Anti-Hyperuricemic Potential of Kalunay (*Amaranthus spinosus Linn*) Extract on Potassium-Oxonate-Induced Hyperuricemia in Albino Mice and Characterization of the Formulated Syrup

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Abstract

Hyperuricemia, a condition characterized by elevated uric acid levels, presents a growing global health concern, particularly among older adults and specific ethnic groups, including Filipinos. Conventional treatments often have significant adverse effects, prompting a search for alternative therapies, particularly from traditional medicinal plants. Kalunay (*Amaranthus spinosus Linn*) is a Philippine herb traditionally used for various ailments and contains bioactive compounds like flavonoids and glycosides, suggesting potential anti-hyperuricemic properties. This study scientifically examined the anti-hyperuricemic potential and safety of Kalunay syrup. The research involved phytochemical screening of the plant extract, acute oral toxicity and in vivo evaluation of its anti-hyperuricemic effects in BALB/c mice, and formulation and evaluation of the resulting syrup. Acute oral toxicity testing established a significant toxicity at 2000 mg/kg, classifying it under GHS Category 4 and suggesting an estimated LD50 below 500 mg/kg. In vivo evaluation on BALB/c mice revealed that Kalunay extract at doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg effectively reduced serum uric acid levels in potassium oxonate-induced hyperuricemic mice, with 100 mg/kg identified as the minimum effective dose. The formulated Kalunay syrup presented a specific gravity (1.214) within typical ranges for sugar-based syrups, a slightly varied pH (pH 6.25 and pH 6) depending on the measurement method but generally acceptable, a pleasant herbal odor consistent with the plant's profile, and some suspended microparticles common in herbal formulations. These findings suggest that Kalunay has significant anti-hyperuricemic potential, warranting further research for safe and effective herbal remedies for hyperuricemia-related disorders like gout.

Keywords: Hyperuricemia; Kalunay *(Amaranthus spinosus Linn)*; anti-hyperuricemic; herbal syrup; gout.

1. Introduction

Hyperuricemia, characterized by elevated levels of uric acid in the blood, is a significant and growing global health concern (Wen et al., 2024). Studies across diverse populations highlight its substantial prevalence and associated risk factors. In the United States, an analysis of National Health and Nutrition Examination Survey (NHANES) data from 2011 to 2020, encompassing 10,175 participants, indicated a hyperuricemia prevalence of 20.7% in the most recent cycle (2017-2020) (Kim et al., 2024). This research identified elevated blood pressure as the strongest associated factor, with a notably weaker association in the Asian population. The highest prevalence was observed in individuals aged 60 and over, alongside a significant increase from 12.6% to 18.2% among the Hispanic population. Similarly, a 2021 cross-sectional study of 48,988 urban Chinese health check-up subjects reported an age- and sex-adjusted hyperuricemia prevalence of 13.6%, revealing a marked gender disparity with 24.3% in men versus 2.6% in women (Feng et al., 2024). Risk factors identified in this cohort included male sex, aging, hypertension, obesity, abdominal obesity, hypertriglyceridemia, and hypercholesterolemia.

In the Philippines, hyperuricemia also poses a considerable public health challenge. A study by Yasay et al. (2014) found prevalence rates of 31.7% in men and 17.3% in women aged 20 years and older, with a mean serum uric acid level of 5.5 mg/dL. Significant predictors for hyperuricemia among Filipino men included hypertension (odds ratio [OR] 1.54), current smoking (OR 1.42), and obesity (OR 2.3). For Filipino women, hypertension (OR 1.88), abnormal creatinine (OR 7.06), obesity (OR 2.6), and menopausal status (OR 2.37) were key risk factors. This chronic elevation of uric acid often leads to gout, which is the most common inflammatory arthritis in the Philippines, affecting 1.6% of the population. Gout predominantly affects males, with an incidence 2.6-fold higher than in females, and its occurrence increases with age, typically plateauing after 70 years. Postmenopausal women are also susceptible as the uricosuric protection offered by estrogen wanes (De Vera et al., 2022).

Uric acid, a natural byproduct of purine metabolism from diet and endogenous production, circulates in the bloodstream as urate at physiological pH (Richette et al., 2019). Normal serum uric acid levels typically range from 1.5 to 6.0 mg/dL in women and 2.5 to 7.0 mg/dL in men, with monosodium urate crystal formation occurring above 6.8 mg/dL (George et al., 2023). Unlike most mammals that further metabolize uric acid to allantoin via uricase, humans and great apes exhibit significantly higher urate levels (240–360 μM in humans compared to 30–50 μM in mice) due to ancestral genetic mutations that silenced the uricase gene (Zhou et al., 2024). The kidneys play a primary role in uric acid elimination, accounting for approximately two-thirds of excretion through urine, with the remaining one-third eliminated via the intestines. This renal excretion is meticulously regulated by glomerular filtration and proximal tubular exchange, where about 90% of filtered uric acid is reabsorbed, largely facilitated by transporters like URAT1 (Li et al., 2020; Wen et al., 2024).

Hyperuricemia, defined by elevated serum uric acid concentrations (typically exceeding 7.0 mg/dL in men, 6.0 mg/dL in women, and 5.5 mg/dL in children), results from either excessive uric acid production, reduced renal excretion, or a combination of both (Abujbara et al., 2022; George et al., 2023). This condition is a significant risk factor for various non-communicable diseases, including gout, renal dysfunction, hypertension,

hyperlipidemia, diabetes, and obesity (Li et al., 2020). Gout, an inflammatory arthritis, manifests as acute, severe joint pain, redness, and swelling, with men being at higher risk due to naturally higher baseline uric acid levels. The pathogenesis of gout involves the deposition of monosodium urate crystals in joints and tissues, often leading to uric acid renal stones and associations with cardiovascular and metabolic disorders. The enzyme xanthine oxidase plays a crucial role in purine metabolism, contributing to this accumulation (Alghamdi et al., 2021).

Risk factors for hyperuricemia are multifactorial, encompassing age, gender, race, genetics, environment, and diet. Dietary habits, particularly the consumption of purine-rich foods, alcohol, and fructose, can increase uric acid production and impair its excretion. Genetic defects, conditions characterized by high cell turnover (e.g., certain cancers), and medical treatments like chemotherapy can also elevate uric acid levels, potentially leading to acute renal failure. Conversely, reduced uric acid excretion is often linked to genetic disorders, kidney insufficiency, and metabolic syndrome, with diseases such as kidney disease, acidosis, and hyperthyroidism impairing urate secretion (George et al., 2023; Li et al., 2020).

Current pharmacological treatments for hyperuricemia and acute gout, while effective, present notable limitations and potential adverse effects. Non-steroidal anti-inflammatory drugs (NSAIDs), commonly used for acute gout, are contraindicated in patients with conditions such as renal impairment, heart failure, or peptic ulcers, and can lead to gastric toxicity and reduced creatinine clearance (Du et al., 2024). Corticosteroids, though effective, may impact blood pressure and glucose levels, posing risks for individuals with hypertension or diabetes (Wen et al., 2024). Colchicine, another alternative, requires careful monitoring due to its narrow therapeutic index (Conley et al., 2023). Furthermore, long-term hyperuricemia management with xanthine oxidase (XO) inhibitors like allopurinol often involves complex dose adjustments to mitigate adverse effects and gout flares, sometimes resulting in suboptimal urate levels and hypersensitivity reactions (FitzGerald et al., 2020). Febuxostat, another XO inhibitor, is associated with increased cardiovascular mortality and can cause liver function abnormalities, diarrhea, headache, nausea, and rash (Li et al., 2020). These persistent limitations and side effects associated with conventional synthetic anti-hyperuricemic agents (Cicero et al., 2021) highlight a critical need for the exploration of alternative and complementary therapeutic approaches, particularly those derived from traditional medicinal plants (Li et al., 2020).

Among the many herbal plants traditionally used for medicinal purposes, *Amaranthus spinosus Linn*, commonly known as “Uray” in Tagalog, “Spiny Amaranth” in English, and “Kalunay” in Iloko, is recognized for its claimed anti-hyperuricemic properties (Gautam & Khedkar, 2024). This plant, belonging to the Amaranthaceae family, has a long history of use in traditional medicine systems such as Ayurveda, Unani, and Folk Medicine for its diverse health benefits (Basu et al., 2019). It is a stout, branched herb prevalent in tropical and subtropical lowlands, including the Philippines (Stuart, 2021). Characterized by ovate leaves with slender petioles and rigid, sharp spines at each leaf node, this plant produces small, greenish flower clusters and develops a strong, often reddish taproot system. Traditionally, *A. spinosus L.* has been utilized as both a vegetable and a medicinal plant (Okeke et al., 2020), underscoring its historical importance in local cultures.

Its medicinal potential is attributed to its rich composition of bioactive compounds, including alkaloids, flavonoids, glycosides, and phenolic acids. Specifically, its stem bark contains betalains, quercetin, kaempferol glycosides, rutin, and 7-p-coumaroyl apigenin 4-O-beta-D-glucopyranoside (Ganjare & Raut, 2019). The presence of these compounds, particularly flavonols like quercetin and kaempferol, is significant given that compounds such as caffeic acid and various flavonols (apigenin, myricetin, quercetin, and kaempferol) are known to inhibit xanthine oxidase, a key enzyme in uric acid production (Ooi et al., 2021). Therefore, the abundance of quercetin and kaempferol in *A. spinosus L.* strongly suggests its potential as a natural anti-hyperuricemic agent, similar to established xanthine oxidase inhibitors.

The BALB/c mouse, an extensively utilized inbred laboratory strain, is characterized by its albino coat, red eyes, small stature, and reduced whiskers (Inotiv, 2024). Developed in the early 20th century by Dr. Halsey J. Bagg, these mice have become a fundamental animal model in various biomedical fields, including immunology, oncology, microbiology, and infectious diseases, as well as pharmacokinetic and pharmacodynamic studies (Inotiv, 2024; River, 2023). Their consistent genetic makeup, well-documented immunological responses, robust immune system, and susceptibility to tumors make them invaluable for investigating disease mechanisms and evaluating therapeutic interventions (Hartmann et al., 2021; Inotiv, 2024; River, 2023).

Potassium oxonate (PO), a potent uricase inhibitor, is widely employed by researchers to induce hyperuricemia in animal models, particularly mice and rats, serving as a valuable alternative to genetically modified animals (Da Silva et al., 2023). This method allows for flexible experimental protocols, with PO administered orally or intraperitoneally at varying doses (e.g., 200-300 mg/kg) over durations ranging from 7 to 14 days, often in conjunction with or without a high-purine diet (Wen et al., 2024). The PO-induced model effectively elevates uric acid levels and can lead to renal injury, making it a critical tool for investigating the pathophysiology of hyperuricemia and evaluating potential anti-hyperuricemic agents (Lin et al., 2022). However, a limitation of this model is that it typically induces only mild hyperuricemia, potentially not fully replicating the severity seen in humans, and associated renal injury may not always present significant changes under light microscopy. Despite these limitations, the PO model remains extensively used for studying both hypertension and hyperuricemia, complementing other models like those involving mutant animals, adenine, ethambutol, or fructose induction (Lin et al., 2022; Su et al., 2020).

The formulation of plant extracts into syrups offers significant advantages for patient administration and compliance, particularly for populations such as children and the elderly who may have difficulty swallowing solid dosage forms (Meshram et al., 2023). Syrups, with their liquid base, can facilitate rapid absorption and potentially improve the bioavailability of active compounds, leading to a quicker onset of action and enhanced therapeutic efficacy, as anticipated for Kalunay (*Amaranthus spinosus L.*) (Kolling & Ghosh, 2021). This dosage form also allows for precise and flexible dosing, catering to individual patient needs, and can mask unpleasant tastes, thereby improving palatability (Wang et al., 2022). Among various preparation methods, percolation is often preferred in commercial settings for its ability to ensure uniform distribution of active ingredients, enhance stability, reduce microbial

contamination, and extend shelf life, while also preserving heat-sensitive components (Kolling & Ghosh, 2021; Zhang et al., 2021).

Despite the recognized traditional uses and documented medicinal properties of Kalunay (*Amaranthus spinosus L.*), comprehensive scientific investigations into its anti-hyperuricemic effects, particularly in formulated pharmaceutical dosage forms like syrup, remain limited. While existing research has focused on isolating and characterizing bioactive compounds such as flavonoids and alkaloids that suggest anti-hyperuricemic potential, there is a notable lack of evidence-based studies confirming its efficacy and safety as an anti-hyperuricemic agent. Therefore, this study aims to bridge this knowledge gap by conducting *in vivo* experiments to evaluate the anti-hyperuricemic activity and potential toxicity of Kalunay (*Amaranthus spinosus L.*), formulated as a syrup. This research seeks to provide scientific validation for the traditional use of Kalunay, thereby contributing to the development of safe and effective herbal remedies for hyperuricemia-related conditions, especially gout.

2. Objectives

This study primarily aims to evaluate the potential of Kalunay *(Amaranthus spinosus L.)* crude extract as an anti-hyperuricemic agent using Bagg Albino (BALB/c) mice. Specifically, the research seeks to determine the extract's minimum toxic dose and Globally Harmonised System (GHS) category via the OECD guidelines for acute oral toxicity testing using the Acute Toxic Class (ATC) method. Moreover, the study intends to identify its lowest effective dosage for anti-hyperuricemic activity, and assess if there is a significant difference in efficacy when compared to the standard reference drug, Allopurinol. Ultimately, the study aims to characterize the formulated syrup in terms of its specific gravity, pH, color, odor, and clarity, and compare these characteristics with the herbal syrup formulation by Landge & Kalyane (2023).

3. Materials and Methods

This section details the specific research materials and methods employed to evaluate the potential anti-hyperuricemic properties of Kalunay *(Amaranthus spinosus L.)* extract, which was formulated as a syrup. It comprehensively covers the entire research process, including the research design, study duration and locale, data gathering tools and procedures, and data treatment methods.

Research Design

The study employed an experimental methodology, a scientific approach designed to investigate cause-and-effect relationships by systematically manipulating independent variables and observing their impact on dependent variables, while diligently controlling extraneous factors to predict or determine outcomes (Okoye & Hosseini, 2024). The independent variables included plant extract concentration, treatment groups, drug administration, the induced hyperuricemia model in test animals, treatment/observation duration, and syrup formulation method. The dependent variables assessed were the toxicity levels of the plant material (determined via acute oral toxicity testing), its anti-hyperuricemic

activity (measured by serum uric acid levels), phytochemical constituents (detected through phytochemical screening), and syrup characteristics (evaluated using a set of parameters).

Duration and Locale of the Study

The study was conducted at the Pharmacy Skills Laboratories (S3501-502), Lorma Colleges-Campus for Health Sciences, Carlatan, City of San Fernando, La Union. The Lorma Nursery-Animal Laboratory served as the site for acclimatization and experimentation on Bagg Albino (BALB/c) mice. This research was carried out from the First Semester through the Second Semester of Academic Year 2024-2025.

Data Gathering Tools

The tools and apparatuses utilized in this study, including mortar and pestle, flasks, beakers, and a rotary evaporator, were obtained from the Pharmacy Skills Laboratories (S3501-502) at Lorma Colleges-Campus for Health Sciences, Carlatan, City of San Fernando, La Union. Following the establishment of the extract's anti-hyperuricemic activity, it was formulated into a syrup, which was then subjected to physicochemical evaluation. This assessment specifically included parameters such as specific gravity, pH, color, taste, odor, viscosity, and clarity.

Collection and Authentication of the Plant Sample

The aerial parts (leaves, stems, and flowers) of Kalunay (*Amaranthus spinosus L.*) were collected from Barangay Lantag, Tagudin, Ilocos Sur, a site chosen due to the plant's abundance and ideal growth conditions. Formal authorization for collection was obtained from the relevant land proprietor or designated caretaker intended for thesis purposes. Following collection, the plant samples were submitted for authentication at the Don Mariano Marcos Memorial State University-North La Union Campus (DMMMSU-NLUC), located in Sapilang, Bacnotan, La Union.

Preparation and Extraction of the Plant Sample

The fresh aerial parts (leaves, stems, and flowers) of Kalunay (*Amaranthus spinosus L.*) were thoroughly washed, garbled, air-dried, weighed, and then ground into a powder. This powdered material was stored in air-tight containers until extraction.

The dried plant powder underwent exhaustive cold maceration using 80% ethanol at room temperature for 3 days, a process repeated 10 times to maximize the extraction of potential antihyperuricemic bioactive compounds (Rahmi et al., 2020). The resulting organic filtrate was concentrated using a rotary evaporator to remove excess ethanol. To confirm complete ethanol removal, an Iodoform Test was performed post-evaporation. The absence of a yellow precipitate indicates no residual ethanol, which was critical to prevent interference with subsequent animal testing.

Phytochemical Screening of the Plant Sample

A confirmatory phytochemical screening of Kalunay (*Amaranthus spinosus L.*) was performed to identify various constituents, specifically alkaloids, flavonoids, saponins, and tannins, which may contribute to the plant's potential anti-hyperuricemic properties. Alkaloids were detected using Dragendorff's, Hager's, Mayer's, and Wagner's tests.

Flavonoids were identified via the Alkaline Reagent test and Lead Acetate test. Saponins were confirmed with the Froth test, and tannins were detected using the Gelatin test (Shaikh & Patil, 2020).

Bagg Albino or BALB/c Mice as Test Animals

A total of thirty-three (33) male Bagg Albino (BALB/c) mice, weighing 25-38 grams and approximately 11 weeks old (Abdullahi et al., 2024), were utilized in this study. Twelve (12) mice were designated for acute oral toxicity testing, while the remaining twenty-one (21) were used for the in-vivo evaluation of the anti-hyperuricemic potential of the *Kalunay (Amaranthus spinosus L.)* ethanol-free plant extract.

Acclimatization of Test Animals

All test animals were subjected to an acclimatization period to facilitate their adaptation to the experimental environment and laboratory conditions, ensuring reliable results. The mice were housed in eleven (11) cages within the Animal Laboratory at Lorma Nursery, Lorma Colleges, Carlatan City of San Fernando, La Union. Four (4) cages were allocated for acute oral toxicity tests, and seven (7) for the in-vivo investigation of anti-hyperuricemic potential. The laboratory maintained a controlled environment at 25 ± 2°C and 50% humidity, free from disturbances.

During acclimatization, the animals received a regular rodent diet consisting of food pellets and grain mix, with distilled water supplied *ad libitum*. They were also provided with appropriate lighting conditions. This acclimatization period lasted for 7-14 days, allowing the mice to fully adapt to their new surroundings before experimental procedures commenced.

Acute Oral Toxicity Testing Based on the OECD Test Guideline 423 (2001)

For acute oral toxicity testing of the ethanol-free plant extract, a total of twelve (12) randomly selected male albino mice were utilized. These mice were divided into four (4) groups, with three (3) mice per group, each receiving a fixed dose level of the extract: 5, 50, 300, and 2000 mg/kg body weight, respectively . Prior to dosing, all test animals underwent a 4-hour fasting period, with access to water *ad libitum*. The protocol allowed for the inclusion of additional mice if necessitated by observed mortality rates related to the compound at any given dosing step.

Test substances were administered at a constant volume, adjusted by varying the dosing preparation concentration. The maximum volume administered to rodents generally did not exceed 1 mL per 100 grams of body weight; however, for aqueous solutions, up to 2 mL per 100 grams of body weight is permissible (OECD Test Guideline 423, 2001). In this study, the maximum administered volume was strictly adhered to 2 mL per 100 grams of body weight, in accordance with the OECD Test Guideline 423 (2001).

The ethanol-free plant extract was administered starting with an initial dose of 300 mg/kg body weight, selected from fixed dose levels of 5, 50, 300, 2000, and exceptionally, 5000 mg/kg, to identify doses inducing signs of toxicity or mortality. Prior to dosing, mice were fasted for 4 hours with *ad libitum* water access. Each animal was weighed, and a single oral dose of the Kalunay (*Amaranthus spinosus L.*) extract, prepared at 10 mg/mL concentration, was delivered via gavage using a stomach tube. The individual animal's dose

(in mg) was calculated by multiplying its weight (in kg) by the target dose (e.g., 300 mg/kg). This modified dose (in mg) was then converted into a volume (in mL) by dividing it by the extract concentration (i.e., 10 mg/mL) (Sexton, 2021). Following administration, food was withheld for an additional 1-2 hours.

Dose adjustments were based on the observed number of moribund or deceased animals at each step, utilizing the remaining fixed dose levels (5, 50, and 2000 mg/kg body weight), following the OECD Test Guideline 423 (2001). If 2-3 out of 3 animals were moribund or died at a given dose, the next dose level was decreased. If 1 out of 3 animals was affected, an additional group of 3 animals was dosed at the same level; if subsequently 2-3 out of these 3 were affected, the dose was decreased, but if 1 out of 3 was affected, the dose was increased. This pattern continued until the lowest dose level of 5 mg/kg body weight was reached. The onset, duration, and severity of toxic signs were meticulously monitored, and subsequent doses were delayed until the survival of previously dosed animals was confirmed.

In Vivo Evaluation of Anti-Hyperuricemic Potential of Kalunay (*Amaranthus spinosus L.*)

For the *in vivo* evaluation of anti-hyperuricemic activity, a total of twenty-one (21) male Bagg Albino (BALB/c) mice were divided into seven (7) groups, each comprising of three (3) mice, including a Negative Control, Positive Control, Normal Control, and four (4) Experimental Groups. Hyperuricemia was induced in all groups except the Normal Control by an intraperitoneal injection of potassium oxonate (250 mg/kg) dissolved in 0.9% normal saline solution, administered one hour before test substance administration. All treatments were given orally and monitored for fourteen (14) days. The groups were designated as follows: Experimental Groups I-V received ethanolic crude Kalunay (*Amaranthus spinosus L.*) extract at oral doses of 50, 100, 200, or 400 mg/kg, respectively (Abdullahi et al., 2024). The Positive Control Group received allopurinol (20 mg/kg orally). The Negative Control (Hyperuricemic) Group received potassium oxonate (250 mg/kg) intraperitoneally and 1% carboxymethyl cellulose (CMC) orally (50 mg/kg). The Normal Control Group received only 1% CMC orally (50 mg/kg) to eliminate vehicle-related variations.

To evaluate the anti-hyperuricemic potential of *Kalunay* (*Amaranthus spinosus* L.), various dosages of its ethanol-free extract were prepared, specifically 50, 100, 200, and 400 mg/kg. These doses were precisely adjusted for each animal's individual weight using the formula:

$$Animal's\ Dose\ =\ Animal's\ Weight\ (in\ kg)\ \times \frac{Drug\ Dose\ (in\ mg)}{1\ kg}$$

Subsequently, the dose in milligrams was converted to its equivalent volume in milliliters using following equation (Sexton, 2021):

$$Animal's\ Dose\ (in\ mL)\ =\ \frac{Modified\ Dose\ (in\ mg)}{Concentration\ of\ the\ Extract\ (in\ mg/mL)}$$

The study incorporated both negative and positive controls. The negative control involved the administration of potassium oxonate, a potent and selective uricase inhibitor, to consistently induce hyperuricemia in the mice. For the positive control, Allopurinol, a xanthine oxidase inhibitor commonly used to reduce uric acid levels, was utilized. Veterinary dosing adjustments, crucial due to species-specific variations, were implemented as per

principles outlined in "Ansel's Pharmaceutical Calculations and Drug Delivery Systems" (11th ed.), by applying the formula:

$$Animal's\ Dose\ =\ Animal's\ Weight\ (in\ kg)\ \times \frac{Drug\ Dose\ (in\ mg)}{1\ kg}$$

Hyperuricemia was experimentally induced in the laboratory mice through an intraperitoneal administration of potassium oxonate at a dose of 250 mg/kg. As a potent uricase inhibitor, potassium oxonate reliably elevated uric acid levels. This induction was consistently performed one hour prior to the administration of any test animals throughout the entire study period. By establishing a controlled hyperuricemic state, the researchers aimed to observe the therapeutic responses of the test animals to varying dosages of the Kalunay (*Amaranthus spinosus L.*) extract. This approach allowed for a direct and quantifiable assessment of the extract's effectiveness in mitigating elevated uric acid levels.

Blood Sampling from the Tail Vein

Blood samples were collected from the tail vein of mice one hour after the final administration of the test substances. This procedure was executed by a licensed veterinarian at Seika Veterinary Clinic, located at Bagulin St., Barangay Ortiz, Naguilian, La Union. The methodology for blood sample preparation and collection was adapted from Lee & Goosens (2019), with minor modifications to align with the specific requirements of this study.

For blood sampling preparation, catheters and syringes were heparinized (1,000 USP units/ml) by aspirating and expelling heparin, ensuring only trace amounts remained. A butterfly catheter was attached to the syringe, keeping the needle shielded. Each mouse was then secured in a clean cloth or conventional holder to ensure comfortable positioning and unrestricted breathing, without constricting external genitalia. An assistant restrained the rodent on a solid surface, allowing its tail to hang freely.

The blood sampling process began by immersing the tail in 42°C water for 40-50 seconds to induce vessel dilation, followed by drying. The rodent's body was rotated to prevent tail twisting. The lateral tail veins were targeted, avoiding the mid-dorsal artery, and the area was cleaned with a 2% chlorhexidine solution. Negative pressure was created in the syringe, and the catheter was inserted into the vein at a shallow angle, approximately 5 cm from the tail tip. Blood was then collected at a rate of about 20 µl/sec, with gentle finger pressure along the vein to facilitate flow. If collection was unsuccessful, the needle was reinserted further up the vein, avoiding multiple penetrations to prevent vein collapse.

Following sample collection, pressure in the syringe was released, and a slight aspiration was performed. Pressure was applied to the insertion site to stop bleeding, the area was cleaned with antiseptic, and the mouse was returned to its cage. Throughout the entire process, strict adherence to maximum blood volume guidelines was maintained, ensuring that the total volume collected did not exceed 15% of the animal's total blood volume within a 14-day period, in consultation with veterinary staff.

Serum Uric Acid Analysis

For serum uric acid analysis, blood samples collected from the mice were allowed to clot at room temperature for approximately one hour without anticoagulant, followed by centrifugation at 3000 rpm for 10 minutes at Seika Veterinary Clinic. The resulting serum

was then submitted to Hi-Precision Diagnostics (2nd Floor CYA Centrum, Military Cut-Off, Kennon Road, Baguio City, Benguet) for comprehensive blood chemistry analysis. The primary focus of this analysis was to determine serum uric acid levels, which were compared against established normal mouse reference values of approximately 30.06-50.4 µmol/L (0.34-0.57 mg/dL) (Bai et al., 2024; Zhou et al., 2024), serving as the main basis for the study's findings.

Formulation of Herbal Syrup

The herbal syrup was formulated using the percolation method, a technique where purified water (or an aqueous phase) is passed through a bed of crystalline sucrose. This process, often regulated by a flowmeter to optimize interaction, requires a cylindrical or conical percolator, coarse sugar for adequate porosity, and a carefully placed cotton plug to ensure proper flow and prevent turbidity. The collected percolate is then agitated with additional sucrose and ingredients to create the final syrup (Desu et al., 2023).

For the simple syrup preparation, a moistened cotton plug was initially placed at the neck of the percolator, followed by 66.7 g of sucrose. A filter paper was positioned atop the sucrose to prevent clogging. Purified water was then slowly poured over the sugar bed, and approximately 60 mL of the percolate (syrup) was collected.

The concentrated ethanol-free Kalunay (*Amaranthus spinosus* L.) extract was gradually added to the prepared simple syrup, ensuring uniform mixing. This final syrup formulation was then subjected to physicochemical property assessment and quality control standards, with comparisons drawn against the herbal syrup formulation described by Landge & Kalyane (2023).

To enhance the syrup's stability and effectiveness, specific excipients were incorporated. Glycerin, acting as a humectant, was added to prevent sugar crystallization and maintain a smooth, consistent texture (Chen et al., 2022). Sodium benzoate was included as a preservative, effectively inhibiting the growth of bacteria, yeast, and other microbes to prevent spoilage and extend the syrup's shelf life (De Carvalho et al., 2024).

Evaluation Tests for Kalunay Syrup

The formulated syrup underwent various evaluation tests to ensure compliance with regulatory requirements, including specific gravity, pH, color, taste, odor, and clarity. For specific gravity, a pycnometer was used to compare the syrup's density to water. The procedure involved weighing the dry, empty pycnometer, then weighing it filled with distilled water (W1), and finally weighing it filled with the formulated syrup (W2). Specific gravity was calculated using the following formula (Landge & Kalyane, 2023):

$$Specific\ Gravity\ = \frac{W2}{W1}$$

$Where$:

$W1\ =\ Weight\ of\ Water$;

$W2\ =\ Weight\ of\ Formulated\ Syrup$

The pH of the syrup was determined using both pH paper (by matching color changes to a reference chart) and a pH meter (by immersing an electrode and recording the digital reading). Color and odor were assessed by visual inspection and olfactory evaluation, respectively. To ensure objectivity, these parameters were additionally evaluated by three independent respondents: one faculty member, one lower-year pharmacy student, and one student from another department at Lorma Colleges, utilizing a structured questionnaire.

Finally, the clarity of the formulated syrup was assessed through visual inspection to ensure it was free from floating particles and contaminants, and by passing a beam of light through the sample solution. Consistent with the color and odor evaluations, clarity was also objectively evaluated by the aforementioned three independent respondents using the structured questionnaire tool.

Data Treatment Methods

Responsible research mandates the meticulous handling of data through both statistical and non-statistical measures to uphold scientific integrity and public trust. Accurate data treatment is crucial, as mishandling can introduce inaccuracies that potentially misrepresent risks, particularly in areas like medical treatments, thereby ensuring the reliability of research findings and safeguarding public health.

For Statement of the Problem Number 1, an Acute Oral Toxicity Test was conducted following the OECD Test Guideline 423 (2001). The minimum toxic concentration of the plant extract was determined using the LD50 cut-off values (in mg/kg body weight) as defined by this guideline, based on the Globally Harmonized System (GHS) classification of the extract's toxicity profile.

For Statement of the Problem Number 2, statistical analysis involved expressing results as mean ± SEM (standard error of the mean) to identify the lowest dosage exhibiting the most effective anti-hyperuricemic activity. This approach allowed for a clear quantitative assessment of dose-response relationships.

For Statement of the Problem Number 3, it was addressed using one-way analysis of variance (ANOVA), followed by Post-hoc analysis and Tukey's test for simultaneous comparisons. These statistical tools were employed to determine if significant differences existed in anti-hyperuricemic efficacy between the *Kalunay (Amaranthus spinosus L.)* extract, Allopurinol, and the control groups (negative and positive), thereby identifying the relationships between the dependent and independent variables.

Finally, for Statement of the Problem Number 4, various physicochemical and organoleptic evaluation tests were performed on the formulated syrup, specifically assessing pH level, specific gravity, color, taste, odor, and clarity. A pH paper and pH meter were used for pH determination, and a pycnometer for specific gravity. For an objective evaluation of the syrup's organoleptic properties (color, odor, and clarity), a structured questionnaire was disseminated to three independent respondents: a Lorma Colleges-College of Pharmacy faculty member, a lower-year Lorma Colleges-College of Pharmacy student, and a student from another Lorma Colleges department.

4. Results

Table 1. Phytochemical Screening Results of Kalunay (*Amaranthus spinosus L.*) Extract

Phytochemical Group	Test Performed	Observation	Result
Alkaloids	**Dragendorff's Test**	Formation of Reddish-Brown Precipitate	Positive (+)
	Hager's Test	Formation of Creamy White Precipitate	Positive (+)
	Mayer's Test	Formation of Creamy White/Yellow Precipitate	Positive (+)
	Wagner's Test	Formation of Brown/Reddish Precipitate	Positive (+)
Flavonoids	**Alkaline Reagent Test**	No Formation of Yellow Color	Negative (-)
	Lead Acetate Test	Formation of Yellow Precipitate	Positive (+)
Saponins	**Froth Test**	Persistent Foam (approx. 10 minutes)	Positive (+)
Tannins	**Gelatin Test**	Formation of White Precipitate	Positive (+)
	Ferric Chloride-Potassium Ferricyanide test	Formation of Deep Blue-Black Colored Solution	Positive (+)

Table 2.1. Acute Oral Toxicity Testing Results at 300 mg/kg Body Weight in 2 Cycles of Administration

Mouse No.	Weigh (in kg)	Calculated Dose (in mL)	Observations (First 24 hrs)
Mouse #1	0.032	0.96	Retching post-administration, normal activity resumed. No observed moribundity or death.
Mouse #2	0.033	0.99	
Mouse #3	0.034	1.02	

Table 2.2. Acute Oral Toxicity Testing Results at 2000 mg/kg Body Weight in 1 Cycle of Administration

Mouse No.	Weigh (in kg)	Calculated Dose (in mL)	Observations (First 24 hrs)	Outcome
Mouse #1	0.032	6.4	Severe respiratory distress, labored breathing, impaired mobility, lack of responsiveness.	Moribund/ Death (approx. 5-7 mins)
Mouse #2	0.033	6.6		
Mouse #3	0.034	6.8		

Table 3. Serum Uric Acid Analysis Results Across Various Treatment Groups Using Mean ± SEM (Standard Error of the Mean)

Treatment Group	Average SUA Level (in µmol/L)	Average SUA Level (in mg/dL)	Interpretation
Group I	55.03	0.62	High
Group II	48.1	0.54	Normal
Group III	42.5	0.48	Normal
Group IV	38.5	0.44	Normal
Group V	40.02	0.45	Normal
Group VI	65.8	0.74	Alert Value High
Group VII	35.2	0.4	Normal

Note. Reference Value: 30.06 - 50.4 µmol/L (0.34 - 0.57 mg/dL)

Table 4.1. Comparative Analysis Results of the Anti-Hyperuricemic Efficacy Among Various Treatment Groups Using One-Way ANOVA

One Factor ANOVA		
Mean	**n**	**Group Description**
55.03	3	Group I: Experimental Group I
48.10	3	Group II: Experimental Group II
42.50	3	Group III: Experimental Group III
38.50	3	Group IV: Experimental Group IV

40.03	3	Group V: Positive Control Group
65.80	3	Group VI: Negative Control Group
35.20	3	Group VII: Normal Control Group
46.5	21	Total
ANOVA Table		
Source	**SS**	**df**
Treatment	2,092.079	6
Error	91.433	14
Total	2,183.512	20

Note. A p value of <0.05 indicates there is a significant difference.

Table 4.2. Post-Hoc Analysis Results of the Significant Differences Among Treatment Groups

Experimental Groups	**Vs. Group V: Positive Control Group**	
	p-value	**Interpretation**
Group I: Experimental Group 1	4.65×10^{-6}	There is a significant difference
Group II: Experimental Group 2	0.0017	There is a significant difference
Group III: Experimental Group 3	0.2568	There is no significant difference
Group IV: Experimental Group 4	0.4746	There is no significant difference

Note. Post-hoc analysis (pairwise t-tests) significance levels (p-values): 0.05 significance level; 0.01 significance level.

Table 4.3. Tukey's Test Results of the Significant Differences Among Treatment Groups

Experimental Groups	**Vs. Group V: Positive Control Group**	
	t-value	**Interpretation**
Group I: Experimental Group 1	7.19	There is a significant difference

Group II: Experimental Group 2	3.87	There is a significant difference
Group III: Experimental Group 3	1.18	There is no significant difference
Group IV: Experimental Group 4	0.73	There is no significant difference

Note. Tukey's test critical values for experimentwise error rate (t-values): 0.05 significance level (3.42); 0.01 significance level (4.30).

Table 5. Physicochemical and Organoleptic Characteristics of the Formulated Kalunay (Amaranthus spinosus L.) Syrup

Characteristics	Reference Standard	Formulated Syrup	Remarks
a. Specific Gravity	0.5-0.6	1.214	Failed
b. pH	pH 5-6	pH 6.26 (using pH meter) pH 6 (using pH paper)	Passed
c. Color	Yellowish Green	Yellowish Green	Passed
d. Odor	Aromatic	Herbal	Failed
e. Clarity	Translucent	Translucent	Passed

Note. Adapted from the formulation of the herbal syrup by Landge & Kalyane (2023).

5. Discussions

Table 1 showcases the acquired results of the confirmatory phytochemical screening that revealed a complex profile of secondary metabolites in the Kalunay (Amaranthus spinosus L.) extract.

Alkaloids were consistently detected across all four tests employed. Specifically, Dragendorff's test resulted in the formation of a reddish-brown precipitate, while Hager's, Mayer's, and Wagner's tests yielded creamy white, creamy white/yellow, and brown/reddish precipitates, respectively. These positive results across multiple tests strongly indicate the presence of alkaloids in the plant extract. The detection of alkaloids in Kalunay (*Amaranthus spinosus L.*) extract is significant for its potential anti-hyperuricemic effects. Alkaloids are known bioactive compounds that can influence various physiological processes, including metabolism and excretion, which are crucial in managing hyperuricemia. Studies, from Cheng-Yuan and Jian-Gang (2023), for instance, have shown that certain alkaloids can modulate enzymes involved in uric acid production, such as xanthine oxidase, thereby

reducing serum urate levels. Thus, the presence of alkaloids in Kalunay extract suggests it may have similar effects, enhancing its potential as a natural remedy for hyperuricemia.

Flavonoids exhibited mixed results. The Lead Acetate test yielded a positive result, indicated by the formation of a yellow precipitate, suggesting the presence of flavonoids. However, the Alkaline Reagent test showed a negative result, as no yellow color was observed, and the solution did not become colorless upon the addition of diluted acid. This discrepancy could be attributed to the specific types of flavonoids present, or the sensitivity differences between the tests. The lack of a positive result in the Alkaline Reagent test may also indicate that the flavonoids present are of a specific concentration that does not react with the alkaline reagent in the standard manner. Despite this discrepancy, Liu et al. (2024) notes that flavonoids are known for their uric acid-lowering effects by inhibiting xanthine oxidase (XO) activity and regulating uric acid transporters. The presence of flavonoids, even in varying forms or concentrations, could contribute to the extract's potential anti-hyperuricemic effects, as seen in studies like the one from Xue et al. (2023) where flavonoids like quercetin and kaempferol significantly reduced serum uric acid levels in hyperuricemic models.

Saponins, characterized by their foaming ability, were confirmed through the Froth test, which resulted in the formation of a persistent foam lasting approximately 10 minutes. This positive result indicates the presence of saponins, which are known for their potential to modulate various biological processes. Jiang et al. (2020) states that saponins are known for their ability to reduce uric acid levels by inhibiting xanthine oxidase (XO) activity and enhancing uric acid excretion by modulating transporters like OAT1 and URAT1. Studies have demonstrated that saponins from various plants, such as quinoa bran and Dioscorea, exhibit anti-hyperuricemic properties by reducing serum uric acid levels and alleviating renal damage (Lin et al., 2023). Similarly, the presence of saponins in Kalunay extract suggests it may have similar therapeutic benefits, contributing to its potential as a natural remedy for hyperuricemia.

Tannins were also detected in the extract. The Gelatin test produced a white precipitate, and the Ferric Chloride-Potassium Ferricyanide test resulted in a deep blue-black colored solution, both confirming the presence of tannins. (Appendix M). Tannins, as described by Hatano et al. (2020), particularly hydrolyzable and condensed tannins, have been identified to inhibit xanthine oxidase (XO) activity, a key enzyme in uric acid production. This inhibitory action can lead to reduced serum uric acid levels, which is beneficial in managing hyperuricemia. Studies on various plant extracts have demonstrated that tannins contribute to anti-hyperuricemic effects by modulating uric acid metabolism (Sutyarso et al., 2020).

These findings collectively support the idea that the Kalunay (*Amaranthus spinosus L.*) extract contains a range of phytochemicals that could contribute to its potential anti-hyperuricemic properties. The presence of alkaloids, flavonoids, saponins, and tannins suggests that the plant may exert its therapeutic effects through multiple mechanisms.

Table 2.1 presents the acute oral toxicity testing results of Kalunay extract in mice at a starting dose of 300 mg/kg, accounting for two (2) sets of administration.

The acute oral toxicity test of the extract at this dose level showed that all three mice experienced retching shortly after being given the extract. This reaction is mild and temporary, as the mice quickly returned to normal activity without showing any unusual or concerning behaviors. This observation suggests an initial physiological response from the extract (Lundstrom et al., 2019), indicating the activation of chemoreceptor trigger zones or mild gastrointestinal irritation (MacDougall & Sharma, 2023). No signs of severe distress, organ failure, or other critical conditions observed within the first 24 hours of monitoring. Additionally, there were no cases of moribund or death during the first four hours of observation.

The retching observed at 300 mg/kg was short and mild. It was consistently observed as initial physiological responses to plant extracts. For example, *Grewia lasiodiscus* root extract caused decreased respiratory rate and abdominal contractions in mice at 100 mg/kg which were also short in duration and non-lethal (Okhale et al., 2008). The brief retching may be potentially linked to saponins or trace alkaloids identified in the existing phytochemical analyses of Kalunay extract, which stimulate mucosal receptors at lower doses before adaptation occurs (Girija et al., 2011).

In *Gnidia stenophylla*, the oral administration of 400 mg/kg caused no gastrointestinal (GIT) discomfort in mice. When 800 mg/kg was administered, mucosal erosion was observed as dose-dependent effects (Nigatu et al., 2017). This parallels the observation of mild signs observed at a lower dose (300 mg/kg). Furthermore, retching may arise from activation of chemoreceptor trigger zones or mild GIT irritation, as observed in copper sulfate-induced emesis models (Nallamadan et al., 2024). These responses are often temporary and do not reflect systemic toxicity.

These findings suggest that the observed retching in mice administered Kalunay extract at 300 mg/kg is a mild reaction to bioactive compounds, consistent with safety profiles of related species and other plant extracts. The absence of any moribund or death at 300 mg/kg in two (2) cycles of administration with a manifestation of retching, suggests that the minimum toxic dose of the Kalunay extract is higher than this initial dose. Adhering to the OECD 423 (2001) guidelines, since no mortality was observed at the initial dose, increasing the dosage from 300 mg/ kg to 2000 mg/kg for the next tests is necessary to determine the median lethal dose (LD50).

Table 2.2 presents the observations and outcomes of the acute oral toxicity test conducted at 2000 mg/kg using the same set of three (3) mice, following the initial testing at 300 mg/kg, which showed no mortality.

It is important to note the calculated volumes administered already exceeded the recommended safety limit of 2 mL per 100 grams of body weight according to OECD Test Guideline 423 (2001). Therefore, severe toxicity and death will occur in the test animals, at this dose level, as a result is expected.

The acute oral toxicity test at 2000 mg/kg showed severe toxicity in all mice within 5-7 minutes. Signs of respiratory distress, labored breathing, responsiveness decreased, impaired mobility, and their death followed shortly afterwards. These signs observed indicate a high degree of acute toxicity at this dosage (Gemeda et al., 2025). These symptoms suggest

a rapid compromise of vital functions, potentially due to major organ failure or severe neurological effects (Nugroho et al., 2020).

Based on Kojima et al. (2022), an oral dose of 2000 mg/kg is a standard limit dose in acute toxicity testing, often used to classify substances as slightly toxic or severely toxic, depending on observed effects and mortality. Studies from Nath and Yadav (2014), Saleem et al. (2017), and Brígido et al. (2021), have shown that substances causing mortality at 2000 mg/kg are considered to have significant acute toxicity, with signs reflecting major organ or neurological compromise.

In summary, the observed signs of acute toxicity at 2000 mg/kg aligns with established toxicological data indicating high toxicity, rapid systemic compromise, and lethality in mice, showing the critical impact on vital functions such as on respiration and on neurological control.

Based on OECD Test Guideline 423 (2001), the minimum toxic dose of Kalunay is classified under GHS Category 4, corresponding to an oral LD50 of >300-2000 mg/kg. The deaths suggest an LD50 cut-off value under 500 mg/kg, as per the guideline's diagram. However, data from similar studies, like Abba et al. (2023), showed no mortality at 2000 mg/kg using a methanolic extract of Kalunay in rats, while Shahrul (2021) reported an aqueous bark extract with an LD50 of 1450 mg/kg in rodents, is classified under GHS Category 4. These findings show the differences in species-specific responses, extract compositions, and plant parts used.

Table 3 presents the statistical findings of the serum uric acid (SUA) levels among seven treatment groups, expressed as mean ± SEM (standard error of the mean). The table presents both the average SUA levels in micromoles per liter (µmol/L) and milligrams per deciliter (mg/dL), with the interpretation of these levels related to the provided reference range. The reference range for SUA levels in mice blood is 30.06 - 50.4 µmol/L (0.34 - 0.57 mg/dL).

Based on this reference range, the treatment groups exhibit varying SUA levels. Among the experimental groups treated with Kalunay extract, Group I (50 mg/kg) presented the highest average SUA level at 55.03 µmol/L (0.62 mg/dL), classified as "High," suggesting that this dosage was not effective in lowering uric acid levels. Groups II (100 mg/kg), III (200 mg/kg), and IV (400 mg/kg) all demonstrated average SUA levels within the normal range: 48.1 µmol/L (0.54 mg/dL), 42.5 µmol/L (0.48 mg/dL), and 38.5 µmol/L (0.44 mg/dL), respectively. These findings imply that Kalunay extract at dosages of 100 mg/kg, 200 mg/kg, and 400 mg/kg potentially possesses anti-hyperuricemic activity in this experimental model, as evidenced by the normalization of SUA levels compared to the hyperuricemic control group.

The Positive Control Group (Group V), treated with allopurinol, also showed a normal average SUA level of 40.02 µmol/L (0.45 mg/dL), indicating the efficacy of the standard drug in managing hyperuricemia. The Negative Control Group (Group VI), which only received potassium oxonate, exhibited a marked elevation average in SUA level of 65.8 µmol/L (0.74 mg/dL), categorized as "Alert Value High," confirming the successful induction of hyperuricemia. While the Normal Control Group (Group VII) displayed a normal average

SUA level of 35.2 µmol/L (0.40 mg/dL), based on the reference value 30.06 - 50.4 µmol/L (0.34 - 0.57 mg/dL).

Considering these results, the three tested dosages of the Kalunay extract (100 mg/kg, 200 mg/kg, and 400 mg/kg) appear to have exerted an anti-hyperuricemic effect, as the average SUA levels in all treated groups decreased but within normal range despite the initial induction of hyperuricemia using potassium oxonate. To determine the minimum effective anti-hyperuricemic dosage among these three groups, the researchers determine the lowest dosage that successfully maintained the SUA levels within the normal range. In this case, the lowest tested dosage was 100 mg/kg administered to Group II, resulting in an average SUA level within the normal range. Although higher doses (200 mg/kg and 400 mg/kg) have also exhibited anti-hyperuricemic effects, the 100 mg/kg dose demonstrated the optimal balance between efficacy and minimal dosage, making it the minimum effective anti-hyperuricemic dosage.

This finding aligns with similar studies on plant extracts, where lower doses effectively normalized uric acid levels while higher doses also showed efficacy but without additional benefits proportional to the increased dosage. For example, extracts from *Marantodes pumilum* leaves at 100 mg/kg significantly reduced serum uric acid (Rahmi et al., 2020). Similarly, *Chaenomeles speciosa* fruit extracts showed dose-dependent hypouricemic effects with doses ranging from 100 to 500 mg/kg reducing uric acid and protecting kidney function (Xu et al., 2024). These corroborations support that plant-based extracts can exert anti-hyperuricemic effects at relatively low dosages, with the minimum effective dose being critical for therapeutic efficiency and safety. Thus, the 100 mg/kg dose of Kalunay extract is consistent with literature findings as the minimal effective anti-hyperuricemic dose that normalizes SUA levels without unnecessary dose escalation.

Table 3.1 presents the one-way ANOVA results of the comparison among the treatment groups regarding their anti-hyperuricemic efficacy.

The data shows a statistically significant difference in serum uric acid (SUA) levels exists among the various treatment groups ($F_{(6,14)} = 53.39$, $p<0.05$), indicating that the treatments had different effects on hyperuricemia, as interpreted from the ANOVA Table. The p-value of 7.54×10^{-9} is substantially below the significance threshold of 0.05, strongly suggesting that at least one treatment group's mean SUA level is significantly different from the others.

Table 4.2 presents the p-values for comparisons of the different experimental groups (Group I - IV) against the positive control group (Group V), based on the results of the Post-hoc analysis (pairwise t-tests), assessing the anti-hyperuricemic efficacy of Kalunay extract against allopurinol.

The focus of the comparisons involved the experimental groups that only yielded a significant difference, particularly Group I (50 mg/kg plant extract) and Group II (100 mg/kg plant extract), against Group V (100 mg/kg allopurinol).

The comparison between Group I and Group V yielded a highly significant p-value of 4.65×10^{-6} ($p<0.05$; $p<0.01$), suggesting a substantial difference in their anti-hyperuricemic activities.Similarly, the p-value between Group II and Group V is 0.0017, indicating a

statistically significant difference ($p<0.05$; $p<0.01$) in their effects on serum uric acid (SUA) levels.

Group I has an average SUA level of 55.03 μmol/L (0.62 mg/dL), classified as "High." Despite the statistically significant difference observed between Group I and the positive control (allopurinol), the elevated SUA level in Group I indicates that this specific dosage of Kalunay extract was ineffective in lowering uric acid levels to classify as within the normal range. The statistical significance suggests that the effect of the 50 mg/kg dosage is different from allopurinol regarding the desired effect in reducing hyperuricemia.

In contrast, Group II, receiving a 100 mg/kg dose of Kalunay extract, has an average SUA level which is within the normal range. While higher doses of Kalunay extract 200 mg/kg and 400 mg/kg, exhibited anti-hyperuricemic effects, the 100 mg/kg dose proved to be the minimum effective dosage, achieving SUA levels within the normal range. This suggests that while the 50 mg/kg dose had a statistically different effect compared to allopurinol, it lacked the therapeutic efficacy to normalize SUA levels in the hyperuricemic mice, highlighting the importance of considering both statistical significance and biological relevance when evaluating drug efficacy.

Particularly, effective uric acid reduction involves dual mechanisms: inhibiting reabsorption (via URAT1 suppression) and enhancing excretion (via OAT1 upregulation) (Y. Lee et al., 2019). At 50 mg/kg, the plant extract's inability to normalize SUA implies insufficient modulation of these transporters, similar to low-dose *Cichorium intybus* extracts that require higher doses to upregulate OAT3 and downregulate ABCG2 (Cheng-Yuan & Jian-Gang, 2023). The observed statistical significance in Group I, based on the results of Post-Hoc Analysis and Tukey's Test, reflects minor pharmacological activity rather than therapeutic relevance. Therefore, while statistically different from the positive control group, the 50 mg/kg dosage did not demonstrate a practical anti-hyperuricemic effect.

On the other hand, comparing Group II (100 mg/kg plant extract) and Group V (100 mg/kg allopurinol) directly, Tukey's test results provide a t-value of 3.87. This obtained t-value indicates a statistically significant difference ($p<0.05$) between the anti-hyperuricemic effects of Kalunay and allopurinol. This implies that while both treatments are effective in lowering uric acid, their efficacies are significantly different under the tested conditions.

Studies on various medicinal plants, including Kalunay, have demonstrated significant anti-hyperuricemic activity in animal models, often attributed to phytochemicals like alkaloids, flavonoids, saponins, tannins, and other constituents like polyphenols and sterols (Prince et al., 2021). Research on Kalunay has documented that these bioactive compounds are known to possess diuretic and metabolic effects, which may contribute to its potential uric acid-lowering activity (Jan et al., 2023; Stuart, 2021). These compounds can inhibit xanthine oxidase or enhance uric acid excretion, mechanisms similar to allopurinol but with potentially different efficacy profiles (Bakar et al., 2018).

Reviews of anti-gout medicinal plants highlight that while some plant extracts can significantly reduce uric acid levels, their efficacy often differs from standard drugs like allopurinol, sometimes being less potent or acting through additional mechanisms (Bakar et al., 2018). Thus, the observed statistical difference in the data aligns with these findings, suggesting that while Kalunay is effective, its anti-hyperuricemic potency is not identical to

allopurinol at the tested doses. Differences in mechanism, bioactive compounds, and potency explain the observed statistical significance in efficacy between the two treatments.

The lowest tested dosage of Kalunay extract at 100 mg/kg, administered to Group II, resulted in an average SUA level that fell well within the defined normal parameters for mice. Although the study also evaluated higher doses of Kalunay extract (200 mg/kg and 400 mg/kg), the 100 mg/kg dose represents the minimum effective dosage, achieving a balance between efficacy in lowering uric acid and the amount of extract administered. This finding underscores a dose-dependent response for the anti-hyperuricemic activity of Kalunay extract. Moreover, this suggests that a threshold dosage is necessary for the Kalunay extract to exert its uric acid-lowering effects.

Studies on plant-based anti-hyperuricemic agents consistently demonstrate dose-dependent effects, where lower doses can be effective but higher doses may yield greater or similar efficacy up to a certain threshold. For example, from the study of Y. Lee et al. (2019), *Alpinia oxyphylla* seed extract at 100–400 mg/kg significantly reduced serum uric acid (SUA) in hyperuricemic rats, while lower doses showed limited efficacy. Similarly, celery (*Apium graveolens* var. *dulce*) seed extracts at 500 mg/kg reduced plasma and urinary uric acid levels effectively, while suboptimal doses may not achieve therapeutic thresholds, as found by Mohamed and Al-Okbi (2008). Further, the finding that 100 mg/kg of Kalunay extract is the minimum effective dose aligns with this pattern, as similar studies on other medicinal plants (e.g., *Phyllanthus niruri*, *Zingiber officinale*), for instance by Bakar et al. (2018), demonstrated significant uric acid-lowering effects at specific threshold doses, with diminishing returns or safety concerns at much higher concentrations.

The concept of a “threshold dosage” is well-supported in phytotherapy literature, for example, by J. Chen et al. (2019), where a minimum effective dose is necessary to achieve the desired pharmacological effect without unnecessary increase in dosage, which could raise the risk of side effects or toxicity. Therefore, in the case of Kalunay, the 100 mg/kg dose achieving normal SUA levels in mice indicates this threshold, and is consistent with findings from other plant-based anti-hyperuricemic interventions.

Table 5 presents the determined evaluation characteristics of the formulated Kalunay syrup in comparison with the established standards by Landge & Kalyane (2023), thus evaluating its potential as a viable herbal syrup formulation.

The physicochemical analysis of the herbal syrup revealed a specific gravity of 1.214, which notably exceeded the reference standard range of 0.5 to 0.6. This significant deviation suggests a higher density in the developed syrup compared to the standard, leading to a "Failed" remark for this characteristic.

Particularly, this measured density is typical for syrups due to their high sugar content. Specific gravity values <1.0 indicate substances less dense than water (e.g., oils: 0.88–0.94), while syrups inherently exceed 1.0 due to dissolved sugars (Bhobet & Baluyot, 2024). The 0.5–0.6 range might mistakenly reference alcohol-based solutions (e.g., ethanol: ~0.79) or misinterpret density units.

Aqueous sucrose solutions at 60% concentration exhibit densities of ~1.286 g/cm³ (Nordzucker, 2022), consistent with the syrup’s measured specific gravity of 1.214 (equivalent to 1.214 g/cm³). Moreover, studies on polyherbal syrups report specific gravities

of 1.205–1.293, such as those by Kumar and Prasan (2013) and Dauda et al. (2023), with other optimal herbal syrup formulations targeting ~1.3, for example, by Nugraha and Satar (2021). With this, the value of 1.214 falls within this expected range. Therefore, the analysis suggests the syrup's specific gravity is typical for its formulation type, and the "failed" designation likely stems from a probable discrepancy with the reference standard rather than a product defect.

In terms of pH, the formulated syrup was evaluated using two methods. A pH meter showed a value of 6.26, falling slightly outside the reference standard range of pH 5-6. However, when assessed using pH paper, the syrup registered a pH of 6, which aligns with the upper limit of the standard. Consequently, the pH characteristic was marked as "Passed," indicating that while the more precise measurement showed a slight difference, a less precise method placed it within the acceptable range.

This discrepancy highlights methodological considerations in pH assessment, as pH paper provides a less precise but practical alternative in resource-limited settings (Kumadoh et al., 2024). Studies, such as those of Goswami and Srivastava (2016) and Sharma et al. (2020), used digital pH meters for precise measurements, similar to the Landge & Kalyane's (2023) protocol. However, pH paper remains a validated qualitative tool in pharmaceutical evaluations (Kumadoh et al., 2024).

Specifically, the measured pH characteristics position the formulated herbal syrup within a slightly acidic to neutral range, a factor crucial for stability and palatability (Noorulla et al., 2024). Medicated syrups are typically formulated within pH 3–6 to ensure drug stability and preservative efficacy (Vázquez-Blanco et al., 2018). For example, a polyherbal syrup, formulated by Goswami and Srivastava (2016), with a pH of 5.87 demonstrated stability and quality, while another hepatoprotective herbal syrup, produced by Gujar et al. (2024), registered a pH of 4.91. These pH values accordingly align with the standard's reference range of pH 5-6.

In summary, slightly acidic to neutral pH (near 6) enhances palatability and minimizes degradation of bioactive compounds (Noorulla et al., 2024). The formulated syrup's pH of 6 (via pH paper) falls within this stability range, supporting its suitability for oral administration.

To further confirm the organoleptic properties, a structured questionnaire was administered to three independent respondents: a pharmacy faculty member, a lower-year pharmacy student, and a student from another department within Lorma Colleges. All three respondents consistently described the syrup's color as "yellowish green" and confirmed the color's uniformity throughout the solution, which matched the reference standard, resulting in a "Passed" assessment.

This consistency suggests a homogenous distribution of the plant extracts within the syrup matrix. The observed uniform coloration aligns with methodologies for evaluating sensory attributes in herbal formulations, such studies on mango leaf syrup (Raj et al., 2024) and carrot-pineapple combination syrups (Rahayu & Sipado, 2024) used structured questionnaires and hedonic scales (1–5 ratings) to assess color, emphasizing homogeneity as a key quality indicator. Similar evaluations of Kalunay-based products., such as that of Prince et al. (2021), reported characteristic green hues due to chlorophyll and flavonoid content.

The odor of the formulated syrup was characterized as “herbal”, differing from the “aromatic” scent specified in the reference standard. This discrepancy led to a "Failed" remark for the odor characteristic, suggesting a notable difference in the volatile compounds present in the developed syrup compared to the standard formulation.

Phytochemical studies on Kalunay, such as that of a local research by Queddeng (2009), confirm the presence of alkaloids, saponins, and glycosides in its roots and leaves, but not aromatic oils or resins, which are often responsible for “aromatic” scents in herbal preparations. This supports the finding that the formulated syrup’s odor is “herbal” rather than “aromatic,” as the volatile compounds present in Kalunay differ from those in standard aromatic formulations. Further, the absence of aromatic oils in the plant sample does not diminish its therapeutic potential, as the active constituents responsible for anti-hyperuricemic effects are unrelated to scent (Prince et al., 2021).

On the other hand, based on the findings of the objective evaluation, the odor of the formulated syrup was unanimously described as "pleasant" and "herbal." This uniformity in olfactory perception highlights the distinct odor profile derived from the phytochemical profile of Kalunay, which lacks strong aromatic compounds but contains bioactive constituents typical of herbal scents (Queddeng, 2009). This is consistent with literature that describes the plant as an herb with a mild, green, or earthy odor, rather than a sweet or spicy aromatic profile (Stuart, 2021), and deviations from the reference standard are common when using different plant species or extraction methods. The acceptability of a “herbal” scent, especially when judged as “pleasant,” is often considered favorable in ethnomedicinal contexts, even if it does not match pharmaceutical standards for “aromatic” syrups (Sharma et al., 2020).

Regarding clarity, all respondents agreed that the syrup was "translucent,” which is consistent with the reference standard, and therefore also "Passed.” However, they also consistently noted the presence of "suspended microparticles of powdered plant material." This observation indicates that while the syrup is translucent and allows light to pass through, as confirmed by both light beam testing and visual inspection, as well as the objective evaluation, it is not entirely free from particulate matter, most likely the undissolved plant components, affecting its overall clarity.

The consistent observation of “suspended microparticles of powdered plant material” is a common issue in plant-based liquid formulations (Tian et al., 2023). Literature on herbal syrups and extracts notes that undissolved plant material can remain as visible particulates, especially when whole plant extracts or powders are used rather than purified compounds (Da Silva et al., 2023; Gomes et al., 2025).

As mentioned previously, Kalunay contains certain bioactive constituents, as confirmed by phytochemical analysis of both leaves and roots (Queddeng, 2009). The presence of suspended microparticles in the syrup likely reflects these undissolved phytochemical-rich plant materials, which are not fully soluble in the syrup matrix (Boro et al., 2023).

Generally, herbal syrups often face challenges in achieving complete clarity due to the complex mixture of active and inert plant constituents (Hajimehdipoor et al., 2021). According to pharmaceutical and nutraceutical formulation literature, such as those of Nerkar

et al. (2023) and Chaudhari et al. (2025), a translucent appearance with some suspended matter is acceptable if it does not indicate contamination or instability and if the product passes light transmission and visual inspection tests.

6. Conclusions

The Kalunay extract demonstrated promising antihyperuricemic potential, effectively reducing serum uric acid (SUA) levels in hyperuricemic mice, with dose-dependent efficacy observed at 400 mg/kg, 200 mg/kg, and 100 mg/kg, with SUA levels of 38.5 µmol/L, 42.5 µmol/L, and 48.1 µmol/L, respectively. While 400 mg/kg achieved the greatest reduction in SUA, the 100 mg/kg dosage was identified as the minimum effective dose, achieving an optimal balance between efficacy and minimal administration. However, the extract exhibited toxicity at doses exceeding 500 mg/kg, emphasizing the critical role of dosage in safety and the need for further investigation into active compounds and varying extraction methods. Furthermore, the formulated Kalunay syrup generally displayed acceptable physicochemical characteristics for a herbal preparation, despite minor deviations in specific gravity and pH measurements, which are attributable to measurement variability and inherent properties of herbal formulations. Overall, these findings collectively support Kalunay's potential as a viable natural remedy for hyperuricemia, with considerations for optimal dosage and formulation stability.

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9. Appendices

Appendix A

TIMETABLE

Tasks/Parts	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
Chapter 1: Introduction									
Chapter 2: Research Methodology									
Plant Authentication and Certification									
Bureau of Animal Industry (BAI) Application for Authorization									
Lorma Colleges-Research Ethics Committee Application for Review and Certification									
Collection of *Amaranthus spinosus L.* (Uray) Aerial Parts									
Extraction of *Amaranthus spinosus L.* (Uray)									
Phytochemical Screening for *Amaranthus spinosus L.* (Uray) Crude Extract									
Acquisition and Acclimatization of Test Animals									
Acute Oral Toxicity Testing									
In Vivo Evaluation of Anti-Hyperuricemic Potential									
Formulation of Syrup									

Evaluations Tests for Formulated Syrup									
Chapter 3: Results and Discussions									
Chapter 4: Conclusions and Recommendations									

Appendix B
MATERIALS, EQUIPMENTS, AND REAGENTS

Plant Specimen	Aerial Plant Parts *Amaranthus spinosus L.* (Leaves, Stems, Flowers)
Laboratory Apparatus and Equipment	Beakers Graduated Cylinders Erlenmeyer Flasks Dry Incubator or Oven Test Tubes Test Tube Racks Droppers Weighing Balance Stirring Rods Knife Mortar and Pestle Sieves Distilled Water Rotary Evaporator Percolator Pycnometer Iron Stand Water Bath
Reagents	Ethanol Dilute Sodium Hydroxide Glacial Acetic Acid Concentrated Sulfuric Acid Potassium Dichromate Solution Dragendorff's Reagent (Potassium Bismuth Iodide) Hager's Reagent (Saturated Picric Acid) Mayer's Reagent (Mercuric-Potassium Iodide) Wagner's Reagent (Iodine in Potassium Iodide) 2% Sodium Hydroxide Solution Dilute Hydrochloric Acid Lead Acetate Solution Ferric Chloride Solution

	Ammonia Methanol Magnesium Ribbon Concentrated Hydrochloric Acid 1% Gelatin Solution 10% Sodium Chloride Solution Potassium Oxonate 1% Carboxymethyl Cellulose 0.9% Normal Saline Solution Sucrose Glycerin Sodium Benzoate Allopurinol

Appendix C

DETAILED PROCEDURES OF THE IDENTIFICATION TESTS FOR THE PRESENCE OF ETHANOL

Test	Reagent	Procedure	Positive (+) Result
Iodoform Test	Dilute Sodium Hydroxide	1. Measure 5 mL of the sample extract. 2. Add a few drops of the Dilute Sodium Hydroxide followed by adding 2 mL of 0.05 M Iodine. 3. Observe the color of the precipitate.	Formation of Yellow Precipitate

Appendix D

DETAILED PROCEDURES OF PHYTOCHEMICAL SCREENING TESTS

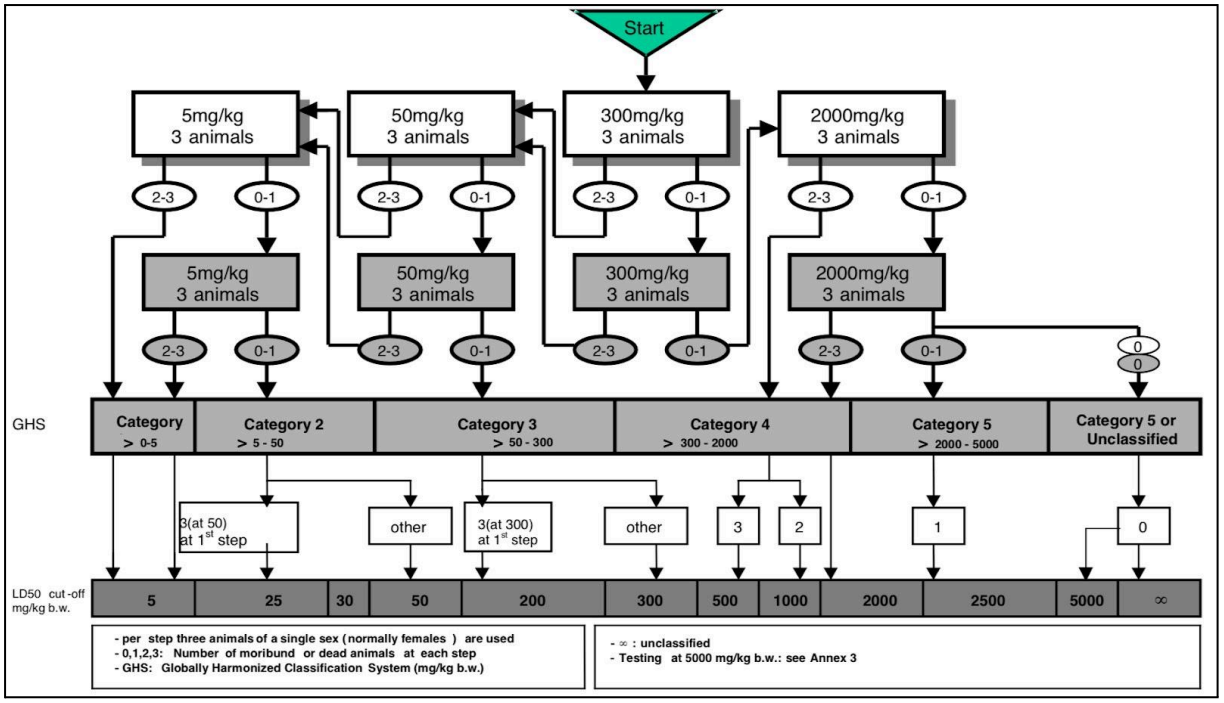
Test for Alkaloids			
Test	**Reagent**	**Procedure**	**Positive (+) Result**
Dragendorff's Test	Dragendorff"s Reagent (Potassium Bismuth Iodide)	1. Measure 2-3 mL of the sample extract. 2. Add a few drops of the reagent. 3. Observe the color of the precipitate.	Formation of Reddish-Brown Precipitate
Hager's Test	Hager's Reagent (Saturated Picric Acid)	1. Measure 2-3 mL of the sample extract. 2. Add a few drops of the reagent. 3. Observe the color of the precipitate.	Formation of Creamy White Precipitate
Mayer's Test	Mayer's Reagent (Mercuric-Potassium Iodide)	1. Measure 2-3 mL of the sample extract. 2. Add a few drops of the reagent. 3. Observe the color of the precipitate.	Formation of Creamy White/Yellow Precipitate
Wagner's Test	Wagner's Reagent (Iodine in Potassium Iodide)	1. Measure 2-3 mL of the sample extract. 2. Add a few drops of the reagent. 3. Observe the color of the precipitate.	Formation of Brown/Reddish Precipitate
Test for Flavonoids			
Test	**Reagent**	**Procedure**	**Positive (+) Result**
Alkaline	2% Sodium	1. Measure 1 mL of the	Formation of Yellow

Reagent Test	Hydroxide Solution + Dilute Hydrochloric Acid	sample extract. 2. Add 2 mL of the 2% Sodium Hydroxide Solution and a few drops of Dilute Hydrochloric Acid. 3. Observe the color of the solution.	Color (becomes colorless on addition of diluted acid)
Lead Acetate Test	Lead Acetate Solution	1. Measure 1 mL of the sample extract. 2. Add a few drops of Lead Acetate Solution. 3. Observe the color of the precipitate.	Formation of Yellow Precipitate
Test for Saponins			
Test	**Reagent**	**Procedure**	**Positive (+) Result**
Froth Test	Distilled Water	1. Dissolve 1 mL of sample extract in 2 mL of water. 2. Vigorously shake the mixture until a stable foam will form.	Persistent Foam (for 10 minutes)
Test for Tannins			
Test	**Reagent**	**Procedure**	**Positive (+) Result**
Gelatin Test	1% Gelatin Solution + 10% Sodium Chloride Solution	1. Dissolve 3 mL of sample extract in 5 mL of water. 2. Add a few drops of 1% Gelatin Solution followed by	Formation of White Precipitate

		adding a few drops of + 10% Sodium Chloride. 3. Observe the color of the precipitate.	
Ferric Chloride-Potassium Ferricyanide test	5% Ferric Chloride Solution	1. Measure 2-3 mL of the sample extract. 2. Add a few drops of 5% Ferric Chloride Solution. 3. Observe the color of the solution.	Formation of Deep Blue-Black Colored Solution

Appendix E

DETAILED DIAGRAM OF OECD ACUTE ORAL TOXICITY TESTING PROCEDURE WITH A STARTING DOSE OF 300 MG/KG BODY WEIGHT



Appendix F

IDENTIFICATION CERTIFICATE OF PLANT MATERIAL

DON MARIANO MARCOS MEMORIAL STATE UNIVERSITY
North La Union Campus
Sapilang, Bacnotan 2515, La Union
COLLEGE OF AGROFORESTRY AND FORESTRY

BAGONG PILIPINAS

IDENTIFICATION CERTIFICATE OF PLANT MATERIAL

RELEASED
NLUC-RECORDS
FO4-1624
DATE: 11/28/2024

This is to certify that **Danielle John A. Untalasco, Maricris Q. Baruela, Irene Mae L. Frigillana, Annaben B. Galvez,** and **Sol Myra D. Gines** of the College of Pharmacy, Lorma Colleges, City of San Fernando, La Union have brought plant species for proper authentic identification. After a thorough and closer examination on the morphological and botanical characteristics of the specimen, it was identified and described as follows.

Common Name - Uray
Scientific Name - *Amaranthus spinosus* Linn.
Family Name - Amaranthaceae

This certification is issued to **Danielle John A. Untalasco, Maricris Q. Baruela, Irene Mae L. Frigillana, Annaben B. Galvez,** and **Sol Myra D. Gines** for all legal intentions and purposes.

Issued this 26th day of November 2024, College of Agroforestry and Forestry, Don Mariano Mariano Marcos Memorial State University, North La Union Campus, Bacnotan, La Union.

Prepared and examined by:

FOR. RUBY ANNE G. OLBINADO
Dendrologist/Faculty, CAFF

Noted:

FOR. JAY MARK G. CORTADO
Dean, CAFF

DR. JUNIFER REY E. TABAFUNDA
Chancellor

WURI The WORLD UNIVERSITY RANKINGS for INNOVATION
PRIME-HRM BRONZE AWARDEE
QS STARS RATING SYSTEM

Appendix G

BAI ANIMAL RESEARCH CLEARANCE

Republic of the Philippines
Department of Agriculture
BUREAU OF ANIMAL INDUSTRY
Visayas Avenue, Brgy. Vasra, Quezon City

ANIMAL RESEARCH CLEARANCE

NAME OF INSTITUTION : **LORMA COLLEGES**	**REFERENCE NO :** *AR - 2024 - 0524*
	DATE/VENUE : November 2024 - April 2025 Lorma Colleges
BUSINESS ADDRESS : Carlatan, San Fernando City, La Union	**LEAD RESEARCHER/VETERINARIAN/IACUC CHAIR:** **Danielle John A. Untalasco** – Lead Researcher **Conrado A. Apusen III, RPh** – Veterinarian/IACUC Chair

Pursuant to the provisions of Republic Act 8485 or the Animal Welfare Act of 1998 as amended by RA 10631 and DA-Administrative Order (AO) No. 40, series of 1999, on the Rules and Regulations on the Scientific Procedure Using Animals, this Permit is hereby issued to **LORMA COLLEGES** after completing the requirements to conduct the research entitled **"The Anti-Hyperuricemic Potential of Uray (*Amaranthus spinosus Linn*) Syrup Extract on Potassium-Oxonate-induced Hyperuricemia in Albino Mice"** on the date and venue stipulated above.

The Institution is hereby reminded to observe the provisions of DA-AO no. 40 s.1999.

Prepared on December 04, 2024

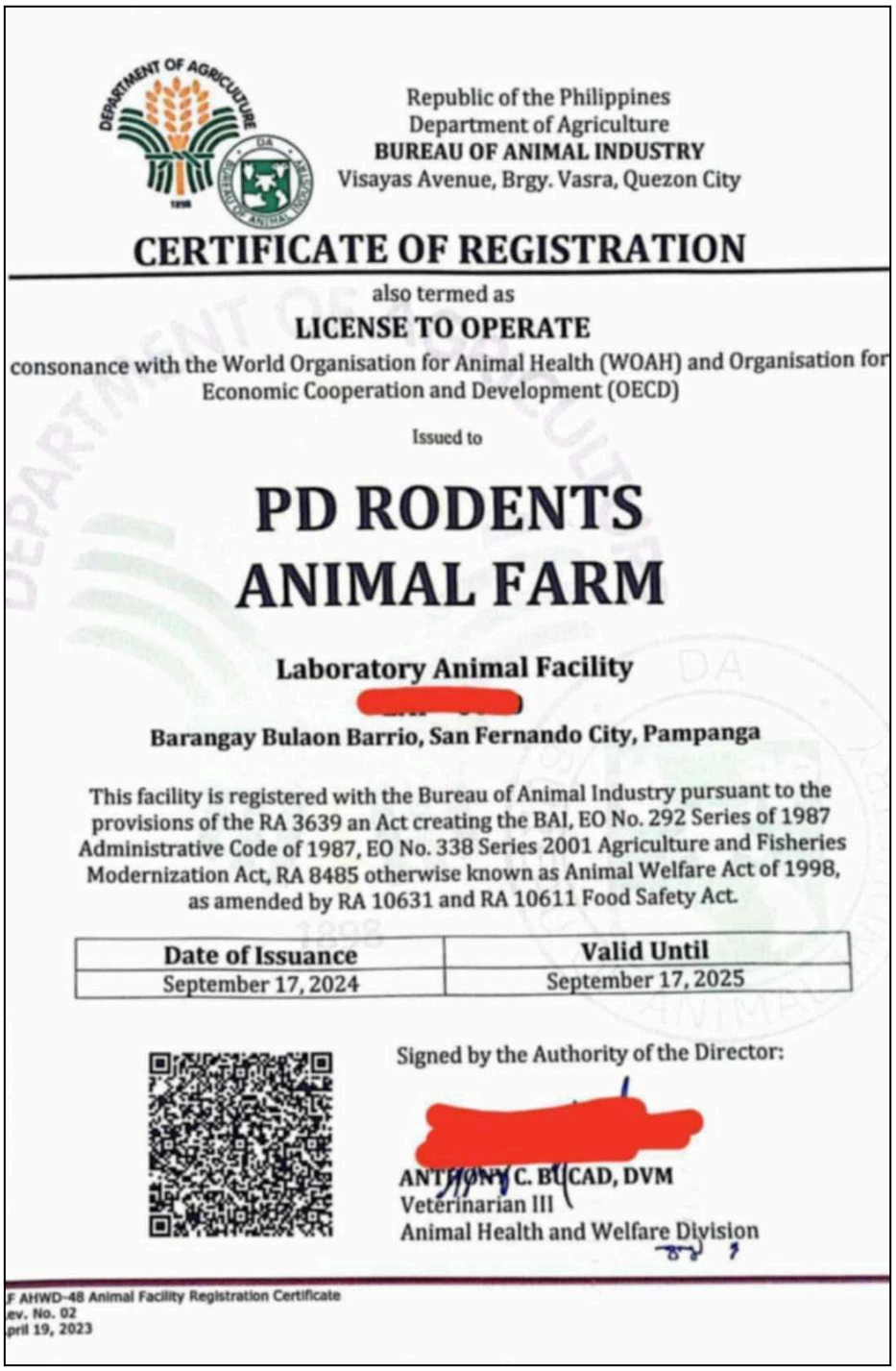
Approved By Authority of the Director

HYACINTH G. NAPILOY, DVM, MPS-PA
Chief,
Animal Health and Welfare Division

RF AHWD-49 Animal Research Clearance
Rev. No. 03
April 19, 2023

Appendix H

BAI CERTIFICATION OF THE SUPPLIER OF THE TEST ANIMALS



Republic of the Philippines
Department of Agriculture
BUREAU OF ANIMAL INDUSTRY
Visayas Avenue, Brgy. Vasra, Quezon City

CERTIFICATE OF REGISTRATION

also termed as

LICENSE TO OPERATE

consonance with the World Organisation for Animal Health (WOAH) and Organisation for Economic Cooperation and Development (OECD)

Issued to

PD RODENTS ANIMAL FARM

Laboratory Animal Facility

[redacted]

Barangay Bulaon Barrio, San Fernando City, Pampanga

This facility is registered with the Bureau of Animal Industry pursuant to the provisions of the RA 3639 an Act creating the BAI, EO No. 292 Series of 1987 Administrative Code of 1987, EO No. 338 Series 2001 Agriculture and Fisheries Modernization Act, RA 8485 otherwise known as Animal Welfare Act of 1998, as amended by RA 10631 and RA 10611 Food Safety Act.

Date of Issuance	Valid Until
September 17, 2024	September 17, 2025

Signed by the Authority of the Director:

[redacted]

ANTHONY C. BUCAD, DVM
Veterinarian III
Animal Health and Welfare Division

F AHWD-48 Animal Facility Registration Certificate
ev. No. 02
pril 19, 2023

Appendix I

LORMA COLLEGES-RESEARCH ETHICS COMMITTEE CERTIFICATION OF EXEMPTION FROM REVIEW

LORMA COLLEGES

LC-REC Form #040
CERTIFICATE OF EXEMPTION FROM REVIEW

CERTIFICATION OF EXEMPTION FROM REVIEW

To: Maricris Baruela, Irene Mae Frigilana, Annaben Galvez, Sol Myra Gines and Danielle John Untalasco

From: LORMA Colleges - Research Ethics Committee

Date: February 13, 2025

This is to certify that the Research Proposal entitled, "ANTI-HYPERURICEMIC POTENTIAL OF KALUNAY *(AMARANTHUS SPINOSUS LINN)* EXTRACT ON POTASSIUM -OXONATE-INDUCED HYPERURICEMIA IN ALBINO MICE AND CHARACTERIZATION OF THE FORMULATED SYRUP" submitted by Maricris Q. Baruela, Irene Mae L. Frigilana, Annaben B. Galvez, Sol Myra D. Gines and Danielle John A. Untalasco of College of Pharmacy has been reviewed by the Research Ethics Committee of LORMA Colleges and found that all ethical considerations are in place to conduct the research in the stated locale of the study. Hence, this research proposal is exempted from review.

RYAN JAY G. MOSTOLES, MASE, RMT
Interim Chairman, LC-REC 2/14/25

Appendix J
CERTIFICATION OF STATISTICIAN

LORMA COLLEGES

GENERAL EDUCATION DEPARTMENT
Campus for Health Sciences
Carlatan, San Fernando City, La Union

May 7, 2025

CERTIFICATION

This is to certify that the research entitled **"Anti-Hyperuricemic Potential of Kalunay (*Amaranthus spinosus Linn)* extract on Potassium-Oxonate-Induced Hyperuricemia in Albino Mice and Characterization of the Formulated Syrup"** by Maricris Q. Baruela, Irene Mae L. Frigillana, Annaben B. Galvez, Sol Myra D. Gines, and Danielle John A. Untalasco, third year students taking the degree Bachelor of Science in Pharmacy of LORMA Colleges, had been checked and statistically analyzed by the undersigned.

This certification is issued to ensure that the institution received quality research work. Signed this 7th day of May, 2025.

JEROME P. VERA
Statistician

Appendix K

QUESTIONNAIRE TOOL FOR EVALUATION OF COLOR, ODOR, & CLARITY

LORMA COLLEGES

Lorma Colleges
Campus for Health Sciences
Carlatan, City of San Fernando, La Union
College of Pharmacy

Evaluation Questionnaire for Formulated Herbal Syrup

Greetings with a LORMA Smile!

We are currently conducting a study titled, "**Anti-Hyperuricemic Potential of Kalunay (*Amaranthus spinosus Linn*) Extract on Potassium-Oxonate-Induced Hyperuricemia in Albino Mice and Characterization of the Formulated Syrup,**" in partial fulfillment of the requirements in the subject PHARMACY RESEARCH METHODS AND THESIS WRITING.

We kindly invite you to participate in the evaluation of our formulated herbal syrup. Your insights and feedback on parameters such as color, taste, odor, and clarity are invaluable to our study. Your responses will significantly contribute to the advancement of our research.

Thank you for your time and cooperation.

Best Regards,
Untalasco, Danielle John A.
Baruela, Maricris Q.
Frigillana, Irene Mae L.
Galvez, Annaben B.
Gines, Sol Myra D.
The Researchers
College of Pharmacy
Lorma Colleges

Instructions for Evaluators: *Please complete each section based on your sensory experience with the syrup. Evaluate the formulated herbal syrup based on the following parameters. Mark the appropriate box for each question and specify the answers if needed.*

Parameter	Question
Color	1. How would you describe the color? ☐ Pale Green ☐ Yellowish Green ☐ Brownish Green ☐ Dark Green
	2. Is the color consistent throughout the syrup? ☐ Yes ☐ No
Odor	1. Is the odor: ☐ Pleasant; ☐ Unpleasant?
	2. How would you describe the overall odor? ☐ Herbal ☐ Aromatic ☐ Fruity ☐ Chemical ☐ Others (please specify):
Clarity	1. How would you describe the overall clarity? ☐ Clear ☐ Cloudy

	☐ Translucent ☐ Opaque ☐ Others (please specify):
	2. Are there any visible particles or sediments? ☐ Yes ○ If yes, please specify: ☐ No
Additional Comments: *Please provide any additional comments, observations, and suggestions regarding the syrup's appearance, taste, or odor.*	

Appendix L
PHOTO-DOCUMENTATION OF THE PREPARATION OF THE PLANT SAMPLE

Preparation of Plant Sample

Collection

Washing

Garbling

Air-Drying

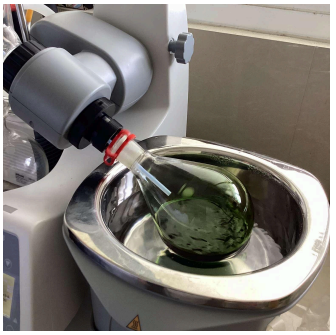
Grinding

Sieving

Storing

Appendix M

PHOTO-DOCUMENTATION OF THE EXTRACTION OF THE PLANT SAMPLE

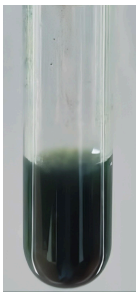
Extraction (Exhaustive Ethanolic Cold Maceration and Rotary Evaporation Technique)		
 Weighing the plant sample	 Cooling the plant extract to be macerated using an ice bath.	 Storing the macerated sample in refrigerator under cold temperature for 3 days
 Gentle swirling/agitation of the macerated sample	 Filtering and plant extraction	 Transferring the plant extract on the Rotary Evaporator to remove excess ethanol
	 Iodoform Test to confirm the presence of Ethanol	

Appendix N

PHOTO-DOCUMENTATION OF PHYTOCHEMICAL SCREENING RESULTS OF THE PLANT EXTRACT

Test for Alkaloids		
Test	**Photo-Documentation**	**Result**
Dragendorff's Test		Positive (+)
Hager's Test		Positive (+)
Mayer's Test		Positive (+)
Wagner's Test		Positive (+)

Test for Flavonoids		
Test	**Photo-Documentation**	**Result**
Alkaline Reagent Test		Negative (-)
Lead Acetate Test		Positive (+)
Test for Saponins		
Test	**Photo-Documentation**	**Result**
Froth Test		Positive (+)

Test for Tannins		
Test	**Photo-Documentation**	**Result**
Gelatin Test		Positive (+)
Ferric Chloride-Potassium Ferricyanide test		Positive (+)

Appendix O

CALCULATION OF MODIFIED DOSES FOR ACUTE ORAL TOXICITY TESTING OF PLANT EXTRACT

Equation		
$Animal's\ Dose\ =\ Animal's\ Weight\ (in\ kg)\ \times \frac{300\ mg}{1\ kg}$ $Animal's\ Dose\ (in\ mL)\ =\ \frac{Modified\ Dose\ (in\ mg)}{Concentration\ of\ the\ Extract\ (in\ mg/mL)}$		
Modified Doses for 300 mg/kg Body Weight		
Mouse 1	**32 g**	$Animal's\ Dose\ =\ 0.032\ (in\ kg)\ \times \frac{300\ mg}{1\ kg} = 9.6\ mg$ $Animal's\ Dose\ (in\ mL)\ =\ \frac{9.6\ mg}{10\ mg/mL} =\ 0.96\ mL$
Mouse 2	**33 g**	$Animal's\ Dose\ =\ 0.033\ (in\ kg)\ \times \frac{300\ mg}{1\ kg} = 9.9\ mg$ $Animal's\ Dose\ (in\ mL)\ =\ \frac{9.9\ mg}{10\ mg/mL} =\ 0.99\ mL$
Mouse 3	**34 g**	$Animal's\ Dose\ =\ 0.034\ (in\ kg)\ \times \frac{300\ mg}{1\ kg} = 10.2\ mg$ $Animal's\ Dose\ (in\ mL)\ =\ \frac{9.6\ mg}{10\ mg/mL} =\ 1.02\ mL$
Modified Doses for 2000 mg/kg Body Weight		
Mouse 1	**32 g**	$Animal's\ Dose\ =\ 0.032\ (in\ kg)\ \times \frac{2000\ mg}{1\ kg} = 64\ mg$ $Animal's\ Dose\ (in\ mL)\ =\ \frac{9.6\ mg}{10\ mg/mL} =\ 6.6\ mL$
Mouse 2	**33 g**	$Animal's\ Dose\ =\ 0.032\ (in\ kg)\ \times \frac{2000\ mg}{1\ kg} = 66\ mg$ $Animal's\ Dose\ (in\ mL)\ =\ \frac{9.6\ mg}{10\ mg/mL} =\ 6.4\ mL$
Mouse 3	**34 g**	$Animal's\ Dose\ =\ 0.032\ (in\ kg)\ \times \frac{2000\ mg}{1\ kg} = 68\ mg$ $Animal's\ Dose\ (in\ mL)\ =\ \frac{9.6\ mg}{10\ mg/mL} =\ 6.8\ mL$

Appendix P

CALCULATION OF MODIFIED DOSES FOR *IN VIVO* EXPERIMENTAL EVALUATION OF ANTIHYPERURICEMIC POTENTIAL

Equation

$$Animal's\ Dose\ =\ Animal's\ Weight\ (in\ kg)\ \times \frac{Drug\ Dose\ (in\ mg)}{1\ kg}$$

$$Animal's\ Dose\ (in\ mL)\ =\ \frac{Modified\ Dose\ (in\ mg)}{Concentration\ of\ the\ Extract\ (in\ mg/mL)}$$

Group I - Experimental Group I (50 mg/kg Plant Extract; 250 mg/kg Potassium Oxonate)

Mouse No.	Weight	Potassium Oxonate	Plant Extract
Mouse 1	28 g	$0.028\ kg\ \times \frac{250\ mg}{1\ kg} = 7\ mg$ $\frac{7\ mg}{10\ mg/mL} = 0.7\ mL$	$0.028\ kg\ \times \frac{50\ mg}{1\ kg} = 1.4\ mg$ $\frac{1.4\ mg}{10\ mg/mL} = 0.14\ mL$
Mouse 2	28 g	$0.028\ kg\ \times \frac{250\ mg}{1\ kg} = 7\ mg$ $\frac{7\ mg}{10\ mg/mL} = 0.7\ mL$	$0.028\ kg\ \times \frac{50\ mg}{1\ kg} = 1.4\ mg$ $\frac{1.4\ mg}{10\ mg/mL} = 0.14\ mL$
Mouse 3	26 g	$0.026\ kg\ \times \frac{250\ mg}{1\ kg} = 6.5\ mg$ $\frac{6.5\ mg}{10\ mg/mL} = 0.65\ mL$	$0.026\ kg\ \times \frac{50\ mg}{1\ kg} = 1.3\ mg$ $\frac{1.3\ mg}{10\ mg/mL} = 0.13\ mL$

Group II - Experimental Group II (100 mg/kg Plant Extract; 250 mg/kg Potassium Oxonate)

Mouse No.	Weight	Potassium Oxonate	Plant Extract
Mouse 1	27 g	$0.027\ kg\ \times \frac{250\ mg}{1\ kg} = 6.75\ mg$ $\frac{6.75\ mg}{10\ mg/mL} = 0.675\ mL$	$0.027\ kg\ \times \frac{100\ mg}{1\ kg} = 2.7\ mg$ $\frac{2.7\ mg}{10\ mg/mL} = 0.27\ mL$
Mouse 2	32 g	$0.032\ kg\ \times \frac{250\ mg}{1\ kg} = 8\ mg$ $\frac{8\ mg}{10\ mg/mL} = 0.8\ mL$	$0.032\ kg\ \times \frac{100\ mg}{1\ kg} = 3.2\ mg$ $\frac{3.2\ mg}{10\ mg/mL} = 0.32\ mL$
Mouse 3	27 g	$0.027\ kg\ \times \frac{250\ mg}{1\ kg} = 6.75\ mg$ $\frac{6.75\ mg}{10\ mg/mL} = 0.675\ mL$	$0.027\ kg\ \times \frac{100\ mg}{1\ kg} = 2.7\ mg$ $\frac{2.7\ mg}{10\ mg/mL} = 0.27\ mL$

Group III - Experimental Group III (200 mg/kg Plant Extract; 250 mg/kg Potassium Oxonate)

Mouse No.	Weight	Potassium Oxonate	Plant Extract
Mouse 1	27 g	$0.027\ kg\ \times \frac{250\ mg}{1\ kg} = 6.75\ mg$	$0.027\ kg\ \times \frac{200\ mg}{1\ kg} = 5.4\ mg$

		$\frac{6.75\ mg}{10\ mg/mL} = 0.675\ mL$	$\frac{5.4\ mg}{10\ mg/mL} = 0.54\ mL$
Mouse 2	**33 g**	$0.033\ kg \times \frac{250\ mg}{1\ kg} = 8.25\ mg$ $\frac{8.25\ mg}{10\ mg/mL} = 0.825\ mL$	$0.033\ kg \times \frac{200\ mg}{1\ kg} = 6.6\ mg$ $\frac{6.6\ mg}{10\ mg/mL} = 0.66\ mL$
Mouse 3	**36 g**	$0.036\ kg \times \frac{250\ mg}{1\ kg} = 9\ mg$ $\frac{9\ mg}{10\ mg/mL} = 0.9\ mL$	$0.036\ kg \times \frac{200\ mg}{1\ kg} = 7.2\ mg$ $\frac{7.2\ mg}{10\ mg/mL} = 0.72\ mL$

Group IV - Experimental Group IV (300 mg/kg Plant Extract; 250 mg/kg Potassium Oxonate)

Mouse No.	Weight	Potassium Oxonate	Plant Extract
Mouse 1	**31 g**	$0.031\ kg \times \frac{250\ mg}{1\ kg} = 7.75\ mg$ $\frac{7.75\ mg}{10\ mg/mL} = 0.775\ mL$	$0.031\ kg \times \frac{300\ mg}{1\ kg} = 9.3\ mg$ $\frac{9.3\ mg}{10\ mg/mL} = 0.93\ mL$
Mouse 2	**36 g**	$0.036\ kg \times \frac{250\ mg}{1\ kg} = 9\ mg$ $\frac{9\ mg}{10\ mg/mL} = 0.9\ mL$	$0.036\ kg \times \frac{300\ mg}{1\ kg} = 10.8\ mg$ $\frac{10.8\ mg}{10\ mg/mL} = 1.08\ mL$
Mouse 3	**37 g**	$0.037\ kg \times \frac{250\ mg}{1\ kg} = 9.25\ mg$ $\frac{9.25\ mg}{10\ mg/mL} = 0.925\ mL$	$0.037\ kg \times \frac{300\ mg}{1\ kg} = 11.1\ mg$ $\frac{11.1\ mg}{10\ mg/mL} = 1.11\ mL$

Group V - Positive Control Group V (100 mg/kg Allopurinol; 250 mg/kg Potassium Oxonate)

Mouse No.	Weight	Potassium Oxonate	Allopurinol
Mouse 1	**37 g**	$0.037\ kg \times \frac{250\ mg}{1\ kg} = 9.25\ mg$ $\frac{9.25\ mg}{10\ mg/mL} = 0.925\ mL$	$0.037\ kg \times \frac{100\ mg}{1\ kg} = 3.7\ mg$ $\frac{3.7\ mg}{10\ mg/mL} = 0.37\ mL$
Mouse 2	**27 g**	$0.027\ kg \times \frac{250\ mg}{1\ kg} = 6.75\ mg$ $\frac{6.75\ mg}{10\ mg/mL} = 0.675\ mL$	$0.027\ kg \times \frac{100\ mg}{1\ kg} = 2.7\ mg$ $\frac{2.7\ mg}{10\ mg/mL} = 0.27\ mL$
Mouse 3	**25 g**	$0.025\ kg \times \frac{250\ mg}{1\ kg} = 6.25\ mg$ $\frac{6.25\ mg}{10\ mg/mL} = 0.625\ mL$	$0.025\ kg \times \frac{100\ mg}{1\ kg} = 2.5\ mg$ $\frac{2.5\ mg}{10\ mg/mL} = 0.25\ mL$

Group VI - Negative Control Group VI (50 mg/kg Carboxymethyl Cellulose; 250 mg/kg Potassium Oxonate)

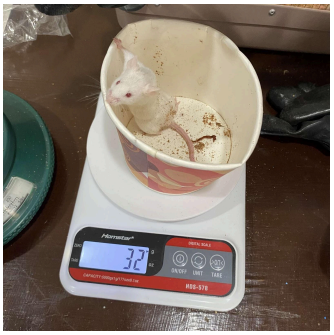
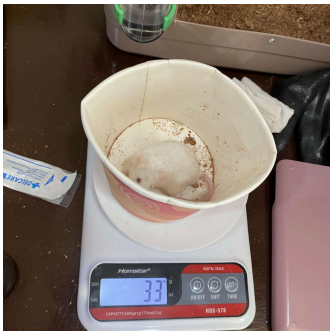
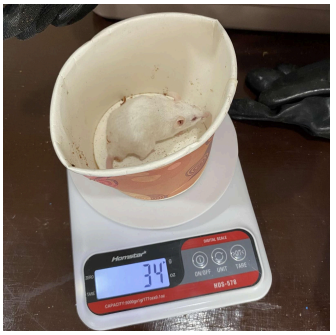
Mouse No.	Weight	Potassium Oxonate	Carboxymethyl Cellulose
Mouse 1	**31 g**	$0.031\ kg \times \frac{250\ mg}{1\ kg} = 7.75\ mg$	$0.031\ kg \times \frac{50\ mg}{1\ kg} = 1.55\ mg$

		$\frac{7.75\ mg}{10\ mg/mL} = 0.775\ mL$	$\frac{1.55\ mg}{10\ mg/mL} = 0.155\ mL$
Mouse 2	**32 g**	$0.032\ kg \times \frac{250\ mg}{1\ kg} = 8\ mg$ $\frac{8\ mg}{10\ mg/mL} = 0.8\ mL$	$0.032\ kg \times \frac{50\ mg}{1\ kg} = 1.6\ mg$ $\frac{1.6\ mg}{10\ mg/mL} = 0.16\ mL$
Mouse 3	**32 g**	$0.032\ kg \times \frac{250\ mg}{1\ kg} = 8\ mg$ $\frac{8\ mg}{10\ mg/mL} = 8\ mL$	$0.032\ kg \times \frac{50\ mg}{1\ kg} = 1.6\ mg$ $\frac{1.6\ mg}{10\ mg/mL} = 0.16\ mL$

Group VII - Normal Control Group VII (50 mg/kg Carboxymethyl Cellulose)		
Mouse No.	**Weight**	**Carboxymethyl Cellulose**
Mouse 1	**32 g**	$0.032\ kg \times \frac{50\ mg}{1\ kg} = 1.6\ mg$ $\frac{1.6\ mg}{10\ mg/mL} = 0.16\ mL$
Mouse 2	**25 g**	$0.025\ kg \times \frac{50\ mg}{1\ kg} = 1.25\ mg$ $\frac{1.25\ mg}{10\ mg/mL} = 0.125\ mL$
Mouse 3	**34 g**	$0.034\ kg \times \frac{50\ mg}{1\ kg} = 1.7\ mg$ $\frac{1.7\ mg}{10\ mg/mL} = 0.17\ mL$

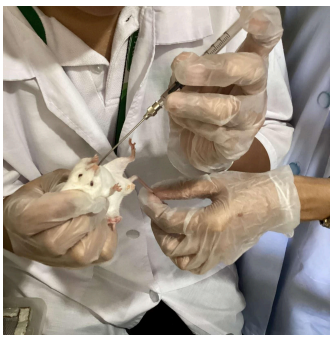
Appendix Q

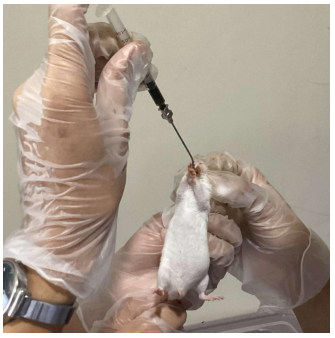
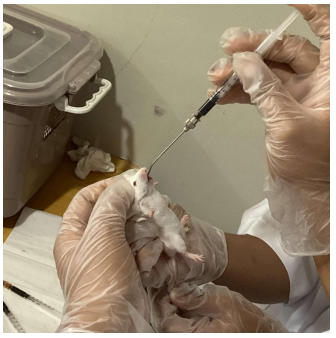
PHOTODOCUMENTATION OF ACUTE ORAL TOXICITY TESTING

Acute Oral Toxicity Testing		
		
Weighing the first set of 3 test animals		
		
Administration of Plant Extract (300 mg/kg)		
		
Administration of Plant Extract (2000 mg/kg)		
		

Appendix R

PHOTODOCUMENTATION OF THE *IN VIVO* EVALUATION OF THE ANTI-HYPERURICEMIC POTENTIAL OF KALUNAY EXTRACT AMONG TREATMENT GROUPS

<table>
<tr><td>  </td><td>  </td><td>  </td></tr>
<tr><td colspan="3">Administration of Potassium Oxonate Solution on the Experimental (Groups 1-4), Positive Control (Group 5), and Negative Control (Group 6) Groups via Intraperitoneal Injection for the Induction of Hyperuricemia</td></tr>
<tr><td>  </td><td>  </td><td>  </td></tr>
<tr><td colspan="3">Administration of Carboxymethylcellulose on the Negative Control (Group 6) and Normal Control (Group 7) Groups via Oral Gavage</td></tr>
<tr><td>  </td><td>  </td><td>  </td></tr>
<tr><td colspan="3">Administration of Allopurinol on the Positive Control (Group 5) Group via Oral Gavage</td></tr>
</table>

		
Administration of the Kalunay Extract on the Experimental (Groups 1-4) Groups via Oral Gavage		

Appendix S

PHOTODOCUMENTATION OF THE BLOOD EXTRACTION PROCESS IN SEIKA VETERINARY CLINIC

Appendix T

PHOTODOCUMENTATION OF THE SERUM URIC ACID ANALYSIS IN HI-PRECISION DIAGNOSTIC LABORATORY

Appendix U

SERUM URIC ACID ANALYSIS RAW DATA

HI-PRECISION diagnostics

2nd Floor CYA Centrum, Military Cut-Off, Kennon Road, Baguio City Benguet

Name : **GROUP 1 (EXPERIMENTAL GROUP)** Lab. Number : **2536009879**
PID : 00 Dispatch : EMAIL
Age : 0 Sex : MALE Page : 1 of 1
Source : REFERRAL (ANIMAL)
Clinician : N/A
Date Requested : 04-14-2025 04:01 PM Received : 04-14-2025 04:22 PM

TEST		Result (SI Unit) Reference Interval		Result (Conv. Unit) Reference Interval	
CHEMISTRY					
Uric Acid (ENZYMATIC)		↑ **55.03** 30.06-50.4	umol/L	↑ **0.62** 0.34-0.57	mg/dL
	Sample 1	**52.5**	umol/L	**0.59**	mg/dL
	Sample 2	**55.1**	umol/L	**0.62**	mg/dL
	Sample 3	**57.5**	umol/L	**0.65**	mg/dL

NOTE : *PAB Accredited test/s.
The results are best interpreted by a healthcare professional and are not intended to be used as the sole means for diagnosis or management.
Specimen rechecked, result/s verified.

LEGEND: ↑ - High ↑↑ - Alert Value High ↓ - Low ↓↓ - Alert Value Low

CH: DIAZ, MARIA ELOISA M. Lic. #0088884 — REGISTERED MEDICAL TECHNOLOGIST
CHUA, RODEL L. Lic. # 0026297 — QUALITY CONTROL
ASUNCION, BABY LYNNE C.,MD Lic. # 0105047 — PATHOLOGIST

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HI-PRECISION diagnostics

2nd Floor CYA Centrum, Military Cut-Off, Kennon Road, Baguio City Benguet

Name : **GROUP 2 (EXPERIMENTAL GROUP)**
PID : 00
Age : 0 Sex : MALE
Source : REFERRAL (ANIMAL)
Clinician : N/A
Date Requested : 04-14-2025 04:01 PM

Lab. Number : **2536009880**
Dispatch : EMAIL
Page : 1 of 1
Received : 04-14-2025 04:22 PM

TEST		Result Reference Interval	(SI Unit)	Result Reference Interval	(Conv. Unit)
CHEMISTRY					
Uric Acid (ENZYMATIC)		**48.1** 30.06-50.4	umol/L	**0.54** 0.34-0.57	mg/dL
	Sample 1	**45.8**	umol/L	**0.52**	mg/dL
	Sample 2	**48.5**	umol/L	**0.55**	mg/dL
	Sample 3	**50.0**	umol/L	**0.57**	mg/dL

NOTE : *PAB Accredited test/s.
The results are best interpreted by a healthcare professional and are not intended to be used as the sole means for diagnosis or management.
Specimen rechecked, result/s verified.

LEGEND: ↑ - High ↑↑ - Alert Value High ↓ - Low ↓↓ - Alert Value Low

CH: DIAZ, MARIA ELOISA M.
Lic. #0088884
REGISTERED MEDICAL TECHNOLOGIST

CHUA, RODEL L.
Lic. # 0026297
QUALITY CONTROL

ASUNCION, BABY LYNNE C.,MD
Lic. # 0105047
PATHOLOGIST

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HI-PRECISION diagnostics

2nd Floor CYA Centrum, Military Cut-Off, Kennon Road, Baguio City Benguet

Name : **GROUP 3 (EXPERIMENTAL GROUP)**
PID : 00
Age : 0 Sex : MALE
Source : REFERRAL (ANIMAL)
Clinician : N/A
Date Requested : 04-14-2025 04:01 PM

Lab. Number : **2536009881**
Dispatch : EMAIL
Page : 1 of 1
Received : 04-14-2025 04:22 PM

TEST		Result (SI Unit) Reference Interval		Result (Conv. Unit) Reference Interval	
CHEMISTRY					
Uric Acid (ENZYMATIC)		**42.5** 30.06-50.4	umol/L	**0.48** 0.34-0.57	mg/dL
	Sample 1	**39.8**	umol/L	**0.45**	mg/dL
	Sample 2	**42.7**	umol/L	**0.48**	mg/dL
	Sample 3	**45.0**	umol/L	**0.51**	mg/dL

NOTE : *PAB Accredited test/s.
The results are best interpreted by a healthcare professional and are not intended to be used as the sole means for diagnosis or management.
Specimen rechecked, result/s verified.

LEGEND: ↑ - High ↑↑ - Alert Value High ↓ - Low ↓↓ - Alert Value Low

CH: DIAZ, MARIA ELOISA M.
Lic. #0088884
REGISTERED MEDICAL TECHNOLOGIST

CHUA, RODEL L.
Lic. # 0026297
QUALITY CONTROL

ASUNCION, BABY LYNNE C.,MD
Lic. # 0105047
PATHOLOGIST

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HI-PRECISION diagnostics

2nd Floor CYA Centrum, Military Cut-Off, Kennon Road, Baguio City Benguet

Name : **GROUP 4 (EXPERIMENTAL GROUP)**
PID : 00
Age : 0 Sex : MALE
Source : REFERRAL (ANIMAL)
Clinician : N/A
Date Requested : 04-14-2025 04:01 PM

Lab. Number : **2536009882**
Dispatch : EMAIL
Page : 1 of 1
Received : 04-14-2025 04:22 PM

TEST		Result (SI Unit) Reference Interval		Result (Conv. Unit) Reference Interval	
CHEMISTRY					
Uric Acid (ENZYMATIC)		**38.5** 30.06-50.4	umol/L	**0.44** 0.34-0.57	mg/dL
	Sample 1	**36.2**	umol/L	**0.41**	mg/dL
	Sample 2	**38.8**	umol/L	**0.44**	mg/dL
	Sample 3	**40.5**	umol/L	**0.46**	mg/dL

NOTE : *PAB Accredited test/s.
The results are best interpreted by a healthcare professional and are not intended to be used as the sole means for diagnosis or management.
Specimen rechecked, result/s verified.

LEGEND: ↑ - High ↑↑ - Alert Value High ↓ - Low ↓↓ - Alert Value Low

CH: DIAZ, MARIA ELOISA M.
Lic. #0088884
REGISTERED MEDICAL TECHNOLOGIST

SYSTEM VALIDATED
QUALITY CONTROL

ASUNCION, BABY LYNNE C.,MD
Lic. # 0105047
PATHOLOGIST

** Report Electronically Signed Out **
HPD

HI-PRECISION diagnostics

2nd Floor CYA Centrum, Military Cut-Off, Kennon Road, Baguio City Benguet

Name : **GROUP 5 (POSITIVE CONTROL GROUP)** Lab. Number : **2536009883**
PID : 00 Dispatch : EMAIL
Age : 0 Sex : MALE Page : 1 of 1
Source : REFERRAL (ANIMAL)
Clinician : N/A
Date Requested : 04-14-2025 04:01 PM Received : 04-14-2025 04:22 PM

TEST		Result Reference Interval	(SI Unit)	Result Reference Interval	(Conv. Unit)
CHEMISTRY					
Uric Acid (ENZYMATIC)		**40.02** 30.06-50.4	umol/L	**0.45** 0.34-0.57	mg/dL
	Sample 1	**37.5**	umol/L	**0.42**	mg/dL
	Sample 2	**40.1**	umol/L	**0.45**	mg/dL
	Sample 3	**42.5**	umol/L	**0.48**	mg/dL

NOTE : *PAB Accredited test/s.
The results are best interpreted by a healthcare professional and are not intended to be used as the sole means for diagnosis or management.
Specimen rechecked, result/s verified.

LEGEND: ↑ - High ↑↑ - Alert Value High ↓ - Low ↓↓ - Alert Value Low

CH: DIAZ, MARIA ELOISA M.
Lic. #0088884
REGISTERED MEDICAL TECHNOLOGIST

SYSTEM VALIDATED
QUALITY CONTROL

ASUNCION, BABY LYNNE C.,MD
Lic. # 0105047
PATHOLOGIST

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HI-PRECISION diagnostics

2nd Floor CYA Centrum, Military Cut-Off, Kennon Road, Baguio City Benguet

Name : **GROUP 6 (NEGATIVE CONTROL GROUP)** Lab. Number : **2536009884**
PID : 00 Dispatch : EMAIL
Age : 0 Sex : MALE Page : 1 of 1
Source : REFERRAL (ANIMAL)
Clinician : N/A
Date Requested : 04-14-2025 04:01 PM Received : 04-14-2025 04:22 PM

TEST		Result (SI Unit) Reference Interval		Result (Conv. Unit) Reference Interval	
CHEMISTRY					
Uric Acid (ENZYMATIC)		↑↑ **65.8** 30.06-50.4	umol/L	↑↑ **0.74** 0.34-0.57	mg/dL
	Sample 1	**62.1**	umol/L	**0.7**	mg/dL
	Sample 2	**65.9**	umol/L	**0.75**	mg/dL
	Sample 3	**69.4**	umol/L	**0.78**	mg/dL

NOTE : *PAB Accredited test/s.
The results are best interpreted by a healthcare professional and are not intended to be used as the sole means for diagnosis or management.
Specimen rechecked, result/s verified.

LEGEND: ↑ - High ↑↑ - Alert Value High ↓ - Low ↓↓ - Alert Value Low

CH: DIAZ, MARIA ELOISA M.
Lic. #0088884
REGISTERED MEDICAL TECHNOLOGIST

SYSTEM VALIDATED
QUALITY CONTROL

ASUNCION, BABY LYNNE C.,MD
Lic. # 0105047
PATHOLOGIST

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HI-PRECISION diagnostics

2nd Floor CYA Centrum, Military Cut-Off, Kennon Road, Baguio City Benguet

Name : **GROUP 7 (NORMAL CONTROL GROUP)**
PID : 00
Age : 0 Sex : MALE
Source : REFERRAL (ANIMAL)
Clinician : N/A
Date Requested : 04-14-2025 04:01 PM

Lab. Number : **2536009885**
Dispatch : EMAIL
Page : 1 of 1
Received : 04-14-2025 04:22 PM

TEST		Result	(SI Unit)	Result	(Conv. Unit)
		Reference Interval		Reference Interval	
CHEMISTRY					
Uric Acid (ENZYMATIC)		**35.2**	umol/L	**0.4**	mg/dL
		30.06-50.4		0.34-0.57	
	Sample 1	**33.1**	umol/L	**0.37**	mg/dL
	Sample 2	**35.5**	umol/L	**0.4**	mg/dL
	Sample 3	**37.0**	umol/L	**0.42**	mg/dL

NOTE : *PAB Accredited test/s.
The results are best interpreted by a healthcare professional and are not intended to be used as the sole means for diagnosis or management.
Specimen rechecked, result/s verified.

LEGEND: ↑ - High ↑↑ - Alert Value High ↓ - Low ↓↓ - Alert Value Low

CH: DIAZ, MARIA ELOISA M.
Lic. #0088884
REGISTERED MEDICAL TECHNOLOGIST

SYSTEM VALIDATED
QUALITY CONTROL

ASUNCION, BABY LYNNE C.,MD
Lic. # 0105047
PATHOLOGIST

** Report Electronically Signed Out **
HPD

Appendix V

RAW DATA OF THE SERUM URIC ACID ANALYSIS RESULTS USING MEAN ± SEM

Group I: Experimental Group I	Individual SUA Level (in µmol/L)	Individual SUA Level (in mg/dL)
Subject 1	52.5	0.59
Subject 2	55.1	0.62
Subject 3	57.5	0.65
Average SUA Level	55.03	0.62
Interpretation	High	
Reference Value	30.06 - 50.4	0.34 - 0.57

Group II: Experimental Group II	Individual SUA Level (in µmol/L)	Individual SUA Level (in mg/dL)
Subject 1	45.8	0.52
Subject 2	48.5	0.55
Subject 3	50	0.57
Average SUA Level	48.1	0.54
Interpretation	Normal	
Reference Value	30.06 - 50.4	0.34 - 0.57

Group III: Experimental Group III	Individual SUA Level (in µmol/L)	Individual SUA Level (in mg/dL)
Subject 1	39.8	0.45
Subject 2	42.7	0.48
Subject 3	45	0.51
Average SUA Level	42.5	0.48
Interpretation	Normal	
Reference Value	30.06 - 50.4	0.34 - 0.57

Group IV: Experimental Group IV	Individual SUA Level (in µmol/L)	Individual SUA Level (in mg/dL)
Subject 1	36.2	0.41
Subject 2	38.8	0.44
Subject 3	40.5	0.46
Average SUA Level	38.5	0.44
Interpretation	Normal	
Reference Value	30.06 - 50.4	0.34 - 0.57

Group V: Positive Control Group	Individual SUA Level (in µmol/L)	Individual SUA Level (in mg/dL)
Subject 1	37.5	0.42
Subject 2	40.1	0.45
Subject 3	42.5	0.48
Average SUA Level	40.02	0.45
Interpretation	Normal	
Reference Value	30.06 - 50.4	0.34 - 0.57

Group VI: Negative Control Group	Individual SUA Level (in µmol/L)	Individual SUA Level (in mg/dL)
Subject 1	62.1	0.7
Subject 2	65.9	0.75
Subject 3	69.4	0.78
Average SUA Level	65.8	0.74
Interpretation	Alert Value High	
Reference Value	30.06 - 50.4	0.34 - 0.57

Group VII: Normal Control Group	Individual SUA Level (in µmol/L)	Individual SUA Level (in mg/dL)
Subject 1	33.1	0.37
Subject 2	35.5	0.4
Subject 3	37	0.42
Average SUA Level	35.2	0.4
Interpretation	Normal	
Reference Value	30.06 - 50.4	0.34 - 0.57

Appendix W

RAW DATA OF THE COMPARATIVE ANALYSIS USING ONE WAY-ANOVA AND POST HOC ANALYSIS AND TUKEY'S TEST OF THE SIGNIFICANT DIFFERENCES AMONG THE TREATMENT GROUPS

One factor ANOVA

Mean	*n*	*Std. Dev*	
55.03	3	2.501	Group I: Experimental Group I
48.10	3	2.128	Group II: Experimental Group II
42.50	3	2.606	Group III: Experimental Group III
38.50	3	2.166	Group IV: Experimental Group IV
40.03	3	2.501	Group V: Positive Control Group
65.80	3	3.651	Group VI: Negative Control Group
35.20	3	1.967	Group VII: Normal Control Group
46.45	21	10.449	Total

ANOVA table

Source	*SS*	*df*	*MS*	*F*	*p-value*	
Treatment	2,092.079	6	348.6798	53.39	7.54E-09	There is a significant Difference
Error	91.433	14	6.5310			
Total	2,183.512	20				

Post hoc analysis

p-values for pairwise t-tests

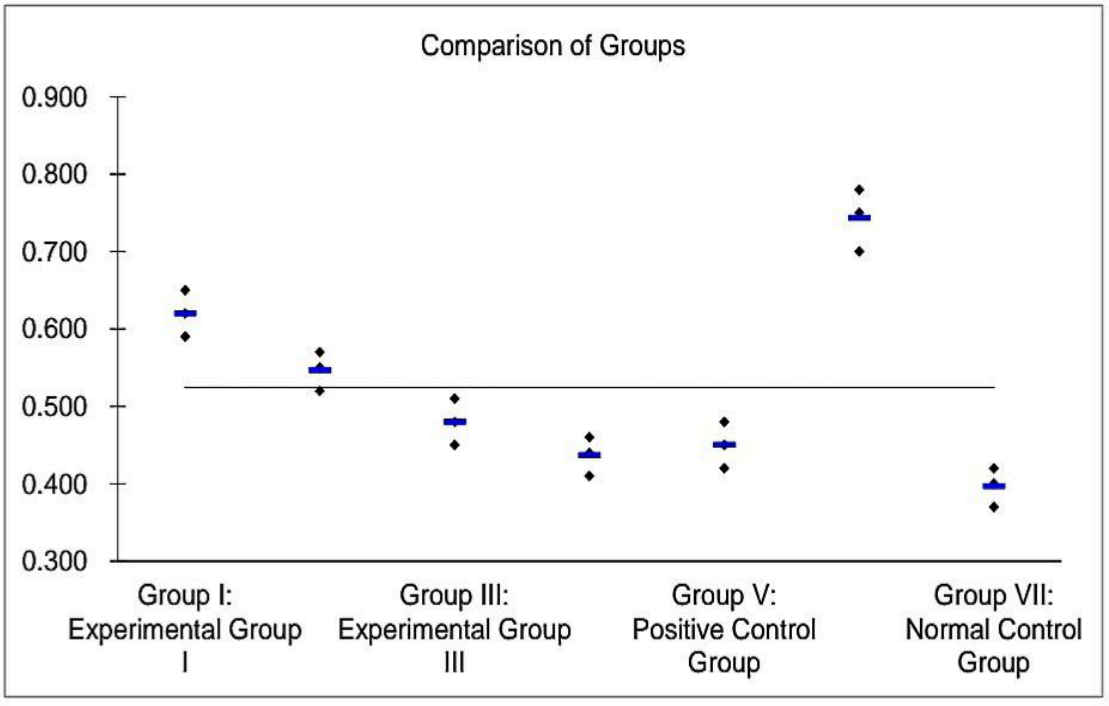
		Group VII: Normal Control Group 35.20	Group IV: Experimental Group IV 38.50	Group V: Positive Control Group 40.03	Group III: Experimental Group III 42.50	Group II: Experimental Group II 48.10	Group I: Experimental Group I 55.03	Group VI: Negative Control Group 65.80
Group VII: Normal Control Group	35.20							
Group IV: Experimental Group IV	38.50	.1361						
Group V: Positive Control Group	40.03	.0362	.4746					
Group III: Experimental Group III	42.50	.0035	.0759	.2568				
Group II: Experimental Group II	48.10	2.38E-05	.0004	.0017	.0178			
Group I: Experimental Group I	55.03	1.75E-07	1.53E-06	4.65E-06	3.22E-05	.0050		
Group VI: Negative Control Group	65.80	6.86E-10	3.06E-09	6.47E-09	2.34E-08	6.88E-07	.0001	

Tukey simultaneous comparison t-values (d.f. = 14)

		Group VII: Normal Control Group	Group IV: Experimental Group IV	Group V: Positive Control Group	Group III: Experimental Group III	Group II: Experimental Group II	Group I: Experimental Group I	Group VI: Negative Control Group
		35.20	38.50	40.03	42.50	48.10	55.03	65.80
Group VII: Normal Control Group	35.20							
Group IV: Experimental Group IV	38.50	1.58						
Group V: Positive Control Group	40.03	2.32	0.73					
Group III: Experimental Group III	42.50	3.50	1.92	1.18				
Group II: Experimental Group II	48.10	6.18	4.60	3.87	2.68			
Group I: Experimental Group I	55.03	9.51	7.92	7.19	6.01	3.32		
Group VI: Negative Control Group	65.80	14.66	13.08	12.35	11.17	8.48	5.16	

critical values for experimentwise error rate:

0.05	3.42
0.01	4.30



One factor ANOVA

Mean	*n*	*Std. Dev*	
0.620	3	0.0300	Group I: Experimental Group I
0.547	3	0.0252	Group II: Experimental Group II
0.480	3	0.0300	Group III: Experimental Group III
0.437	3	0.0252	Group IV: Experimental Group IV
0.450	3	0.0300	Group V: Positive Control Group
0.743	3	0.0404	Group VI: Negative Control Group
0.397	3	0.0252	Group VII: Normal Control Group
0.525	21	0.1183	Total

ANOVA table

Source	*SS*	*df*	*MS*	*F*	*p-value*
Treatment	0.2673	6	0.04454	50.02	1.16E-08
Error	0.0125	14	0.00089		
Total	0.2797	20			

Post hoc analysis

p-values for pairwise t-tests

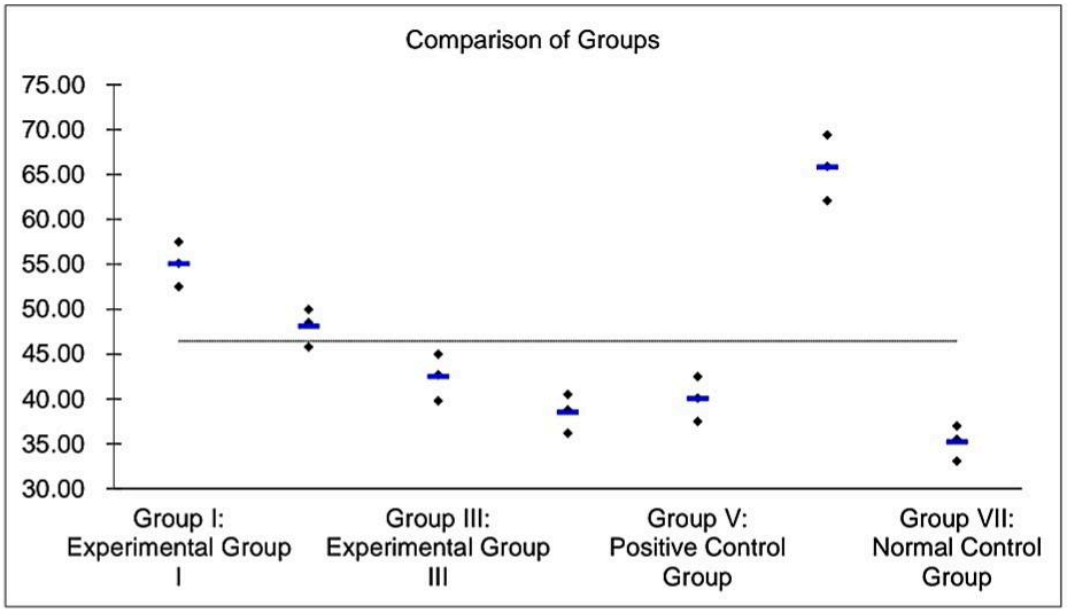
		Group VII: Normal Control Group 0.397	Group IV: Experimental Group IV 0.437	Group V: Positive Control Group 0.450	Group III: Experimental Group III 0.480	Group II: Experimental Group II 0.547	Group I: Experimental Group I 0.620	Group VI: Negative Control Group 0.743
Group VII: Normal Control Group	0.397							
Group IV: Experimental Group IV	0.437	.1229						
Group V: Positive Control Group	0.450	.0460	.5928					
Group III: Experimental Group III	0.480	.0041	.0970	.2385				
Group II: Experimental Group II	0.547	2.49E-05	.0005	.0014	.0161			
Group I: Experimental Group I	0.620	2.72E-07	2.77E-06	6.48E-06	.0001	.0094		
Group VI: Negative Control Group	0.743	1.02E-09	5.06E-09	8.97E-09	3.54E-08	1.23E-06	.0002	

Tukey simultaneous comparison t-values (d.f. = 14)

		Group VII: Normal Control Group	Group IV: Experimental Group IV	Group V: Positive Control Group	Group III: Experimental Group III	Group II: Experimental Group II	Group I: Experimental Group I	Group VI: Negative Control Group
		0.397	0.437	0.450	0.480	0.547	0.620	0.743
Group VII: Normal Control Group	0.397							
Group IV: Experimental Group IV	0.437	1.64						
Group V: Positive Control Group	0.450	2.19	0.55					
Group III: Experimental Group III	0.480	3.42	1.78	1.23				
Group II: Experimental Group II	0.547	6.16	4.51	3.97	2.74			
Group I: Experimental Group I	0.620	9.17	7.52	6.98	5.75	3.01		
Group VI: Negative Control Group	0.743	14.23	12.59	12.04	10.81	8.07	5.06	

critical values for experimentwise error rate:

0.05	3.42
0.01	4.30

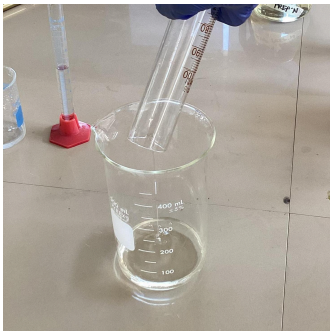


Appendix X

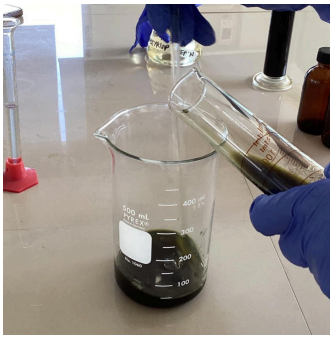
PHOTODOCUMENTATION OF THE FORMULATION OF THE HERBAL SYRUP

Formulation of Herbal Syrup

Collection of Simple Syrup



Pouring the Simple Syrup into a beaker



Addition of the plant extract to the simple syrup and mixing it uniformly

Addition of Glycerin (Humectant)

Addition of Sodium benzoate (Preservative)

Stirring the mixture to ensure uniform mixing

Storing the syrup in a amber-colored bottle

Appendix Y

PHOTODOCUMENTATION OF THE CHARACTERIZATION OF THE HERBAL SYRUP THROUGH PHYSICOCHEMICAL EVALUATION TESTS

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<tr><th colspan="3">Specific Gravity</th></tr>
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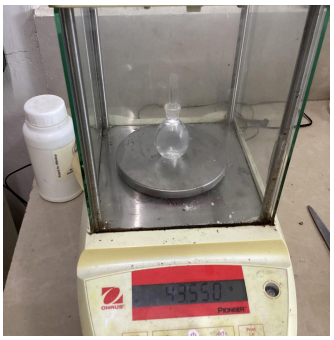
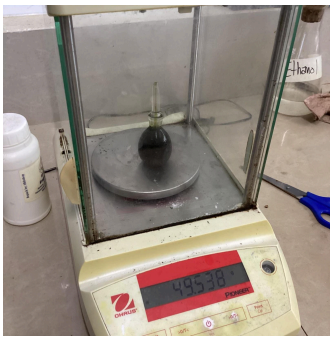
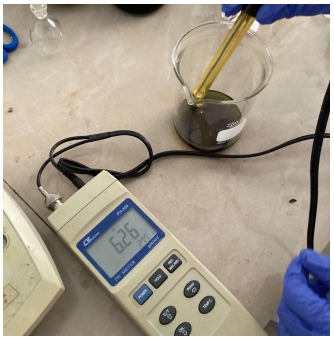
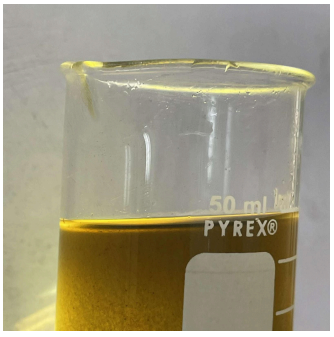
<td> 
Weighing the empty pycnometer</td>
<td> 
Weighing the pycnometer filled with water</td>
<td> 
Weighing the pycnometer filled with the herbal syrup</td>
</tr>
<tr><th colspan="3">pH Strips and pH Meter</th></tr>
<tr>
<td>  </td>
<td> 
</td>
<td>  </td>
</tr>
<tr><th colspan="3">Clarity (via Light Beam Test and Visual Inspection)</th></tr>
<tr>
<td colspan="2"> 
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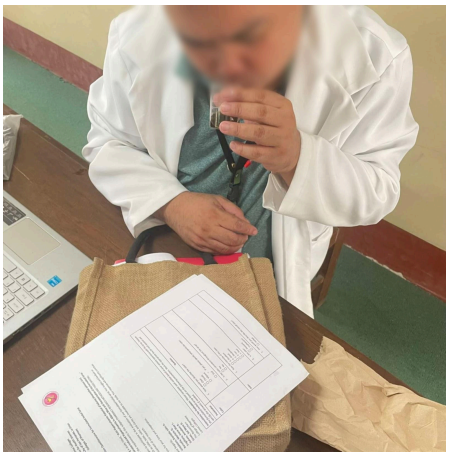
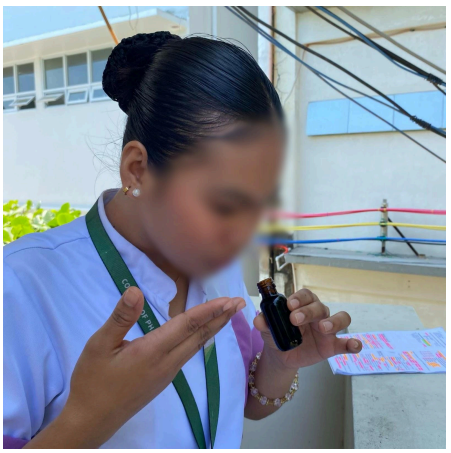
Appendix Z

PHOTODOCUMENTATION OF THE OBJECTIVE EVALUATION OF THE HERBAL SYRUP



One (1) teacher from the faculty of Lorma Colleges-College of Pharmacy



One (1) student enrolled in the Lorma Colleges-College of Pharmacy from the lower year levels

One (1) student from the other departments of Lorma Colleges

Appendix AA

RESULTS OF THE OBJECTIVE EVALUATION TEST

LORMA COLLEGES

Lorma Colleges
Campus for Health Sciences
Carlatan, City of San Fernando, La Union
College of Pharmacy

Evaluation Questionnaire for Formulated Herbal Syrup

Greetings with a LORMA Smile!

We are currently conducting a study titled, "**Anti-Hyperuricemic Potential of Kalunay (*Amaranthus spinosus Linn*) Extract on Potassium-Oxonate-Induced Hyperuricemia in Albino Mice and Characterization of the Formulated Syrup,**" in partial fulfillment of the requirements in the subject PHARMACY RESEARCH METHODS AND THESIS WRITING.

We kindly invite you to participate in the evaluation of our formulated herbal syrup. Your insights and feedback on parameters such as color, taste, odor, and clarity are invaluable to our study. Your responses will significantly contribute to the advancement of our research.

Thank you for your time and cooperation.

Best Regards,
Untalasco, Danielle John A.
Baruela, Maricris Q.
Frigillana, Irene Mae L.
Galvez, Annaben B.
Gines, Sol Myra D.
The Researchers
College of Pharmacy
Lorma Colleges

Instructions for Evaluators: *Please complete each section based on your sensory experience with the syrup. Evaluate the formulated herbal syrup based on the following parameters. Mark the appropriate box for each question and specify the answers if needed.*

Parameter	Question
Color	1. How would you describe the color? ☐ Pale Green ☑ Yellowish Green ☐ Brownish Green ☐ Dark Green
	2. Is the color consistent throughout the syrup? ☑ Yes ☐ No
Odor	1. Is the odor: ☑ Pleasant; ☐ Unpleasant?
	2. How would you describe the overall odor? ☑ Herbal ☐ Aromatic ☐ Fruity ☐ Chemical ☐ Others (please specify):
Clarity	1. How would you describe the overall clarity? ☐ Clear ☐ Cloudy

LORMA COLLEGES

Lorma Colleges
Campus for Health Sciences
Carlatan, City of San Fernando, La Union
College of Pharmacy

	☑ Translucent ☐ Opaque ☐ Others (please specify):
	2. Are there ~~any~~ visible particles or sediments? ☑ Yes o If yes, please specify: small particles ☐ No
Additional Comments: *Please provide any additional comments, observations, and suggestions regarding the syrup's appearance, taste, or odor.*	

Lorma Colleges
Campus for Health Sciences
Carlatan, City of San Fernando, La Union
College of Pharmacy

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The Researchers
College of Pharmacy
Lorma Colleges

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	2. Is the color consistent throughout the syrup? ☑ Yes ☐ No
Odor	1. Is the odor: ☑ Pleasant; ☐ Unpleasant?
	2. How would you describe the overall odor? ☑ Herbal ☐ Aromatic ☐ Fruity ☐ Chemical ☐ Others (please specify):
Clarity	1. How would you describe the overall clarity? ☐ Clear ☐ Cloudy

LORMA COLLEGES

Lorma Colleges
Campus for Health Sciences
Carlatan, City of San Fernando, La Union
College of Pharmacy

	☑ Translucent ☐ Opaque ☐ Others (please specify):
	2. Are there any visible particles or sediments? ☑ Yes o If yes, please specify: fine particles ☐ No
Additional Comments: *Please provide any additional comments, observations, and suggestions regarding the syrup's appearance, taste, or odor.*	

[illegible]

LORMA COLLEGES

Lorma Colleges
Campus for Health Sciences
Carlatan, City of San Fernando, La Union
College of Pharmacy

Evaluation Questionnaire for Formulated Herbal Syrup

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The Researchers
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Parameter	Question
Color	1. How would you describe the color? ☐ Pale Green ☑ Yellowish Green ☐ Brownish Green ☐ Dark Green
	2. Is the color consistent throughout the syrup? ☑ Yes ☐ No
Odor	1. Is the odor: ☑ Pleasant; ☐ Unpleasant?
	2. How would you describe the overall odor? ☑ Herbal ☐ Aromatic ☐ Fruity ☐ Chemical ☐ Others (please specify):
Clarity	1. How would you describe the overall clarity? ☐ Clear ☐ Cloudy

LORMA COLLEGES

Lorma Colleges
Campus for Health Sciences
Carlatan, City of San Fernando, La Union
College of Pharmacy

	☑ Translucent ☐ Opaque ☐ Others (please specify):
	2. Are there any visible particles or sediments? ☑ Yes o If yes, please specify: Small particles ☐ No
Additional Comments: *Please provide any additional comments, observations, and suggestions regarding the syrup's appearance, taste, or odor.*	

10. Authors

This academic endeavor was collaboratively undertaken by a dedicated team of third-year Bachelor of Science in Pharmacy students from Lorma Colleges: Maricris Q. Baruela, Irene Mae L. Frigillana, Annaben B. Galvez, Sol Myra D. Gines, and Danielle John A. Untalasco. Their collective commitment was instrumental in the various phases of this research.

Maricris Q. Baruela, an accomplished Dean's Lister during her first semester (A.Y. 2021-2022), contributed significantly through her foundational understanding of pharmaceutical sciences. Her participation in seminars such as "Safe Animal Handling and Training for Experimental Research and Thesis Writing" (2024) underscored her preparedness for experimental protocols.

Irene Mae L. Frigillana brought a focused approach to the study, strengthened by her attendance at relevant symposia including "Safe Animal Handling and Training for Experimental Research and Thesis Writing" (2024) and "9th IPS Research Hour: Pronisomes for the Skin Delivery of Drugs and Bioactives" (2024), which provided insights into advanced drug delivery systems.

Annaben B. Galvez, a registered nurse with prior experience in the medical field, including roles as a Medical Representative (January 2010 - June 2010), brings a unique perspective and maturity to the team, currently pursuing her BS Pharmacy degree. Her attendance at seminars such as "Safe Animal Handling and Training for Experimental Research and Thesis Writing" (2024) was valuable for methodological precision.

Sol Myra D. Gines, an academically distinguished student with consistent honors since primary education and prior involvement in high school research on alternative products, served as the College of Pharmacy Student Body Organization's 3rd Year Class Representative (2024-Present), demonstrating leadership and a proactive research mindset.

Danielle John A. Untalasco played a pivotal role, marked by an exceptional academic record as a consistent Dean's Lister across multiple academic years (A.Y. 2022-2024) and receiving numerous academic excellence awards with high and highest honors throughout his secondary education (Curriculum Vitae). His extensive background in research, including projects like "State of Happiness: An Intergenerational and Intercultural Study" (A.Y. 2021-2022) and various studies on the acceptability of natural products as alternatives during high school, coupled with his role as a Junior Philippine Pharmacists Association (JPPhA) officer (2022-2023), provided a strong foundation for the study's design and execution. He attended multiple relevant seminars on research writing and scientific inquiry, further enhancing his contributions.

The research was conducted under the expert guidance of Griselle Ann A. Marquez, RPh, CIP, the thesis adviser/faculty researcher and an instructor from the College of Pharmacy at Lorma Colleges. As a Registered Pharmacist and Certified Immunizing Pharmacist, Ms. Marquez’s professional background includes significant experience as a Staff Pharmacist at St. Luke's Medical Center-Quezon City (June 2019 - December 2021) and Lorma Medical Center (February 2015 - February 2018), along with previous faculty appointments. Her extensive involvement in various pharmacy-related seminars and experience advising previous student research projects, such as "Testing the Binding

Properties of Cellulose Obtained from Jute Leaves" and "The Effectiveness of Alugbati Leaf Extract Shampoo on Hair Growth Promotion," were crucial in shaping the study's objectives, methodology, and overall academic excellence.

Collectively, the student researchers contributed their diligent efforts in data collection, analysis, and manuscript preparation, demonstrating a strong grasp of pharmaceutical concepts and research principles. Ms. Marquez provided invaluable mentorship, ensuring the scientific integrity and successful completion of the study. This collaborative synergy between experienced faculty guidance and the students' enthusiastic engagement was fundamental to the accomplishment of this research.