An Experimental Study on the Antihyperuricemic Effect of *Ficus Nota* (Tibig) Leaf Extract in a Hyperuricemic Male Sprague-Dawley Rat Model

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ABSTRACT

Gout, an inflammatory arthropathy associated with substantial global health and economic burdens, primarily results from hyperuricemia—an abnormal elevation of serum uric acid concentration. The limitations of conventional urate-lowering agents in terms of efficacy, safety, and accessibility underscore the need for alternative therapeutic strategies. This study evaluated the antihyperuricemic potential of the ethanolic leaf extract of *Ficus nota* (Tibig) using a potassium oxonate–induced hyperuricemic rat model. Phytochemical analysis confirmed the presence of bioactive constituents within the extract. Statistical evaluation using one-way ANOVA demonstrated a significant reduction in serum uric acid levels among the treatment groups, with *F. nota* extract exhibiting effects comparable to those of allopurinol. The observed antihyperuricemic activity may be attributed to xanthine oxidase inhibition and/or enhanced uric acid excretion. These findings suggest that *F. nota* possesses significant potential as a locally sourced, cost-effective phytotherapeutic candidate for hyperuricemia management. Further studies are warranted to elucidate its underlying mechanisms and long-term safety profile.

Keywords: Allopurinol; Antihyperuricemic activity; Ficus nota; Hyperuricemia; Potassium oxonate; Sprague-Dawley rats

INTRODUCTION

Hyperuricemia is a biochemical state characterized by abnormally elevated serum uric acid and is the principal substrate for gout, the most common inflammatory arthritis worldwide. Sustained hyperuricemia not only precipitates monosodium urate crystal deposition and recurrent gouty flares but is also strongly associated with hypertension, chronic kidney disease, type 2 diabetes mellitus, and cardiovascular events, thereby amplifying its public health impact. Global estimates indicate tens of millions of individuals affected by gout, with age-standardized prevalence rising over recent decades and projected to continue increasing in the coming decades.

The burden of hyperuricemia and gout has grown particularly rapidly in Asia, where demographic transitions, urbanization, obesogenic diets, and sedentary lifestyles have contributed to rising incidence and prevalence. Regional analyses show that East Asia and several rapidly urbanizing economies have experienced marked increases in hyperuricemia and gout, often in parallel with obesity and metabolic syndrome. In the Philippines, rheumatology data suggest a substantial national burden, with millions of individuals living with gout or hyperuricemia despite limited specialist availability and out-of-pocket payment for most chronic care. Local studies highlight suboptimal attainment of target serum urate levels among patients receiving conventional urate-lowering therapy (ULT), underscoring persistent gaps in dosing, adherence, tolerability, and long-term management.

Established pharmacologic options such as xanthine oxidase inhibitors (e.g., allopurinol, febuxostat) are effective for many patients but are constrained by hypersensitivity reactions, the need for cautious dosing in renal impairment, drug interactions, and challenges with sustained adherence to lifelong therapy. In low- and

middle-income settings, these clinical limitations are compounded by economic barriers and fragmented follow-up, leading to recurrent flares, progressive joint damage, and substantial direct and indirect costs for affected households. These realities have stimulated growing interest in plant-derived, mechanism-based antihyperuricemic agents that could serve as complementary or alternative therapies with favorable safety, cost, and cultural acceptability.

The Philippines possesses rich indigenous flora and a longstanding tradition of ethnomedicine, providing a promising source of potential phytotherapeutics. *Ficus nota* (Blanco) Merr., locally known as Tibig, is a native fig species traditionally used in rural communities, where decoctions of its roots and bark are taken for hypertension and urinary tract disorders. Contemporary phytochemical investigations have reported that *F. nota* leaves contain high levels of phenolic acids, flavonoids, and other secondary metabolites associated with antioxidant, anti-inflammatory, and xanthine oxidase–inhibitory effects. In vitro assays have shown that Tibig leaf extracts can inhibit xanthine oxidase, suggesting a capacity to reduce uric acid synthesis via mechanisms analogous to conventional XO inhibitors. However, despite these encouraging in vitro data, there remains a critical gap: the antihyperuricemic efficacy of *F. nota* has not yet been demonstrated in vivo using validated animal models.

Hyperuricemia can be reproducibly induced in rodents by administering potassium oxonate, a selective uricase inhibitor that blocks the conversion of uric acid to allantoin and thereby elevates serum uric acid levels. This model has been widely used to assess the urate-lowering effects of standard ULTs and numerous plant extracts and purified phytochemicals. Sprague–Dawley rats are frequently employed because their physiology is well characterized and they respond predictably to pharmacologic interventions. Within this experimental framework, evaluating the uric acid–lowering effect of *F. nota* leaf extract in potassium oxonate–induced hyperuricemic rats represents a logical next step toward bridging the gap between in vitro evidence and potential clinical application.

In view of the growing burden of hyperuricemia, the limitations of existing therapy, and the preliminary phytochemical and in vitro evidence supporting *Ficus nota*, the present study aimed to evaluate the antihyperuricemic effect of *F. nota*leaf extract in potassium oxonate–induced hyperuricemic male Sprague–Dawley rats.

Theoretical Framework

In 1948, the World Health Organization (WHO) stated health as a complete physical, mental and social well-being and not merely the absence of disease or infirmity.

The primary theories guiding this research include **the Xanthine Oxidase (XO) Inhibition Theory** and **the Free Radical Theory of Oxidative Stress.** These two theories are closely interlinked and provide two different but compatible approaches to understanding how to treat hyperuricemia. The XO Inhibition Theory states that the best method of controlling hyperuricemia is to inhibit the production of uric acid through the inhibition of the XO enzyme that catalytically converts hypoxanthine into xanthine, which is then converted into uric acid. Elevated levels of XO produce increased rates of purine catabolism that lead to elevated levels of serum uric acid, which in turn leads to the precipitation of monosodium urate crystals and subsequent gouty inflammation. Allopurinol has previously demonstrated efficacy in reducing uric acid production through competitive inhibition of XO when metabolized into oxypurinol. However, allopurinol has several limitations, including a risk of hypersensitivity reactions, the need to be carefully dosed in patients with impaired renal function, and the requirement for long-term adherence to therapy.

A complementary theoretical approach to understand how to treat hyperuricemia includes the free radical theory of oxidative stress. XO is a dual-function enzyme: while it produces uric acid, it also produces reactive oxygen species (ROS) during the conversion of purines into uric acid. In hyperuricemic individuals, elevated levels of both urate and ROS contribute to endothelial dysfunction, lipid peroxidation, and the activation of pro-inflammatory signaling pathways, leading to additional joint and organ damage. Compounds with antioxidant properties have the ability to reduce hyperuricemia-related damage through the reduction of oxidative stress by scavenging ROS, enhancing endogenous antioxidant defenses, and modulating downstream inflammatory pathways. Both theories are related in that elevated XO activity is a common upstream mechanism that drives

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both excessive production of uric acid and excessive production of ROS; therefore, a compound that both inhibits XO and reduces oxidative stress could potentially impact both biochemical and inflammatory aspects of the disease. *Ficus nota* (Tibig) is situated within this integrated XO-oxidative stress paradigm. *Ficus nota* extracts contain flavonoids, phenolic compounds, beta-sitosterol, lupeol, and other secondary metabolites known to exhibit XO-inhibitory, antioxidant, and anti-inflammatory activities. Mechanistically, these secondary metabolites can bind to XO and inhibit its enzymatic activity, thus reducing uric acid synthesis in a similar manner to allopurinol, while neutralizing ROS and modulating inflammatory pathways that further exacerbate hyperuricemia-related damage. *Ficus nota* has been used empirically to treat hypertension, urinary or kidney disorders in Filipino traditional medicine and may be recognized to possess cardiovascular and renal benefits that align with contemporary molecular mechanisms underlying hyperuricemia. To assess whether the theoretical proposals outlined above regarding the dual-mechanism action of Tibig extracts in reducing oxidative stress and XO activity are supported by empirical data, this study utilizes a controlled animal model of hyperuricemia induced by potassium oxonate in male Sprague-Dawley rats. If Tibig leaf extract is capable of lowering serum uric acid and improving other hyperuricemia-related measures in this model, it would suggest that the dual-mechanism of action of Tibig extracts (i.e., XO inhibition and antioxidant effect) provides a rational basis for its use as a natural, mechanism-based adjunct or alternative treatment for hyperuricemia.

Conceptual Framework

Ficus nota (Tibig) served as both the primary source of information and the main input for the experiment. Throughout the study, the *F. nota* leaf extract shall undergo various experimental procedures to determine its blood uric acid–lowering potential in male Sprague-Dawley rats induced with hyperuricemia using potassium oxonate.

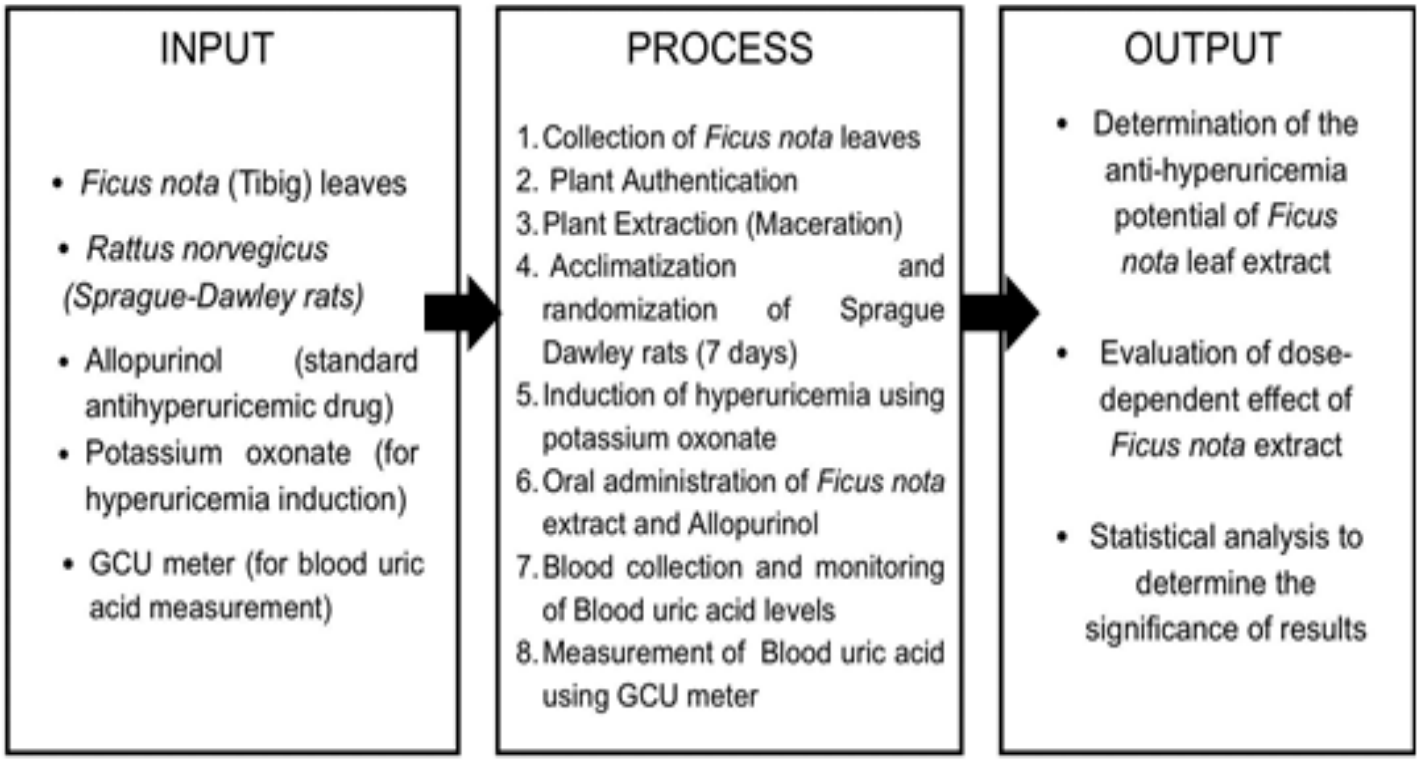

Figure 1. Conceptual Framework of the Study

Figure 1 illustrates the study's framework, showing the systematic approach in evaluating the Antihyperuricemic effect of F. nota (Tibig) leaf extract in Male Sprague-Dawley rats models.

Statement Of The Problems

This study aims to determine the therapeutic effect of *Ficus nota* (Tibig) on blood uric acid in male Sprague-Dawley rats.

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Specifically, this study sought to answer the following questions:

1. What is the percentage yield of *F. nota* leaf extract using ethanol as the solvent?

2. What phytochemical compounds are present in the ethanol extract of *F. nota* leaves?

3. Which treatment group achieves the greatest reduction in blood uric acid levels induced by Potassium Oxonate and as measured by a GCU Meter, compared to both the negative and positive controls?

3.1 Positive Control (Allopurinol)

3.2 Negative group (Carboxymethyl cellulose)

3.3 Extract Treatment Group

Low Dose (100mg/kg *F. nota* Extract)

High Dose (200mg/kg *F. nota* Extract)

Research Hypothesis

The hypotheses were tested at 0.05 level of significance.

The alternative hypothesis is **accepted** since there is a statistically significant difference between the effects of *Ficus nota* extract (100 mg/kg and 200 mg/kg) and the positive control on blood uric acid levels in male Sprague-Dawley rats.

However, the detailed results show that:

- At 100 mg/kg, *F. nota* did not differ significantly from the negative control and did not achieve a clinically relevant reduction in uric acid.

- At 200 mg/kg, *F. nota* produced a significant reduction in blood uric acid and was statistically equivalent to allopurinol (positive control) after 5 days.

So, while the overall test found a significant difference among groups (thus supporting the alternative hypothesis), only the high-dose (200 mg/kg) extract matched the positive control in antihyperuricemic effect, whereas the low-dose (100 mg/kg) did not.

Significance Of The Study

The researchers believe that the result will provide data that are beneficial to the following:

Community and Patients: Individuals suffering from hyperuricemia may benefit from a natural, safe, and cost-effective alternative for managing high uric acid levels. This could improve accessibility to affordable treatment options, especially in communities with limited access to conventional medicines.

Pharmacists and Health Professionals: The findings may aid in the development and promotion of plant-based formulations as potential alternatives for hyperuricemia management. It may also enhance their understanding of the therapeutic potential of *Ficus nota* (Tibig) and its role in integrative medicine.

Scientists and Researchers: This study can provide new insights into the bioactive compounds and pharmacological properties of Tibig leaves, supporting future research on natural xanthine oxidase inhibitors and herbal-based therapies for hyperuricemia.

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Students and Future Researchers: The study can serve as a reference and foundation for those who wish to explore Tibig or other medicinal plants with similar healing properties, encouraging further investigation into the use of local herbs for therapeutic applications.

Pharmaceutical and Herbal Industries: Manufacturers of herbal products and pharmaceuticals may use the generated evidence as a basis for developing standardized Tibig-based formulations, supporting product innovation, quality assurance, and potential commercialization of local plant resources.

Policy Makers and Public Health Agencies: Regulatory bodies and health authorities may draw on the results to inform guidelines, advocacy, and policy initiatives promoting safe, evidence-based use of indigenous medicinal plants as adjuncts or alternatives in the management of hyperuricemia, particularly in resource-limited settings.

Scope And Limitation

This study is confined to evaluating the antihyperuricemic activity of the ethanol leaf extract of *Ficus nota* (Tibig) in male Sprague-Dawley rats. It aims to determine the effectiveness of the extract at various concentrations compared with a positive control (a standard anti-gout drug) and a negative control. The inclusion of all rats available at the time of experimentation ensures consistency and reliability of data, while the use of a standard anti-gout drug allows for comparison with a scientifically recognized treatment.

However, the study does not extend to human trials or to other animal species, as it solely focuses on the Sprague-Dawley rats model. Additionally, other plant parts such as the fruit, bark, or roots of *F. nota*, as well as other solvents for extraction, are excluded to maintain uniformity in the sample preparation. Furthermore, this research does not include the long-term toxicity, safety assessment, or evaluation of potential side effects of the extract, as it is limited to short-term antihyperuricemic testing. The isolation and identification of specific bioactive compounds responsible for the observed effects are also beyond the study's scope. Therefore, the results are primarily intended to provide preliminary scientific evidence on the potential of *F. nota* leaf extract as an antihyperuricemic agent under controlled experimental conditions.

Definition Of Terms

The following terms were defined to avoid ambiguity and to guide the readers in interpreting the findings of this research:

Allopurinol – A standard medication used to lower uric acid levels in the blood by inhibiting xanthine oxidase, an enzyme responsible for uric acid production.

Ethanolic Extract – The concentrated solution obtained after soaking plant material in ethanol to extract its active compounds.

***Ficus nota* (Tibig)** – A native Philippine plant belonging to the family Moraceae, used in this study for its potential uric acid–lowering activity.

Hyperuricemia – A medical condition characterized by an abnormally high level of uric acid in the blood, which can lead to gout and other metabolic disorders.

Induction – The process of artificially producing a specific condition or disease in experimental animals, such as hyperuricemia, for research purposes.

Pharmacological Effect – The observable biological response or change that occurs after the administration of a substance or extract.

Plant Authentication – The process of scientifically verifying and confirming the identity of a plant species used in research

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Potassium Oxonate – A chemical compound used to induce hyperuricemia in experimental animals by inhibiting the enzyme uricase.

Reagents – Substances or chemicals used in laboratory experiments to cause reactions or detect the presence of other substances.

Sprague-Dawley rats– A breed of albino laboratory rats commonly used in biomedical research due to their docile nature and predictable growth patterns.

Therapeutic Effect – Refers to the blood uric acid–lowering activity of *Ficus nota* (Tibig) extract that helps reduce elevated uric acid levels in hyperuricemic rats.

Uric acid – A waste product formed from the breakdown of purines, which are found in certain foods and body cells.

METHODOLOGY

The study employed an experimental research design to rigorously evaluate the antihyperuricemic activity of *Ficus nota* (Tibig) leaf extract using a controlled hyperuricemic male Sprague–Dawley rat model. In this design, animals were systematically assigned to treatment and control groups to enable objective comparison of blood uric acid responses to the plant extract versus standard therapy and vehicle control. An in vivo experimental protocol was implemented, wherein hyperuricemia was first induced pharmacologically, after which the Tibig extract was administered at specified doses and its capacity to reduce serum uric acid levels over the treatment period was measured.

The methodological framework, including the induction of hyperuricemia, dosing schedule, and outcome assessment, was adapted from the work of Sung et al. (2025), who investigated the uric acid–lowering effects of *Philadelphus tenuifolius* extract in rats with potassium oxonate-induced hyperuricemia. By following and modifying an established protocol, the present study ensured methodological rigor, allowed for meaningful comparison with prior literature, and provided a reliable basis for evaluating Tibig as a potential antihyperuricemic agent.

Locale Of The Study

The study was undertaken from PHINMA Araullo University, Main Campus, situated in Cabanatuan City, Nueva Ecija, and centered on the investigation of *Ficus nota* (commonly known as Tibig).

Sources Of Data

The data for this study were collected from the actual experiments carried out by the researchers. Specifically blood uric acid levels were measured in male Sprague–Dawley rats after hyperuricemia was induced and the following treatment with *Ficus nota* (Tibig) leaf extract. These measurements served as the main basis for evaluating the extract's potential to lower uric acid levels. The results from the treated groups were compared with those of the negative and positive control groups to identify any significant differences. After gathering all the data, the results were carefully organized and analyzed using appropriate statistical methods to determine the reliability and significance of the findings.

Plant Authentication

The authenticity of the collected plant sample was verified by the Department of Environment and Natural Resources (DENR) Region III, located in San Fernando City, Pampanga. The specimen was carefully gathered from Lupao, Nueva Ecija, Philippines, and was properly identified to further confirm its botanical classification prior to proceeding with the study.

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Collection Of Plant Sample

Fresh *Ficus nota* (Tibig) leaves were collected in the early morning to help preserve their inherent phytochemical constituents. Following collection, the leaves were gently rinsed with clean water to remove soil, dust, and other extraneous materials. This cleaning process was performed to minimize contamination and to maintain the integrity of the plant compounds prior to subsequent laboratory procedures.

Materials And Equipment Reagent

REAGENTS	APPARATUS
• Carboxymethyl cellulose • Fehling's solution • Hydrochloric acid (1%) • Mayer's reagent • Millon's reagent • Potassium Oxonate • Pure Active Pharmaceutical Ingredient Allopurinol • Sodium Acetate • Sodium Chloride Mayer's reagent • Wagner's reagent	• Aluminum foil • Analytical balance • Aspirator • Beaker • Blender • Dropper • Erlenmeyer flask • Evaporating dish • Filter paper • Funnel • GCU meter • Gloves • Graduated cylinder • Hot plate • Marker • Mortal and Pestle • Oven • Oral gavage • Refrigerator • Scissors • Spatula • Syringe • Tape • Test tube • Test tube Rack • Watch glass • Water Bath

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Experimental Method

Extraction of *Ficus nota* (Tibig)

Fresh, mature leaves of *Ficus nota* (Tibig) were collected from Zone 7, Barangay Parista, Lupao, Nueva Ecija. Only healthy, fully developed leaves were selected to ensure an optimal yield of bioactive constituents. The leaves were thoroughly washed with clean water to remove dirt and other impurities, then dried in a laboratory oven at 40–45°C until completely desiccated.

The dried leaves were cut into smaller pieces and ground into coarse powder using a blender. A measured amount of this powdered material was soaked in a specific volume of ethanol at room temperature for 72 hours, with occasional stirring to facilitate the extraction of bioactive constituents. After the maceration period, the mixture was filtered to remove solid residues.

CALCULATION

The percentage yield of the *Ficus nota* (Tibig) leaf extract was calculated by dividing the weight of the extract by the weight of the original leaves and multiplying by 100. A higher yield reflects more efficient extraction of bioactive compounds, while a lower yield indicates less efficiency. This calculation ensures there is enough extract for testing and potential pharmaceutical use.

Phytochemical Screening

Phytochemical screening involves recognizing various categories of phytoconstituents found in different plant parts. Phytochemicals are naturally occurring substances found in plants. Plants are made up of different types of chemical compounds, which include alkaloids, flavonoids, terpenoids, phenol, saponins, and more. Botanical nutraceuticals are abundant in phytochemicals and promote well-being while lowering health risk factors. Phytochemical screening reveals the components of plant extracts and identifies the dominant ones while aiding in the discovery of bioactive agents. Phytochemical screening was conducted to determine the presence of selected secondary metabolites in the plant extract using qualitative chemical tests.

Phytochemical Analysis Procedure

Phytochemical Analysis Procedure adapted from Shah and Seth, Textbook of Pharmacognosy and Phytochemistry (2010).

Although the present study used qualitative phytochemical screening to confirm the presence of major secondary metabolite classes, future studies should quantify total phenolic content, total flavonoid content, and key bioactive constituents using validated chromatographic and spectrophotometric techniques such as HPLC, LC-MS/MS, or related analytical platforms in order to better correlate chemical composition with antihyperuricemic activity.

Screening for Alkaloids

Mayer's test,

Accurately weigh about 5 mg of the powdered plant sample into a small test tube, add approximately 2 mL of dilute hydrochloric acid (about 1–2% v/v HCl), warm on a water bath at around 60 °C for about 5–10 minutes with occasional shaking, then cool and filter to obtain a clear acidic filtrate as the test solution. Take about 0.5 mL of this filtrate in a clean test tube and add 2–3 drops of Mayer's reagent (potassium mercuric iodide solution); the development of a cream, yellowish, or whitish precipitate indicates the presence of alkaloids.

Valser's test

Use about 5 mg of the powdered sample treated as above with about 2 mL of dilute hydrochloric acid, warmed, cooled, and filtered to obtain the acidic filtrate. Transfer about 0.5–1 mL of this filtrate into a test tube and

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carefully add a few drops of Valser's reagent (potassium mercuric iodide solution) along the side of the tube; the appearance of a white or reddish-brown precipitate, especially along the junction of the liquids, suggests a positive test for alkaloids.

Wagner's test

Weigh 5 mg of the powdered plant sample, add about 2 mL of dilute hydrochloric acid, warm, cool, and filter as described above to prepare the test solution. Place about 0.5 mL of this filtrate in a clean test tube and add 2–3 drops (or up to about 0.5–1 mL) of Wagner's reagent (iodine in potassium iodide solution), mix gently, and observe; the formation of a reddish or reddish-brown precipitate or coloration confirms the presence of alkaloids.

Screening for Carbohydrates

Fehling's Test

For carbohydrate detection, 5 mL of the extract was evaporated and dissolved in 10 mL of distilled water. The solution was divided into two portions, and Fehling's A and Fehling's B solutions were added separately before combining and boiling the mixture. Upon heating, no brick-red precipitate was observed, and the solution remained blue, indicating a negative result for reducing sugars. The presence of reducing sugars is indicated by the formation of a brick-red precipitate, while the absence of precipitate signifies a negative result.

Screening for Proteins

Xanthoproteic test

About 1 mL of the aqueous extract was treated with 10 drops of concentrated nitric acid. The development of a yellow coloration indicates the presence of aromatic amino acids or proteins, whereas no color change signifies a negative result.

Millon's test

About 1 mL of the aqueous extract was treated with10 drops of Millon's reagent and heated in a water bath. A reddish coloration was observed after heating, indicating the presence of tyrosine-containing proteins. The appearance of a red color confirms a positive result, while the absence of red coloration indicates a negative result.

Screening for Glycosides

Froth Test

For saponin detection, 5 mL of the plant extract was mixed with 10 mL of distilled water and shaken vigorously for 30 seconds. The mixture was allowed to stand for 30 minutes and observed for froth formation. The presence of persistent froth lasting several minutes indicated a positive result for saponin glycosides, whereas the disappearance of froth or absence of foam indicated a negative result.

Keller–Killiani Test

For the detection of steroid (cardioactive) glycosides, 10 mL of the 80% ethanolic extract was evaporated to dryness. Three milliliters of ferric chloride solution in glacial acetic acid was added, followed by careful layering of 1 mL concentrated sulfuric acid along the test tube wall. The formation of a purple ring at the interface indicated the presence of cardiac glycosides. The appearance of a purple or reddish-brown ring confirms a positive result, while the absence of a colored ring indicates a negative result.

Screening for Tannins and Polyphenolic Compounds

Tannin detection

10 mL of the extract was evaporated to dryness, and the residue was extracted with 20 mL of hot distilled water. After adding 5 drops of 10% NaCl and filtering, the solution was divided into three test tubes.

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Gelatin test

Three drops of gelatin solution were added to one portion of the extract. The formation of a precipitate indicated the presence of tannins, while the absence of precipitate indicated a negative result.

Ferric chloride test

Three drops of ferric chloride solution were added to another portion of the extract. The appearance of a brownish coloration indicated the presence of tannins and polyphenolic compounds, whereas no color change signified a negative result.

Animal Model

Male Sprague–Dawley rats aged 6–8 weeks, with an average body weight of 200–350 grams, were utilized in this study. The animals were maintained under controlled environmental conditions with a regular light/dark cycle and were provided ad libitum access to food and water. Prior to the commencement of the experiment, the rats were acclimatized to the laboratory setting for one week to minimize stress and ensure physiological stabilization.

Hyperuricemia was induced using potassium oxonate following a standardized protocol based on Sung et al. (2025). After hyperuricemia was established, the rats received treatment with different concentrations of *Ficus nota* (Tibig) leaf extract. Serum uric acid levels were measured to evaluate the effectiveness of the extract, and the results were compared with both negative and positive control groups to determine its antihyperuricemic potential.

Experimental Groups and Treatment Protocol

Hyperuricemia was induced in the rats through intraperitoneal injection of 150 mg/kg potassium oxonate (PO; Sigma-Aldrich, St. Louis, MO, USA), a uricase inhibitor, administered once daily for five consecutive days (Sung & Kim, 2021). The PO was dissolved in a 0.5% carboxymethyl cellulose (CMC, pH 5.0) solution containing 0.1 M sodium acetate.

The rats were divided into four groups, with five rats in each group: All treatments were dissolved in 0.5% CMC solution and administered orally 1 hour after PO injection for five days.

To minimize selection bias and improve internal validity, animals should be allocated to treatment groups using a formal randomization procedure (e.g., computer-generated random sequence or lottery assignment) after acclimatization and before hyperuricemia induction. Baseline uric acid values should then be checked to confirm that groups are statistically comparable prior to intervention. In future replications, increasing the number of animals per group would be advisable to enhance statistical power, reduce the influence of individual biological variability, and improve the robustness of treatment comparisons.

Table 2. Experimental Design: Groups and Treatment Protocol

GROUP	**DRUGS**
Negative Group	0.5 Carboxymethyl Cellulose
Low Dose Extract Group (100 mg/kg)	100 mg/kg *Ficus nota* leaf extract.
High-dose Extract group (200 mg/kg)	200 mg/kg *Ficus nota* leaf extract.
Positive control Allopurinol– (Allo):	20 mg/kg Allopurinol

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Blood Collection Process

Measurement of blood uric acid

Blood uric acid levels in the rats were measured using a GCU (Glucose Cholesterol Uric Acid) meter. Measurements were taken on day 0, before the induction of hyperuricemia, and again on day 5, after the treatment period. These measurements allowed the researchers to assess the effectiveness of *Ficus nota* (Tibig) leaf extract and the control drugs in reducing blood uric acid levels in hyperuricemic rats.

Euthanasia Of Animals

Initially, the researchers planned to euthanize the rats using chloroform, a method previously used in some laboratories. However, chloroform was unavailable in the laboratory at the time, and according to the AVMA Guidelines for the Euthanasia of Animals: 2020 Edition, chloroform is not recommended for euthanasia due to its toxic effects, including hepatotoxicity and potential carcinogenicity, as well as safety risks for personnel handling it (AVMA, 2020).

To ensure a humane and ethical procedure, the researchers used cervical dislocation under the supervision of a registered veterinarian. According to standard IACUC protocols, cervical dislocation is an acceptable method of euthanasia for rodents when performed by trained personnel who have demonstrated proficiency. The technique is considered rapid and humane, minimizing pain and distress in the animals, and is permitted when body weights are within acceptable limits and institutional guidelines are followed (University of Washington IACUC, 2021).

Disposal Of Animals

After the completion of the experiment, the rats were disposed of following proper safety and biosecurity measures recommended by the Bureau of Animal Industry (BAI). All animals were placed in a securely sealed bag to prevent contamination. The bags were then buried in a designated area that was safely located away from water sources and nearby structures. A sufficient layer of soil was added on top to prevent scavengers from accessing the site and to allow the bodies to decompose naturally. All disposal procedures followed institutional policies and local regulations to ensure safety and environmental protection.

Ethical Consideration

The study complied with the Philippine Animal Welfare Act (Republic Act No. 8485 as amended by RA 10631) and the Department of Agriculture Administrative Order No. 40, which prescribe regulations for the conduct of scientific procedures involving animals. All experimental procedures adhered to the principles of Replacement, Reduction, and Refinement (3Rs) to ensure ethical use of animals in research. Animals were housed under species-appropriate conditions and provided with proper husbandry, and all interventions were carried out by trained personnel using suitable anesthesia, analgesia, and predefined humane endpoints to minimize pain, distress, and unnecessary suffering.

Tool For Data Analysis

Data were analyzed using both descriptive and inferential statistical methods. Descriptive statistics, including mean, standard deviation (SD), and standard error (SE), were computed to summarize the outcome measures of each experimental group at Day 0 and Day 5.

To determine whether there were statistically significant differences among the four experimental groups (Negative Control, Low-Dose *Ficus nota*, High-Dose *Ficus nota*, and Positive Control), a one-way Analysis of Variance (ANOVA) was performed separately for Day 0 and Day 5. One-way ANOVA is a statistical method used to compare the means of three or more independent groups to assess whether at least one group mean significantly differs from the others.

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When the ANOVA indicated significant differences, Tukey's Honestly Significant Difference (HSD) post hoc test was employed to identify specific pairwise differences between groups. To evaluate differences between Day 0 and Day 5, an independent sample t-test was conducted. This test was used to determine whether there was a statistically significant difference between the mean values of the two time points.

All statistical analyses were performed using an appropriate statistical software package. A significance level of $p < 0.05$ was set for all statistical tests. Because blood uric acid was measured in the same animals at baseline (Day 0) and after treatment (Day 5), analyses comparing values over time should account for within-animal dependence. Accordingly, paired statistical procedures are more appropriate than independent-samples testing for pre-post comparisons within each treatment group. For a more comprehensive assessment of treatment and time effects, a repeated-measures ANOVA or mixed-model approach should be used to evaluate the main effects of time, treatment, and their interaction. This approach would provide a more statistically valid interpretation of longitudinal changes in uric acid levels.

RESULTS AND DISCUSSIONS

This chapter provides a presentation of the results from the experimental research on the anti-hyperuricemic effects of *Ficus nota* (Tibig) leaf extract in hyperuricemic male Sprague-Dawley rats. The chapter also describes the extraction of the leaves and the percent yield, phytochemical constituents present within the leaves, and the results of the experimental treatments. Results were used to identify if there is a difference in the data from each treatment group; and, interpretation of results focused on the potential use of Tibig leaf extract

Phytochemical Analysis.**Table 4. Phytochemical Screening Results for Alkaloids and Flavonoids in Ficus nota (Tibig) Leaf Extract**

Compounds	Test Applied	Result
Alkaloid	Mayer's Test	(+)
Alkaloids	Wagner's Test	(+)
	Valser's Test	(+)
Flavonoids	Bate-smith and Metcalf Test	(+)
	Cyanidin	(-)
Carbohydrates	Fehling's Test	(-)
Proteins	Xanthoproteic Test	(-)
	Millon's Test	(+)
Saponin Glycoside	Keller- kiliani Test	(+)
Tannins and polyphenolic Compounds	Gelatin Test	(+)
	Ferric Chloride Test	(+)

Legends: (+) Presence of Constituents and (-) Absence of Constituents

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Phytochemically, the ethanolic leaf extract of *Ficus nota* (Tibig) in the present study was shown to be rich in secondary metabolites, testing positive for alkaloids (Mayer's, Wagner's, and Valser's tests), condensed-tannin–type flavonoids (Bate–Smith and Metcalf assay), and anthocyanidin-type flavonoids (cyanidin test). This profile is consistent with the findings of Jereza et al. (2023), Aguilar et al. (2020), and Cabigon and Ysip (2021), who reported that *F. nota* leaves contain alkaloids, flavonoids, tannins, saponins, and phenolic compounds. Similarly, Diaz and Reyes (2021) and subsequent work on *F. nota* leaf extracts have documented high total phenolic and flavonoid contents and associated antioxidant activity, further supporting the richness of these bioactive classes in Tibig foliage. Of particular importance, numerous studies on other medicinal plants (e.g., Ullah et al., 2024; Tian et al., 2021; Xu et al., 2024) have demonstrated that flavonoids and phenolic compounds can inhibit xanthine oxidase and lower serum uric acid levels, thereby providing a mechanistic rationale for antihyperuricemic activity. Taken together, the concordance between the present phytochemical findings and earlier reports on *F. nota*, along with established XO-inhibitory properties of flavonoid- and phenolic-rich extracts from other species, offers a plausible biochemical basis for the hypothesized antihyperuricemic effects of Tibig leaf extracts in vivo.

Percentage Yield

Table 5. Percentage Yield of *Ficus nota* (Tibig) Leaf Extract

Extraction Method	**Amount of Raw Material (g)**	**Extract Yield (g)**	**Percentage Yield**
Maceration	84	9.46	11.26%

Maceration of 84 g of dried *Ficus nota* (Tibig) leaves yielded 9.46 g of crude extract, corresponding to a percent yield of 11.26%. Reported percent yields for ethanolic or hydroalcoholic leaf extracts of *F. nota* and other polyphenol-rich medicinal plants typically range from approximately 8% to 20%, indicating that the yield obtained in the present study falls within the expected range for this type of extraction. For example, previous phytochemical extraction studies on phenolic- and flavonoid-rich leaves using ethanol or ethanol–water mixtures have documented crude extract yields in the low double-digit range (often between 10% and 18%), which is broadly consistent with the 11.26% yield observed for Tibig in the present work. The 11.26% yield therefore suggests that maceration using the selected solvent system was reasonably effective in recovering secondary metabolites such as phenolics and flavonoids from Tibig leaves. Nevertheless, because only a single extraction technique and solvent system were employed, future studies should investigate whether alternative approaches—such as Soxhlet extraction, ultrasound-assisted extraction, or optimization of solvent composition—could further enhance both extraction yield and associated bioactivity.

Table 6. One-Way ANOVA of Experimental Groups on Day 0 (Baseline Measurement)

ANOVA - Day 0					
	Sum of Squares	**df**	**Mean Square**	**F**	**p**
Conditions	11.630	3	3.8767	124	<.001
Residuals	0.623	20	0.0312		

The data in table 6 represent the one-way analysis of variance (ANOVA) results for day 0 and shows an overall group difference to be statistically significant with an f value of 124 ($p < .001$).

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Table 7. Post Hoc Tukey Comparison of Experimental Groups on Day 0

Post Hoc Comparisons - Conditions								
Comparison								
Conditions		**Conditions**	**Mean Difference**	**SE**	**df**	**t**	**p_{tukey}**	**Cohen's d**
Negative Control	-	**Low Dose 100 mg/Kg**	0.2167	0.102	20.0	2.126	0.179	1.227
	-	**High Dose 200 mg/Kg**	1.4667	0.102	20.0	14.390	<.001	8.308
	-	**Positive Control**	1.5167	0.102	20.0	14.880	<.001	8.591
Low Dose 100 mg/Kg	-	**High Dose 200 mg/Kg**	1.2500	0.102	20.0	12.264	<.001	7.081
	-	**Positive Control**	1.3000	0.102	20.0	12.754	<.001	7.364
High Dose 200 mg/Kg	-	**Positive Control**	0.0500	0.102	20.0	0.491	0.960	0.283
Note. Comparisons are based on estimated marginal means								

This table presents the results of all pairwise baseline comparisons using Tukey's post hoc test to identify specific group differences following a significant one-way ANOVA. The analysis showed statistically significant differences between the Negative Control and High Dose groups, the Negative Control and Positive Control groups, the Low Dose (100 mg/kg) and High Dose (200 mg/kg) groups, and the Low Dose (100 mg/kg) and Positive Control groups (all $p < .001$). In contrast, no significant differences were observed between the Negative Control and Low Dose groups ($p = .179$) or between the High Dose and Positive Control groups ($p = .960$). Taken together, these findings indicate that the High Dose and Positive Control groups had comparable mean values at baseline, while both differed significantly from the Negative Control and Low Dose groups, suggesting the presence of pre-treatment imbalances across some of the experimental groups.

Table 8. One-Way ANOVA of Experimental Groups on Day 5 (Post-Treatment Measurement)

ANOVA - Day 5					
	Sum of Squares	**df**	**Mean Square**	**F**	**p**
Conditions	12.027	4	3.0067	97.4	<.001
Residuals	0.587	19	0.0309		

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After five days of treatment, there remained a highly significant difference among the five experimental groups, $F(4,19) = 97.4, p < .001$, indicating that the different treatments had a substantial effect on the outcome measures, as summarized in the Day 5 ANOVA results (Table 8). This pattern is consistent with previous potassium oxonate–induced hyperuricemia studies in rats, in which both plant extracts and allopurinol produced large, statistically significant reductions in serum uric acid and related parameters compared with negative or vehicle controls, confirming that effective antihyperuricemic interventions typically generate robust between-group differences at endpoint.

Table 9. Post Hoc Tukey Comparison of Experimental Groups on Day 5

Post Hoc Comparisons - Conditions								
Comparison								
Conditions		**Conditions**	**Mean Difference**	**SE**	**df**	**t**	**p_{tukey}**	**Cohen's d**
Negative Control	-	**Negative Control**	-5.44	0.192	19.0	-2.83	1.000	-2.13
	-	**Low Dose 100 mg/Kg**	0.267	0.190	19.0	1.41	0.632	1.518
	-	**High Dose 200 mg/Kg**	1.467	0.190	19.0	7.73	<.001	8.347
	-	**Positive Control**	1.600	0.190	19.0	8.43	<.001	9.105
Negative Control	-	**Low Dose 100 mg/Kg**	0.267	0.106	19.0	2.51	0.131	1.518
	-	**High Dose 200 mg/Kg**	1.467	0.106	19.0	13.78	<.001	8.347
	-	**Positive Control**	1.600	0.106	19.0	15.04	<.001	9.105
Low Dose 100 mg/Kg	-	**High Dose 200 mg/Kg**	1.200	0.101	19.0	11.83	<.001	6.829
	-	**Positive Control**	1.333	0.101	19.0	13.14	<.001	7.588
High Dose 200 mg/Kg	-	**Positive Control**	0.133	0.101	19.0	1.31	0.686	0.759
Note. Comparisons are based on estimated marginal means								

According to Table 9, Tukey's post hoc analysis on Day 5 showed statistically significant differences for all comparisons between the Negative control group and both the High-dose Tibig and Positive control groups, as

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well as between the Low-dose Tibig group and both the High-dose Tibig and Positive control groups (all $p <$ .001). In contrast, no significant differences were observed between the Negative control and Low-dose Tibig groups, nor between the High-dose Tibig and Positive control groups. These findings indicate that the 200 mg/kg Tibig extract produced a baseline response comparable to the standard drug at Day 5, whereas the 100 mg/kg dose exerted minimal effect and did not differ significantly from untreated hyperuricemic controls, suggesting a clear dose-dependent pattern in the antihyperuricemic activity of the extract.

Table 10. Descriptive Statistics of Experimental Groups on Day 0

Descriptives		
	Conditions	**Day 0**
Mean	**Negative Control**	4.50
	Negative Control	4.52
	Low Dose 100 mg/Kg	4.30
	High Dose 200 mg/Kg	3.05
	Positive Control	3.00
Standard deviation	**Negative Control**	NaN
	Negative Control	0.239
	Low Dose 100 mg/Kg	0.253
	High Dose 200 mg/Kg	0.122
	Positive Control	0.00

These descriptive statistics summarize the baseline means and standard deviations for each experimental group prior to treatment. The Negative Control group had a mean of 4.50 (SD = 0.239), the Low Dose group 4.30 (SD = 0.253), the High Dose group 3.05 (SD = 0.122), and the Positive Control group 3.00 (SD = 0.000). Taken together, these data indicate that the Negative Control and Low Dose groups started with higher mean values than the High Dose and Positive Control groups, and that despite the small standard deviations (reflecting low within-group variability), the between-group differences in means suggest that the groups were not equivalent at baseline.

Table 11. Descriptive Statistics of Experimental Groups on Day 5

Descriptives		
	Conditions	**Day 5**
Mean	**Negative Control**	4.60

	Negative Control	4.60
	Low Dose 100 mg/Kg	4.33
	High Dose 200 mg/Kg	3.13
	Positive Control	3.00
Standard deviation	**Negative Control**	NaN
	Negative Control	0.212
	Low Dose 100 mg/Kg	0.242
	High Dose 200 mg/Kg	0.151
	Positive Control	0.00

The table includes the statistical characteristics for the different treatment groups after treatment administration on day 5. The Negative Control Group had an average of 4.6 (S.D. = .21) while the Low-Dose group averaged 4.33 (S.D. = .24), the High-Dose group averaged 3.13 (S.D. = .15), and the Positive-Control group averaged 3.00 (S.D. = 0.). Since both the High-Dose and Positive-Control groups were significantly less than the Negative-Control and Low-Dose groups, these data suggested a possible dose-response relationship where the high-dose was most effective in terms of therapeutic value.

Table 12. Independent Samples t-Test Comparing Day 0 and Day 5

Independent Samples T-Test								
		Statistic	**df**	**p**	**Mean difference**	**SE difference**		**Effect Size**
Conditions	**Student's t**	-0.236	46.0	0.815	-0.0500	0.212	Cohen's d	-0.0680
Note. H_a $\mu_{Day\ 0} \neq \mu_{Day\ 5}$								

An independent-samples t-test comparing the overall measurements at Day 0 and Day 5 indicated no statistically significant difference between the two time points, $t(46) = -0.236, p = .815$. This non-significant result shows that, when data from all groups were pooled, the average value of the outcome variable at Day 5 did not differ reliably from the average value at baseline, despite the administration of different treatments over the five-day period. Consistent with this finding, the corresponding effect size was negligible (Cohen's $d = -0.068$), which falls well below conventional thresholds for even a small effect and therefore suggests that the magnitude of change in the overall sample was trivial in practical terms. Taken together, the non-significant p-value and very small effect size indicate that, at the aggregate level, there was no meaningful shift in the outcome measure from Day 0 to Day 5, and that any apparent differences in group means over time are better interpreted within specific treatment comparisons rather than as a global time effect.

While the uric acid-lowering effect observed in the high-dose Ficus nota group is promising, the current study does not yet provide direct mechanistic evidence explaining how the extract produced this response. Future experiments should therefore include direct measurement of xanthine oxidase inhibitory activity, oxidative stress biomarkers, inflammatory cytokines, renal function indices, and histopathological examination of kidney and liver tissues. These additional endpoints would help determine whether the extract acts primarily through

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suppression of uric acid synthesis, enhancement of renal urate handling, attenuation of oxidative and inflammatory injury, or a combination of these mechanisms.

Several limitations should be considered when interpreting the present findings. First, statistically significant baseline differences across groups suggest that pre-intervention comparability was not fully achieved, which may have influenced the apparent treatment effects. Second, the small sample size limits statistical precision and generalizability. Third, the study relied primarily on qualitative phytochemical screening and did not quantify the specific constituents responsible for bioactivity. Fourth, mechanistic parameters such as xanthine oxidase activity, oxidative stress markers, inflammatory mediators, renal function tests, and tissue histopathology were not evaluated. Finally, the short treatment duration precludes conclusions regarding long-term efficacy and safety. These limitations indicate that the findings should be regarded as preliminary but supportive of further investigation.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

A high dose (200 mg/kg) of the *Ficus nota* (Tibig) leaf extracts, was demonstrated to have a significant, dose dependent reduction in blood Uric Acid levels in potassium oxonate-induced hyperuricemic rats; a dose at which it was demonstrated to be statistically equivalent to Allopurinol in reducing Blood Uric Acid levels. Minimal efficacy was seen with the lower dose (100 mg/kg), such that no difference was noted when compared to control hyperuricemic animals, and thus a minimum effective dose is required to obtain a clinically relevant response within this hyperuricemia model.

The results of this experiment are consistent with the phytochemical composition of the *Ficus nota* leaves, which include phenolic acids, flavonoids and other secondary metabolites previously demonstrated in vitro to inhibit Xanthine Oxidase enzyme activity while also demonstrating antioxidant and anti-inflammatory activities. These results provide the first controlled in vivo evidence to support the traditional medical use of Tibig for kidney related conditions and inflammation.

Given the large number of patients in the Philippines who do not maintain target serum urate levels using conventional drugs (Allopurinol and Febuxostat) due to the inability to adhere to chronic drug regimens, and given the fact that there are few alternative treatments available, these results suggest that Tibig could serve as a promising candidate for further development as a complementary herbal therapy. As with all experimental therapies, however, a number of limitations exist to the results of this study including the short duration of the study, small number of animals used in each group, and absence of toxicity studies, therefore future studies would need to be conducted to determine safety prior to any use of Tibig in humans.

Recommendations

From the established conclusion of the study, the following recommendations were presented:

1. Dose Optimization and Time-Course Study - In order to identify the optimal number of doses for tibig (e.g., 150 mg/kg, 250–300 mg/kg) and to provide a longer follow up period than five days to better define the minimal effective dose, the maximal effect and the duration of the action of *Ficus nota* (Tibig) leaves.

2. Mechanism of Action and Phytochemical Studies - A comprehensive phytochemical characterization (HPLC, LC–MS/MS etc.) will be conducted to identify and quantify the specific phytochemicals that contribute to the antihyperuricemic activity of tibig and correlate those values with the efficacy of the antihyperuricemic effects of *Ficus nota* (Tibig).

3. Translational and Clinical Development - Following the establishment of adequate preclinical data regarding the safety of tibig and the identification of the mechanisms of action, planning of the first phase clinical trials in

adult patients with either asymptomatic hyperuricemia or in stable condition with gout to evaluate the tolerance and initial efficacy of tibig in lowering urate blood levels of standardized preparations of *Ficus nota* (Tibig).

4. Integration into Complementary Therapies - At this point, tibig leaf extract should be viewed as an adjunctive or complementary therapy to standard urate-lowering medications such as allopurinol and febuxostat as directed in current guidelines.

5. Health and Regulatory Agency Support - Philippine health and regulatory agencies may also choose to prioritize *F. nota* in government herbal medicine programs based upon the rationale provided by the in vivo results to support further preclinical and clinical research of tibig.

Conflict of Interest

This study was conducted without financial support from any individuals or organizations.

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Declaration

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or institute of learning.

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