified Ulasimang bato *(P. pellucida)* leaves were obtained at a certain area in Impalambong, Malaybalay City, Bukidnon. After the collection of the samples, the plant leaves were given a thorough cleaning with tap water. Once the cleaning process was completed, the plant leaves were left to air dry naturally for a duration of 14 days. This period allowed the removal of any residual moisture and preserving the plant's structural integrity. The room temperature provided an optimal environment for the drying process, avoiding any potential damage that could occur with excessive heat or cold. Once fully dried, the leaves were finely blended until they reached a powdered consistency [5]. This process ensures that the plant material is prepared consistently and standardized.

2.2.3. Extraction Process

Extraction processes were conducted for the leaves of *P. pellucida*, using the maceration process. To begin, three jars containing 20 g of powdered leaf samples were soaked using water in a ratio of 1:3 and macerated for 3 days. Afterward, the distilled water-based extracts were filtered via filter paper and stored in specimen jars. Each sample was then replicated thrice for each test. The researchers consulted an expert from NPRDC concerning the procedure.

2.3. Ethical Consideration

The data were handled with the utmost concern and attention to ensure accuracy and integrity. Followed safety measures to protect the data from any unauthorized access or misuse. The researchers understood the importance of maintaining confidentiality and took all necessary precautions to safeguard the data. Furthermore, the researchers adhered to the standards and principles set forth by the study. They made sure that all ethical rules were observed and carried out the inquiry with the highest level of expertise. By taking responsibility for their actions and following the study's standards and principles, the researchers demonstrated their commitment to upholding the integrity of the data and conducting a thorough and reliable investigation

3. RESULTS

Ulasimang bato (*P. pellucida*) Taxonomy

Kingdom *Plantae* - Plants

Subkingdom*Tracheobionta* - Vascular plants

Superdivision*Spermatophyta* - Seed plants

Division *Magnoliophyta* - Flowering plants

Class *Magnoliopsida* – Dicotyledons

Subclass *Magnoliidae*

Order *Piperales*

Family *Piperaceae* Giseke - Pepper family

Genus *Peperomia* Ruiz & Pav. - *peperomia*

Species *Peperomia pellucida* (L.) Kunth [6]

Ulasimang bato (*P. pellucida*) Morphology

Ulasimang Bato, or pansit-pansitan, with the English identification "*Peperomia*," is a small herb about 1 to 1.5 feet long. It is green, has a heart-shaped leaf, and tiny florals on a thorn, which later bears fruit. Interestingly, this herbal plant can grow almost anywhere that is moist [7].

3.1. Total Phenolic Compound

Table1. *Test for the Total Phenolic Content (mg Gallic acid equivalent/g sample) of Ulasimang bato (P. pellucida) plant leaf extract using Folin-Ciocalteu Colorimetric Assay*

Part	Sample	Result
Leaves	ULB T1	0.773 ±0.006 mg GAE/g sample
	ULB T2	0.782 ±0.005 mg GAE/g sample
	ULB T3	0.747 ±0.007 mg GAE/g sample

Table 1 presents the Total Phenolic Content (mg gallic acid equivalent/g sample) of Ulasimang bato (P. pellucida) plant leaf extract utilizing the Folin-Ciocalteu Colorimetric Assay in all three trials. Among all trials, the second trial yielded the highest, with 0.782 ±0.005 mg GAE/g sample. This indicates that the studied plant part had almost equal results throughout the trials. The study of [9] showed that the plant species affected the testing results and its plant part.

Moreover, it is possible to extract phenolic compounds from *P. sarmentosum* using both standard and non-conventional methods. Maceration showed the highest TPC recovery among all the tested techniques, including infusion, reflux, WBE, MAE, UAE, MAI, and agitation extraction. Although maceration had more readily available extraction equipment than the other methods, the unconventional approach had significant advantages because of its quicker extraction time and lower solvent consumption [10]

In addition, selecting the right solvent is essential to guaranteeing a high recovery rate of phenolic substances. The most suitable solvents for phenolic component extraction include polar solvents such as water, methanol, and ethanol, which are frequently employed in the majority of *P. sarmentosum* studies. However, employing methanol as the extracting solvent produced the most notable TPC value [10]

Chemicals such as phenol, tannins, terpenoids, and carbohydrates are found in the study of the phytochemical analysis of the *Piper nigrum* leaves. The cold extraction of methanol extract revealed 360µg/ml of GAE of phenol compound, which indicates a higher total phenolic content [19]

In the study of [21], acetone is a more effective extraction method than methanol and petroleum ether for phenolic, flavonoid, and tannin components in *Piper betle.* Also, this solvent is known to be highly miscible and can combine with water.

3.2. Total Flavonoid Content

Table2. *Test for the Total Flavonoid Content (mg Quercetin equivalents/g sample) of Ulasimang bato (P. pellucida) plant leaf extract using Aluminum chloride Colorimetric Assay*

Part	Sample	Result
Leaves	ULB T1	3.8 ±0.9 mg QE/g sample
	ULB T2	4.83 ±0.01 mg QE/g sample
	ULB T3	4.8 ±0.2 mg QE/g sample

Table 2 presents the Total Flavonoid Content (mg Quercetin equivalents/g sample) of Ulasimang bato (*P. pellucida*) plant leaf extract. The second and third trials were the values nearest and yielded high among all trials. This indicates that the TFC of the plant yielded high among the three parameters that were used. Hence, the study of [12] stated that the watery extract contained less value than ethyl alcohol (EtOH), which signifies that solvent is a factor that may affect the flavonoid content. In which it has been determined that using distilled water over any other solvent has the potential to vary the results.

According to the study of [13], 96% ethanol is suggested as the best solvent for extracting more polyphenols, flavonoids, and tannins from cubeb fruit based on the extraction findings using other solvents. Solvents used to extract the bioactive components were 70% ethanol and distilled water. The substantial potential of cubeb fruit as a rich source of bioactive chemicals was observed.

According to the phytochemical analysis conducted on the Kankola (P. cubeb), the methanol, aqueous, and ethanolic extracts exhibited the highest concentration of phytoconstituents in the study of [17]. The ethanol extract exhibits the most abundant value among methanol, water, and n-hexane, and it was tested using Schnida's test to assess the presence of flavonoids. From this, It would appear that the solvent significantly impacts the outcome.

Differences in cultivar, growing conditions (temperature, altitude, etc.), and harvest maturation stage may have contributed to extracting *P. betle* differences in the TFC of leaves [22]. This implies that the samples' condition and growth are major factors in influencing the results of the analysis.

3.3. Total Antioxidant Capacity

Table3. *Test for the Total Antioxidant Capacity (mg Ascorbic acid equivalents/g sample) of Ulasimang bato (P. pellucida) plant leaf extract using Phosphomolybdenum Colorimetric Assay*

Part	Sample	Result
Leaves	ULB T1	1.38 ±0.01 mg AAE/g sample
	ULB T2	1.110 ±0.006 mg AAE/g sample
	ULB T3	0.957 ±0.008 mg AAE/g sample

Table 3 shows the Total Antioxidant Capacity (mg Ascorbic acid equivalents/g sample) of Ulasimang bato (*P. pellucida*) plant leaf extract. The first trial had the highest value of the three trials, with the result of 1.38 ±0.01 mg AAE/g sample. Similarly, the measurement of the filtered extract may vary the garnered results. Meanwhile, according to the study of [16], the extraction process can impact the antioxidant activity by increasing the polarity.

In order to create new sources of antioxidants, *Piper* species are being investigated as potential therapeutic plants. The extract levels, fractions, and compounds of the *Piper* species, which showed strong antioxidant activity, were the starting point for a number of investigations. The DPPH method, a spectrophotometric technique that assesses the antioxidant potential of compounds in herbal extracts and many other materials, was also applied. Ethanol, methanol, dichloromethane, ethyl acetate, hexane, aqueous, and hydroethanol are the solvents that were employed. Methanol has the highest antioxidant value among them. Another important component is the plant; according to the data, the leaves had the most value when compared to the roots, seeds, and fruits [18]

With certain adjustments, the Blois (1958) approach was used to assess the antioxidant activity or radical scavenging activity. Extracts were produced at the following concentrations: 40 µg/mL, 80 µg/mL, 120 µg/mL, 160 µg/mL, and 200 µg/mL. Ethanol is the most effective solvent for obtaining the antioxidant value of *Piper cubeba* and *Piper nigrum*, with water and methanol coming in second and third, respectively. Because it is less harmful, which signifies that ethanol is thought to be more efficient than other solvents [23].

Furthermore, the active antioxidant can be used to describe the efficiency of the Suruhan leaf ethanol extract. There is a lot of potential for using Suruhan (*P. pellucida*) leaf ethanol extract as an antioxidant and antimalarial medication. Moreover, the value of chloroquine extract was deemed to be quite low [24]. The use of this solvent may also lead to degradation of compounds, which leads to the efficacy of the extract itself.

Bioactive Constituents of Ulasimang bato (*P. pellucida*) as Anti-gout Compound

The bioactive compounds that support Ulasimang bato (*P. pellucida*) as anti-gout compounds are phenols and flavonoids. However, antioxidants are properties that these compounds contain. These assess in reducing oxidative stress and inflammation in gout pain. This can be a natural alternative medication to anti-gout drug prescriptions. Furthermore, *P. pellucida*, commonly called silver bush, thrives in some South American and Asian nations as a perennial plant. It is a member of the *Piperaceae* family. Historically, the plant species has been used ethnomedically to treat various ailments, including gout, rheumatic pain, acne, boils, exhaustion, headaches, and renal illnesses. According to the researchers, the crude extract of *P. pellucida* contained tannins, alkaloids, flavonoids, resins, phenols, steroids, and carbohydrates [27].

The plant *P. pellucida's* stems and leaves can be considered green. *P. pellucida* has been used in ethnomedicine to treat rheumatic joint pain, headaches, gout, colic, exhaustion, acne, boils, and abdominal abscesses. Consequently, the herb's decoction method is used in the Philippines to help renal function and lower uric acid levels predicaments. This study's bioactive constituents are saponins, glycosides, flavonoids, tannins, and carbohydrates [28].

In the study [29], fruits and leafy vegetables include phytochemicals called phenolic compounds, which have the most potent anti-oxidative activity. Phenolic chemicals are found in wood, roots, stems, leaves, seeds, and many other elements of plants. Phenolic chemicals have antioxidative properties because they can give free radicals hydrogen atoms. Also, oxidative problems, including autoimmunity and neuromuscular disorders, can be prevented from developing and spreading by using antioxidants that scavenge ROS. The human body needs antioxidants to stabilize health, particularly to maintain strong bodily systems and defense against cell damage. Consequently, antioxidants are beneficial to people. Daily consumption of edible medicinal plants, such as *Morinda elliptical* (*Rubiaceae*) leaves and *Piper sarmentosum* can help the said diseases naturally.

4. CONCLUSION

The phytochemical analysis revealed that phenols and flavonoids are both present in the Ulasimang bato (*P. pellucida*) leaf extracts. This implies that these bioactive compounds contain various health benefits, mainly anti-inflammation against joint pains such as gout. Moreover, the Ulasimang bato *(P. pellucida)* lead extracts have antioxidant capacity. Hence, the bioactive compounds of the Ulasimang bato *(P. pellucida*) that support the anti-gout properties are phenols and flavonoids. These components are said to lessen inflammation and joint pain. Analgesic plants such as the Ulasimang bato (*P. pellucida)* have the property to relieve pain or discomfort. This may be an alternative to drug-store anti-gout medications.

5. RECOMMENDATIONS

The researchers suggest utilizing different extraction methods besides distilled water extracts to gain comparative analysis among the various solvents that help gain results on Phytochemical Analysis. Also, it is better to use different plant parts to differentiate its efficacy. Doing more than one thermal treatment or drying procedure is highly recommended as it may influence specific analytic tests. And to observe and use proper gear and equipment when handling chemicals or such solvents to avoid any possible accidents or alteration of the study results. It is better to conduct more phytochemical analysis tests to quantify the different compounds that the Ulasimang bato (*P. pellucida*) obtains and to determine its minimum and maximum values.

ACKNOWLEDGMENT

The researchers would like to express their gratitude to everyone who assisted in the success of this research. To Noel Lagunday, Ph.D. from the Department of Zoologist Member, Pool of Taxonomist, Central Mindanao University, for his guidance. To Sir Regie Novem P. Idulsa, Natural Products Research and Development Center, Central Mindanao University, for his invaluable assistance regarding Phytochemical analysis on Ulasimang bato (*P. pellucida*) leaf extracts. Their technical expertise and resources have played a significant role in the extraction process. The same acknowledgment is given to the researcher's parents and family for the unwavering support in all aspects that allowed the researchers to pursue and accomplish this study. The same gratitude is also extended to San Isidro College for all the support provided.

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Citation: *Juris Alexandra A. Pagasian, et.al., "Phytochemical Analysis and Antioxidant Capacity of Ulasimang Bato (Peperomia pellucida) Leaf Extracts" International Journal of Medicinal Plants and Natural Products (IJMPNP), vol 11, no. 1, 2025, pp. 1-08 DOI: https://doi.org/10.20431/2454- 7999.1101001.*