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Antimitotic activity of Anahaw (*Saribus rotundifolius*, Lam. Blume) leaf crude extract topical gel

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Abstract

This study aimed to assess the antimitotic properties of a topical gel formulated from the crude leaf extract of anahaw (*Saribus rotundifolius* Lam. Blume). Anahaw leaves contain bioactive compounds such as saponins, cardiac glycosides, and flavonoids, which are known for their antiproliferative properties and potential role in preventing abnormal cell development. The crude extract was incorporated into a topical gel formulation and evaluated for biological activity using the meristematic onion (*Allium cepa*) root tip assay. The leaves of *Saribus rotundifolius* were extracted and screened for the presence of phytochemical constituents, including alkaloids, saponins, carbohydrates, tannins, flavonoids, and cardiac glycosides. The antimitotic activity of both the crude leaf extract and the formulated topical gel was assessed by determining the mitotic index (MI) using the onion root tip assay. The mitotic index served as a quantitative indicator of mitotic activity, with lower MI values indicating greater inhibition of cell division. Among all treatments, methotrexate (positive control) consistently demonstrated the strongest antimitotic effect, producing the lowest mean MI values. In contrast, distilled water (negative control) showed the highest MI values, indicating minimal inhibition of mitotic activity. Among the test samples, the crude leaf extract topical gel treatments showed varying inhibitory effects. Treatment C exhibited the most pronounced antimitotic activity, approaching the effectiveness of methotrexate, while Treatments A and B showed moderate reductions in mitotic activity. The results suggest that *Saribus rotundifolius* leaf extract, particularly in Treatment C, has significant antimitotic potential. This study highlights anahaw as a promising natural source of antimitotic compounds with possible anticancer applications.

Keywords: *crude leaf extract, phytochemicals, mitotic index, anticancer applications*

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1. Introduction

Cancer remains a pressing global health problem faced by humans in the 21st century. According to the Philippine Statistics Authority (PSA), the World Health Organization's International Agency for Research on Cancer—Global Cancer Observatory (IARC-GCO) identified cancer as the second leading cause of death in 2022, with lung, breast, and liver cancers ranking highest in mortality. However, cancer drug development remains complex despite significant advances and the identification of numerous targeted therapies. Studies show that combination therapy may represent a promising future approach for cancer treatment (Wei et al., 2024; Palmer & Sorger, 2017; Damodaran et al., 2025; Mokhtari et al., 2017).

Cancer is widely recognized as a multifaceted disease with a highly complex origin and progression. One of the major challenges in developing anticancer drugs is the increasing incidence of multidrug resistance and relapse (Emran et al., 2022; Li et al., 2025). Conventional chemotherapeutic agents directly target cellular DNA; however, mutations may allow cancer cells to develop resistance (Meegan & O'Boyle, 2019). Because of these challenges, this study aims to identify and curate a new candidate for cancer drug research and development, one that is naturally sourced and readily accessible in the Philippines.

Anahaw (*Saribus rotundifolius* Lam. Blume) is a hermaphroditic fan palm that is evergreen, erect, and grows with a single trunk reaching a height of approximately 15 to 25 meters. It is native to the Philippines and is widely regarded as the country's "unofficial" national leaf. The plant is also known for its antimicrobial properties. A phytochemical screening conducted by Dagalea et al. (2021) at the University of Eastern Philippines revealed that anahaw leaves contain saponins along with other phytochemicals such as tannins, cardiac glycosides, and terpenoids. Another phytochemical screening conducted by Dr. Bucao at Mariano Marcos State University also identified the presence of flavonoids in anahaw leaves (Agbigay, 2023).

Studies have shown that these phytochemicals exhibit potential anticancer properties. For instance, Tian et al. (2020) reported that saponins can prevent and treat cancer through multiple mechanisms, including apoptosis induction, cell cycle arrest, anti-angiogenesis, autophagy induction, inhibition of cell migration, and tumor cell differentiation. Similarly, flavonoids have been found to induce necrosis, arrest the cell cycle, inhibit tumor angiogenesis, and suppress the migration and invasion of cancer cells (Zhang et al., 2015). Kopustinskiene et al. (2020) further demonstrated that flavonoids possess a broad spectrum of anticancer

activities, such as modulating enzymes that scavenge reactive oxygen species (ROS), inducing apoptosis and autophagy, and inhibiting the proliferation and invasiveness of cancer cells, which contribute to their antimitotic potential.

Additionally, anahaw contains cardiac glycosides (Dagalea et al., 2021), compounds traditionally used to treat heart diseases. Osman et al. (2017) reported that these bioactive compounds exhibit antimitotic properties and are being investigated as potential anticancer agents. Cardiac glycosides may induce cell death or inhibit cancer cell growth by blocking the Na^+/K^+-ATPase pump, which plays a crucial role in cell signaling and proliferation. Based on these evidence, *Saribus rotundifolius* (Lam. Blume) demonstrates significant potential as a source of antimitotic compounds.

2. Literature Review

2.1 Skin Cancer

Cancer remains one of the leading causes of death in the Philippines. In 2020, more than 150,000 cancer cases were diagnosed, with over 90,000 deaths reported (Sung et al., 2020). Specifically, the Philippine Dermatological Society documented 2,102 cases of basal cell carcinoma and 614 cases of squamous cell carcinoma between 2011 and 2021 (Tan et al., 2022). One of the primary causes of skin cancer is excessive exposure to ultraviolet (UV) radiation from the sun, sunlamps, or tanning beds (Schulman & Fisher, 2009). UV radiation damages the DNA of skin cells, and the accumulation of DNA damage over time can lead to uncontrolled cell growth and the development of cancer.

Mitosis is a fundamental cellular process in which a cell replicates its chromosomes and separates them to produce two identical nuclei prior to cell division. This process ensures that two daughter cells receive identical genetic material (McIntosh, 2016). The cell cycle consists of interphase and the mitotic (M) phase. Interphase accounts for approximately 90% of the cell cycle and includes the G1, S, and G2 phases. During these stages, the cell grows, synthesizes proteins and organelles, and replicates its DNA in preparation for mitosis (Kaul et al., 2019).

Mitosis consists of several stages: prophase, prometaphase, metaphase, anaphase, telophase, and cytokinesis (Rehman et al., 2023). During prophase, chromatin condenses into visible chromosomes, each consisting of two sister chromatids connected at the centromere. The mitotic spindle begins to form as centrosomes move toward opposite poles of the cell. In

prometaphase, the nuclear envelope breaks down and spindle microtubules attach to kinetochores located at the centromeres of chromosomes. In metaphase, chromosomes align along the metaphase plate at the center of the cell. During anaphase, sister chromatids separate and migrate toward opposite poles. Telophase follows, during which two new nuclear envelopes form around the separated chromosomes. Finally, cytokinesis divides the cytoplasm, producing two genetically identical daughter cells (Rehman et al., 2023). Errors during mitosis can result in abnormal chromosome segregation, which may lead to uncontrolled cell proliferation and the formation of cancer cells (Bakhoum et al., 2014).

2.2 Saribus rotundifolius (Anahaw): Origin, Classification, Morphology, and Cultivation

Traditional herbal medicine has long been practiced in the Philippines. Historical records indicate that Filipinos used medicinal plants to treat various diseases even before Spanish colonization. Early Catholic missionaries such as Fr. Pablo Clain, SJ; Fr. Francisco Ignacio Alcina, SJ; and Fray José de Valencia documented medicinal plants used in the Philippines as early as 1669 (Meñiza et al., 2024).

Anahaw is traditionally used in Benguet, Philippines, where crushed leaves are applied to wounds and leaf decoctions are consumed to treat diarrhea (Balangcod et al., 2015). The plant originated in Southeast Asia, including Malaysia, Indonesia, Java, the Moluccas, the Philippines, Sulawesi, and the Lesser Sunda Islands, but it is now cultivated worldwide as an ornamental plant in tropical and subtropical regions (NParks, n.d.). Anahaw belongs to the Arecaceae family, which includes approximately 2,500 arboreal species distributed in tropical and subtropical regions (Hunter & Bystriakova, 2004). The genus Saribus is native to Southeast Asia, Papuasia, and the Pacific Islands (Govaerts, 2019). Members of this genus are known as fan palms because their leaves form rounded, costapalmate fans composed of numerous leaflets (Bacon & Baker, 2011).

Morphologically, anahaw is a medium-to-large palm that can grow 10–15 meters tall and may reach up to 24 meters in natural habitats. It has a slender, solitary trunk marked with rings of leaf scars. Its large fan-shaped leaves, measuring about 1–2 meters in diameter, are supported by long petioles that allow them to sway in the wind. The plant produces cream-colored flowers arranged in panicles, followed by small spherical fruits that change from bright red to black when ripe (Dowe, 2009). Anahaw is widely cultivated due to its horticultural,

economic, and ecological importance. It thrives in humid tropical climates and grows best in clay or clay-loam soils. Its leaves are commonly used for roofing and fan-making, while its trunk is utilized in constructing low-cost houses in rural communities. Additionally, the plant has ornamental, nutritional, and medicinal value.

Anahaw has long been valued for its medicinal properties. The young buds are consumed as vegetables, while the young green nuts and ripe fruits are edible. Traditionally, extracts from the leaves are used to treat wounds and diarrhea and have been reported to possess antibacterial properties (Essien et al., 2017). Phytochemical screening conducted by Dagalea et al. (2021) revealed that anahaw leaves contain several bioactive compounds, including alkaloids, tannins, flavonoids, terpenoids, cardiac glycosides, and saponins, which may contribute to the plant's therapeutic potential.

2.3 Saponins: Characteristics, Applications, and Antimitotic Activity

Saponins are amphiphilic glycosides composed of hydrophilic sugar chains attached to hydrophobic steroid or triterpene aglycones. They are widely distributed in plants and are known for their diverse pharmacological activities (Yang et al., 2021). Structurally, saponins consist of an aglycone nucleus containing approximately 27–30 carbon atoms and one or more sugar moieties (Ashour et al., 2019). These compounds exhibit unique physicochemical properties such as foaming and hemolytic activity due to their amphiphilic nature. Their ability to produce stable foam when shaken with water results from the presence of both hydrophilic and lipophilic components, making them useful as natural surfactants in industrial applications.

Saponins have also demonstrated significant anticancer and antimitotic properties. Studies have shown that these compounds can inhibit tumor growth through mechanisms such as anti-angiogenesis, induction of autophagy, inhibition of cell migration, promotion of tumor cell differentiation, and induction of apoptosis (Podolak et al., 2023; Majnooni et al., 2023; Khan et al., 2022; Elsori et al., 2025; Elekofehinti et al., 2021). Additionally, saponins have shown cytotoxic effects against various cancer cell lines and may enhance immune responses when used as adjuvants (Timilsena et al., 2023).

2.4 Flavonoids: Characteristics, Applications, and Antimitotic Activity

Flavonoids are naturally occurring polyphenolic compounds characterized by a 15-carbon structure consisting of two aromatic rings connected by a heterocyclic ring. These

compounds are widely distributed in plants and play roles in pigmentation, UV protection, nitrogen fixation, and defense against pathogens (Stachelska et al., 2025; Mierziak et al., 2014; Roy et al., 2022). Flavonoids possess several biological activities, including antioxidant, anti-inflammatory, antiviral, and anticancer effects. They are classified into various subclasses such as flavones, flavonols, flavanones, isoflavones, anthocyanidins, and flavanols.

In pharmacology, flavonoids have gained attention for their potential role in cancer therapy. They may inhibit tumor growth by inducing apoptosis, causing cell cycle arrest, and suppressing tumor progression. For instance, quercetin promotes apoptosis by regulating signaling pathways such as Akt and NF-κB and modulating apoptotic regulators including p53 and Bcl-2 (Nguyen et al., 2017). Similarly, naringenin induces apoptosis in HepG2 liver cancer cells through ROS-mediated inhibition of the JAK2/STAT3 signaling pathway (Zhang et al., 2015). Flavonoids may also act as antimitotic agents by disrupting mitosis and inducing cell cycle arrest. Eupatorin, for example, inhibits Aurora B kinase involved in the spindle assembly checkpoint, leading to mitotic defects, polyploidy, and eventual apoptosis of cancer cells (Salmela et al., 2009).

2.5 Cardiac Glycosides: Characteristics, Applications, and Antimitotic Activity

Cardiac glycosides are steroidal compounds that enhance the force of heart contractions by inhibiting the sodium–potassium ATPase pump. These compounds have long been used in the treatment of heart failure and cardiac rhythm disorders (Fu et al., 2019). Their chemical structure typically includes a steroid nucleus linked to a lactone ring and one or more sugar moieties. Common examples include digoxin, digitoxin, ouabain, and oleandrin (El-Seedi et al., 2022).

Beyond their cardiovascular applications, cardiac glycosides have demonstrated antiproliferative and anticancer activities. These compounds can induce cell cycle arrest and inhibit cancer cell growth by disrupting Na^+/K^+-ATPase–mediated signaling pathways (Osman et al., 2017). For example, bufalin induces G2/M phase arrest in malignant melanoma cells (Yang et al., 2006) and activates tumor suppressor pathways involving p53 and p21 while suppressing cyclin D expression (Jiang et al., 2010). Similarly, ouabain has shown antiproliferative effects against breast and prostate cancer cells through increased expression of cell cycle inhibitors and alterations in intracellular calcium signaling (Weidemann et al., 2025).

2.6 Evaluation of Antimitotic Activity Using Allium cepa

The Allium cepa assay is widely used to evaluate the cytotoxic and antimitotic effects of chemical compounds. Onion root cells are large and easily observable under low magnification, allowing researchers to determine the number of cells undergoing mitosis (Gupta, 2023; Suslov et al., 2009). Using this method, the mitotic index, the percentage of cells undergoing mitosis, is calculated. The mitotic index is an indicator of cell proliferation and helps identify tissues with active cell division (Campbell, 1983). A reduction in the mitotic index often indicates cytotoxic effects or cell cycle arrest (Evangelista et al., 2006).

The meristematic onion root tip (MORT) assay involves germinating onion bulbs in water and exposing the developing roots to different concentrations of plant extracts. After exposure, the root tips are stained and observed under a microscope to count dividing cells and determine the mitotic index.

2.7 Topical Gels as Drug Delivery Systems

Topical gels are semisolid pharmaceutical formulations consisting of a liquid phase entrapped within a three-dimensional polymeric matrix (Rout et al., 2024; Pawar et al., 2024; Alaghawani et al., 2024; Sastri et al., 2022). Due to their unique physical properties, gels are widely used for topical drug delivery. These formulations offer several advantages, including ease of application, prolonged contact with the skin, and reduced systemic side effects compared with oral or other topical preparations (Dixit et al., 2013).

The effectiveness of topical drug delivery depends on several factors, including the area of application, frequency of application, viscosity of the formulation, and the condition of the skin. Drug penetration into the dermis is influenced by the solubility of the active compound within the formulation, which determines the rate of drug diffusion across the skin barrier (Bhuyan et al., 2021).

3. Methodology

3.1 Study Design

This study utilized a quantitative research design, specifically an experimental research design, as statistical methods were employed to evaluate the factors influencing the mitotic index. The crude extracts obtained from anahaw leaves, together with the anahaw leaf crude

extract formulated as a topical gel at varying concentrations, were tested for their antimitotic activity. The study included both a negative control group and a positive control group.

3.2 Setting of the Study

Anahaw leaves were collected from Caiyang, Batan, Aklan, where the plant is readily available. The extraction, identification, formulation, and pharmacological assay for evaluating the antimitotic activity of anahaw were conducted at the Pharmacy and Research Laboratories of Saint Gabriel College (PRL-SGC), located in Old Buswang, Kalibo, Aklan. Similarly, the evaluation of antimitotic activity using the Meristematic Onion Root Tip (MORT) assay was also performed at the same laboratory.

3.3 Experimental Protocols

Collection and preparation of materials. Saribus rotundifolius (Lam. Blume) is the only species of the genus Saribus used in this study.

Apparatus and instruments. The following apparatus and instruments were used in the study: blender, microscope, scissors, slides, shot glass, knife, stirring rod, test tubes, reagent bottles, beakers, droppers, filter paper, funnel, digital balance, pH meter, rotary evaporator, graduated cylinder, disposal bags, glass panels, a 500 g metal weight, and a lyophilizer.

Test materials. The test materials used in the assay included the following:

Test Treatments. The study used anahaw crude leaf extract at concentrations of 12.5 mg, 25 mg, and 50 mg, as well as anahaw leaf crude extract formulated as a topical gel at concentrations of 12.5 mg/g, 25 mg/g, and 50 mg/g.

Chemicals and solutions. The chemicals and solutions used in the study included distilled water, plant extract solutions, methotrexate injection, Carbopol 940, triethanolamine, disodium edetate, deionized water, propylene glycol, sodium hydroxide, hydrochloric acid, Molisch's reagent, sulfuric acid, and ferric chloride.

Formulation. The topical gel formulation utilized in this study was based on the herbal topical gel formulation described by Aiyalu et al. (2016). The formulation was adopted with slight modifications. The topical gel consisted of the following components:

Carbopol 940	1.5g
Deionized water	100g qs.
Disodium edetate	0.005g
Triethanolamine	1.5g
Propylene glycol	5g
Anahaw (Saribus rotundifolius, Lam. Blume) leaf crude extract	[A] 1250 mg [B] 2,500 mg [C] 5,000 mg

The plant extract used in the original formulation was replaced with anahaw leaf crude extract while maintaining the same quantity of 1 g. The amount of Carbopol used in the formulation was also adjusted to 0.5 g to increase the spreadability of the dosage form. In the original formulation, the Carbopol concentration was 1.5 g. Higher spreadability of a dosage form enhances drug diffusion through the skin (Forestryana et al., 2022).

Test media. The test medium employed in this study was the meristematic onion root tip assay for evaluating antimitotic activity.

Groupings. The experiment was conducted using a Completely Randomized Design (CRD). Distilled water was used for dilution and served as the negative control, while methotrexate was used as the standard or positive control. The antimitotic assay was performed in triplicate using onion bulbs treated with the following test samples: anahaw leaf crude extract and the topical gel formulated from anahaw leaf crude extract.

3.4 Preparation and Extraction

The preparation and extraction procedures used in this study were based on the methods described by Dagalea et al. (2021), with slight modifications. The Saribus rotundifolius leaves were garbled, washed, and dried using a dehydrator at 60°C instead of 45°C. This adjustment was made because saponins are stable at temperatures up to 90°C (Li et al., 2025), cardiac glycosides are stable up to 350°C (Higuchi et al., 1986b), and flavonoids are stable up to 110°C (Hardinasinta et al., 2021). Instead of air-drying the leaves for five days in an ambient room, the leaves were dried using a dehydrator. The use of a dehydrator is more efficient and time-

saving because it provides even heat distribution and a controlled environment for drying plant samples (Delica-Balagot et al., 2024).

After drying, the leaves were ground into smaller pieces and soaked in 95% ethanol for three (3) days at ambient room temperature. The solution was then filtered using filter paper. A rotary evaporator was used to concentrate the macerate at 75°C instead of 45°C, as specified in the original method. This temperature was chosen because it is closer to the boiling point of ethanol (78°C), which facilitates a faster concentration process (Wade & G., 2025). The resulting extract was stored at 4°C for future use.

3.5 Phytochemical Screening

A preliminary phytochemical screening was conducted using established procedures to determine the presence or absence of specific phytochemical constituents (Raheel et al., 2017).

Test for saponins. Approximately 0.25 mg of crude plant extract was mixed with 20 mL of distilled water and shaken in a graduated cylinder for 15 minutes. The formation of a stable foam layer measuring approximately 1 cm indicated the presence of saponins (Marami et al., 2022).

Test for carbohydrates (Molisch's Test). Three to four drops of Molisch's reagent were added to 2 mL of the extract and mixed thoroughly. Concentrated sulfuric acid was then carefully added along the side of the test tube. The formation of a blue or purple ring at the interface of the two layers indicated the presence of carbohydrates.

Test for alkaloids (Dragendorff's Test). Two milliliters of extract were treated with 1 mL of Dragendorff's reagent. The formation of an orange-red precipitate indicated the presence of alkaloids (Kancherla et al., 2019).

Test for tannins (Ferric Chloride Test). Two milliliters of the aqueous extract were mixed with a few drops of 10% ferric chloride solution. A greenish-black color indicated the presence of catechol tannins, while a blue-black color indicated the presence of gallic tannins.

Test for flavonoids (Alkaline Reagent Test). Two milliliters of extract were mixed with two to three drops of sodium hydroxide solution, producing a bright yellow color. Upon

the addition of a few drops of diluted hydrochloric acid, the solution turned colorless, confirming the presence of flavonoids (Kancherla et al., 2019).

Test for glycosides (Keller–Kiliani Test). Approximately 0.25 g of aqueous extract was dissolved in distilled water. Five milliliters of the extract solution were mixed with 2 mL of glacial acetic acid containing one drop of ferric chloride solution. This mixture was carefully layered with 1 mL of concentrated sulfuric acid. The formation of a brown ring at the interface indicated the presence of cardiac glycosides (Meharie & Tunta, 2020).

3.6 Solubility Test

This test determines the ability of the anahaw leaf crude extract and the anahaw leaf crude extract topical gel to dissolve in the various solvents that are commonly used as extraction solvents: water, acetone, methanol, and ethanol (Abubakar & Haque, 2020). Table 1 contains the parameters used to determine solubility.

Table 1

Parameters of solubility

Descriptive term	Part of solvent required per part of solute
Very Soluble	Less than 1
Freely Soluble	From 1 to 10
Soluble	From 10 to 30
Sparingly Soluble	From 30 to 100
Slightly Soluble	From 100 to 1000
Very Slightly Soluble	From 1000 to 10,000
Practically Insoluble	From 10,000 and over

3.7 Antimitotic Assay

The Meristematic Onion Root Tip Assay used in this study followed the same technique described by Kuruppuarachchi et al. (2023), with slight modifications. Instead of harvesting onion roots at a length of 1 cm, the roots in this study were harvested when they reached a length of 1–3 cm. For the permanent mounting and storage of onion root specimens, Entellan™, a rapid mounting medium for microscopy, was used. It is a ready-to-use, anhydrous mounting medium composed of a polymer and a mixture of acrylates dissolved in toluene. It can be used manually or automatically in diagnostic laboratories and is registered as an in vitro diagnostic (IVD) product with Conformité Européenne (CE) certification (Entellan™ Rapid Mounting Medium for Microscopy | Sigma-Aldrich, n.d.).

Part A: Growing of allium cepa roots. Allium cepa bulbs were visually inspected and confirmed to be in good condition. The bottom plates and dead scales of the onion bulbs were carefully removed. The bulbs were then suspended in dechlorinated water, ensuring that only their basal plates (approximately 0.1–0.3 cm) were in contact with the water to prevent rotting. The bulbs were placed in an ambient room without direct exposure to sunlight for one week. The water was replenished every 24 hours to maintain optimal growth conditions.

Part B: Treatment. Once the roots reached approximately 1–3 cm in length, the onion bulbs were transferred to treatment solutions containing different concentrations of anahaw leaf crude extract (12.5 mg/mL, 25 mg/mL, and 50 mg/mL), anahaw leaf crude extract topical gel (12.5 mg/g, 25 mg/g, and 50 mg/g), and methotrexate (0.10 mg/mL) as the standard reference drug (Bayuran, 2017). The bulbs were exposed to these treatments for 48 hours at 27 ± 2°C. Each test concentration was prepared in three replicates using separate containers. The treatment solutions were renewed every 24 hours. The positive control consisted of methotrexate injection (25 mg/mL), while the negative control consisted of dechlorinated water, the same type of water used during the initial root-growth stage.

After the 48-hour exposure period, one root tip (approximately 0.5–2 cm) was collected from each bulb and placed in a 1:3 solution of 1N HCl and glacial acetic acid. The samples were then heated in a water bath at 60 ± 2°C for 10 minutes. The root tips were subsequently transferred to another container containing 1% acetocarmine solution for 15–20 minutes, or until proper staining was observed, indicated by the presence of deeply stained ends. A stained root tip was placed on a glass slide with a drop of distilled water, covered with a coverslip, and gently pressed to release the cells. The onion root cells were examined using an OMAX LED compound microscope. Visualization and documentation were performed using an OMAX 18-megapixel microscope camera with TopView imaging software running on the Windows platform.

Using a data table created in Microsoft Excel, the total number of cells in each mitotic phase, prophase, metaphase, anaphase, and telophase, was recorded. The total number of cells and the number of non-dividing cells (interphase) were also quantified (Bayuran, 2017). In total, 30 root tips were analyzed using this procedure. The total number of cells and the number of dividing cells in each mitotic stage were counted manually using ImageJ software, and the mitotic index was calculated using the following formula:

$$\text{Mitotic Index} = \frac{\text{Number of dividing cells}}{\text{Total number of cells}} \times 100$$

3.8 pH testing of anahaw leaf crude extract

The method used to test the pH of the anahaw leaf crude extract followed the Laqua Horiba pH testing method, which is derived from US EPA Method 9045D. In a beaker or similar container, 20 g of the sample was combined with 20 mL of water and stirred for five minutes. The solution was then centrifuged at 100 rpm for five minutes to separate the sample from the water phase. The pH of the water phase was measured using a pH meter, and the pH value was recorded.

3.9 Spreadability Test

The method used to determine the spreadability of the anahaw leaf crude extract topical gel was adapted from the procedure described by Forestryana et al. (2022). In this test, 0.5 g of the topical gel was placed at the center of a 2 cm diameter circle pre-marked on a glass plate. A second glass plate was then placed on top of the gel, and a 500 g weight was applied to the upper glass plate for five minutes. After the specified time, the diameter of the gel spread was measured to determine its spreadability.

3.10 Acceptability Test (Hedonic Test)

Hedonic tests are used to measure the degree of liking or acceptance of a product. The level of liking is expressed through a hedonic scale, which evaluates consumer preferences (Pakpakan, 2025). This study used a 7-point hedonic scale to evaluate the acceptability of three formulations of anahaw leaf crude extract topical gel at concentrations of 12.5 mg/g, 25 mg/g, and 50 mg/g. The following characteristics of each formulation were evaluated: appearance, color, odor, texture, ease of application, feel on the skin after application, and overall acceptability. The scoring scale used was as follows: Like very much (7); Like moderately (6); Like slightly (5); Neither like nor dislike (4); Dislike slightly (3); Dislike moderately (2); and Dislike very much (1).

The respondents consisted of 30 randomly selected students from the Bachelor of Science in Pharmacy. All participants were asked to sign an informed consent form explaining the purpose of the study, the criteria for participant selection, and the potential risks and

benefits associated with participation. Participants were also informed that no financial compensation would be provided for participating in the study.

3.11 Statistical Treatment of Data

Both descriptive and inferential statistical analyses were performed on the collected data. For descriptive statistics, the mean was used to determine the average results of the extract, positive control, and negative control groups. For inferential statistics, Analysis of Variance (ANOVA) was used to evaluate the differences among the various concentrations of anahaw leaf crude extract used in the antimitotic assays (Bayuran, 2019). Specifically, the researchers employed One-Way ANOVA, followed by the Post Hoc Scheffé test to determine significant differences between treatment groups.

3.12 Waste Disposal

Since this study consumed a large quantity of onions, pit burial was used as a waste disposal method. According to the Sharma et al. (2016), this method is inexpensive and efficient for discarding large quantities of onions.

4. Findings and Discussion

4.1 Yield determination of Saribus rotundifolius extract

The percentage yield is significant in determining the amount of extract that can be obtained from a specific weight of *Saribus rotundifolius* (Lam. Blume) leaves. Extraction temperature and solvent contact time are critical parameters for optimizing the extraction process of anahaw leaves. As shown in Table 2, 1,013 g of *Saribus rotundifolius* (Lam. Blume) leaves yielded 126.73 g of extract, which is equivalent to 12.51% w/w.

Table 2

Percentage yield of Saribus rotundifolius extract

Sample	Weight of Saribus rotundifolius leaves (g)	Weight of Saribus rotundifolius leaf extract (g)	Percentage yield (%)
Anahaw (Saribus rotundifolius) Leaves	1,013	126.73	12.51

The study adopted the extraction method for anahaw leaves used in the study by Dagalea et al. (2021), with slight modifications. Specifically, the temperature used during rotary evaporation was adjusted. In this study, the rotary evaporator temperature was set to 75°C instead of 45°C, as indicated in the original method. This modification was made to improve the efficiency of the extraction process and reduce processing time.

The adjustment is supported by the findings of Li et al. (2023), which reported that saponins are stable at temperatures up to 90°C. Additionally, cardiac glycosides are stable at temperatures up to 350°C (Higuchi et al., 1986b), while flavonoids remain stable at temperatures up to 110°C (Hardinasinta et al., 2021). These findings suggest that increasing the evaporation temperature would not significantly degrade the phytochemical constituents of interest.

Instead of air-drying the plant material in the shade, the study used a dehydrator set at 60°C to ensure faster and more uniform drying of the anahaw leaf samples. This modification is supported by the study conducted by Delica-Balagot et al. (2024), indicating that the use of a dehydrator better preserved the properties of plant samples compared to air-drying methods, which showed poorer preservation results. The study used 95% ethanol as the extraction solvent, with a maceration period of three (3) days, as specified in the original method.

4.2 Physicochemical Identification of Anahaw Leaf Extract

Organoleptic evaluation was conducted to determine the physical characteristics of the anahaw leaf extract. Table 3 presents the results of the organoleptic evaluation of the *Saribus rotundifolius* (Lam. Blume) leaf extract. The anahaw leaf crude extract exhibited a dark olive-green color, a strong, sour, herbal odor, and a viscous consistency.

Table 3

Organoleptic test for the anahaw (Saribus rotundifolius, Lam. Blume) leaf extract

Properties	**Results**
Color	Dark olive green
Smell	Strong, sour, herbal odor
Consistency	Viscous

Phytochemical screening was conducted on the anahaw leaf extract to determine the presence of various phytochemicals, including saponins, carbohydrates, alkaloids, tannins, flavonoids, and cardiac glycosides. The results of the screening showed that the anahaw leaf crude extract tested positive for saponins, flavonoids, and cardiac glycosides.

Table 4

Phytochemical screening

Phytochemical	Name of test	Actual result	Positive result
Saponins	Froth test	Stable froth measuring 1.31 cm	Froth with a height of 1 cm.
Carbohydrates	Molisch test	No reactions observed	Appearance of blue or purple ring.
Alkaloids	Dragendorff's test	No reactions observed	Formation of orange-red precipitate.
Tannins	Ferric chloride test	No reactions observed	Greenish-black/ Blackish-blue colored solution.
Flavonoids	Alkaline Reagent Test	Yellow solution upon the addition of NaOH that turned clear after a few drops of HCL was added.	Yellow solution upon the addition of NaOH that turns clear after a few drops of HCL is added.
Cardiac Glycosides	Keller-Kiliani test	Formation of brown ring	formation of brown ring.

The anahaw leaf extract underwent pH testing with 3 replicates. The results of the test are shown in Table 5.

Table 5

pH determination

Trials	pH
1	4.1
2	4.4
3	4.3
Average	**4.3**

The average of the three trials was 4.3, indicating that the extract was acidic. Moreover, it was more acidic than the reference standard, Methotrexate IV, which had a pH value of 8.5.

The solubility of the anahaw leaf extract was examined in different solvents: methanol, ethanol, acetone, and distilled water. The parameter used to evaluate its solubility was the USP criteria for solubility.

Table 6

Solubility test

Solvent	Solubility description
Methanol	Freely soluble
Ethanol	Very soluble
Acetone	Very soluble
Distilled water	Freely soluble

The solubility test showed that the anahaw leaf extract was freely soluble in methanol, as it required 10 parts of methanol to dissolve the extract. The extract was very soluble in ethanol, requiring only 0.5 parts of ethanol for complete dissolution. Similarly, it was very soluble in acetone, as 0.5 parts of acetone were sufficient to dissolve the extract. Lastly, the extract was freely soluble in distilled water, requiring 10 parts of water for complete dissolution.

4.3 Quality Control Tests for the Anahaw Leaf Crude Extract Topical Gel

The anahaw leaf crude extract topical gels at different concentrations were tested for their spreadability. The results of these tests are presented in Table 7.

Table 7

Spreadability test

Formulation	Weight (g)	Spreadability (mm)
Formulation A (12.5mg/g)	0.5	59.5
Formulation B (25mg/g)	0.5	64.45
Formulation C (50mg/g)	0.5	80.1

The anahaw leaf crude extract topical gels at different concentrations were tested for their spreadability. Formulation A had a spreadability of 59.5 mm, Formulation B had a spreadability of 64.45 mm, and Formulation C had a spreadability of 80.1 mm.

Spreadability testing is conducted to determine the ability of a topical gel to spread easily on the skin. Higher spreadability of a dosage form allows greater drug diffusion through the skin (Forestryana et al., 2022). According to Nurman et al. (2019), the acceptable spreadability range for topical gels is 5–7 cm. Based on the results, Formulation B, with a spreadability of 64.45 mm (6.45 cm), exhibited the most optimal spreadability among the three formulations because it falls within the recommended range.

The pH of the topical gel formulations at varying concentrations was also examined. The results showed that Formulation A had a pH of 4.9, Formulation B had a pH of 4.8, and Formulation C had a pH of 4.9.

Table 8

pH of the anahaw leaf crude extract topical gel

Formulation	pH
Formulation A (12.5mg/g)	4.9
Formulation B (25mg/g)	4.8
Formulation C (50mg/g)	4.9

According to Lukić et al. (2021), the recommended pH range for topical formulations is 4–6. Therefore, it can be concluded that all three formulations fall within the recommended pH range for topical applications.

The organoleptic properties of the topical gel formulations were evaluated at different concentrations. Formulations A and B exhibited an olive green color, while Formulation C showed a dark olive green color. All three formulations had a strong, sour, herbal odor and exhibited a viscous consistency.

Table 9

Organoleptic test of the anahaw leaf crude extract topical gel

Formulation	Color	Smell	Consistency
Formulation A (12.5mg/g)	Olive green	Strong, sour, herbal odor	Viscous
Formulation B (25mg/g)	Olive green	Strong, sour, herbal odor	Viscous
Formulation C (50mg/g)	Deep olive green	Strong, sour, herbal odor	Viscous

4.4 Evaluation of Antimitotic Activity of Anahaw Leaf Extract

Table 10

Mitotic index of different treatments

Trial	Treatment A (12.5 mg)	Treatment (25 mg)	Treatment C (50 mg)	Distilled Water	Methotrexate
1	62.35	40.0	13.95	97.0	11.76
2	34.78	52.0	7.0	94.38	10.71
3	43.75	38.02	8.14	96.91	0.0
Mean Mitotic Index	**46.96**	**43.34**	**9.70**	**96.10**	**7.49**

The Meristematic Onion Root Tip Assay was used to evaluate the antimitotic activity of *Saribus rotundifolius* (Lam. Blume) leaf crude extract. The mitotic index percentages were analyzed to determine the effectiveness of the different treatments in inhibiting cell division, where a lower mitotic index indicates greater inhibitory activity.

As shown in Table 10, the positive control (Methotrexate) exhibited the lowest mean mitotic index (7.49), indicating the strongest inhibitory effect on cell division among all treatments. In contrast, the negative control (distilled water) had the highest mean mitotic index (96.10), suggesting the least effectiveness in suppressing mitosis.

Among the treatments, Treatment C showed a mean mitotic index of 9.70, indicating a strong inhibitory effect on cell division. Treatment B and Treatment A exhibited mean mitotic indices of 43.34 and 46.96, respectively, demonstrating moderate antimitotic activity compared to Treatment C. The results suggest that Methotrexate and Treatment C were the most effective in reducing cell division, while distilled water showed the least inhibitory effect on mitosis.

Table 11

Analysis of Variance

Source	Sum of square SS	Degrees of freedom vv	Mean square MS	F statistic	p-value
Treatment	15, 537.1718	4	3, 884. 2929	61.9629	5. 0937e-07
Error	626, 8741	10	62. 6874		
Total	**16, 164.0459**	**14**			

$\alpha = 0.01$

A one-way ANOVA was conducted to examine the effect of different treatments on the observed variable. The results indicated a significant effect of treatment, $F(4, 10) = 61.96$, $p < 0.001$. Given that the p-value (5.0937×10^{-7}) is well below the alpha level of 0.01, the null hypothesis, which states that there is no difference among the treatment means, is rejected. This result suggests that there is a statistically significant difference among at least two of the treatment groups. Furthermore, the large F-statistic indicates that the variability between treatment groups is substantially greater than the variability within groups. Therefore, post hoc comparisons are necessary to determine which specific treatment groups differ significantly from each other.

Table 12

Post hoc Scheffé test

Treatment pairs	Scheffé TT-Statistic	Scheffé P-Value	Scheffé Interference
12.5 mg vs 25 mg	0.5600	0.9872210	Insignificant
12.5 mg vs 50 mg	5.7642	0.0032094	** p<0.01
12.5 mg vs Distilled water	7.6008	0.0003680	** p<0.01
12.5 mg vs Methotrexate	6.1055	0.0020925	** p<0.01
25 mg vs 50 mg	5.2042	0.0066327	** p<0.01
25 mg vs Distilled water	8.1608	0.0002028	** p<0.01
25 mg vs Methotrexate	5.5455	0.0042462	** p<0.01
50 mg vs Distilled water	13.3650	2.4019 e-06	** p<0.01
50 mg vs Methotrexate	0.3413	0.9980706	Insignificant
Distilled water vs Methotrexate	13.7064	1.8951 e-06	** p<0.01

To further investigate the significant differences observed in the ANOVA results, a Scheffé post hoc test was conducted to determine which specific treatment pairs exhibited statistically significant differences in the tested variable. The results, as shown in Table 12, indicate that most treatment comparisons yielded p-values less than 0.01, confirming statistically significant differences between the majority of the treatment pairs.

Significant differences were observed between the following pairs: 12.5 mg vs. 50 mg, 12.5 mg vs. distilled water, 12.5 mg vs. methotrexate, 25 mg vs. 50 mg, 25 mg vs. distilled water, 25 mg vs. methotrexate, 50 mg vs. distilled water, and distilled water vs. methotrexate.

However, the treatment pairs 12.5 mg vs. 25 mg and 50 mg vs. methotrexate were found to be statistically insignificant ($p > 0.01$), indicating that there was no substantial difference in their effects. These findings suggest that most treatments differed significantly in their effects, except for the two aforementioned.

4.5 Evaluation of Antimitotic Activity of Anahaw Leaf Extract Topical Gel

The Meristematic Onion Root Tip Assay was used to evaluate the antimitotic activity of the *Saribus rotundifolius* (Lam. Blume) leaf crude extract topical gel. The percentage mitotic index (% MI) values were analyzed to determine the effectiveness of the different treatments in inhibiting cell division, where a lower % mitotic index indicates greater inhibitory activity on mitosis.

Table 13

Mitotic index of different treatments

Trial	Treatment A (12.5mg)	Treatment B (25mg)	Treatment C (50mg)	Distilled Water	Methotrexate
1	47.17	60.53	14.2	95.35	9.09
2	52.78	50.0	9.93	85.59	7.14
3	54.74	11.76	14.79	81.44	11.54
Mean Mitotic Index	**51.55**	**40.76**	**12.97**	**87.46**	**9.26**

As shown in Table 13, the mean % mitotic index varied across the five treatments (Treatment A to Methotrexate). Distilled water exhibited the highest mitotic index (87.4600), indicating the least effectiveness in inhibiting cell division. In contrast, methotrexate showed the lowest mitotic index (9.2567), suggesting that it was the most effective in reducing mitotic activity. Meanwhile, Treatment A and Treatment B showed moderate inhibitory effects, with mean mitotic index values of 51.5633 and 40.7633, respectively. Treatment C (12.9733) demonstrated a stronger inhibitory effect on cell division compared to Treatments A and B.

The results suggest that Treatment C and methotrexate were the most effective in reducing the mitotic index, whereas distilled water exhibited the least inhibitory effect on mitosis.

A one-way ANOVA was conducted to examine the effect of different treatments on the observed variable (% mitotic index). The results indicated a significant effect of treatment, $F(4, 10) = 20.67$, $p < 0.001$. Given that the p-value (7.9897×10^{-5}) is well below the alpha level of 0.01, the null hypothesis, which states that there is no difference among the treatment means, is rejected.

Table 14

Analysis of Variance

Source	Sum of square SS	Degrees of freedom vv	Mean square MS	F statistic	p-value
Treatment	12, 184.5745	4	3, 046.1436	20.6675	7.9897e-05
Error	1, 473.8813	10	147.3881		
Total	13, 658.4557	14			

α =0.01

This finding suggests that there is a statistically significant difference among at least two of the treatment groups. The large F-statistic further supports the conclusion that the variability between treatment groups is substantially greater than the variability within groups. Therefore, post hoc comparisons are necessary to determine which specific treatment groups differ significantly from each other.

Table 15

Post hoc Scheffé

Treatment pairs	Scheffé TT-Statistic	Scheffé P-Value	Scheffé Interference
12.5 mg vs 25 mg	1.0895	0.8735083	Insignificant
12.5 mg vs 50 mg	3.8930	0.0398303	*p<0.05
12.5 mg vs Distilled water	3.6213	0.0581554	Insignificant
12.5 mg vs Methotrexate	4.2680	0.0236414	Insignificant
25 mg vs 50 mg	2.8035	0.1761302	Insignificant
25 mg vs Distilled water	4.7109	0.0128616	Insignificant
25 mg vs Methotrexate	3.1785	0.1070006	Insignificant
50 mg vs Distilled water	7.5144	0.0004046	Insignificant
50 mg vs Methotrexate	0.3749	0.9972220	Insignificant
Distilled water vs Methotrexate	7.8893	0.0002698	**p<0.05

To further investigate the significant differences observed in the ANOVA results, a Scheffé post hoc test was conducted to determine which specific treatment pairs exhibited statistically significant differences in absorbance (clarity). The results, as shown in Table 15, reveal that some treatment comparisons yielded p-values below the 0.01 threshold, indicating significant differences. Specifically, a significant difference was observed between Treatment D and Treatment E ($p < 0.01$). However, the other treatment comparisons did not show statistically significant differences ($p > 0.01$), indicating that the treatments involved in these pairs did not differ significantly in terms of their effects.

4.6 Hedonic Test of Anahaw Leaf Crude Extract Topical Gel

A 7-point hedonic scale was used to evaluate the acceptability of the formulated gels at varying concentrations (Formulation A: 12.5 mg/g, Formulation B: 25 mg/g, Formulation C: 50 mg/g). According to Lawless and Heymann (2010), hedonic testing primarily relies on consumer judgments to evaluate sensory attributes such as taste, aroma, texture, appearance, and overall acceptability. This type of testing provides valuable insight into consumer preferences, which can guide product development and marketing strategies. In this study, the topical gels were evaluated based on appearance, color, odor, texture, ease of application, feel on the skin after application, and overall acceptability.

Table 16

Average of the evaluation characteristics

Evaluation Characteristics	Formulation A (12.5 mg/g)	Formulation B (25 mg/g)	Formulation C (50mg/g)
Appearance	6.07	6.33	6.3
Color	6.10	6.13	5.7
Odor	5.07	5.37	5.57
Texture	6.03	6.10	6.27
Ease of application	6.00	5.93	6.27
Feel on the skin after application	6.00	6.20	6.23
Overall acceptability	**6.27**	**6.27**	**6.43**

Table 16 presents the mean scores of the evaluation characteristics for each of the three topical gel formulations. For Formulation A, the mean score for appearance was 6.07, indicating that it was “liked moderately” by the respondents. This suggests that the formulation

generally looked appealing but may have had minor imperfections. The color had a mean score of 6.10, also categorized as “liked moderately,” indicating that the color was acceptable and generally pleasant. The odor had a mean score of 5.07, which corresponds to “liked slightly,” suggesting that the odor was somewhat pleasant but not particularly appealing. The texture had a mean score of 6.03, indicating that it was “liked moderately,” meaning that the texture was generally acceptable with minor roughness. The ease of application, feel on the skin after application, and overall acceptability had mean scores of 6.00, 6.00, and 6.27, respectively, all corresponding to “liked moderately.” These results suggest that the formulation was relatively easy to apply, produced an acceptable skin feel with minimal discomfort, and was generally well accepted by the respondents.

For Formulation B, the appearance, color, texture, feel on the skin after application, and overall acceptability had mean scores of 6.13, 6.33, 6.10, 6.20, and 6.27, respectively. These values correspond to “liked moderately,” indicating that the formulation had a generally pleasant color, an acceptable appearance with minor imperfections, a fairly acceptable texture with slight roughness, and an overall favorable response from the respondents. Meanwhile, the odor and ease of application had mean scores of 5.37 and 5.93, respectively, both categorized as “liked slightly.” This suggests that the odor was moderately acceptable but not particularly pleasant, and that the gel required some effort during application, although it remained manageable.

Lastly, Formulation C showed mean scores of 6.30 for appearance, 6.27 for texture, 6.27 for ease of application, 6.23 for feel on the skin after application, and 6.43 for overall acceptability. These scores indicate that these characteristics were “liked moderately” by the respondents. This implies that the formulation generally appeared visually appealing, had an acceptable texture, was relatively easy to apply, and produced an acceptable sensation on the skin after application. The color and odor of Formulation C had mean scores of 5.70 and 5.57, respectively, corresponding to “liked slightly.” This indicates that the color and odor were somewhat pleasant but may not have been ideal for some respondents.

The results of the hedonic evaluation indicate that all three formulations were generally acceptable to the respondents, with most evaluation parameters falling within the “liked moderately” category.

5. Conclusion

The results showed that among all treatments applied to the crude leaf extract samples, methotrexate (positive control) consistently demonstrated the most pronounced antimitotic effect, yielding the lowest mean mitotic index (MI) values (7.49 and 9.26 in separate trials). In contrast, distilled water (negative control) exhibited the highest MI values (96.10 and 87.46), indicating minimal inhibition of mitotic activity.

For the crude leaf extract topical gel samples, the treatments showed varying degrees of inhibitory effects. Treatment C demonstrated a strong antimitotic effect, with MI values of 9.70 and 12.97, which were comparable to those of methotrexate. Meanwhile, Treatments A and B showed moderate reductions in mitotic activity, with mean MI values ranging from approximately 40.76 to 51.56. These findings suggest that Treatment C possesses significant antimitotic potential comparable to methotrexate, while Treatments A and B exhibited intermediate inhibitory effects. As expected, distilled water showed the least inhibitory effect on mitosis.

Based on the tests performed and the results obtained, the study concluded that 50 mg/mL of anahaw leaf crude extract (Concentration C) exhibited notable antimitotic activity. Additionally, the anahaw leaf crude extract topical gel formulations at concentrations of 12.5 mg/g (Formulation A), 25 mg/g (Formulation B), and 50 mg/g (Formulation C) also demonstrated antimitotic properties. Therefore, the leaf crude extract and topical gel formulation of *Saribus rotundifolius* (Lam. Blume) may serve as a potential alternative source of antimitotic agents.

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The conduct of this study was approved by Saint Gabriel College. All experiments were carried out at the Pharmacy and Research Laboratories of Saint Gabriel College (PRL-SGC) under the strict supervision and guidance of the school authorities. All participants involved in the trial were asked to sign an informed consent form and were informed of the potential risks. Strict supervision and assistance were provided throughout the trial in case of emergencies.

AI Declaration

The authors declare the use of Artificial Intelligence (AI) in writing this paper. In particular, the author used Quillbot and Grammarly in grammar and plagiarism checking. The authors take full responsibility in ensuring proper review and editing of contents generated using AI.

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