Phytochemical Screening and In-Vitro Comparative Assessment of the Extracted Triterpenoids from the Leaves of *Hoya meliflua* Merr. (Wax Plant) and *Telosma procumbens* (Latok) for their Potential Antioxidant and Antiproliferative Properties Against Breast Cancer

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ABSTRACT

Introduction: Breast cancer was among the most prevalent diseases globally in 2022, accounting for 31.2% of all cancer diagnoses in the Philippines—the highest across both sexes (GLOBOCAN, 2024). This study investigated the antioxidant and antiproliferative potential of *Hoya meliflua* Merr. (Wax plant) and *Telosma procumbens* (Latok) against breast cancer.

Method: This study utilized an experimental research design, wherein this study characterized the therapeutic potentials of *Hoya meliflua* Merr. (Wax Plant) and *Telosma procumbens* (Latok). The methodology included qualitative determination of the phytochemical constituents and extraction of triterpenoid metabolites, with functional groups identified via Fourier Transform Infrared (FTIR) spectroscopy. Total Triterpenoid Content (TTC) was quantified, while biological efficacy was evaluated in-vitro through 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays to assess potential antioxidant and antiproliferative properties.

Results and Discussion: From the analysis of phytochemical properties of *Hoya meliflua* Merr. (Wax Plant) and *Telosma procumbens* (Latok), the two were found to contain alkaloids, phenolic compounds, tannin, and triterpenoids; *Hoya meliflua* Merr. (Wax Plant) contained fixed oil and *Telosma procumbens* (Latok) contained volatile oil. From the FTIR analysis, it was found out that the two samples contained functional groups such as hydroxyl group, aromatic double bond, and methyl groups; hence, indicating similarity in chemical composition in both species from the same plant family (Apocynaceae). It was discovered that *Telosma procumbens* (Latok) sample contained higher amounts of triterpenoid compounds (104.27 mg UAE/g extract) compared to *Hoya meliflua* Merr. (Wax Plant) sample (34.67 mg UAE/g extract). From the DPPH radical scavenging activity, *Telosma procumbens* (Latok) sample showed better antioxidant activity (IC50 value = 335.47) as compared to *Hoya meliflua* Merr. (Wax Plant) sample (IC50 value = 10,042.39). Antioxidant activity was dependent on concentration. Extracts of both samples: *Hoya meliflua* Merr. (Wax Plant) and *Telosma procumbens* (Latok) had antiproliferative effects; with the highest percentage inhibition recorded at 11.51% at 128 µg/mL. IC_{50} was however the IC_{50} value was not attained within the tested concentration range.

Conclusion: This study demonstrated the phytochemical composition and bioactivity of triterpenoids extracted from the leaves of *Hoya meliflua* Merr. and *Telosma procumbens*, demonstrating their potential as natural antioxidants, with *Telosma procumbens* showing higher triterpenoid content. Also, *Telosma procumbens* demonstrated modest antiproliferative properties against the MCF-7 breast cancer cell line, suggesting a pharmacological basis for future research.

Key words: triterpenoids, antioxidant, antiproliferative

From the School of Pharmacy

Cancer encompasses a group of diseases caused by complex biological processes (World Health Organization, 2019; Majérus, 2022). Due to its evolving and progressive nature, Brown et al. (2023) established an updated definition of it as "...a disease of uncontrolled proliferation by transformed cells subject to evolution by natural selection." This genetic variability poses a major challenge in developing a universal treatment that is effective for all patients (National Cancer Institute, 2021). Hence, cancer remains among the leading causes of mortality globally (WHO, 2025).

In 2022, breast cancer is the most prevalent malignancy in the Philippines, with 33,079 new cases accounting for 31.2% of all female cancer diagnoses (Global Cancer Observatory, 2022). According to the same statistics, it also had the highest 5-year prevalence, at 100,556 cases (179 people per 100,000 population), suggesting both high incidence and survivorship. Despite advances in prognosis and treatment, it remains the second leading cause of tumor-related deaths, with 11,587 fatalities reported in 2022. While most cases of breast cancer occur in women over 50, an increasing number are seen in younger, premenopausal women, which makes this a significant public health challenge (Chen et al., 2025). Accordingly, recent studies indicate that the burden of breast cancer in the Western Pacific region will remarkably affect low- and middle-income countries, emphasizing the urgent need to prioritize breast cancer prevention efforts at this critical time (Chen et al., 2025; Houssami, 2025).

The National Cancer Institute (2024) cites different types of treatment for breast cancer, such as surgery as the primary therapy and adjuvant therapies, including radiation therapy, chemotherapy, hormone/endocrine therapy, targeted therapy, and immunotherapy. While several therapy options exist, many people find it challenging to even get standard diagnostic testing, especially in remote areas, particularly in the Philippines. Due to the concentration of treatment centers in urban areas, accessing specialist care can be expensive and time-consuming (Eala et al., 2022). On average, treatment costs range from PHP 120,000 to over PHP 1 million, which is why the use of alternative medicines is now of growing interest, especially in cancer treatment (Garcia, 2023).

Approximately 80% of the population in the Philippines, as well as in other developing nations, relies heavily on traditional medical practices, particularly the use of plants for primary healthcare (Sampang, 2024). As a matter of fact, plants make a substantial contribution to the list of natural product anticancer medications and constitute a significant source of some common anticancer medications, including topotecan, etoposide, and vincristine (Newman & Cragg, 2020).

According to the World Health Organization (2022), the use of alternative medicinal plants has significantly increased among the population from 20%% to 88% over the past 20 years. Given the growing use of medicinal plants as a valuable foundation for drug development and discovery, it is essential to explore local botanical families with significant therapeutic potential, including the Apocynaceae. It includes varieties of trees, shrubs, herbs, and flowering plants located in tropical and subtropical regions. They exhibit antibacterial and antiproliferative properties linked to their secondary metabolites, such as indole alkaloids and terpenes (Bamidele, 2024). Interestingly, many of these medicinal plants are found in the Philippines, which is one of the world's biodiversity hotspots. However, many of these natural resources have not yet been sufficiently scientifically explored.

Among the Apocynaceae species identified in the Philippines are *Hoya meliflua* Merr., which is locally referred to as Wax Plant, and *Telosma procumbens*, which is known as Latok. Latok is an edible plant that comprises flavonoids, terpenoids, and glycosides, which exhibit cancer cell growth inhibiting capabilities (Cajuday & Amparado, 2014). On the other hand, *Hoya meliflua* Merr. (Wax Plant) shows antiproliferative capabilities, and this is mainly because of the β-sitosterol and stigmasterol found in the plant. The plant also contains triterpenoids such as lupeol, α-amyrin, oleanone, ursenone, and lupenone. However, despite being rich in phytochemicals with biological significance, this plant is not known to be used for any form of medication. Therefore, there is a need for further studies on the phytochemical makeup and biological activities of these two plants. In this context, this study aimed at determining the phytochemical contents and functional groups in the ethanolic crude extract of leaves from *Hoya meliflua* Merr. (Wax Plant) and *Telosma procumbens* (Latok). Also, the triterpenoids content in the hexane extract will be quantified.

In addition, as triterpenoids were found to have antioxidant and anticancer properties, the

study intends to find out the concentrations of the triterpenoids extracts that have the best antioxidant property using the DPPH test and the maximum antiproliferative property against the MCF-7 breast cancer cells using the MTT test. In order to confirm their biological properties, the antioxidant activity of the extracts will be evaluated against Ascorbic Acid, whereas the antiproliferative activity will be evaluated against Doxorubicin, by comparing their IC50 values. Through this evaluation, it is expected that the study will be able to compare the antioxidant and antiproliferative activity of Hoya meliflua Merr. (Wax Plant) and Telosma procumbens (Latok).

MATERIALS AND METHODS

This experimental study aimed to determine and compare the phytochemical constituents and functional groups present in the crude ethanolic and n-Hexane extracts of *Hoya meliflua* Merr. (Wax Plant) and *Telosma procumbens* (Latok). Functional group analysis was performed using the Fourier Transform Infrared Spectrometer (FTIR). The study also quantified total triterpenoid content using the vanillin–glacial acetic acid colorimetric assay, evaluated the potential antioxidant properties through the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, and assessed the potential antiproliferative properties using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay of the extracted triterpenoids.

Collection, Authentication, and Preparation of the Plant Sample

The fresh leaves of *Hoya meliflua* Merr. (Wax Plant) and *Telosma procumbens* (Latok) were collected in September, 2025. The *Hoya meliflua* Merr. (Wax Plant) was harvested in Lipa, Batangas, with coordinates of 13.9414° N, 121.1642° E; *Telosma procumbens* (Latok) was harvested in Dalig, Batangas, with coordinates of 13.7719° N, 121.0865° E. Both plants were collected under controlled conditions with clear, dry weather. After that, the plants were authenticated at the Jose Vera Santos Memorial Herbarium of the Institute of Biology, College of Science, located at Regidor Street, University of the Philippines Diliman, Quezon City.

The leaves of *Hoya meliflua* Merr. (Wax Plant) and *Telosma procumbens* (Latok) were washed repeatedly until the dirt was removed. After that, the leaves were air-dried for 2 weeks at room temperature in the University Laboratory Room. After that, to ensure uniform size distribution, the dry samples were crushed into a coarse powder using an electric blender and sieved using a twenty-mesh sieve.

Crude Extract Preparation

The crude ethanolic extracts were prepared using maceration, wherein equal portions of the powdered leaves of *Hoya meliflua* Merr. (Wax Plant) and *Telosma procumbens* (Latok), weighing 100 grams (g) each, were dissolved separately with concentrated ethanol in a 1:10 (weight/volume) ratio (Fiardilla et al., 2020). Each mixture was placed in amber glass containers and left to stand for 48 hours at ambient room temperature, with occasional stirring to further enhance the extraction. Then, to separate the solvent from the solid residue, the extract underwent filtration first through a muslin cloth, followed by filter paper. Furthermore, the filtrates were evaporated to half of its initial volume under reduced pressure using a rotary evaporator at 40°C and 50 revolutions per minute (rpm), following the extraction protocol. The resulting filtrates were transferred to two separate amber jars and stored at a cold room temperature (2°C to 8°C) until subjected to phytochemical screening (Jiregna Gari Negasa et al., 2024).

Phytochemical Screening of the Leaf Extract

The qualitative phytochemical testing and screening were used for the preliminary examination of both *Hoya meliflua* Merr. (Wax Plant) and *Telosma procumbens* (Latok). The screening was performed for alkaloids, flavonoids, glycosides, phenolics, tannins, saponins, triterpenoids, carbohydrates, proteins, fixed oils, and volatile oils. The intensity of the color or the precipitate generated acted as the basis for whether the constituent was present or absent.

Determination of Functional Group of the Crude Ethanolic Extract using Fourier Transform Infrared Spectrometer (FTIR)

Each dried plant sample was equilibrated to room temperature. The sample was directly dropped onto the ATR crystal without further sample preparation. It was scanned using the wavelength range of 4000 cm^{-1} to 650 cm^{-1} with a resolution of 4cm^{-1} (Vieira et al.,

2026). The analysis was performed at Centro Escolar University - Manila in the Science Laboratories of the Biological/Physical Sciences Department using the Agilent Cary 630 FTIR-ATR manufactured by Agilent Technologies (2019).

Liquid-Liquid Extraction of Triterpenoids

The 10 mL crude ethanolic extract of *Hoya meliflua* Merr. (Wax Plant) and *Telosma procumbens* (Latok) were dissolved in 50 mL of methanol and separated in a separatory funnel using 100 mL of n-Hexane as the solvent. A lower methanol layer and an upper n-Hexane layer were obtained. The n-Hexane layer was evaporated to half of its initial volume using a rotary evaporator operating at 70°C and 40 rpm (Sinurat, 2020)

Confirmatory Tests for Triterpenoids

The crude ethanolic and n-Hexane extracts of *Hoya meliflua* Merr. (Wax Plant) and *Telosma procumbens* (Latok) underwent confirmatory tests to determine the presence of triterpenoids, namely: Liebermann-Burchard Test and Salkowski's Test (Pollo et al., 2025). All of these were utilized to determine which solvents produced a higher presence of triterpenoids in a solution.

Determination of Functional Group of the n-Hexane Extract using Fourier Transform Infrared Spectrometer (FTIR)

Each dried plant sample was equilibrated to room temperature. For the n-hexane extract, the sample was directly dropped onto the ATR crystal without further sample preparation. It was scanned using the wavelength range of 4000 cm^{-1} to 650 cm^{-1} with a resolution of 4 cm^{-1} (Vieira et al., 2026). The analysis was performed at Centro Escolar University – Manila in the Science Laboratories of the Biological/Physical Sciences Department using the Agilent Cary 630 FTIR-ATR manufactured by Agilent Technologies (2019).

Total Triterpenoid Content (TTC)

Total Triterpenoid Content analysis indicated the presence of triterpenoids, which were used in evaluating the antioxidant and antiproliferative potential of the plant extracts. This was performed through the vanillin-glacial acetic acid colorimetric assay by creating a colored complex that is measured using a Ultraviolet-Visible (UV/Vis) Spectrophotometer. Vanillin (4-hydroxy-3-methoxybenzaldehyde) functions as a chromogenic reagent in this reaction, which is frequently accelerated by acidic environments such as glacial acetic acid (Naraparaju et al., 2024).

Ursolic acid (3β-hydroxy-urs-12-en-28-oic acid) was used as a reference standard due to its vital accurate measurement of triterpenoid content. Ursolic acid is a pentacyclic triterpenoid molecule characterized by five interconnected six-membered carbon rings with multifaceted pharmacological activity, such as anticancer, antioxidant, antifungal, antidiabetic, antibacterial, anti-inflammatory, and antiviral properties. In the structure of ursolic acid, the hydroxyl group at C-3 and the carboxylic acid group at C-17 are the key functional group that possesses antioxidant properties (Khwaza & Aderibigbe, 2024).

Preparation of Ursolic Acid Standard Stock Solution

Five (5) mg of ursolic acid was dissolved in 60% ethanol to reach a volume of 5 mL creating a solution with a 1 mg/mL concentration of ursolic acid.

Creation of a Ursolic Acid Calibration Curve

Working standard solutions were prepared from the stock solution to create solutions of 20 µg/mL (micrograms per milliliter), 40 µg/mL, 60 µg/mL, 80 µg/mL, and 100 µg/mL (0.2 mL, 0.4 mL, 0.6 mL, 0.8 mL, and 1.0 mL of ursolic acid stock solution followed by 60% ethanol to reach a volume of 10 mL). A combination of 1 mL solution per concentration was transferred to an evaporating dish and was heated in a water bath until evaporated. The solution was mixed with 1 mL 5% (w/v) vanillin-glacial acetic acid solution and 1.8 mL of sulfuric acid solution, and heated at 70ºC for 5 minutes, then cooled and diluted with a sufficient quantity of glacial acetic acid to make it 10 mL. The mixed solution was kept in complete darkness for 2 minutes. The absorbance measurements were evaluated using a UV-Vis spectrophotometer at 573 nm, the maximum wavelength. The calibration curve and linear regression were determined using:

$$y = mx + b$$

Where:

y is the dependent variable, which represents the measured total predicted triterpenoid content in the sample

x is the concentration that influences the total triterpenoid content; m is the slope of the line, which indicates how responsive triterpenoid yield is to changes in x

b is the y-intercept, which represents the baseline triterpenoid level when concentration is zero

Determination of Total Triterpenoid Content Level

A 1 mL extract solution was transferred to an evaporating dish and heated in a water bath until it evaporated. The heated solution was then mixed with 1 mL 5% (w/v) vanillin-glacial acetic acid solution and 1.8 mL of sulfuric acid. The mixed solution was heated again at 70ºC for 5 minutes, then cooled and diluted with glacial acetic acid to make it to 10 mL. The mixed solution was kept in complete darkness for 2 minutes. A UV-Vis spectrophotometer was used to analyze the absorbance of the solution at a wavelength of 573 nm. The total triterpenoid content was determined using the equation:

$$TTC = \frac{C \times V}{sample\ (m)}$$

Where:

C is the concentration of the sample from the calibration curve (μg/mL)

V is the volume of extract solution in the assay (mL)

m is the mass of the extract used in the assay (g)

Determination of the Antioxidant Property using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

A deep-violet molecule that is stable and soluble in methanol with a maximum absorption wavelength of 515 nm is known as the 2,2-Diphenyl-1-picrylhydrazyl radical or DPPH. Using this test, the antioxidant property of crude extract components is frequently assessed (Njoya, 2021). The DPPH test was conducted with the methodology provided in the study of Baliyan et al. (2022).

Preparation of DPPH Standard Stock Solution

Twenty (20) mg of DPPH was transferred to a 10 mL volumetric flask and dissolved with methanol (2 mg/mL).

Preparation of Blank Solution

Two (2) mL of the DPPH standard stock solution was obtained to fill a 10 mL volumetric flask. Methanol was used to fill the volumetric flask up to the mark line.

Preparation of Sample Solution

Each plant sample of 50 mg was weighed and dissolved in 50 mL of methanol in two separate volumetric flasks until it reached the mark line to serve as the sample stock solution, which was completely used for the triplication of results. Each sample stock was measured using a micropipette with 1 mL, 2 mL, 3 mL, 4 mL, and 5 mL to obtain a concentration of 100 parts per million (ppm), 200 ppm, 300 ppm, 400 ppm, and 500 ppm into 10 mL volumetric flasks.

Measurement of Antioxidant

Two (2) mL of the DPPH standard stock solution was added to each concentration. Finally, it was volumed to 10 mL with methanol and was homogenized. The solutions were kept in complete darkness for 40 minutes, and the absorption was measured using a UV-Vis spectrophotometer at the maximum absorption wavelength of 515 nm. The test was done in triplicate trials.

Data Analysis

The percent inhibition is calculated by the following formula:

$$\%\ of\ Antioxidant\ Activyt = [(Ac - As) \div Ac] \times 100$$

Where:

Ac is the Control Reaction Absorbance

As is the Testing Specimen Absorbance

IC_{50} was used to present the results. A lower IC_{50} indicates higher antioxidant activity.

Determination of the Antiproliferative Property using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) Assay

The (MTT) assay was used to assess the potential antiproliferative property of the n-Hexane extracts from *Hoya meliflua* Merr. (Wax Plant) and *Telosma*

procumbens (Latok) on MCF-7. MCF-7 cell lines were cultured using Dulbecco's Modified Eagle Medium (DMEM) with 10% Fetal Bovine Serum (FBS) and 1% penicillin (100 U/mL), following the methodology of De Guzman et al. (2023).

All procedures were carried out at the Mammalian Tissue Culture Laboratory under the Research Center for the Natural and Applied Sciences at the University of Santo Tomas, located along España Boulevard, Sampaloc, Manila. The cells were seeded in 96-well plates at 1 x 10^5 cells/mL. The cells were treated with the n-Hexane extracts and doxorubicin, which were stored in 0.1% aqueous DMSO (dimethyl sulfoxide) at concentrations up to 128 µg/mL. Doxorubicin was used as the positive control, while 0.1% aqueous DMSO was used as the blank control. All plates were incubated at 37°C with 5% CO_2 for 72 hours.

After incubation, media from each well were removed, and 10 µL of 3-(4,5 dimethylethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) with a 5 mg/mL concentration dissolved in phosphate buffer saline pH 7.2 was added into each well. The 96-well plate containing the cells was then incubated for 4 hours at 37°C in a humidified incubator with 5% CO_2. After incubation, 100 µL of DMSO was added to each well. The plate was left in the dark for 30 minutes at room temperature, then the absorbance was read at 570 nm using a microtiter plate reader.

The MTT assay is based on the principle that metabolically active (viable) cells reduce MTT into a purple formazan product, and the intensity of this color was directly proportional to the number of living cells present. Thus, higher absorbance values indicate greater cell viability, while lower absorbance reflects reduced cell survival (Peeva et al., 2024). This was used to compute the % inhibition of each concentration of plant extracts, as well as for Doxorubicin, with the following formula:

$$\% \text{ inhibition: } 100 - \text{Cell Viability}$$

$$\text{Cell Viability (\%)} = \left(\frac{\textit{Mean Abs. of Sample - Mean Abs. of Blank}}{\textit{Mean Abs. of 0(–) – Mean Abs. of Blank}} \right) \times 100$$

Statistical Treatment of Data

Statistical analyses of the antioxidants and antiproliferative activities of the extracts have been done for a thorough evaluation and comparison. The descriptive statistics of each extract were conducted using the software Microsoft Excel to gain preliminary knowledge about the activity of each extract. In order to test if the differences between the groups were statistically significant, One Way ANOVA test followed by Tukey's post-hoc test was employed to identify the significantly different groups.

Furthermore, IC_{50} values were determined using the 4-Parameter Logistic (4PL) model.

RESULTS AND DISCUSSION

Identification Tests and Screenings

The crude ethanolic extracts of *Hoya meliflua* Merr. (Wax Plant) and *Telosma procumbens* (Latok) indicate the presence of several phytochemical constituents based on the test results. Both extracts yielded positive results for Alkaloids using Wagner's Test, Phenolic Compounds and Tannins through the Ferric Chloride Test, and Triterpenoids with the Liebermann-Burchard and Salkowski's Tests. For Fixed Oils, *Hoya meliflua* Merr. (Wax Plant) showed positive results in both the Spot Test and Saponification Test. On the other hand, *Telosma procumbens* (Latok) showed a positive result in the Filter Paper Test and Sudan III Test, indicating the presence of Volatile Oils.

Characterization of the Functional Group of Crude Ethanolic Extract

Figure 1 shows the analysis of the FTIR spectrum representing the functional groups of *Hoya meliflua* Merr. (Wax Plant) and *Telosma procumbens* (Latok) crude ethanolic extract showing a similar phytochemical profile, marked by a broad O–H peak (3200–3500 cm^{-1}). Both exhibit C–H stretching (2850–3000 cm^{-1}), however, *Hoya meliflua* Merr. (Wax Plant) shows higher absorbance and a stronger C=O peak at 1045.5 cm^{-1}.

Table 2 shows the FTIR spectrum analysis of *Hoya meliflua* Merr. (Wax Plant) crude ethanolic extract, wherein 3334.1 cm^{-1} (a) corresponds to hydrogen-bonded O–H stretching, typical of triterpene alcohols (Li et al., 2023); 2970.7 and 2926.0 cm^{-1} (b) suggesting C–H alkane, and correlating regions of O–H carboxylic acid stretching, indicating a saturated backbone (Ng et al., 2019). The peak at 1559.9 cm^{-1} (c) correlated to C=C stretching, typically found in amyrin skeletons or aromatic rings that are often isolated from the Hoya genus (Panajon et al., 2016). Additionally, peaks at 1271.0 and 1045.4 cm^{-1} (d)

Table 1. Phytochemical screening of crude ethanolic extracts of *Hoya meliflua* Merr. (Wax Plant) and *Telosma procumbens* (Latok).

Test	*Hoya meliflua* Merr. (Wax Plant)	*Telosma procumbens* (Latok)
Alkaloids		
Wagner's Test	+	+
Phenolic Compounds and Tannins		
Ferric Chloride Test	+	+
Triterpenoids		
Liebermann-Burchard Test	+	+
Salkowski's Test	+	+
Fixed Oils		
Spot Test	+	-
Saponification Test	+	-
Volatile Oils		
Filter Paper Test	-	+
Sudan III Test	-	+

Note: (+) Present; (-) Absent

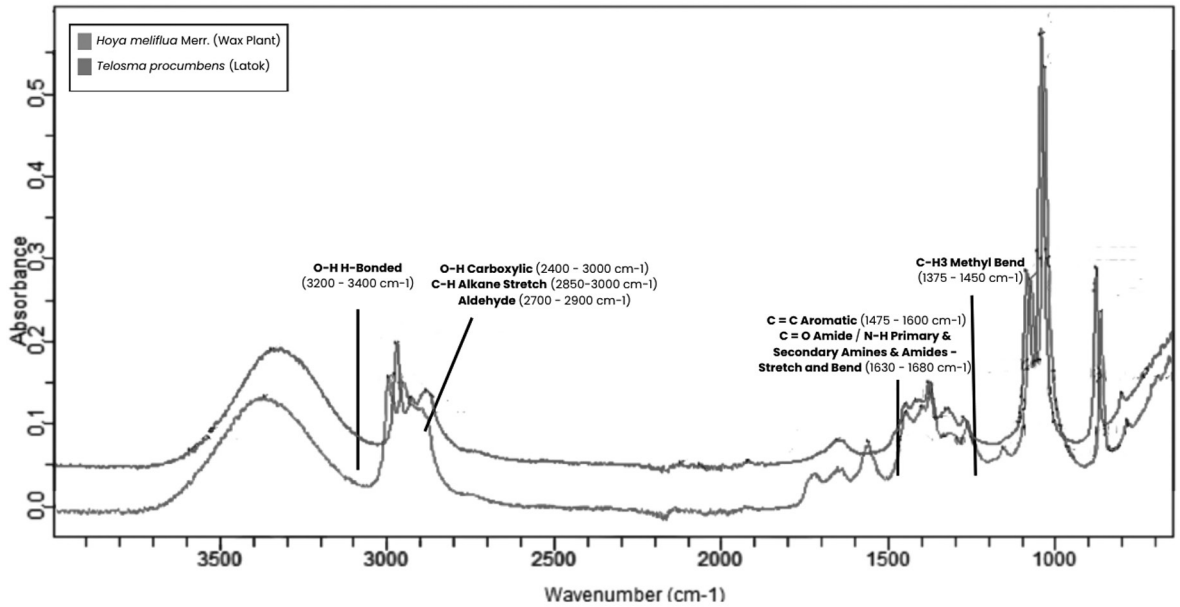

Figure 1. FTIR spectra representing potential bands in the crude ethanolic extract of *Telosma procumbens* (Latok) and *Hoya meliflua* Merr. (Wax Plant).

represent C–O stretching in alcohols and esters in the fingerprint region. The presence of hydroxyl (O–H), aliphatic (C–H), and alkene (C=C) groups confirms the presence of triterpenoids, as these functional groups are primary chemical markers used for the rapid identification of bioactive secondary metabolites in medicinal plant extracts (Mane & Khilare, 2021). These functional groups are recognized for their antioxidant and antiproliferative properties which allows them to neutralize free radicals and inhibit the growth of cancer cells.

Table 3 shows the analysis of FTIR spectrum analysis representing the functional groups of the *Telosma procumbens* (Latok) crude ethanolic extract, wherein a peak at 3589 cm^{-1} (a) originates from the stretching vibration of free O–H groups, while a broader and more intense peak at 3345.3 cm^{-1} (b) is attributed to hydrogen-bonded O–H stretching, which is commonly associated in triterpenoid alcohols and sterols relative to the flowering family of Apocynaceae (Cajuday & Amparo, 2014). Additionally, the peaks at 2972.6, 2927.8, and 2883.1 cm^{-1} (c) illustrates the presence of aliphatic structures which is comparable to the C-H stretching vibrations of alkanes an aldehydes demonstrating the cyclic hydrocarbon skeleton and triterpenoid's aliphatic side chains (Nandiyanto, 2026). Moreover, a peak at 1653.1 cm^{-1} (d) 1653.1 cm^{-1} (d) in the double bond region exhibits contributions from C=C aromatic stretching, C=O amide stretching, or N–H vibrations of amines or amides. The peaks at 1448.1 and 1379.1 cm^{-1} (e) located in the fingerprint region corresponds to methyl group bending. Lastly, a peak at 1045.5 cm^{-1} (f) presents a C-O stretching which is a feature of alcohols, esters, ethers, carboxylic acids, or anhydrides. The identified functional groups suggest potential antioxidant and antiproliferative properties (Umar et al., 2021; Nistor et al., 2023).

Table 2. All potential bands, corresponding functional groups, and possible compounds identified in the crude ethanolic extract of *Hoya meliflua* Merr. (Wax Plant) using FTIR spectroscopy.

	Wave Number (cm^{-1})	Wave Number Range (cm^{-1})	Type of Bond and Vibration
(a)	3334.1	3400 - 3200	O–H H-Bonded
(b)	2970.7 2926.0	3000 - 2850 3400-2400	C–H Alkane (Stretch) O–H Carboxylic Acid
(c)	1559.9	1475 - 1600	C=C Aromatic
(d)	1271.0 1045.4	1000 - 1300	C–O Alcohol, Esters, Ethers, Carboxylic Acid, Anhydrides

Table 3. All potential bands, corresponding functional groups, and possible compounds identified in the crude ethanolic extract of *Telosma procumbens* (Latok) using FTIR spectroscopy.

	Wave Number (cm^{-1})	Wave number Range (cm^{-1})	Type of Bond and Vibration
(a)	3589	3600 - 3650	O–H Free
(b)	3345.3	3400 - 3200	O–H H-Bond
(c)	2972.6 2927.8 2883.1	3000 - 2850 2900 - 2700	C–H Alkane (Stretch) Aldehyde
(d)	1653.1	1600 - 1475 1680 - 1630	C = C Aromatic C = O Amide N-H Primary & Secondary Amines & Amides (Stretch and Bend)
(e)	1448.1 1379.1	1450 - 1375	C–H_3 Methyl (Bend)
(f)	1045.5	1300 - 1000	C–O Alcohol, Esters, Ethers, Carboxylic Acid, Anhydrides

Confirmatory Tests for Triterpenoids

The extracted *Hoya meliflua* Merr. (Wax Plant) and *Telosma procumbens* (Latok) plant consistently produced positive findings in all the confirmatory tests for triterpenoids, including the Liebermann-Burchard test and Salkowski's test. The consistency of the results across all tests was performed to support the reliability of the techniques used to successfully identify triterpenoids in the examined plant.

Characterization of Functional Group of n-Hexane Extracts

Figure 2 shows the FTIR spectra of *Hoya meliflua* Merr. (Wax Plant) and *Telosma procumbens* (Latok) n-Hexane extracts, with key bands at 2850–3000 cm^{-1} (C–H alkane stretching), 2400–3000 cm^{-1} (O–H), 1450–1600 cm^{-1} (C=C aromatic), and 1380–1470 cm^{-1} (CH_3 bending).

The analysis of the FTIR spectrum of the n-Hexane extracts for *Hoya meliflua* Merr. (Wax Plant) and *Telosma procumbens* (Latok) demonstrate a high degree of chemical correlation, specifically in the non-polar region where triterpenes and sterols predominate. Both extracts display intense C–H stretching vibrations (2957–2857 cm^{-1}) and methyl bending (1377 cm^{-1}), which align with the aliphatic skeleton and cyclic hydrocarbon framework characteristic of the triterpenoid family as described by Nandiyanto (2026).

A similar reading at 2957-2857 cm^{-1} also suggests contributions to O-H bonding which is relative to antioxidant properties. Given that both plant species are under the Apocynaceae family which is known for the production of secondary metabolites such as

Table 4. Confirmatory Tests for the Extracted Triterpenoids of *Hoya meliflua* Merr. (Wax Plant) and *Telosma procumbens* (Latok).

Test	*Hoya meliflua* Merr. (Wax Plant)	*Telosma procumbens* (Latok)
Liebermann-Burchard test	+	+
Salkowski's test	+	+

Note: (+) Present; (-) Absent

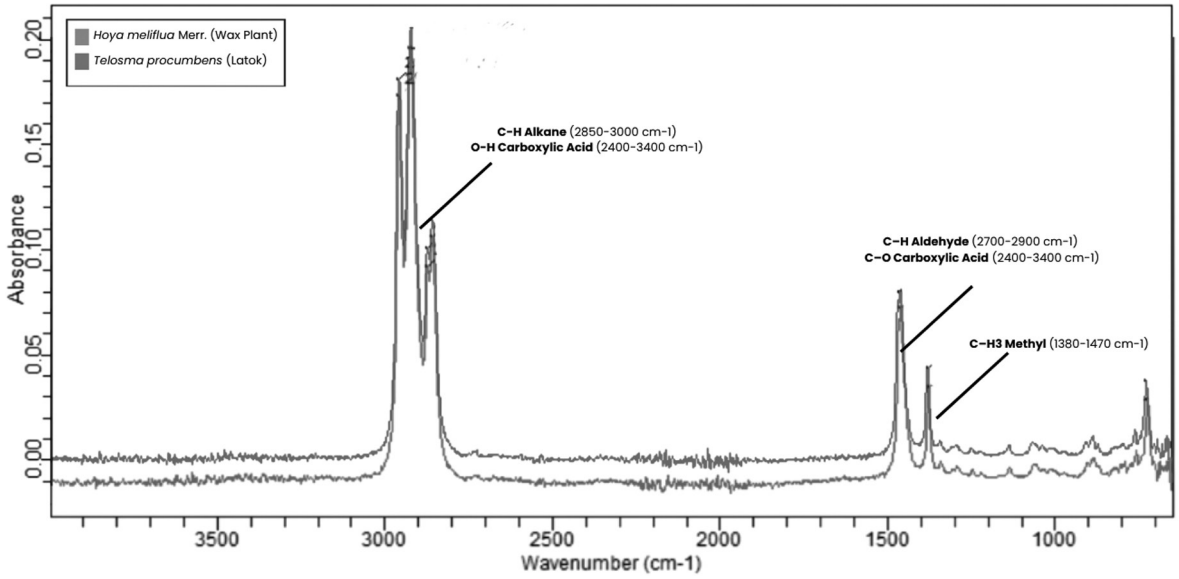

Figure 2. FTIR spectra representing potential bands in the n-Hexane extract of *Hoya meliflua* Merr. (Wax Plant) and *Telosma procumbens* (Latok)

alpha-amyrin, beta-amyrin, and lupeol, it is highly probable that their spectra are similar (Cajuday & Amparado, 2014; Ng et al., 2022).

Structural similarities continue in the fingerprint region, where both plants show C=C aromatic stretching at exactly 1459.3 cm^{-1} and C-H_3 methyl bending at 1377.3 cm^{-1}, indicating a common framework of cyclic or branched hydrocarbons. Consequently, the consistency in these functional groups suggests that both extracts are rich in bioactive triterpenoids, which have been established as natural sources for the antioxidant and antiproliferative properties discussed by Umar et al. (2021) and Nistor et al. (2023).

Table 5. All potential bands, corresponding functional groups, and possible compounds identified in the n-Hexane extract of *Hoya meliflua* Merr. (Wax Plant) using FTIR spectroscopy.

Wave Number (cm^{-1})	Wave number Range (cm^{-1})	Type of Bond and Vibration
2957.6 2924.1 2871.9 2858.9	2850-3000 2400-3000	C–H Alkane (Stretch) O–H Carboxylic Acid
1459.3	1450-1600	C=C Aromatic
1377.3	1380-1470	C–H_3 Methyl

Table 6. All potential bands, corresponding functional groups, and possible compounds identified in the n-Hexane extract of *Telosma procumbens* (Latok) using FTIR spectroscopy.

Wave Number (cm^{-1})	Wavenumber Range (cm^{-1})	Type of Bond and Vibration
2957.6 2922.2 2873.8 2857.0	2850-3000 2400-3400	C–H Alkane (Stretch) O–H Carboxylic Acid
1459.3	1450-1600	C=C Aromatic
1377.3	1380-1470	C–H_3 Methyl

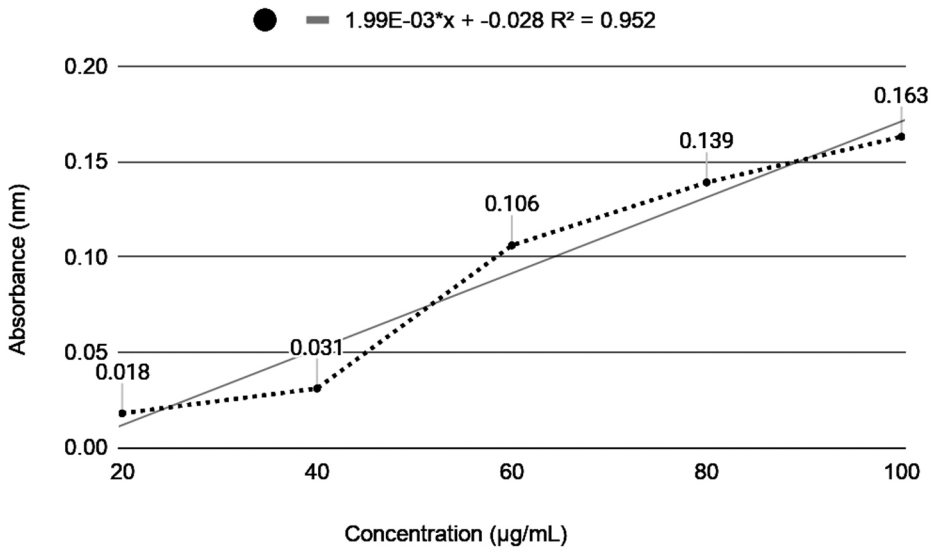

Figure 3. Ursolic acid calibration curve.

A calibration curve with a R^2 value 20.952 was obtained after plotting the recorded absorbance at the wavelength maximum of 573 nm against concentrations of respective ursolic acid standard solutions (Figure 3). These suggested that the model accurately predicted the dependent variable and interpreted all the data points as being close to the regression line. The amount of ursolic acid was determined using the linearity of the graph with a regression equation of y = 0.00199x + (-0.028), which was obtained from the calibration curve. These findings indicate a strong connection between ursolic acid content and absorbance, which shows that the calibration curve is precise and reliable for analytical purposes.

As presented in Table 7, the total predicted triterpenoid content of the plant extracts of *Hoya meliflua* Merr. (Wax Plant) is 34.67 mg UAE/g of extract and 104.27 mg UAE/g of extract for *Telosma procumbens* (Latok). The Standard Deviation (SD) of *Hoya meliflua* Merr. (Wax Plant) and *Telosma procumbens* (Latok) are 0.125 and 0.25 respectively and both exhibit a p-value of <0.001. This indicates that there is a significant difference between *Hoya meliflua* Merr. (Wax Plant) and *Telosma procumbens* (Latok) plant extract.

Moreover, the triterpenoid content of *Telosma procumbens* (Latok) extract is approximately 3.06 times higher than in *Hoya meliflua* Merr. (Wax Plant). According to Pan et al. (2024), the leaves of *Ziziphus jujuba* Mill. have the highest triterpenoid content at 41,570 µg/g. It was found in the specific triterpenoic compound of *Ziziphus jujuba* Mill. (2α, 3β, 28-trihydroxy-urs-20(30)-ene) has the highest antioxidant property. Therefore, it is interpreted as higher triterpenoid content may be associated with greater antioxidant property. Triterpenoids have been studied for their pharmacological properties, especially their role in cancer inhibition. According to Aly et al. (2024), triterpenoids exert anticancer effects through their antiproliferative properties, which interfere with processes such as angiogenesis and cellular differentiation. Therefore, the triterpenoid levels in *Telosma procumbens* (Latok) may indicate greater potential for its anti-cancer properties with processes such as angiogenesis and cellular differentiation. Therefore, the triterpenoid levels in *Telosma procumbens* (Latok) may indicate greater potential for its anti-cancer properties.

Antioxidant Property using DPPH Radical Scavenging Assay

In this assay, ascorbic acid was used as positive control due to its established antioxidant activity, whereas *Hoya meliflua* Merr. (Wax Plant) and *Telosma procumbens* (Latok) were compared to each other as comparator groups to measure their antioxidant potential.

The data showed the antioxidant properties of triterpenoid extracts from *Hoya meliflua* Merr. (Wax Plant) and *Telosma procumbens* (Latok) using percent inhibition values calculated at concentrations ranging from 100 ppm to 500 ppm. As shown in Figure 4, Ascorbic Acid (positive control) maintained a nearly flat, maximal inhibition level of over 92% whereas, the extract with the highest antioxidant property in the DPPH assay was *Telosma procumbens* (Latok). The antioxidant property of T*elosma procumbens* (Latok) extract exhibited 29.01% (SD = 0.740) inhibition at 100 ppm, and reached its peak by 62.93% (SD = 0.016) at 500 ppm. According to Antonio &

Table 7. Total triterpenoid content of *Hoya meliflua* Merr. (Wax Plant) and *Telosma procumbens* (Latok)

Plant Sample	Total Predicted Triterpenoid Content (mgUAE/g extract)	SD	p-value
Hoya meliflua Merr. (Wax Plant)	34.67	0.125	<0.001
Telosma procumbens (Latok)	104.27	0.25	<0.001

Note: mg UAE/g extract: milligrams of ursolic acid equivalents per gram of extract; p-value <0.05 significant, while >0.05 not significant

Vivit (2017), *Telosma procumbens* (Latok) showed a rich array of phytochemicals, notably for its potential antioxidant property, and exhibited the highest DPPH radical scavenging activity. On the other hand, *Hoya meliflua* Merr. (Wax Plant) exhibited weaker antioxidant activity, with the inhibition percentage increasing from 26.33% (SD = 0.875) at 100 ppm to 37.14% (SD = 0.018) at 500 ppm, compared to *Telosma procumbens* (Latok). Despite its lower inhibition values, *Hoya meliflua* Merr. (Wax Plant) extract exhibited a steady increase, which suggests that its antioxidant capacity increases proportionally with concentration. Park et al. (2023) confirmed the antioxidant properties of Lupeol, a pentacyclic triterpenoid found in fruits and vegetables, against nitric oxide (NO), hydroxyl, and superoxide radical scavenging activities. This suggests that triterpenoids were used as potent natural antioxidants, capable of scavenging free radicals.

The antioxidant property of *Hoya meliflua* Merr. (Wax Plant) is lower than that of *Telosma procumbens* (Latok). It yielded an IC50 value of 10,042.39 parts per million, which indicated low radical-scavenging activity, with its response lying beyond the 50% range. Nonetheless, the antioxidant property exhibits a concentration-dependent behavior. Across the tested concentrations of 100 ppm to 500 ppm, the percent inhibition rose steadily from 26.33% to 37.14%. This

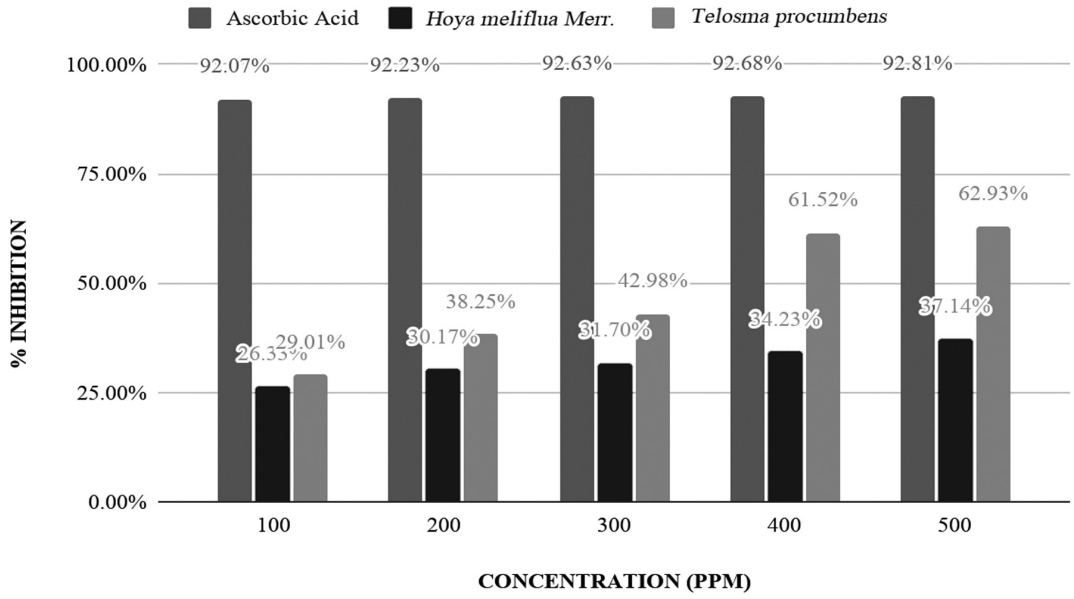

Figure 4. DPPH percent (%) inhibition of *Hoya meliflua* Merr. (Wax Plant) and *Telosma procumbens* (Latok), and ascorbic acid (Positive Control).

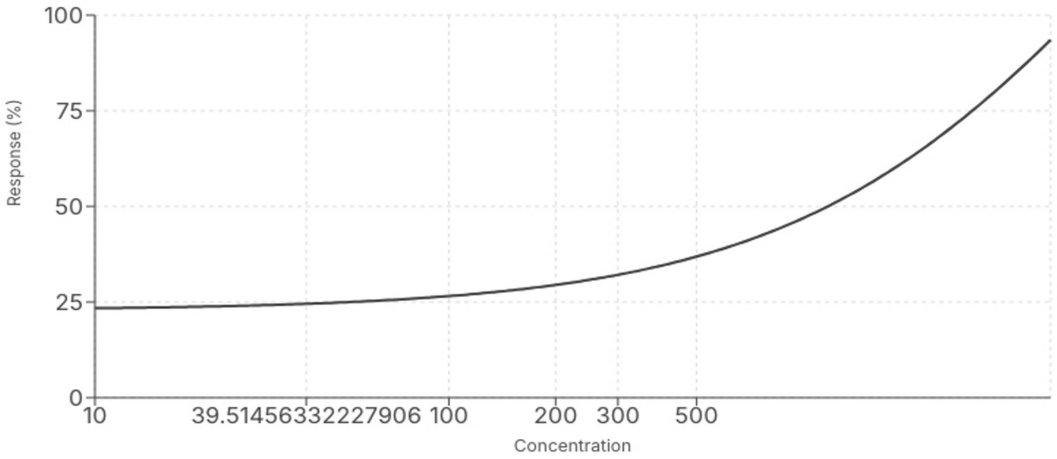

Figure 5. Dose-response curve of *Hoya meliflua* Merr. (Wax Plant) on 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay.

suggests that significantly higher concentrations of the extract will be required to achieve substantial antioxidant effects.

Telosma procumbens (Latok) exhibited significantly greater antioxidant properties than *Hoya meliflua* Merr. (Wax Plant). It yielded an IC50 value of 335.47 ppm. The antioxidant property of *Telosma procumbens* (Latok) exhibits a concentration-dependent behavior. The dose-response curve of its DPPH assay followed a sigmoidal pattern consistent with the Four-Parameter Logistic (4PL) model. At concentrations of 100 ppm and 200 ppm, the response remained relatively low. However, when the concentration increased to about 300 ppm, the response rose steeply and continued to do so until 500 ppm.

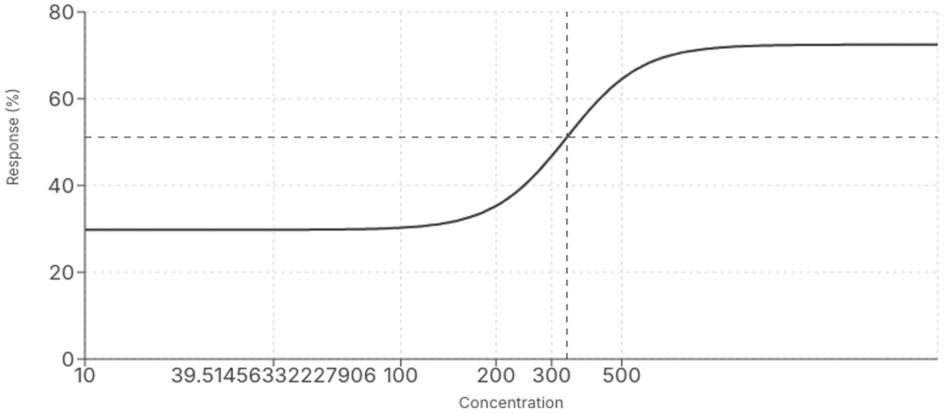

Figure 6. Dose-response curve of *Telosma procumbens* (Latok) on 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay.

Table 8. ANOVA test result of % inhibition on IC50 of antioxidant property per concentration using DPPH assay.

Concentration (ppm)	Source	Sum of Squares	df	Mean Square	F-value	p-value
100 ppm	Between Groups	8306.808	2	4153.404	9117.896	<.001
	Within Groups	2.733	6	0.456		
	Total	8309.542	8			
200 ppm	Between Groups	6831.182	2	3415.591	7267.731	<.001
	Within Groups	2.820	6	0.470		
	Total	6834.002	8			
300 ppm	Between Groups	6305.563	2	3152.782	6136.071	<.001
	Within Groups	3.083	6	0.514		
	Total	6308.646	8			
400 ppm	Between Groups	5132.118	2	2566.059	14743.700	<.001
	Within Groups	1.044	6	0.174		
	Total	5133.162	8			
500 ppm	Between Groups	4653.885	2	2326.942	2732.115	<.001
	Within Groups	5.110	6	0.852		
	Total	4658.995	8			

Note: <0.05 significant, while >0.05 not significant

The One-way Analysis of Variance (ANOVA) was performed on the radical scavenging activity, which showed a highly significant effect of treatment type across 100 ppm, 200 ppm, 300 ppm, 400 ppm, and 500 ppm concentrations. The ANOVA results demonstrated that significant variation exists in the DPPH inhibition properties of *Hoya meliflua* Merr. (Wax Plant) and *Telosma procumbens* (Latok) groups when compared to Ascorbic Acid (Positive Control). The null hypothesis is rejected at all concentration levels because the observed p-value (<.001) is consistently below the established alpha threshold of <0.05. This clearly shows that the type of treatment has a significant effect on antioxidant properties.

Across all concentration levels, the exceptionally high F-value of 14,743.700 at 400 ppm, along with a p-value of <0.001, indicates that statistically significant differences exist in the DPPH percent inhibition values for *Hoya meliflua* Merr. (Wax Plant) and *Telosma procumbens* (Latok). Across the concentration range, the consistency of these findings suggests that the specific plant extract utilized, regardless of concentration, is a key determinant of antioxidant effectiveness.

The data revealed that the treatment groups are primarily responsible for the majority of the variability observed in percent inhibition. At a 100 ppm concentration, the Sum of Squares Between Groups (8306.808) exceeds the Within Groups variance (2.733), indicating a significant effect of the plant species on the results. This observation underscores the characteristics of *Hoya meliflua* Merr. (Wax Plant) and *Telosma procumbens* (Latok) exert distinct effects on their antioxidant properties. It remains statistically significant regardless of variations in concentration.

As treatment conditions consistently influenced antioxidant property measurements across all concentrations, the results indicated a significant change in the relationship under study. The extract composition interacts with concentration levels to yield a particular antioxidant property, indicated in the systematic results. It is clear that the variance in potency of *Hoya meliflua* Merr. (Wax Plant), and *Telosma procumbens* (Latok) are not a result of random occurrence, since the p-values are <.001 at all levels from 100 ppm to 500 ppm, establishing a standardized foundation for their potential therapeutic uses.

This potential antioxidant inhibition analysis provided a detailed comparison at 100 ppm to 500 ppm concentrations between Ascorbic Acid (Positive Control) and the treatment groups *Hoya meliflua* Merr. (Wax Plant) and *Telosma procumbens* (Latok). The Tukey post-hoc test conducted pairwise comparisons to reveal the specific effectiveness levels of treatments, which proved significant in the initial ANOVA analysis.

Pairwise comparisons showed that Ascorbic Acid (Positive Control) exhibited significantly higher antioxidant properties than both plant extracts at all concentrations. The significant mean differences, for instance, at 100 ppm concentration, are 65.75 of Ascorbic Acid (Positive Control) vs. *Hoya meliflua* Merr. (Wax Plant) and 63.06 Ascorbic Acid (Positive Control) vs. *Telosma procumbens* (Latok). However, the comparison between *Hoya meliflua* Merr. (Wax Plant) and *Telosma procumbens* (Latok) revealed a significant difference as concentration increased. Indicated by the mean differences from -2.68 at 100 ppm, increasing to -25.76 at 500 ppm concentration, and a significant p-value of <.007 at 100 ppm and a p-value of <.001 at 500 ppm. This suggests that although both plants have antioxidant properties, *Telosma procumbens* (Latok) is more effective at blocking free radicals than *Hoya meliflua* Merr. (Wax Plant). Moreover, the results indicated that Ascorbic Acid (Positive Control) has higher antioxidant properties between the two plant samples. Moreover, *Telosma procumbens* (Latok) is a more effective plant-derived antioxidant when used for DPPH percent inhibition than *Hoya meliflua* Merr. (Wax Plant) across all concentrations.

Antiproliferative Property using MTT Assay

Doxorubicin served as the positive control whereas the experimental comparator extracts were *Hoya meliflua* Merr. (Wax Plant) and *Telosma procumbens* (Latok), which allowed for a direct comparison of their antiproliferative properties.

Doxorubicin exhibited the lowest cell viability among all treatments, indicating its strong antiproliferative effect on the breast cancer cells. For *Hoya meliflua* Merr. (Wax Plant), at a concentration of 8 µg/mL, *Hoya meliflua* Merr. (Wax Plant) exhibited a cell viability of approximately 79.55%, corresponding to a 20.45% inhibition of breast cancer cell growth. This shows measurable but limited antiproliferative properties, suggesting that both extracts contain bioactive constituents that are capable of producing antiproliferative properties. At the highest

Table 9. Tukey's post-hoc test pair-wise comparison on antioxidant property per concentration using DPPH assay.

Concentration (ppm)	Pair-wise Comparison	Mean Difference	Std. Error	p-value
100 ppm	**Ascorbic acid vs. *Hoya meliflua* Merr.**	65.75	0.551	<.001
	Ascorbic acid vs. *Telosma Procumbens*	63.06	0.551	<.001
	Hoya meliflua* Merr. vs. *Telosma procumbens	-2.68	0.551	<.007
200 ppm	**Ascorbic acid vs. *Hoya meliflua* Merr.**	62.06	0.560	<.001
	Ascorbic acid vs. *Telosma Procumbens*	53.99	0.560	<.001
	***Hoya meliflua* Merr. vs.**	-8.07	0.560	<.001
	Telosma procumbens			
300 ppm	**Ascorbic acid vs. *Hoya meliflua* Merr.**	60.93	0.585	<.001
	Ascorbic acid vs. *Telosma Procumbens*	49.65	0.585	<.001
	Hoya meliflua* Merr. vs. *Telosma procumbens	-11.28	0.585	<.001
400 ppm	**Ascorbic acid vs. *Hoya meliflua* Merr.**	58.45	0.341	<.001
	Ascorbic acid vs. *Telosma procumbens*	31.16	0.341	<.001
	Hoya meliflua* Merr. vs. *Telosma procumbens	-27.29	0.341	<.001
500 ppm	**Ascorbic acid vs. *Hoya meliflua* Merr.**	55.65	0.754	<.001
	Ascorbic acid vs. *Telosma procumbens*	29.88	0.754	<.001
	Hoya meliflua* Merr. vs. *Telosma procumbens	-25.76	0.754	<.001

Note: <0.05 significant, while >0.05 not significant

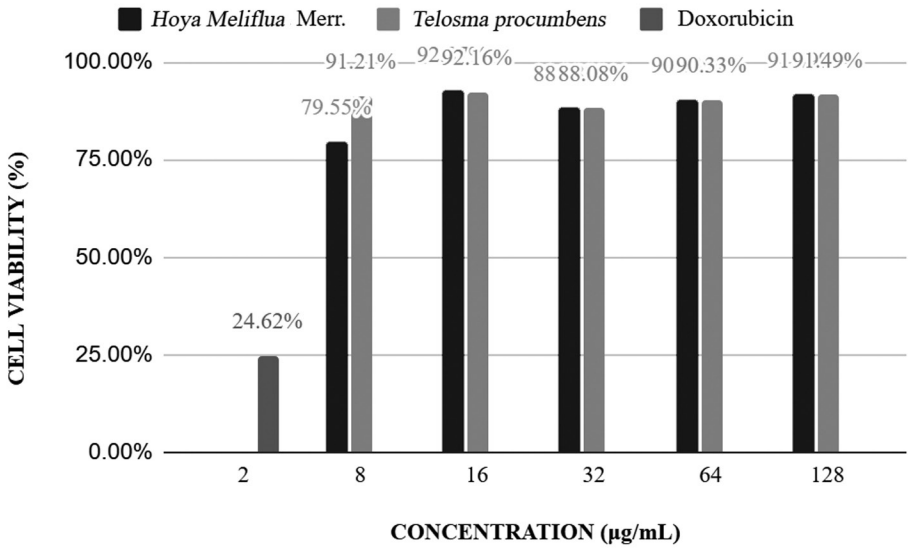

Figure 7. Cell viability (%) of *Hoya meliflua Telosma procumbens* (Latok), and doxorubicin (Positive Control) against breast cancer cell line (MCF-7).

Table 10. % Inhibition of breast cancer cell line (MCF-7) treated with *Telosma procumbens* (Latok) extracts in different concentrations.

Concentration	Percent Inhibition (%)		
	H. meliflua Merr. (Wax Plant)	*T. procumbens* (Latok)	Doxorubicin (positive control) at 2 µg/mL
8 µg/mL	20.45%	8.79%	75.38%
16 µg/mL	7.13%	7.84%	
32 µg/mL	11.92%	4.49%	
64 µg/mL	9.67%	9.73%	
128 µg/mL	8.51%	11.51%	

concentration of 128 µg/mL, *Telosma procumbens* (Latok) exhibited a cell viability of approximately 89% with a %inhibition of approximately 11%. Compared to the positive control, both extracts demonstrated relatively low antiproliferative properties. However, this still suggests the presence of bioactive compounds with potential antiproliferative properties. Further studies particularly optimization of concentration ranges and isolation of the compounds may provide a clearer understanding of the extracts' antiproliferative properties.

As shown in Table 10, Doxorubicin was used as the positive control at a single concentration of 2 µg/mL. Ramazi et al. (2023) identified this concentration as the half-maximal inhibitory concentration for certain conditions, which also exhibited the highest antiproliferative property in this study with a mean % inhibition of 75.38%. This suggests its effectiveness as a positive control for this study.

In comparison, *Hoya meliflua* Merr. (Wax Plant) demonstrated measurable antiproliferative activity across all tested concentrations. The highest % inhibition for *Hoya meliflua* Merr. (Wax Plant) was observed at the concentration of 8 µg/mL (20.45%), indicating an early and notable cytotoxic response even at the lowest concentration. The observed pattern may reflect a non-linear dose–response relationship, which may be resolved by isolating specific constituents.

Overall, the results indicate that *Hoya meliflua* Merr. (Wax Plant) may not be up to par with the positive control, but still possess antiproliferative potential.

The % inhibition of *Telosma procumbens* (Latok) ranged from 4.49% to 11.51%, with the highest activity observed at 128 µg/mL (11.51%), followed by 64 µg/mL (9.73%) and 8 µg/mL (8.79%). The results indicate that *Telosma procumbens* (Latok) may not reach the level of activity exhibited by the positive control, but it demonstrates antiproliferative potential. Although this reflected relatively low antiproliferative activity, previous studies have demonstrated that isolated triterpenoids exhibit stronger cytotoxicity. Nistor et al. (2023) reported that pentacyclic triterpenoids decrease cancer cell proliferation by inducing apoptosis, cell cycle arrest, and modulating signal pathways. Furthermore, Al-Ghruabi et al. (2023) observed that triterpenoid compounds have potent inhibitory activity against MCF-7 breast cancer cells. Despite being known to contain triterpenoids, results could be further enhanced through the isolation and purification of active compounds.

The One-way Analysis of Variance (ANOVA) was performed to evaluate the percent inhibition

Table 11. ANOVA test result of % inhibition on antiproliferative activity per concentration using MTT assay.

Concentration (µg/mL)	Source	Sum of Squares	df	Mean Square	F-value	p-value
8 µg/mL	Between Groups	6829.856	2	3414.928	38.870	<.001
	Within Groups	527.133	6	87.856		
	Total	7356.989	8			
16 µg/mL	Between Groups	8300.650	2	4150.325	405.486	<.001
	Within Groups	61.413	6	10.235		
	Total	8362.063	8			
32 µg/mL	Between Groups	8198.495	2	4099.247	227.432	<.001
	Within Groups	108.144	6	18.024		
	Total	8306.639	8			
64 µg/mL	Between Groups	7766.803	2	3883.402	397.205	<.001
	Within Groups	58.661	6	9.777		
	Total	7825.464	8			
128 µg/mL	Between Groups	7705.814	2	3852.907	616.208	<.001
	Within Groups	37.516	6	6.253		
	Total	7743.329	8			

Note: <0.05 significant, while >0.05 not significant

of the triterpenoid extract of *Telosma procumbens* (Latok) across concentrations of 8 µg/mL, 16 µg/mL, 32 µg/mL, 64 µg/mL, and 128 µg/mL. The results consistently demonstrated a highly significant effect of treatment at all concentration levels, with p-values of less than .001 (p-value <.001). Thus, the null hypothesis is rejected across all tested concentrations, indicating that the type of treatment significantly affects antiproliferative property. At 8 µg/mL, the ANOVA revealed a statistically significant difference among treatment groups, with an p-value of <.001. There was a substantially higher difference between the sum of squares between groups (6829.856) and sum of squares within groups (527.133). This indicates that the observed variation in percent inhibition is significantly relative to treatment effects rather than experimental error.

At 16 µg/mL, the p-value was <.001, similar across all concentrations, still suggesting a significant difference among treatment groups. The between-group variation (8300.650) exceeded the within-group variation (61.413), indicating high consistency in the observed responses and minimal variation within replicates. At 32 µg/mL, the ANOVA maintained significant variation in antiproliferative property, with a p-value of <.001. The measurable gap between the sum of squares between groups (8198.495) and within groups (108.144) suggests that treatment type remains the primary reason for the observed variability.

At 64 µg/mL, it resulted in a p-value of <.001, this indicates a substantial treatment effect. The relatively low within-group variation (58.661) compared to the between-group variation (7766.803) suggests reliable reproducibility and consistent biological response.

At 128 µg/mL, which is the highest concentration, the p-value yielded <.001. This indicates that there is a significant difference among the group. The negligible value of the mean square within groups (6.253) suggests minimal experimental error, while the predominance of the between-group variation (7705.814) verifies that the observed antiproliferative effects are primarily driven by treatment differences.

Overall, the ANOVA results showed that the treatment type has a statistically significant effect on percent inhibition across all of the tested concentrations. Nevertheless, regardless of the strong statistical significance, it is important to note that the extent of inhibition remains relatively low, suggesting that while differences between treatments exist, the extensive antiproliferative activity of the extract is limited under the conditions tested.

The specific differences between treatments were evaluated using Tukey's Post-Hoc Test to determine pairwise comparisons of antiproliferative activity across concentrations in the MTT assay. Across all

Table 12. Tukey's post-Hoc test pair-wise comparison on antiproliferative property per concentration using MTT assay.

Concentration (µg/mL)	Pair-wise Comparison	Mean Difference	Std. Error	p-value
8 µg/mL (Doxorubicin at 2mcg/mL)	**Doxorubicin vs *T. procumbens* (Latok)**	63.175	7.653	<.001
16 µg/mL (Doxorubicin at 2mcg/mL)	**Doxorubicin vs. *T. Procumbens* (Latok)**	64.085	2.612	<.001
32 µg/mL (Doxorubicin at 2mcg/mL)	**Doxorubicin vs. *T. Procumbens* (Latok)**	67.260	3.466	<.001
64 µg/mL (Doxorubicin at 2mcg/mL)	**Doxorubicin vs. *T. Procumbens* (Latok)**	62.285	2.553	<.001
128 µg/mL (Doxorubicin at 2mcg/mL)	**Doxorubicin vs. *T. Procumbens* (Latok)**	60.598	2.042	<.001

Note: <0.05 significant, while >0.05 not significant

concentrations, the positive control (Doxorubicin at 2 µg/mL) consistently showed significantly higher % inhibition than *Telosma procumbens* (Latok), with all comparisons yielding statistically significant differences ($p \leq .001$). This confirms the expected superior cytotoxic efficacy of the standard chemotherapeutic agent relative to the plant extracts.

At 8 µg/mL, Doxorubicin showed a significantly higher antiproliferative activity compared to *Telosma procumbens* (Latok) with a mean difference = 63.175, $p < .001$. At 16 µg/mL, plant extracts remained significantly less cytotoxic than the positive control ($p < .001$). The mean difference between Doxorubicin and *Telosma procumbens* (Latok) was 64.085. At 32 µg/mL, Doxorubicin continued to exhibit significantly higher % inhibition compared to *Telosma procumbens* (Latok) with a mean difference = 67.260, $p < .001$. At 64 µg/mL, the same trend persisted, with Doxorubicin showing significantly greater antiproliferative activity than *Telosma procumbens* (Latok) with a mean difference = 62.285, $p < .001$. At 128 µg/mL, Doxorubicin still demonstrated significantly higher % inhibition compared to *Telosma procumbens* (Latok) with a mean difference = 60.598, $p < .001$.

Overall, Tukey's Post-Hoc analysis demonstrates that while *Telosma procumbens* (Latok) exhibit measurable antiproliferative activity, it does not significantly outperform Doxorubicin across all tested concentrations.

In the final analysis, antioxidants act as a defense mechanism to prevent the buildup of a chain reaction caused by oxidative stress that leads to the development of mutations and DNA damage in the early phases of carcinogenesis. On the other hand, the mechanism of antiproliferative agents is to inhibit the proliferation of cancer cells by processes such as apoptosis or oxidative stress. In this manner, antioxidants effects do not always correlate with antiproliferative properties but, studying both assays provides comprehensive analysis of how bioactive compounds influence both oxidative stress and regulation of cell growth that is critical for developing anticancer therapies with maximal efficacy and minimal adverse effects.

CONCLUSION

This study investigated the phytochemical composition and comparative bioactivity of triterpenoids extracted from the leaves of *Hoya meliflua* Merr. (Wax Plant) and *Telosma procumbens* (Latok). The findings indicate that both plant extracts contain functional groups attributed to significant constituents known for exhibiting antioxidant and antiproliferative properties. A comparative analysis showed that *Telosma procumbens* (Latok) contains a greater amount of triterpenoids than *Hoya meliflua* Merr. (Wax Plant), which is consistent with the findings of the DPPH antioxidant assay. This is further supported by data showing that *Telosma procumbens* (Latok) reduced its performance gap relative to the Ascorbic Acid (Positive Control) by more than 50% at 500 ppm. Consequently, *Telosma procumbens* (Latok) demonstrated a distinct dose-dependent response, with radical scavenging activity increasing in direct proportion to concentration, marking it as a promising candidate for treating oxidative stress-related disorders. Overall, the results suggest the therapeutic potential of *Hoya meliflua* Merr. (Wax Plant) and *Telosma procumbens* (Latok) as sources of natural antioxidants, which may provide a probable pharmacological basis for future therapeutic studies. Furthermore, the findings suggest that the two plant extracts at any concentration (8, 16, 32, 64, and 128 µg/mL) demonstrated comparable antiproliferative effects throughout the assay, despite increasing dose levels, and while both extracts showed measurable but relatively low antiproliferative properties, neither outperformed the other significantly. Particularly, *Hoya meliflua* Merr. (Wax Plant) showed its highest antiproliferative property at 8 µg/mL (20.45%), while *Telosma procumbens* (Latok) reached its peak activity at 128 µg/mL (11.51%). Importantly, IC50 values were not computed for the experimental samples because neither plant extract achieved 50% inhibition within the tested concentration range. This further suggests that, under the conditions of the assay, the extracts did not reach a level of potency sufficient for IC_{50} determination.

RECOMMENDATIONS

- Future studies should proceed with the isolation of triterpenoids to further investigate the reliability of the constituents' activity.
- Future studies should utilize Gas Chromatography-Mass Spectrometry (GC-MS) to further identify, quantify, and differentiate the particular components of triterpenoids.
- Future studies should explore sample concentrations that are higher than the established IC_{50} to determine what concentration of the

extracts may achieve comparable antioxidant activity to the positive control.

- Future studies should assess the potential antiproliferative activity of *Telosma procumbens* (Latok) at lower concentrations to better analyze its dose–response relationship.
- Future studies should explore other plant parts of *Hoya meliflua* Merr. (Wax Plant) and *Telosma procumbens* (Latok), such as the flower, stems, and bark.

Conflict of Interest:

The authors have no conflict of interest to declare.

REFERENCES

1. Agoot, L. "Filipino Patients Now Appreciating Alternative Medicine | Philippine News Agency." Pna.gov.ph, 2023, www.pna.gov.ph/articles/1213382.
2. Al-Ghurabi EH et al. (2023). Antiproliferative and cell cycle arrest potentials of 3-O-acetyl-11-keto-β-boswellic acid against MCF-7 cells in vitro. J Gen Eng Biotechn 21, Article 93. https://doi.org/10.1186/s43141-023-00529-2
3. Aly S, Doghish AS & Heba A, El-Nashar S. (2024). Unveiling the pharmacological potential of plant triterpenoids in breast cancer management: an updated review. Naunyn-Schmiedeberg's Arch Pharmacol https://doi.org/10.1007/s00210-024-03054-2
4. Antonio MA & Vivit MB. (2017). Phytoconstituents and in vitro anti-oxidant activity of selected indigenous vegetables in the Ilocos. MMSU Sci Techn J 7 (2): 16-27. https://www.academia.edu/download/106010737/vol7_2_Phytoconstituents.pdf
5. Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Pandey RP & Chang CM. (2022). Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of *Ficus religiosa*. Molecules 27(4): 1326. https://doi.org/10.3390/molecules27041326
6. Bamidele H. (2024, August 16). Ethnomedicinal uses of Apocynaceae family plants. https://www.researchgate.net/publication/393888563_Ethnomedicinal_Uses_of_Apocynaceae_Family_Plants
7. BC Cancer Drug Manual. (2025). Doxorubicin. Www.bccancer.bc.ca. http://www.bccancer.bc.ca/drug-database-site/Drug%20Index/Doxorubicin_monograph.pdf
8. Brown JS, Amend SR, Austin RH, Gatenby RA, Hammarlund EU & Pienta KJ. (2023). Updating the Definition of Cancer. Molec Cancer Res 21(11): 1142–7. https://doi.org/10.1158/1541-7786.mcr-23-0411
9. Cajuday LA & Amparado E A. (2014). Hypoglycemic property of *Telosma procumbens* (Blanco) Merr. (Apocynaceae) in normal and alloxan-induced diabetic juvenile mice (Mus musculus). J Phytopharmacol 3(2): 113–7. https://doi.org/10.31254/phyto.2014.3206
10. Canadian Cancer Society. (2025). How cancer starts, grows and spreads. Canadian Cancer Society. https://cancer.ca/en/cancer-information/what-is-cancer/how-cancer-starts-grows-and-spreads
11. Cancer Today. (2025). Who.int. https://gco.iarc.who.int/today/en/dataviz/tables?mode=population&sexes=0
12. Chen J, Dominique F, Jay E, Wu JF, Magsanoc-Alikpala K, & Dee EC. (2025). Trends in female breast cancer among adolescent and young adults in Southeast Asia. The Lancet Regional Health - Southeast Asia 34: 100545. https://doi.org/10.1016/j.lansea.2025.100545
13. Chen Z, Leong KF, Xie Y, Gianoncelli A & Ribaudo G. (2025). Triterpenoid compounds and their derivatives: emerging pharmacological agents for arthritis treatment. Mini-Reviews in Medicinal Chemistry 25(15): 1127–48. https://doi.org/10.2174/0113895575389522250825063702
14. Cleveland Clinic. (2023, September 25). Breast cancer: Causes, stage, diagnosis & treatment. Cleveland Clinic. https://my.clevelandclinic.org/health/diseases/3986-breast-cancer
15. Cortaga C. (2023). Sources of resistance and trichome analysis of Philippine indigenous hoya species against the milkweed APHID, Aphis nerii Boyer De Fonscolombe (HEMIPTERA: APHIDIDAE). BIOTROPIA - Southeast Asian J Trop Biol 30(1): 74–81. https://doi.org/10.11598/btb.2023.30.1.1783
16. De Guzman G, Cagayan MSF, Bagaoisan DM & Miranda S. (2023). Cytotoxic and apoptotic properties of the flavonoid-rich ethyl acetate fraction of the crude methanol leaf extract of *Swyzygium samarangense* (Blume) Merr. (Myrtaceae) against MCF-7 breast cancer cells. Acta Medica Philippina 57(11). https://doi.org/10.47895/amp.vi0.6631
17. Fiardilla F, Warsiki E & Sugiarto. (2020). The experiment of activity and stability of antioxidant extracted from Senduduk (*Melastoma malabathricum* L) leaves at various conditions of concentration, pH values, and temperatures. IOP Conference Series: Earth and Environmental Science 472, 012003. https://doi.org/10.1088/1755-1315/472/1/012003
18. FT-IR Sample Preparation - NIU - Department of Chemistry and Biochemistry. (n.d.). Northern Illinois University. https://www.niu.edu/clas/chembio/research/analytical-lab/ftir/sample-preparation.shtml
19. Garcia JF. (2023, June 22). Fintech should be made accessible – PIDS. The Manila Times. https://www.manilatimes.net/2023/06/23/business/top-business/fintech-should-be-made-a ccessible-pids/1897393
20. Hayat J, Akodad M, Moumen A, Baghour M, Skalli A, Ezrari S & Belmalha S. (2020). Phytochemical screening, polyphenols, flavonoids and tannin content, antioxidant activities and FTIR characterization of *Marrubium vulgare* L. from 2 different localities of Northeast of Morocco. Heliyon, 6(11), e05609. https://doi.org/10.1016/j.heliyon.2020.e05609
21. Houssami N. (2025). Breast cancer prevention in the Western Pacific region. The Lancet Regional Health - Western Pacific, 101531. https://doi.org/10.1016/j.lanwpc.2025.101531
22. Jiregna Gari Negasa, Teshome, I., Edilu Jorga Sarba & Daro BS. (2024). Phytochemical screening and in vitro antibacterial activity of *Echinops kebericho* Mesfin tuber extracts: experimental studies. Peer J 12(10.7717/peerj.18554), e18554–e18554. https://doi.org/10.7717/peerj.18554

23. Khwaza V & Aderibigbe BA. (2024). Potential pharmacological properties of triterpene derivatives of ursolic acid. Molecules 29(16): 3884. https://doi.org/10.3390/molecules29163884
24. Li Y, Zhou S, Dong Y, Li Q, Gao Y & Yu X. (2023). Developing method of simultaneously determining content of 11 triterpene alcohols and analyzing the influence factors in camellia oil. J Food Comp Analysis 122: 105494. https://doi.org/10.1016/j.jfca.2023.105494
25. Ling T, Boyd L & Rivas F. (2022). Triterpenoids as reactive oxygen species modulators of cell fate. Chem Res Toxicol 35(4): 569–84. https://doi.org/10.1021/acs.chemrestox.1c00428
26. Mane NB & Khilare CJ. (2021). Phytochemical analysis and study of functional groups by FTIR analysis of Withania Somnifera L Dunal. J Sci Res 65(6): 115–9. https://doi.org/10.37398/jsr.2021.650619
27. Majérus MA. (2022). The cause of cancer: The unifying theory. Adv Cancer Biol Metas 4: 100034. https://doi.org/10.1016/j.adcanc.2022.100034
28. Milestones in Cancer Research and Discovery. (2020, August 31). National Cancer Institute; Cancer.gov. https://www.cancer.gov/research/progress/250-years-milestones
29. Nandiyanto ABD. (2026). How to read and interpret FTIR spectroscope of organic material. ASEAN J Sci Eng Materials 5(2): 323–56. https://doi.org/10.17509/ijost.v4i1.15806
30. Naraparaju S, Barla KD, Chaganti S, Anumolu PKD & Sunkara S. (2024). A comprehensive review of drugs determined by spectrophotometry using vanillin as a chromogenic reagent in the past decade. Curr Pharmaceut Anal 20(5): 299–320. https://doi.org/10.2174/0115734129306714240610070448
31. National Cancer Institute. (2021, October 11). What Is Cancer? National Cancer Institute; National Institutes of Health. https://www.cancer.gov/about-cancer/understanding/what-is-cancer
32. National Cancer Institute. (2024, December 11). Breast Cancer Treatment. National Cancer Institute; Cancer.gov. https://www.cancer.gov/types/breast/patient/breast-treatment-pdq
33. Newman DJ & Cragg GM. (2020). Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. J Nat Prod 83(3): 770–803. https://doi.org/10.1021/acs.jnatprod.9b01285
34. Nistor M, et al. (2023). Pentacyclic triterpenoid phytochemicals with anticancer activity: Updated studies on mechanisms and targeted delivery. Int J Molec Sci 24(16): 12923. https://doi.org/10.3390/ijms241612923
35. Ng VAS, Malabed RS, Aurigue FB & Ragasa CY. (2022). Triterpenes and sterols from leaves of *Hoya meliflua* Merr. Animo Repository. https://animorepository.dlsu.edu.ph/faculty_research/6104/
36. Njoya EM. (2021). Medicinal plants, antioxidant potential, and cancer. Cancer: 349–57. https://doi.org/10.1016/b978-0-12-819547-5.00031-6
37. Nistor M, Rugina D, Diaconeasa Z, Socaciu C & Socaciu M. (2023). Pentacyclic triterpenoid phytochemicals with anticancer activity: Updated studies on mechanisms and targeted delivery. Int J Molec Sci 24(16): 12923. https://doi.org/10.3390/ijms241612923
38. Panajon N, Aurigue F, Shen CC & Ragasa C. (2016). Triterpenes and Sterols from *Hoya diversifolia* Blume. J Applied Pharmaceut Sci 079–082. https://doi.org/10.7324/japs.2016.60614
39. Park JS, Rehman IU, Choe K, Ahmad R, Lee HJ & Kim MO. (2023). A triterpenoid lupeol as an antioxidant and anti-neuroinflammatory agent: impacts on oxidative stress in Alzheimer's disease. Nutrients 15(13): 3059. https://doi.org/10.3390/nu15133059
40. Peeva MI, Georgieva MG, Balacheva AA, Pavlov A & Tzvetkov NT. (2024). In vitro investigation of the cytotoxic and antiproliferative effects of haberlea rhodopensis total extract: A comparative study. Cosmetics 11(2): 46. https://doi.org/10.3390/cosmetics11020046
41. Pollo BA, Bueno PR & Yu G. (2025, July 24). Phytochemical screening. Department of Biochemistry and Molecular Biology, College of Medicine University of the Philippines Manila. https://dx.doi.org/10.17504/protocols.io.bp2l6zb85gqe/v1
42. Priyanka DR & Chandran M. (2024). Screening of bioactive phytocompounds in the leaf of *Coldenia procumbens* using biochemical, FTIR and GCMS. Scope 14: 183–93. https://www.researchgate.net/publication/383291565_Screening_of_Bioactive_Phytocompounds_in_the_Leaf_of_Coldenia_procumbens_Using_Biochemical_FTIR_and_GCMS_Analysis
43. Sampang L. (2024, January 22). Traditional Filipino medicine and the quest to cure the incurable. SCIENCE - University of the Philippines Diliman -. https://science.upd.edu.ph/traditional-filipino-medicine-and-the-quest-to-cure-the-incura ble/
44. Sinurat JP, Krisdianilo V, Karo R.and Berutu R. (2020). Analysis of total terpenoids from *Maniltoa grandiflora* (A. Gray) Scheff leaves using TLC and HPLC Methods. Stannum: Jurnal Sains dan Terapan Kimia 2 (2): 40-4. doi: 10.33019/jstk.v2i2.1976
45. The. (2024). Global Cancer Observatory. Who.int. https://gco.iarc.who.int/media/globocan/factsheets/populations/608-philippines-fact-sheet
46. Umar AH, Ratnadewi D, Rafi M & Sulistyaningsih YC. (2021). Untargeted metabolomics analysis using FTIR and UHPLC-Q-Orbitrap HRMS of two Curculigo species and evaluation of their antioxidant and α-Glucosidase inhibitory activities. Metabolites 11(1): 42. https://doi.org/10.3390/metabo11010042
47. Vieira Â, Nascimento APS, Branco MZ, Martins-Lopes P, Eiras-Dias JE, Brazão J, Ferreira L, Machado N & Barros AN. (2026). FTIR-ATR spectroscopy and chemometrics for varietal screening of PDO douro monovarietal wines: An exploratory feasibility study. Molecules 31(6): 1004. https://doi.org/10.3390/molecules31061004
48 World Health Organization: WHO. (2019, July 12). Cancer. Who.int; World Health Organization: WHO. https://www.who.int/health-topics/cancer
49. World Health Organization. (2023, August 10). Traditional Medicine Has a Long History of Contributing to Conventional Medicine and Continues to Hold Promise. World Health Organization. https://www.who.int/news-room/feature-stories/detail/traditional-medicine-has-a-long-history-of-contributing-to-conventional-medicine-and-continues-to-hold-promise
50. World Health Organization: WHO. (2025, February 3). Cancer. World Health Organization; World Health Organization. https://www.who.int/news-room/fact-sheets/detail/cancer