Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 27s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Comparative GCMS Phytochemical Analysis Antihyperuricemic Efficacy of Romanian, Bangladeshi, and Syrian Nigella sativa Varieties in a Fructose and yeast-Induced Rat Model: Mechanistic Insights by computing Predictive Modelling

Mohamed A Farag Fannosh[1], Md. Gousuddin[2], Dr. Dr Sreemoy[3]

[1,2,3]Lincoln University collage Kuala Lumpur, Malaysia.

***Corresponding Author:**

Dr. Sreemoy

Email: mohamedafannosh@gmail.com

Cite this paper as: Mohamed A Farag Fannosh, Md. Gousuddin, Dr. Dr Sreemoy, (2025) Comparative GCMS Phytochemical Analysis Antihyperuricemic Efficacy of Romanian, Bangladeshi, and Syrian Nigella sativa Varieties in a Fructose and yeast-Induced Rat Model: Mechanistic Insights by computing Predictive Modelling. *Journal of Neonatal Surgery*, 14 (27s), 148-178.

ABSTRACT

Nigella sativa, commonly known as black cumin, is widely recognized for its medicinal properties, including its antihyperuricemic, antioxidant, and anti-inflammatory effects. This study aimed to evaluate and compare the antihyperuricemic potential of three varieties of Nigella sativa seeds from Bangladesh, Syria, and Turkey, as well as their phytochemical composition. Using Gas Chromatography-Mass Spectrometry (GC-MS), key bioactive compounds, such as thymoquinone, p-cymene, and α-phellandrene, were identified and quantified. An animal model of hyperuricemia was established in Sprague-Dawley rats using a fructose-brewer's yeast diet, and therapeutic effects were assessed by administering standardized doses of Nigella sativa and comparing their efficacy to allopurinol, a conventional gout medication.The results revealed significant phytochemical variability among the three origins, with Turkish Nigella sativa exhibiting the highest levels of thymoquinone and p-cymene, while the Syrian variety showed unique bioactivity despite lower thymoquinone content. Statistical analysis demonstrated that all three seed varieties effectively reduced uric acid levels, with Turkish Nigella sativa producing the most significant reduction. In silico predictions using pkCSM , SuperPred and Pro Tox III provided insights into mechanisms of action and pharmacokinetics and their toxcity, highlighting the potential of Nigella sativa as a complementary treatment for hyperuricemia and gout. This research emphasizes the importance of geographical influence on the medicinal properties of Nigella sativa and contributes to the understanding of its pharmacological applications.

Keywords: *hyperuricemia, nigella sativa, gout, uric acid, fructose, rat induced model, brewer yeast*

1. INTRODUCTION

Nigella sativa (NS), commonly known as black cumin or black seed, is a medicinal plant with a rich historical significance in traditional systems of medicine, including Greek, Islamic, and Asian traditions [1]Its therapeutic applications span respiratory, gastrointestinal, and skin diseases, and it is renowned for its antioxidant, anti-inflammatory, and anticancer properties, largely attributed to bioactive components like thymoquinone (TQ) and p-cymene [2] & [3].Modern research highlights NS's antihyperuricemic activity, making it a potential alternative to conventional treatments like allopurinol for hyperuricemia—a condition that can lead to gout and kidney stones if unmanaged (Richette & Bardin, 2010). Moreover, variations in the phytochemical profiles of NS, influenced by geographical cultivation, have raised questions about its pharmacological efficacy across different regions [4]&([5] This study aims to compare the antihyperuricemic effects of NS cultivated in Syria, Turkey, and Bangladesh. By using gas chromatography-mass spectrometry (GC-MS) and rat models of hyperuricemia, the research evaluates the phytochemical composition and pharmacological efficacy of these varieties. Furthermore, computational tools like ProTox-III and pkCSM are employed to predict toxicity, pharmacokinetics, and novel mechanisms of action. This comparative assessment seeks to provide insights into the geographical variability in NS's medicinal properties, offering significant implications for pharmaceutical and agricultural applications.

2. EXPERMINTAL

RESEARCH CONTEXT:

This study is conducted within a controlled laboratory environment, where the primary objective is to compare the antihyperuricemic potential of Nigella sativa seeds [6]from three different geographical regions: Bangladesh, Syria, and Turkey. The research focuses on evaluating and comparing the phytochemical compositions [7]and medicinal efficacy[8] [9]of these varieties in a rat model of hyperuricemia.

Geographical Context of NS

The NS seeds used in this study are sourced from three regions known for their distinct climates and cultivation practices Bangladesh: A tropical region with high humidity and substantial rainfall, which may impact the phytochemical composition of the seeds. Syria: A Mediterranean climate with hot, dry summers and mild winters, which may result in a different phytochemical profile.Turkey: A region with varied climates, particularly the continental climate where black seed is grown, potentially influencing the concentration of active compounds like thymoquinone and p-cymene. These regional differences are expected to influence the medicinal properties of the seeds, particularly their ability to reduce uric acid levels.

Animal Model and Laboratory Setting

The study utilizes rats as the experimental model to simulate hyperuricemia[10]& [11]which is induced using fructose and brewer's yeast over a period of 54 days. The rats are housed in a controlled environment with standardized conditions:

Temperature: 22°C ± 2°C,Humidity: 55% ± 10% ,Light/Dark Cycle: 12 hours light/dark cycle[12],Diet: A specially formulated diet mixed with fructose and brewer's yeast to induce [13]hyperuricemia.

Care is taken to ensure that the animals experience minimal stress, which could otherwise affect the results. The ethical handling of the animals follows institutional guidelines to prevent unnecessary pain or suffering.

Phytochemical Analysis

The research involves a detailed laboratory analysis of the phytochemical composition of the Nigella sativa seeds from each region[2]. The seeds are cleaned, dried, ground, and prepared for analysis using Gas Chromatography-Mass Spectrometry (GC-MS). This analysis allows for the identification and quantification of key active compounds, such as thymoquinone and p-cymene[14], which are hypothesized to contribute to the seeds' antihyperuricemic properties.

Experimental Design

The experimental design is structured to evaluate the effectiveness of Nigella sativa in reducing uric acid levels compared to the conventional drug allopurinol[13]. The study also aims to explore potential novel mechanisms of action that Nigella sativa may employ in lowering uric acid levels, particularly through modulation of oxidative stress and inflammatory pathways.[15]

Participants

The participants of this study consist of 70 male Sprague-Dawley rats, [13]each weighing between 200-250 grams. These rats are sourced from a reputable animal facility and are chosen for their physiological suitability in modeling hyperuricemia. Sprague-Dawley rats are widely used in biomedical research due to their consistent response to metabolic and pharmacological studies, making them ideal for evaluating the antihyperuricemic potential of NS .Experimental Groups:The 70 rats are divided into six groups as follows:[12]

Group 1 (Control Group): Rats with no hyperuricemia induction or treatment (n =14).

Group 2 (Negative Control): Hyperuricemic rats receiving no treatment (n = 14).

Group 3 (Bangladesh NS Group): Hyperuricemic rats treated with Nigella sativa from Bangladesh (n = 14).

Group 4 (Syria NS Group): Hyperuricemic rats treated with Nigella sativa from Syria (n = 14).

Group 5 (Turkey NS Group): Hyperuricemic rats treated with Nigella sativa from Turkey (n = 14).

Group 6 (Allopurinol Group): Hyperuricemic rats treated with allopurinol (positive control group, n = 14).

Each group is designed to assess the comparative antihyperuricemic effects of Nigella sativa from different regions, as well as the standard pharmaceutical treatment (allopurinol).

Selection Criteria :Species: Sprague-Dawley rats ,Sex: Male, Weight: 200–250 grams ,Health Status: The rats are healthy and free from any metabolic or systemic diseases prior to the induction of hyperuricemia.

Ethical Considerations:

All procedures involving the rats are conducted in accordance with institutional and international ethical guidelines for

animal research. The study ensures that the rats are housed in appropriate conditions, with controlled temperature, humidity, and a 12-hour light/dark cycle. Special care is taken to minimize any unnecessary stress or pain to the animals during the experiment. Ethical approval for the study has been obtained from the relevant animal ethics committee. This section outlines the type of animals, their grouping, and the ethical considerations for their use in your experiment[13].

3. INSTRUMENTS OF DATA COLLECTION

Instruments of Data Collection in Your Research

Your research focuses on evaluating the antihyperuricemic potential (reduction of uric acid levels) of three varieties of NS cultivated in Bangladesh, Syria, and Turkey, using a rat model of hyperuricemia induced by fructose and brewer's yeast.[10] To achieve this, a variety of data collection instruments were employed:

1.Animal Models (Rats)

Purpose: The main instrument of data collection in your study is the use of rats to model hyperuricemia. Rats were selected due to their biological similarities to humans in metabolic processes, making them a valuable model for studying hyperuricemia and its treatments.

Method: Hyperuricemia was induced in the rats by administering fructose (10%) and brewer's yeast orally over 54 days. These substances were mixed with their daily food and water to simulate natural consumption and reduce stress on the animals[10]&[12].

Data Collected: Serum uric acid levels, weight, glucose blood levels, and lipid profiles were measured to evaluate the effects of NS and compare it with allopurinol, a standard treatment for hyperuricemia. Additionally, the rats were observed for any side effects or toxicities[16].

It is important to say that the data collection, which is of the continuous type, will be measured at a specific time and in the same way and under the same conditions that exclude bias, reduce distortion and enhance clear results. From this standpoint, we decided to measure uric acid, rat weight, FBS, and lipid profile, once on day 0, another on day 27, and the third before taking the treatment of Nigella sativa and allopiranol, on day 54, and the last after taking the treatments for all the relevant groups after two weeks, taking into account that the period between day 0 and day 54, day 27, is equal in days and is done at the same time of the day and never changes, and the quantities of food and drink and all factors are uniform, taking into account the change in weight.[17]

2.phytochemical Analysis (GC-MS*)*

Purpose: Gas Chromatography-Mass Spectrometry (GC-MS) was used to analyse the phytochemical composition of the three varieties of NS from Bangladesh, Syria, and Turkey, focusing on key bioactive compounds such as thymoquinone and p-cymene[18]&.[2]

Method: After purchasing and preparing the seeds (cleaning, drying, grinding), the oil was extracted and subjected to GC-MS analysis. This allowed the identification and quantification of the active components, providing insight into how the geographical origin of the seeds might affect their medicinal properties.

Data Collected: The GC-MS analysis provided detailed information on the concentration of active compounds like thymoquinone, p-cymene, and other phytochemicals in each variety of NS . This helped in correlating the chemical composition with the pharmacological effects observed in the rat models.

3. Comparative Analysis (NS vs. Allopurinol)

Purpose: One of the main objectives of your study was to compare the efficacy of NS with allopurinol in reducing uric acid levels in hyperuricemic rats.

Method: Both NS and allopurinol were administered to different groups of rats, and their effects on serum uric acid levels were recorded and compared. This allowed for a direct comparison of the natural remedy against a conventional pharmaceutical treatment. Use for that HPLC[19]

Data Collected: Changes in serum uric acid levels, reduction in hyperuricemia symptoms, and overall health markers such as glucose levels were compared between the treatment groups.

4. Mechanism prediction by Superperd and Safety and Toxicity Assessment (pkCSM,) and Pro Tox III

Purpose: Your research also aimed to assess the safety and potential side effects of NS in treating hyperuricemia. Tools like pkCSM,& Superperd ,Pro Tox III were employed to predict the toxicity, pharmacokinetics, and bioavailability of the compounds found in NS .

Method: These computational tools helped predict potential toxic effects, drug-likeness, and absorption, distribution, metabolism, and excretion (ADME) properties of the active compounds in the seed extracts.

Data Collected: Predicted toxicity levels, drug interactions, and ADME properties were collected to ensure that NS is both effective and safe for therapeutic use as well as target indication prediction that will give good insight for therapeutic uses of NS seed .

Blood and Biochemical Analysis

Fasting blood suger by (Glucometer):

Purpose: The study required detailed biochemical analysis of blood samples from the treated rats to monitor health markers and evaluate the effectiveness of the treatment.

Method: Blood samples were taken from the rats, and various biochemical markers were measured, glucometer is used for glucose levels determination to monitor the metabolic effects of the treatments of variant type of NS ,advantage is easy and not harm the animal because small amount of blood taken

Lipid profile :

The best laboratory device for analyzing blood samples collected from rats depends on the purpose of the analysis and the required sample volume. Among the available devices, the Mitra® Microsampling Device, which utilizes Volumetric Absorptive Microsampling (VAMS®) technology, is highly recommended. This device is known for its high accuracy and ease of use, especially in studies requiring small sample volumes.

Advantages of the Mitra® Microsampling Device

- Ease of Use: Blood is collected simply by touching the device's tip to the blood for 2-5 seconds,Reduced Impact on the Animal: It requires very small amounts of blood (approximately 10 microliters), minimizing stress on the rat and allowing for repeated sampling

High Accuracy: Provides a standardized sample volume, enhancing the precision of laboratory results,flexibility in Analysis: The sample can be used for plasma or serum analysis.

How to Use the Mitra® Microsampling Device

1. Preparing the Rat, - Secure the rat using a restrainer or manually to minimize movement.

- Select a blood collection site, such as the tail vein (Tail Vein) or saphenous vein (Saphenous Vein).2. Collecting the Sample: - Clean the collection site with alcohol to ensure sterilization.

- Using a small needle (23G or 25G), draw blood from the vein. - Place the tip of the Mitra® device onto the blood drops for 2-5 seconds until it absorbs the required volume.3. Processing the Sample: - Allow the device to dry at room temperature for 2-4 hours. - Send the device to the laboratory for sample analysis.

- The team responsible for sample collection must be well-trained to avoid any harm to the animal.- Local anesthesia is recommended if collecting blood from sensitive areas, such as the submandibular vein.This device is ideal for research requiring small and repeated samples while maintaining the rat's well-being and reducing stress caused by blood collection procedures.

6. Literature Review and Comparative Data

Purpose: A comprehensive literature review was conducted to compare the findings from your research with previous studies on the medicinal properties of NS and its bioactive components.

Method: Data from existing studies were reviewed to support your findings and to provide a broader context for the potential mechanisms of action of Nigella sativa in reducing uric acid levels and managing hyperuricemia.

Data Collected: Comparative data from previous studies on the antihyperuricemic, anti-inflammatory, and antioxidant properties of NS were integrated into your analysis to strengthen your research conclusions.

4. PROCEDURES OF STUDY

Expermintal Desgin, Animal Model (Rats) for Inducing Hyperuricemia:

Chemicals: Hexane as a solvent used by concentration of 95-99%, purchase,Uric acid 99% from Sigma- Aldrich.

Animal Model: 70 male 200-250 g of Sprague-Dawley Rats in good conditions is divided in to 5 groups , group 1 is control group , group 2 is Syrian NS (S)group , group 3 is Turkish NS(T) group , group 4 is Bangladesh NS (B),group5 is Allopurinol group . Each group was separated from the other groups by labeling and numbering each rat separately. Within each group, each rat was separated separately so that we could give it the specified amount of fructose and yeast, and then we could give it the specified amount of Nigella sativa or Allopurinol. All groups were subject to the same temperature, approximately 25, humidity, and the same amount of water and food that had been prepared according to special equations

Inducing hyperuricemia by Fructose 10% and Brewer’s Yeast:

All groups administer fructose and Brewer yeast to induce hyperuricemia , including the control group.

Preparation of Fructose Solution:

Prepare a 10% fructose solution by dissolving fructose in water. For example, dissolve 10 grams of fructose in 90 milliliters of water to make a total of 100 ml of solution.[20]

Administration Method:

The most common method for administering fructose to rats is through their drinking water. This approach is non-invasive and allows the rats to consume the fructose solution voluntarily throughout the day[21]

Volume Calculation:

Rats typically consume about 10% of their body weight in water daily. For a 150g rat, this would be approximately 15ml of fluid per day[22].

Ensure that the rat has continuous access to the 10% fructose solution as its sole source of drinking water to achieve the desired intake

Brewers Yeast : the dose that we will use 15g/kg[13] that wil mix with the normal food which is Rat chow , Composition of Standard Rat Chow:

Protein: 16-18% – important for growth and repair of tissues.

Carbohydrates: 45-60% – provide energy.

Fats: 3-5% – supply essential fatty acids and aid in the absorption of fat-soluble vitamins.

Fiber: 4-6% – supports digestive health.

Vitamins and Minerals: Includes essential vitamins (A, D, E, K, B-complex) and minerals (calcium, phosphorus, magnesium, etc.) to support metabolic processes and bone health.[15]

For your study on hyperuricemia, the Standard Rat Chow will ensure the rats receive all necessary nutrients while allowing you to manipulate other components (like fructose and brewer's yeast) to induce hyperuricemia.

for example if Rat weight is 150g the dose will be 2.25g daily mix with food , daily food consumption is 15-20 g per day [23]

Measure of the rats weight the mean was 14.5 with SD 0.2 ,Fasting blood suger was 6 ,

Uric acid determination : uric acid will measure three times fist time in the start of the experiment for the control group only second time after 27 days of the experiment (inducing of hyperuricemia will takes 55 days) last time after complete of three types of nigella sativa treatments which are 2 weeks[24]

Determination of Uric acid by HPLC :

Fist sample preparations:

Blood collection from the rat : Tail Vein (Non-Surgical, Non-Terminal)

Procedure: The lateral tail vein is used for blood collection. The tail may be warmed to dilate the veins, and a needle (23G) is inserted into the vein to collect blood using a syringe or capillary tube.

Advantages:

No anesthesia required,suitable for small to moderate volumes (up to 2 mL),can be used for repeated sampling,volume: Up to 2 mL per sample.

Considerations:requires effective restraint of the rat.

Warming of the tail may be necessary to visualize the vein.[25]

Serum: Centrifuge blood samples to obtain serum . Typically, 100 µL of serum is deproteinized using an equal volume of acetonitrile to precipitate proteins. After centrifugation (at 10,000 rpm for 10 minutes), the supernatant is collected for analysis.

The samples are typically filtered through a 0.22 µm filter before injection into the HPLC system.

Mobile Phase :

20 mmol/L sodium acetate, 30 mmol/L acetic acid, and 1% methanol.

This method works well on a C18 column and can achieve rapid separation of uric acid and creatinine in biological samples within a short time (around 4 minutes) [26],detection UV between 260-290nm,Flow rate 1.0 ml/min , retention time

Around 4 minutes on a C18 column with sodium acetate/acetic acid buffer ,total analytical time 10 min per sample [26]

Nigella sativa doses and method of administration to the rats : we evaluate three types of NS .S,T&B , we will givs all doses as normal seeds to the Rats but we will gives in concentration 400mg/kg [27]for three different NS types , and this dose is less than 500 mg/kg which was mensioned in reseach studies

Preparation of allopurinol : Allopurinol is typically administered at doses ranging from 30 mg/kg to100 mg /kg [28] , we use 100mg /kg will .

To prepare a suspension of allopurinol for a rat weighing 250 grams at a dose of 100 mg/kg, follow these steps:

1. Calculate the Amount of Allopurinol Needed

Rat's weight: 250 grams (at the end of the experiment 55 days mean of rat weight is 250 g SD 0.2) = 0.25 kg Dose: 100 mg/kg From the calculation:

Amount of allopurinol=100 mg kg×0.25 kg=25 mgAmount of allopurinol=100mg kg×0.25kg=25mg

So, you will need 25 mg of allopurinol for each rat.

2. Choose the Desired Concentration for the Suspension

if you want a concentration of 10 mg/mL (a common concentration for suspensions):

Volume of vehicle=Amount of allopurinol
desired concentration=25 mg10 mg mL=2.5 mLVolume of vehicle=Desired concentrationAmount of allopurinol
=10mg mL25mg=2.5mL

3. Prepare the Suspension

Weigh 25 mg of allopurinol powder.

Suspend it in 2.5 mL of a suitable vehicle, such as 1% sodium carboxymethyl cellulose (CMC) or distilled water.

Mix thoroughly to ensure even distribution of the drug in the suspension.

4. Administer the Suspension

Administer the prepared suspension via oral gavage to the rat.

Since you have prepared a suspension with a concentration of 10 mg/mL, you would administer the entire 2.5 mL to deliver the required dose of 25 mg

Preparation of Uric acid standard Sigma Aldich:

Uric acid (e.g., Sigma-Aldrich U0881 or U2625)

1 M NaOH (for dissolving uric acid)

Deionized water

Volumetric flask

Sonicator and heating device (if necessary)

Procedure:

Weight the uric acid accurately weigh the required amount of uric acid. For example, if you want to prepare a 50 mg/mL stock solution, weigh 50 mg of uric acid.
Dissolve in NaoH uric acid is not very soluble in water but dissolves well in alkaline solutions like 1 M NaOH. Transfer the weighed uric acid into a beaker and add a small volume of 1 M NaOH (e.g., 5 mL). Stir the solution gently. If necessary, apply heat and sonication to assist dissolution, as recommended by Sigma-Aldrich adjust volume once the uric acid is fully dissolved, transfer the solution to a volumetric flask and dilute to the desired final volume with deionized water. For example, if you are preparing 10 mL of a 50 mg/mL solution, dilute up to the 10 mL mark, Filter the solution to remove any undissolved particles if found, you may filter the solution through a 0.45 µm filter before using it in HPLC.

Storage:
we store the uric acid standard at room temperature or not increase than 25 C .

HPLC Preparations: before injecting into the HPLC system, ensure that your mobile phase and other parameters are optimized for uric acid detection based on your specific method ,Three types of Nigella sativa were purchased from a local store in Syria in a Hama city, in Turkey from a Konya city, and in Bangladesh from a Dhaka city. They were washed and dried for three days in the shade and purified from all impurities.

Sample preparation

The three samples were in good condition. Grinding was done first by carefully weighing them on a sensitive balance in the laboratory, where we weighed 60 grams for each type separately, then the special seed grinder was used Secura Electric grinder . After grinding, we use them directly in the Soxhelt process. Hexane is used as a solvent, and the amount of NS is 60 grams and the solvent is 400 ml. The experiment takes three hours while maintaining a temperature of 59-62 C. We leave the device to cool for half an hour, then the extract is placed in a dark bottle in the refrigerator, waiting for the rest of the steps.

Filtrations

The filtration step will be during the period with emptying with a filter paper Material :Nylon ,pore size 0.45 micrometre, and it is repeated several times to remove the colour that will be an obstacle to GCMS analysis Note that all extraction, filtration and storage processes must be at low temperatures to avoid the decomposition of any chemical substance that will decompose immediately upon rising temperature.

Activated Charcoal discolorations :

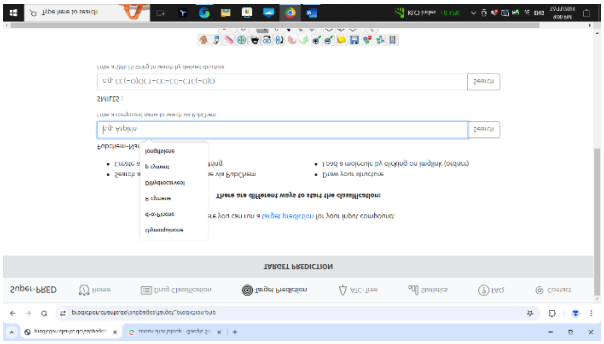
Use of activated charcoal is used with extreme caution and in small concentrations (0.25% of the total volume of the extract) so that the active substances in the extract do not stick to it, but we took this step to obtain a very pure sample for use in GC-MS[29]

GC-MS Procedure :

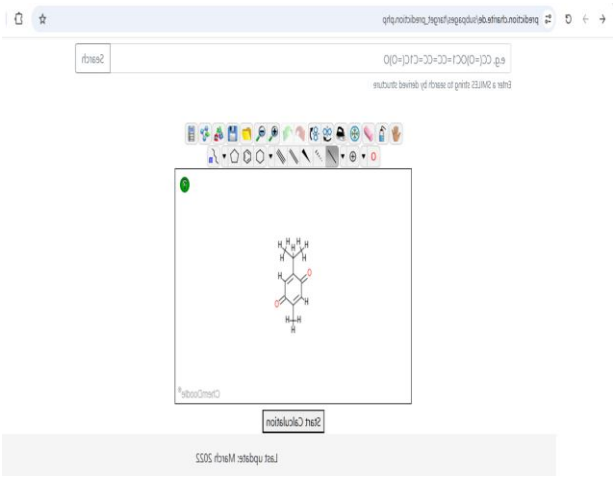
Brand: Shimadzu -Model: GCMS-QP 2010 Plus, Brief description: The GCMS-QP2010 Plus shows the highest sensitivity ever reached in a GCMS system. Ideal for rapid analysis of trace components, the GCMS-QP2010 Plus is the ideal instrument for complex organic mixtures in environment,Column Oven Temperature ,Column SH-Rxi-5Sil MS (Shimadzu) ,length :30m,internal Diameter (ID) :0.25 µm, Film Thickness: 0.25 µm ,Carrier Gas: Helium at a flow rate of 1.0 mL/min, , injection Volume and Mode: Spitless injection of 1 µL sample , Oven Temperature Program: initial temperature of 60°C held for 2 minutes , Ramp at a rate of 10°C/min to a final temperature of 280°C ,hold at final temperature for 10 minutes),Pressure 600 bar, MS Conditions ,ionization mode: Electron Impact (EI),Ion source temperature: 200°c ,mass range scanned: m/z 50–550, solvent Delay(min) 4 mint, library NIST11s.lib contribute to the identification of compounds ,the NIST11s.lib contributes to compound identification in GC-MS by providing, a large database of reference mass spectra for spectral matching, retention index data for cross-verifying compound identity ,enhanced accuracy through combined evaluation of mass spectra and retention indices ,this dual approach ensures more reliable identification of compounds in complex mixtures, as demonstrated in your sample analysis where multiple hits were confirmed with high similarity indices and corresponding retention indices[30]

SuperPred: Target Detection for Drug Discovery

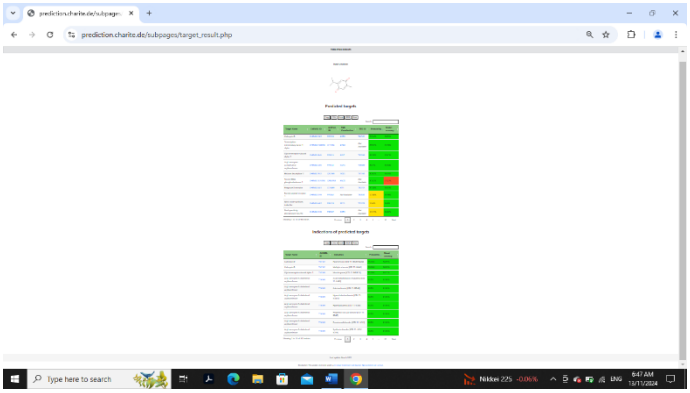
SuperPred is an advanced computational tool designed to predict potential targets and mechanisms of action for bioactive compounds. It leverages a vast database of known drug-target interactions and applies machine learning algorithms to identify likely targets for compounds with unknown or poorly understood mechanisms. This tool is particularly useful in drug discovery and repositioning, helping researchers explore new therapeutic uses for natural products and synthetic compounds, using SuperPred for NS, Compound Identification: Identify the key bioactive compounds present in Nigella sativa, such as thymoquinone, nigellidine, and p-cymene .target Prediction: Input these compounds into SuperPred to predict potential biological targets. SuperPred utilizes structural similarity to known drugs and integrates information from public databases to propose potential targets ,mechanism Elucidation: Analyze the predicted targets to hypothesize new mechanisms of action. This can include interactions with enzymes, receptors, or pathways that have not been previously associated with Nigella sativa, validation: use experimental methods, such as in vitro and in vivo studies, to validate the predicted targets and mechanisms. This step confirms the computational predictions and provides insights into how Nigella sativa exerts its therapeutic effects ,advantages of Using SuperPred, efficiency: Accelerates the identification of potential drug targets compared to traditional experimental methods,cost-Effective: Reduces the need for extensive laboratory resources by narrowing down the list of potential targets,innovative Discovery: Facilitates the discovery of novel mechanisms and therapeutic applications for natural products,by employing SuperPred in the study of Nigella sativa, researchers can uncover new insights into its pharmacological actions and potentially develop new therapeutic applications for its use,method :first write the compound name or there smile structure Figer 1.3 Superperd home page.[31]



Second:after write the name system will drow the structure and ask you if you want to start



Figer 1.4 Superperd search



Figer 1.5 Target result

The system will give result contain name of the target

After calculation the system will give you two results ,first one is peridected tagert with links to information about the gene and disease related and drugs used before , second result is indication of that target after rection and possible syndrome and diseases the compound can treated after affecting , there are percentage of probability and accuracy of each results.

PkCSM :it is perdiction tools in the same way you should write the compound SMILES string like of thymoquinone is CC1=CC(=O)C(=CC1=O)C(C)C , the system will give result ask you to choice between of pharmacokinetic properties = absorption , distribution , metabolism , excretion, toxicity ,or all together ADMET , in the Appindex you will find ADMET of all compound resulted from GCMS In the same way of use and research we can use,

Absorption Distribution Metabolism Excretion Toxicity (ADME),evaluating NS Seed Using, pkCSM, nigella sativa is a

plant with a rich history of medicinal use, and understanding its pharmacokinetics and toxicity is crucial for optimizing its therapeutic potential. Computational tools like pkCSM, can provide valuable insights into these aspects.

1. ProTox-III: Toxicity Prediction

ProTox-II is a web-based tool designed to predict the toxicity of chemical compounds. It uses a machine learning approach to estimate various toxicity endpoints, including acute toxicity, hepatotoxicity, and potential carcinogenicity.

Application: Input the chemical structures of key Nigella sativa compounds, such as thymoquinone, P cymene . α Phellandrene , Nigellidine into ProTox-III.

Toxicity Assessment: Obtain predictions on the LD50 (lethal dose for 50% of the population) This helps in understanding the safety profile and identifying safe dosage ranges. Figer 2.1

2. pkCSM: Pharmacokinetic Predictions

pkCSM is a computational tool that predicts pharmacokinetic properties, including absorption, distribution, metabolism, and excretion (ADME).application: Input the compound structures into pkCSM to predict keypharmacokinetic parameters.distribution and Availability: Analyse predictions for properties such as volume of distribution, blood-brain barrier penetration, and bioavailability. This information helps understand how the compounds distribute in the body and their overall availability at different doses.by combining insights from ProTox-II, pkCSM, researchers can develop a holistic understanding of how Nigella sativa compounds behave in the body, their safety profiles, and how different dosages impact these factors. This approach supports the development of safer and more effective therapeutic applications for NS.

5. RESULTS AND DISCUSSION

Data collection of induced animal model: there ara biochemical data like uric acid level , before and after treatment of both NS and Allopurinol, and body weight and Fasting blood suger level , and lipid profile which important for fructose inducing Model

The research relies entirely on the level of uric acid obtained from the use of HPLC to know the effectiveness of each group that represents a different type of NS, at the beginning of the experiment and on day 27 and day 54, and it is important to compare this effectiveness with the well-known gout drug Allupiranol. because of the symptoms resulting from long-term fructose ingestion, animals must be monitored with vital tests such as blood glucose levels, lipids and Rat body weight. We will use this to create a comparison between the three types of Nigella sativa, so that we know which of the three types of NS can reduce the symptoms that appear as a result of fractose inducing. In next page there are tables of uric acid and other important biochemical parameters in table Table 3.2

Table 3.2

UAL	control	inducing control	27 days	T group	B group	S group	Allopiranol group
	1.09	2.4	1.5	1.6	1.8	2	1.4
	1.1	2.5	1.51	1.6	1.7	1.9	1.3
	1.2	2.6	1.52	1.7	1.9	2	1.2
	1.08	2.4	1.41	1.5	1.8	1.9	1.4
	1.09	2.4	1.5	1.6	1.8	2	1.4
	1.2	2.5	1.5	1.5	1.7	2	1.4
	1.1	2.4	1.5	1.7	1.8	2	1.3
	1.09	2.4	1.41	1.6	1.9	1.8	1.3
	1.2	2.5	1.51	1.6	1.8	1.9	1.4
	1.1	2.6	1.5	1.7	1.8	2	1.2
	1.09	2.4	1.5	1.6	1.8	2	1.4
	1.09	2.5	1.41	1.7	1.8	2	1.4
	1.1	2.4	1.41	1.5	1.8	1.9	1.4
	1.1	2.4	1.5	1.7	1.8	1.9	1.4
	1.1	2.4	1.5	1.7	1.8	1.9	1.4

Rat wieght	control	inducing control	T group	B group	S group
	250	300	280	280	291
	248	292	281	282	292
	250	295	282	281	290
	243	295	283	280	291
	240	299	280	280	292
	252	298	280	282	291
	250	300	280	281	291
	250	300	281	281	291
	245	300	279	282	292
	250	300	279	281	292
	250	300	280	280	291
	245	300	281	280	289
	250	300	275	280	289
	250	300	279	280	288
	250	300	279	280	288

lipid pro	control	induced control	Tgroup	B group	C groyup	Allopiranol group
	65	150	100	122	123	150
	65	150	101	122	124	150
	65	150	100	123	123	150
	65	150	102	119	124	150
	65	140	102	122	125	150
	65	146	100	122	123	150
	65	146	100	125	123	150
	66	149	102	125	123	150
	65	149	101	122	123	150
	66	145	101	122	123	150
	66	150	101	121	123	150
	65	150	100	122	123	150
	65	150	100	122	123	150
	65	150	100	122	123	150
	65	150	100	123	123	150

FBS	control	induced control	T group	B group	S group
	6.2	6.8	6.5	6.6	6.6
	6.2	6.8	6.5	6.7	6.6
	6.3	6.7	6.5	6.6	6.6
	6.3	6.7	6.5	6.7	6.7
	6.2	6.8	6.4	6.6	6.7
	6.3	6.8	6.4	6.6	6.7
	6.2	6.8	6.5	6.4	6.6
	6.3	6.7	6.4	6.5	6.6
	6.2	6.7	6.5	6.6	6.5
	6.2	6.8	6.5	6.6	6.6
	6.2	6.8	6.5	6.5	6.6
	6.4	6.8	6.5	6.6	6.6
	6.4	6.8	6.4	6.7	6.6
	6.2	6.8	6.4	6.7	6.7
	6.2	6.8	6.4	6.7	6.7

Statistical Analysis:

I think I should defined the type of data collected

The type of data collected in my research study appears to be continuous. This is inferred from the focus on measuring serum uric acid levels in rats, which are typically quantified as numerical values (e.g., milligrams per decilitre or similar units). Continuous data is appropriate for this type of pharmacological and biochemical analysis, as it allows for precise comparisons between groups (e.g., the effects of Nigella sativa varieties from different regions).

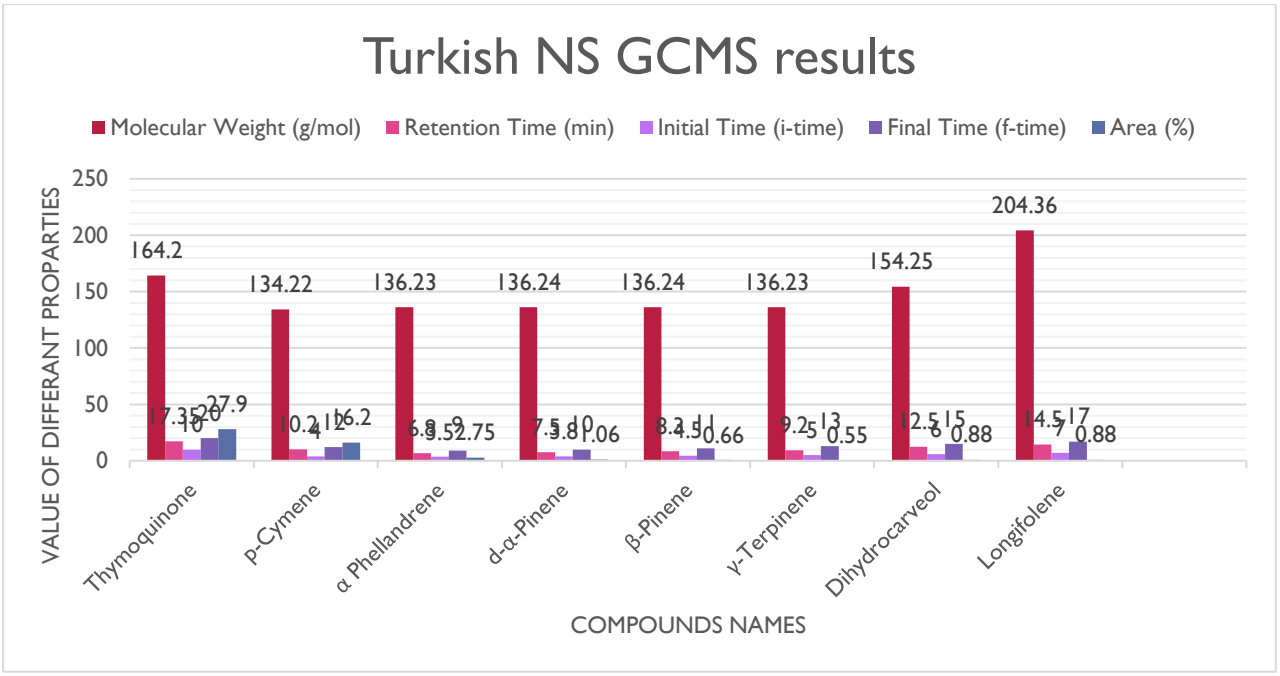
3.5.2 Phytochemical Analysis Using GC-MS (Gas Chromatography-Mass Spectrometry):

The GC-MS analysis provided quantitative data on the concentration of key bioactive compounds, including , Thymoquinone , P-Cymene ,α -Phellandrene α Pinene , γ Terpinene , Dihydrocarveol, Longifolene.

Turkish NS extract GC-MS analysis result Table 3.3

Compound	Molecular Weight (g/mol)	Retention Time (min)	Initial Time (i-time)	Final Time (f-time)	Area (%)
Thymoquinone	164.20	17.35	10.00	20.00	27.9
p-Cymene	134.22 Table 3.3	10.20	4.00	12.00	16.2
α Phellandrene	136.23	6.80	3.50	9.00	2.75
d-α-Pinene	136.24	7.50	3.80	10.00	1.06
β-Pinene	136.24	8.30	4.50	11.00	0.66

Compound	Molecular Weight (g/mol)	Retention Time (min)	Initial Time (i-time)	Final Time (f-time)	Area (%)
γ-Terpinene	136.23	9.20	5.00	13.00	0.55
Dihydrocarveol	154.25	12.50	6.00	15.00	0.88
Longifolene	204.36	14.50	7.00	17.00	0.88



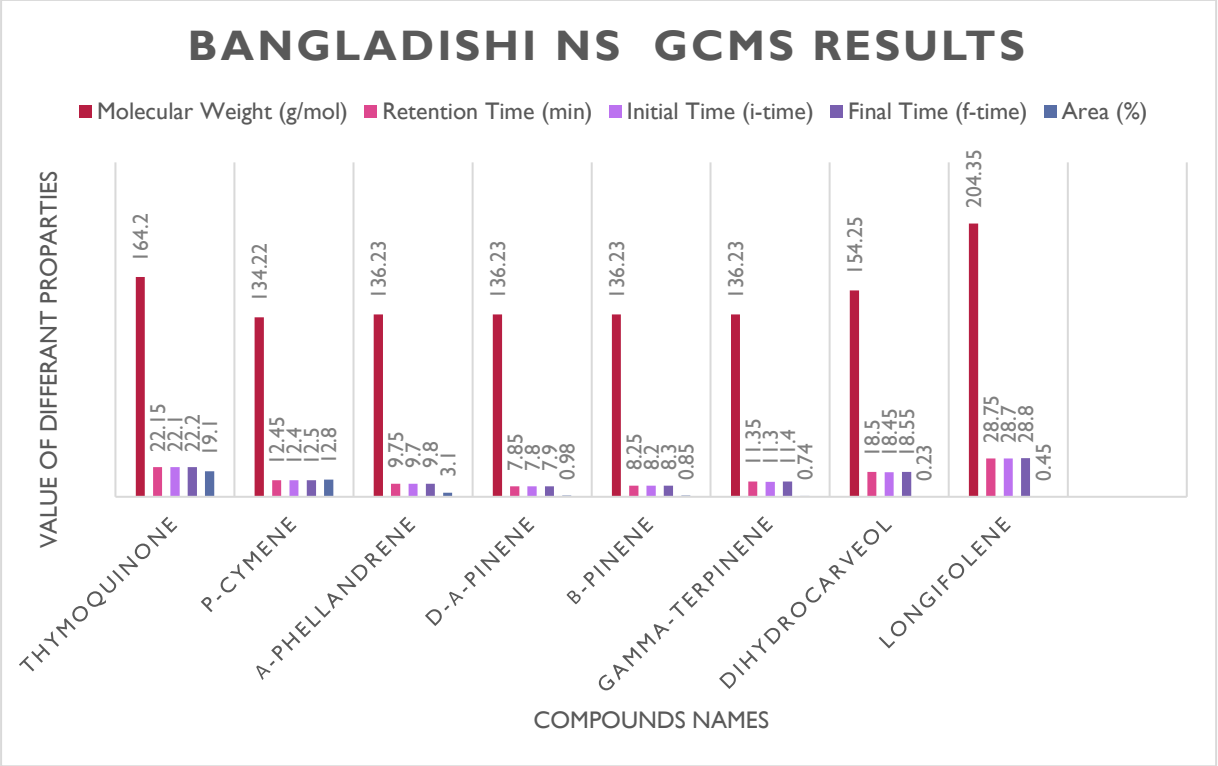
Figer 3.3A

1- T sample THYMOQUINONE 27.9% MW 164.2, P Cymene 10.6% MW134.22, α Phellandrene MW134.23, d-α-Pinene 1.06%,MW136.24 β-Pinene0.66%MW136.24 γ-Terpinene 0.55% MW136.23, Dihydrocarveol 0.88%MW154.25, Longifolene 0.88%MW204.36 -GCMS Figer 3.3 B

Result of Bangladish NS extract by GC-MS analysis Tablet 3.4

Compound	Molecular Weight (g/mol)	Retention Time (min)	Initial Time (i-time)	Final Time (f-time)	Area (%)
Thymoquinone	164.2	22.15	22.10	22.20	19.10
p-Cymene	134.22	12.45	12.40	12.50	12.8
α-Phellandrene	136.23	9.75	9.70	9.80	3.1
d-α-Pinene	136.23	7.85	7.80	7.90	0.98

Compound	Molecular Weight (g/mol)	Retention Time (min)	Initial Time (i-time)	Final Time (f-time)	Area (%)
β-Pinene	136.23	8.25	8.20	8.30	0.85
gamma-Terpinene	136.23	11.35	11.30	11.40	0.74
Dihydrocarveol	154.25	18.50	18.45	18.55	0.23
Longifolene	204.35	28.75	28.70	28.80	0.45



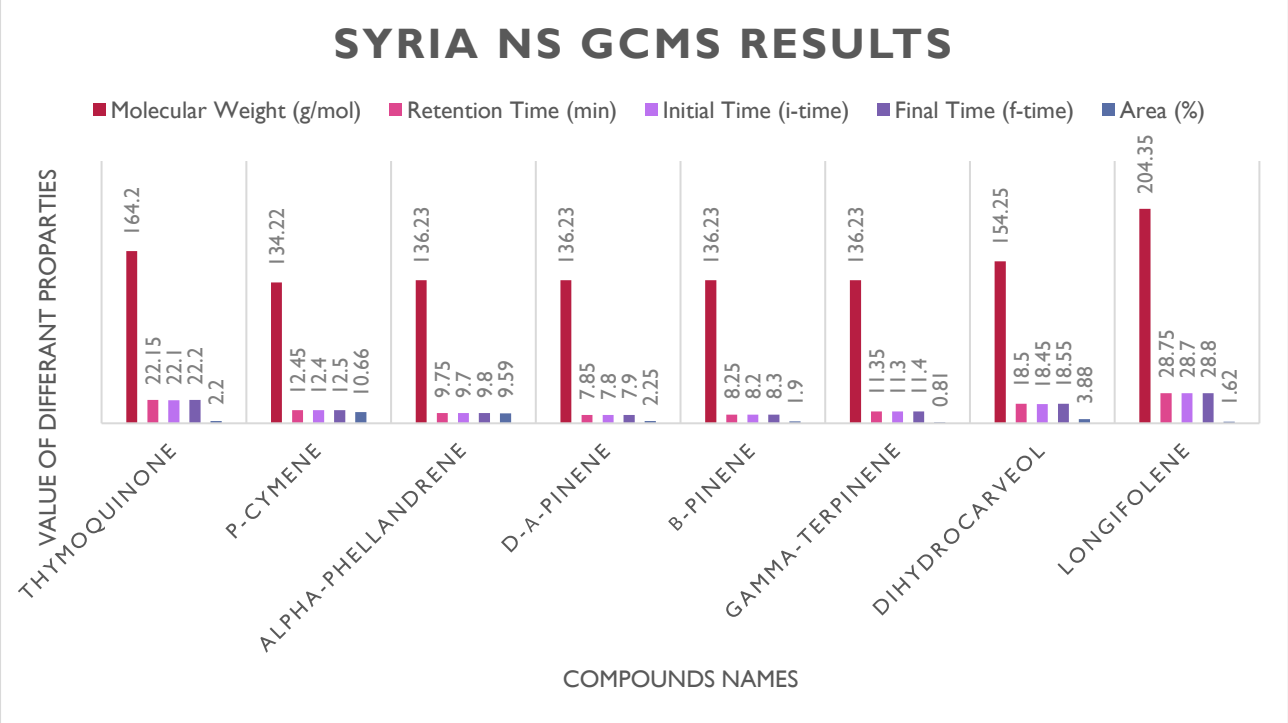
Figer 3.4A

2-B sample THYMOQUINONE 19.1% MW 164.2, P Cymene 12.8% MW134.22, α Phellandrene3.1% MW134.23, d-α-Pinene 0.98%,MW136.24 β-Pinene0.85%MW136.24 γ-Terpinene 0.74% MW136.23, Dihydrocarveol 0.23%MW154.25, Longifolene 0.45%MW204.36 -GCMS Figer 3.4B

Result of Syrian NS extract by GC-MS analysis Tablet 3.5 :

Compound	Molecular Weight (g/mol)	Retention Time (min)	Initial Time (i-time)	Final Time (f-time)	Area (%)
Thymoquinone	164.2	22.15	22.10	22.20	2.20

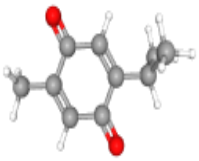
Compound	Molecular Weight (g/mol)	Retention Time (min)	Initial Time (i-time)	Final Time (f-time)	Area (%)
p-Cymene	134.22	12.45	12.40	12.50	10.66
Alpha-Phellandrene	136.23	9.75	9.70	9.80	9.59
d-α-Pinene	136.23	7.85	7.80	7.90	2.25
β-Pinene	136.23	8.25	8.20	8.30	1.90
gamma-Terpinene	136.23	11.35	11.30	11.40	0.81
Dihydrocarveol	154.25	18.50	18.45	18.55	3.88
Longifolene	204.35	28.75	28.70	28.80	1.62



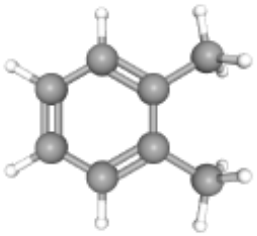
Figer 4.5 A

3-S sample THYMOQUINONE 2.2% MW 164.2, P Cymene 10.66% MW134.22, α Phellandrene 9.59% MW134.23, d-α-Pinene 2.25%,MW136.24 β-Pinene1.90%MW136.24 γ-Terpinene 0.81% MW136.23, Dihydrocarveol 3.88%MW154.25, Longifolene 1.62%MW204.36 -GCMS Figer 4.5B

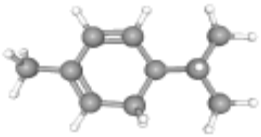
Most important 4 active biochemical that have antihyperuricemic effect in NS seed



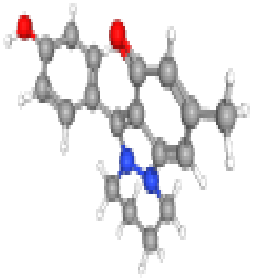
p-cymene



thymoquinone



α phellandrene



Nigellidine alkaloid.

Perdiction of mechanisms Safety and Toxicity Assessment & pharmacokinatics(pkCSM, & SUPERPERD) :

Superperd results of Thymoquinone Tablet 3.6

Target Name	ChEMBL-ID	Indication	Probability	Model accuracy
Formyl peptide receptor 1	T87831	Inflammation [ICD-11: 1A00-CA43.1]	77%	94%
Nitric oxide synthase, inducible	T02703	Inflammation [ICD-11: 1A00-CA43.1]	75.6%	94.8%
Nitric oxide synthase, inducible	T02703	Rheumatoid arthritis [ICD-11: FA20]	75.6%	94.8%
Androgen Receptor	T11211	Pain [ICD-11: MG30-MG3Z]	65%	96%
PI3-kinase p110-delta subunit	T67849	Arthritis [ICD-11: FA20]	62%	96%
Voltage-gated T-type calcium channel alpha-1H subunit	T54644	Pain [ICD-11: MG30-MG3Z]	62%	99%
Voltage-gated N-type calcium channel alpha-1B subunit	T38338	Pain [ICD-11: MG30-MG3Z]	56%	97%
Xanthine dehydrogenase	T40954	Hyperuricaemia [ICD-11: 5C55.Y]	54%	96%
P2X purinoceptor 7	T63414	Inflammation [ICD-11: 1A00-CA43.1]	52.6%	97.5%
P2X purinoceptor 7	T63414	Inflammatory bowel disease	52.6%	97.5%

		[ICD-11: DD72]		
P2X purinoceptor 7	T63414	Pain [ICD-11: MG30-MG3Z]	52.6%	97.5%
P2X purinoceptor 7	T63414	Rheumatoid arthritis [ICD-11: FA20]	52.6%	97.5%
Multidrug resistance-associated protein 1	T11288	Gout [ICD-11: FA25]	52%	96%
Serotonin 2c (5-HT2c) receptor	T83813	Pain [ICD-11: MG30-MG3Z]	52%	90%
Adaptor-associated kinase	T98271	Rheumatoid arthritis [ICD-11: FA20]	52%	83.1%
Tyrosine-protein kinase ITK/TSK	T91761	Inflammation [ICD-11: 1A00-CA43.1]	52%	95%
C-C chemokine receptor type 2	T89988	Arthritis [ICD-11: FA20]	51%	99%
C-C chemokine receptor type 2	T89988	Inflammation [ICD-11: 1A00-CA43.1]	51%	99%
C-C chemokine receptor type 2	T89988	Inflammatory pain [ICD-11: MG30.42]	51%	99%
Xanthine dehydrogenase	T40954	Gout [ICD-11: FA25]	54%	96%

Superperd results of P -Cymene table 3.7

Target Name	ChEMBL-ID	Indication	Probability	Model accuracy
Serotonin 2c (5-HT2c) receptor	T83813	Pain [ICD-11: MG30-MG3Z]	84%	90%
Formyl peptide receptor 1	T87831	Inflammation [ICD-11: 1A00-CA43.1]	84%	94%
Nitric oxide synthase, inducible	T02703	Rheumatoid arthritis [ICD-11: FA20]	77%	94.8%
Cathepsin B	T61746	Rheumatoid arthritis [ICD-11: FA20]	75.6%	94%
Sodium channel protein type III alpha subunit	T76937	Pain [ICD-11: MG30-MG3Z]	64%	96.9%
Transient receptor potential cation channel subfamily A member 1	T84040	Pain [ICD-11: MG30-MG3Z]	59%	92%
Adaptor-associated kinase	T98271	Rheumatoid arthritis [ICD-11: FA20]	59%	83.1%
Multidrug resistance-associated protein 1	T11288	Gout [ICD-11: FA25]	55%	96%
Voltage-gated N-type calcium channel alpha-1B subunit	T38338	Pain [ICD-11: MG30-MG3Z]	55%	97%
Tyrosine-protein kinase ITK/TSK	T91761	Inflammation [ICD-11: 1A00-CA43.1]	54%	95%
PI3-kinase p110-delta subunit	T67849	Arthritis [ICD-11: FA20]	53%	96%

C-C chemokine receptor type 2	T89988	Arthritis [ICD-11: FA20]	51%	99%
P2X purinoceptor 7	T63414	Inflammation [ICD-11: 1A00-CA43.1]	50%	97.5%
P2X purinoceptor 7	T63414	Inflammatory bowel disease [ICD-11: DD72]	50%	97.5%
P2X purinoceptor 7	T63414	Pain [ICD-11: MG30-MG3Z]	50%	97.5%
P2X purinoceptor 7	T63414	Rheumatoid arthritis [ICD-11: FA20]	50%	97.5%

Superperd results of α phellandrene table 3.8

Target Name	ChEMBL-ID	Indication	Probability	Model accuracy
Formyl peptide receptor 1	T87831	Inflammation [ICD-11: 1A00-CA43.1]	85%	94%
PI3-kinase p110-delta subunit	T67849	Arthritis [ICD-11: FA20]	75%	96%
Serotonin 2c (5-HT2c) receptor	T83813	Pain [ICD-11: MG30-MG3Z]	73%	90%
Nitric oxide synthase, inducible	T02703	Inflammation [ICD-11: 1A00-CA43.1]	67%	94.8%
Nitric oxide synthase, inducible	T02703	Rheumatoid arthritis [ICD-11: FA20]	67%	94.8%
Voltage-gated N-type calcium channel alpha-1B subunit	T38338	Pain [ICD-11: MG30-MG3Z]	65%	97%
Voltage-gated T-type calcium channel alpha-1H subunit	T54644	Pain [ICD-11: MG30-MG3Z]	62.1%	99%
Androgen Receptor	T11211	Pain [ICD-11: MG30-MG3Z]	61.1%	96%
Sodium channel protein type III alpha subunit	T76937	Pain [ICD-11: MG30-MG3Z]	61%	96.9%
Multidrug resistance-associated protein 1	T11288	Gout [ICD-11: FA25]	59%	96%
Adaptor-associated kinase	T98271	Rheumatoid arthritis [ICD-11: FA20]	59%	83.1%
Tyrosine-protein kinase ITK/TSK	T91761	Inflammation [ICD-11: 1A00-CA43.1]	57%	95%
C-C chemokine receptor type 2	T89988	Inflammation [ICD-11: 1A00-CA43.1]	57%	99%
C-C chemokine receptor type 2	T89988	Inflammatory pain [ICD-11: MG30.42]	57%	99%
C5a anaphylatoxin chemotactic receptor	T15439	Inflammation [ICD-11: 1A00-CA43.1]	54%	93%
C5a anaphylatoxin chemotactic receptor	T15439	Rheumatoid arthritis [ICD-11: FA20]	54%	93%
Voltage-gated potassium channel subunit Kv1.5	T17569	Pain [ICD-11: MG30-MG3Z]	50%	94%
Amine oxidase, copper containing	T69619	Inflammation [ICD-11: 1A00-CA43.1]	50%	94%

Superperd results of Nigellidine alkaloid. Table 3.9

Target Name	ChEMBL-ID	Indication	Probability	Model accuracy
Thyroid hormone receptor alpha	T79591	High blood cholesterol level [ICD-11: 5C80.00]	80.2%	99%
G-protein coupled bile acid receptor 1	T86273	Type-2 diabetes [ICD-11: 5A11]	77%	94%
Cyclin-dependent kinase 5/CDK5 activator 1	T20973	Obesity [ICD-11: 5B81]	75%	93%
Estrogen receptor beta	T80896	Inflammation [ICD-11: 1A00-CA43.1]	67%	98%
Acetyl-CoA carboxylase 2	T08922	Obesity [ICD-11: 5B81]	61%	98%
Ghrelin receptor	T59604	Obesity [ICD-11: 5B81]	59%	92%
11-beta-hydroxysteroid dehydrogenase 1	T65200	Diabetic complication [ICD-11: 5A2Y]	58%	98%
11-beta-hydroxysteroid dehydrogenase 1	T65200	Obesity [ICD-11: 5B81]	58%	98%
Melanin-concentrating hormone receptor 1	T09572	Obesity [ICD-11: 5B81]	58%	92.5%
Xanthine dehydrogenase	T40954	Gout [ICD-11: FA25]	65%	96%
Hexokinase type IV	T87166	Diabetic complication [ICD-11: 5A2Y]	57%	92%
Hexokinase type IV	T87166	Obesity [ICD-11: 5B81]	57%	92%
Hexokinase type IV	T87166	Type-2 diabetes [ICD-11: 5A11]	57%	92%
Sodium channel protein type III alpha subunit	T76937	Pain [ICD-11: MG30-MG3Z]	55%	96.9%
Acyl-CoA desaturase	T10897	Type-2 diabetes [ICD-11: 5A11]	55%	97.5%
Phosphodiesterase 7A	T39523	Pain [ICD-11: MG30-MG3Z]	54%	99%
GABA-A receptor; alpha-1/beta-3/gamma-2	T08910	Chronic pain [ICD-11: MG30]	52%	95.5%
GABA-A receptor; alpha-1/beta-3/gamma-2	T08910	Inflammation [ICD-11: 1A00-CA43.1]	52%	95.5%
GABA-A receptor; alpha-1/beta-3/gamma-2	T08910	Inflammation [ICD-11: 1A00-CA43.1]	52%	95.5%
GABA-A receptor; alpha-1/beta-2/gamma-2	T08910	Inflammation [ICD-11: 1A00-CA43.1]	50.9%	93%
GABA-A receptor; alpha-1/beta-2/gamma-2	T08910	Inflammation [ICD-11: 1A00-CA43.1]	50.9%	93%
GABA-A receptor; alpha-1/beta-2/gamma-2	T08910	Inflammation [ICD-11: 1A00-CA43.1]	50.9%	93%
Cyclin-dependent kinase 1/cyclin B	T49898	Obesity [ICD-11: 5B81]	50%	96%
Cyclin-dependent kinase 1/cyclin B	T49898	Pain [ICD-11: MG30-MG3Z]	50%	96%

ProTox-III: Toxicity Prediction (Table 3. 10)

COMPOUND NAME	LD50	CLASS
THYMOQUINONE	2400 mg/kg	5
P cymene	3 mg/kg	1 red
α phellandrene	5700 mg/kg	6
Nigellidine alkaloid	1000mg/kg	4

PKCSM of Thymoquinone: (Table 3.11)

Property	Model Name	Predicted Value	Unit
Absorption	Water solubility	**-1.613**	Numeric (log mol/L)
Absorption	Caco2 permeability	**1.271**	Numeric (log Papp in 10^{-6} cm/s)
Absorption	Intestinal absorption (human)	**99.382**	Numeric (% Absorbed)
Absorption	Skin Permeability	**-2.461**	Numeric (log Kp)
Absorption	P-glycoprotein substrate	**No**	Categorical (Yes/No)
Absorption	P-glycoprotein I inhibitor	**No**	Categorical (Yes/No)
Absorption	P-glycoprotein II inhibitor	**No**	Categorical (Yes/No)
Distribution	VDss (human)	**-0.026**	Numeric (log L/kg)
Distribution	Fraction unbound (human)	**0.527**	Numeric (Fu)
Distribution	BBB permeability	**0.326**	Numeric (log BB)
Distribution	CNS permeability	**-2.269**	Numeric (log PS)
Metabolism	CYP2D6 substrate	**No**	Categorical (Yes/No)
Metabolism	CYP3A4 substrate	**No**	Categorical (Yes/No)
Metabolism	CYP1A2 inhibitior	**No**	Categorical (Yes/No)
Metabolism	CYP2C19 inhibitior	**No**	Categorical (Yes/No)

Property	Model Name	Predicted Value	Unit
Metabolism	CYP2C9 inhibitior	**No**	Categorical (Yes/No)
Metabolism	CYP2D6 inhibitior	**No**	Categorical (Yes/No)
Metabolism	CYP3A4 inhibitior	**No**	Categorical (Yes/No)
Excretion	Total Clearance	**0.225**	Numeric (log ml/min/kg)
Excretion	Renal OCT2 substrate	**No**	Categorical (Yes/No)
Toxicity	AMES toxicity	**No**	Categorical (Yes/No)
Toxicity	Max. tolerated dose (human)	**0.89**	Numeric (log mg/kg/day)
Toxicity	hERG I inhibitor	**No**	Categorical (Yes/No)
Toxicity	hERG II inhibitor	**No**	Categorical (Yes/No)
Toxicity	Oral Rat Acute Toxicity (LD50)	**1.743**	Numeric (mol/kg)
Toxicity	Oral Rat Chronic Toxicity (LOAEL)	**2.378**	Numeric (log mg/kg_bw/day)
Toxicity	Hepatotoxicity	**Yes**	Categorical (Yes/No)
Toxicity	Skin Sensitisation	**Yes**	Categorical (Yes/No)
Toxicity	*T.Pyriformis* toxicity	**0.138**	Numeric (log ug/L)
Toxicity	Minnow toxicity	**1.758**	Numeric (log mM)

PKCSM of P-Cymene Table 3.12

Property	Model Name	Predicted Value	Unit
Absorption	Water solubility	**-4.081**	Numeric (log mol/L)
Absorption	Caco2 permeability	**1.527**	Numeric (log Papp in 10^{-6} cm/s)
Absorption	Intestinal absorption (human)	**93.544**	Numeric (% Absorbed)

Property	Model Name	Predicted Value	Unit
Absorption	Skin Permeability	**-1.192**	Numeric (log Kp)
Absorption	P-glycoprotein substrate	**No**	Categorical (Yes/No)
Absorption	P-glycoprotein I inhibitor	**No**	Categorical (Yes/No)
Absorption	P-glycoprotein II inhibitor	**No**	Categorical (Yes/No)
Distribution	VDss (human)	**0.697**	Numeric (log L/kg)
Distribution	Fraction unbound (human)	**0.159**	Numeric (Fu)
Distribution	BBB permeability	**0.478**	Numeric (log BB)
Distribution	CNS permeability	**-1.397**	Numeric (log PS)
Metabolism	CYP2D6 substrate	**No**	Categorical (Yes/No)
Metabolism	CYP3A4 substrate	**No**	Categorical (Yes/No)
Metabolism	CYP1A2 inhibitior	**Yes**	Categorical (Yes/No)
Metabolism	CYP2C19 inhibitior	**No**	Categorical (Yes/No)
Metabolism	CYP2C9 inhibitior	**No**	Categorical (Yes/No)
Metabolism	CYP2D6 inhibitior	**No**	Categorical (Yes/No)
Metabolism	CYP3A4 inhibitior	**No**	Categorical (Yes/No)
Excretion	Total Clearance	**0.239**	Numeric (log ml/min/kg)
Excretion	Renal OCT2 substrate	**No**	Categorical (Yes/No)
Toxicity	AMES toxicity	**No**	Categorical (Yes/No)
Toxicity	Max. tolerated dose (human)	**0.903**	Numeric (log mg/kg/day)
Toxicity	hERG I inhibitor	**No**	Categorical (Yes/No)

Property	Model Name	Predicted Value	Unit
Toxicity	hERG II inhibitor	**No**	Categorical (Yes/No)
Toxicity	Oral Rat Acute Toxicity (LD50)	**1.827**	Numeric (mol/kg)
Toxicity	Oral Rat Chronic Toxicity (LOAEL)	**2.328**	Numeric (log mg/kg_bw/day)
Toxicity	Hepatotoxicity	**No**	Categorical (Yes/No)
Toxicity	Skin Sensitisation	**Yes**	Categorical (Yes/No)
Toxicity	*T.Pyriformis* toxicity	**0.462**	Numeric (log ug/L)
Toxicity	Minnow toxicity	**0.869**	Numeric (log mM)

(Table 3.13) PkCSM of αPhellandrene

Property	Model Name	Predicted Value	Unit
Absorption	Water solubility	**-3.849**	Numeric (log mol/L)
Absorption	Caco2 permeability	**1.414**	Numeric (log Papp in 10^{-6} cm/s)
Absorption	Intestinal absorption (human)	**96.548**	Numeric (% Absorbed)
Absorption	Skin Permeability	**-1.508**	Numeric (log Kp)
Absorption	P-glycoprotein substrate	**No**	Categorical (Yes/No)
Absorption	P-glycoprotein I inhibitor	**No**	Categorical (Yes/No)
Absorption	P-glycoprotein II inhibitor	**No**	Categorical (Yes/No)
Distribution	VDss (human)	**0.408**	Numeric (log L/kg)
Distribution	Fraction unbound (human)	**0.427**	Numeric (Fu)
Distribution	BBB permeability	**0.761**	Numeric (log BB)
Distribution	CNS permeability	**-2.049**	Numeric (log PS)

Property	Model Name	Predicted Value	Unit
Metabolism	CYP2D6 substrate	**No**	Categorical (Yes/No)
Metabolism	CYP3A4 substrate	**No**	Categorical (Yes/No)
Metabolism	CYP1A2 inhibitior	**No**	Categorical (Yes/No)
Metabolism	CYP2C19 inhibitior	**No**	Categorical (Yes/No)
Metabolism	CYP2C9 inhibitior	**No**	Categorical (Yes/No)
Metabolism	CYP2D6 inhibitior	**No**	Categorical (Yes/No)
Metabolism	CYP3A4 inhibitior	**No**	Categorical (Yes/No)
Excretion	Total Clearance	**0.2**	Numeric (log ml/min/kg)
Excretion	Renal OCT2 substrate	**No**	Categorical (Yes/No)
Toxicity	AMES toxicity	**No**	Categorical (Yes/No)
Toxicity	Max. tolerated dose (human)	**0.754**	Numeric (log mg/kg/day)
Toxicity	hERG I inhibitor	**No**	Categorical (Yes/No)
Toxicity	hERG II inhibitor	**No**	Categorical (Yes/No)
Toxicity	Oral Rat Acute Toxicity (LD50)	**1.741**	Numeric (mol/kg)
Toxicity	Oral Rat Chronic Toxicity (LOAEL)	**2.328**	Numeric (log mg/kg_bw/day)
Toxicity	Hepatotoxicity	**No**	Categorical (Yes/No)
Toxicity	Skin Sensitisation	**No**	Categorical (Yes/No)
Toxicity	*T.Pyriformis* toxicity	**0.638**	Numeric (log ug/L)
Toxicity	Minnow toxicity	**Running**	Numeric (log mM)

PkCSM of Nigellidine alkaloide (Table 3.14)

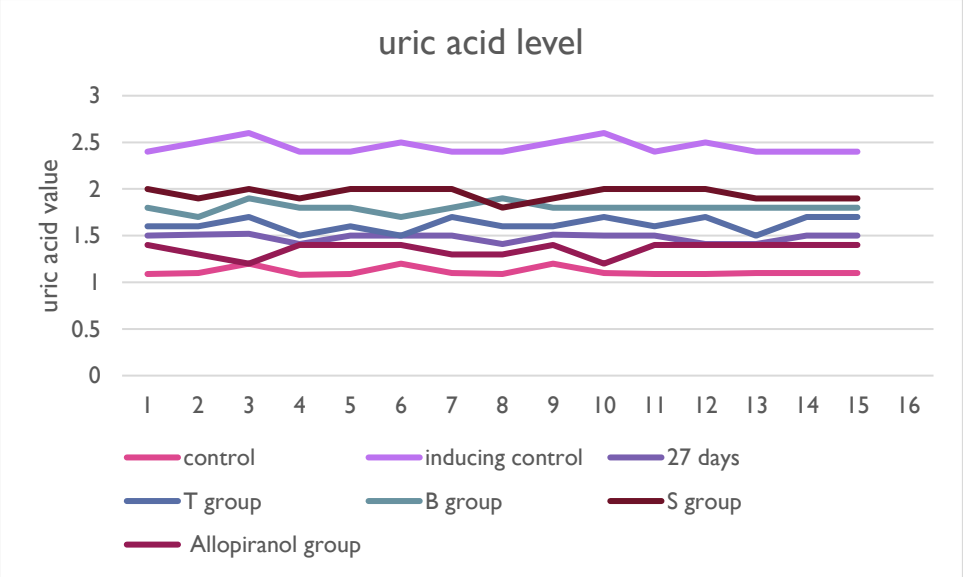
Property	Model Name	Predicted Value	Unit
Absorption	Water solubility	**-2.526**	Numeric (log mol/L)
Absorption	Caco2 permeability	**0.908**	Numeric (log Papp in 10^{-6} cm/s)
Absorption	Intestinal absorption (human)	**100**	Numeric (% Absorbed)
Absorption	Skin Permeability	**-2.732**	Numeric (log Kp)
Absorption	P-glycoprotein substrate	**No**	Categorical (Yes/No)
Absorption	P-glycoprotein I inhibitor	**No**	Categorical (Yes/No)
Absorption	P-glycoprotein II inhibitor	**No**	Categorical (Yes/No)
Distribution	VDss (human)	**-2.287**	Numeric (log L/kg)
Distribution	Fraction unbound (human)	**0.052**	Numeric (Fu)
Distribution	BBB permeability	**0.088**	Numeric (log BB)
Distribution	CNS permeability	**-2.073**	Numeric (log PS)
Metabolism	CYP2D6 substrate	**No**	Categorical (Yes/No)
Metabolism	CYP3A4 substrate	**No**	Categorical (Yes/No)
Metabolism	CYP1A2 inhibitior	**Yes**	Categorical (Yes/No)
Metabolism	CYP2C19 inhibitior	**No**	Categorical (Yes/No)
Metabolism	CYP2C9 inhibitior	**No**	Categorical (Yes/No)
Metabolism	CYP2D6 inhibitior	**No**	Categorical (Yes/No)
Metabolism	CYP3A4 inhibitior	**No**	Categorical (Yes/No)
Excretion	Total Clearance	**0.821**	Numeric (log ml/min/kg)

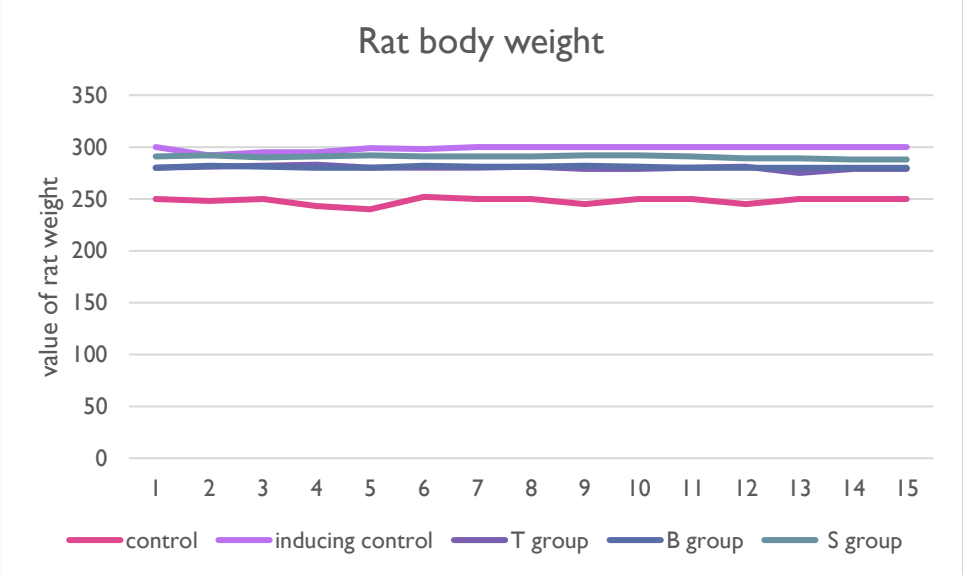
Property	Model Name	Predicted Value	Unit
Excretion	Renal OCT2 substrate	**No**	Categorical (Yes/No)
Toxicity	AMES toxicity	**No**	Categorical (Yes/No)
Toxicity	Max. tolerated dose (human)	**0.62**	Numeric (log mg/kg/day)
Toxicity	hERG I inhibitor	**No**	Categorical (Yes/No)
Toxicity	hERG II inhibitor	**No**	Categorical (Yes/No)
Toxicity	Oral Rat Acute Toxicity (LD50)	**2.323**	Numeric (mol/kg)
Toxicity	Oral Rat Chronic Toxicity (LOAEL)	**1.395**	Numeric (log mg/kg_bw/day)
Toxicity	Hepatotoxicity	**No**	Categorical (Yes/No)
Toxicity	Skin Sensitisation	**No**	Categorical (Yes/No)
Toxicity	*T.Pyriformis* toxicity	**0.285**	Numeric (log ug/L)
Toxicity	Minnow toxicity	**0.992**	Numeric (log mM)

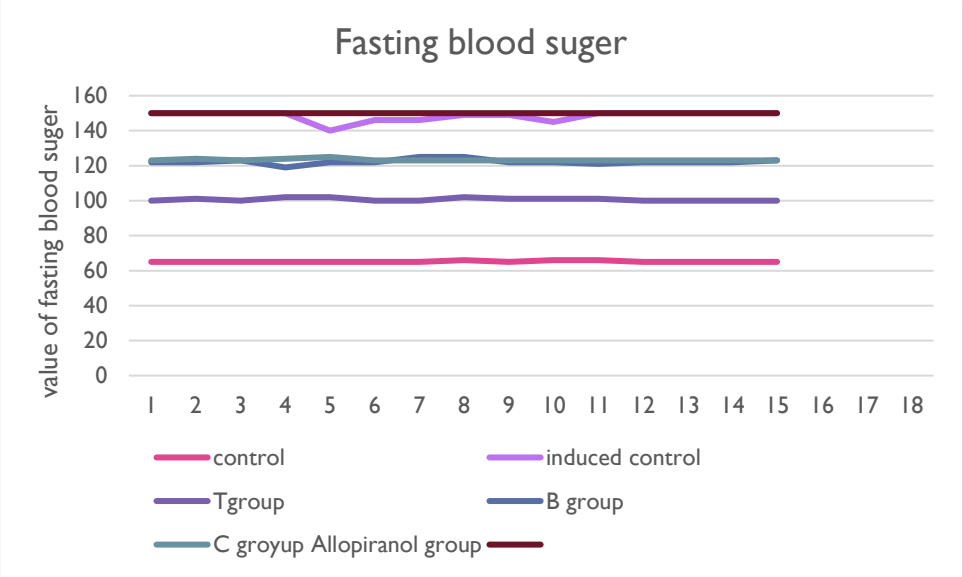
This result for compound which had antihyperuricemic effect or can treat Gout only, not all compounds

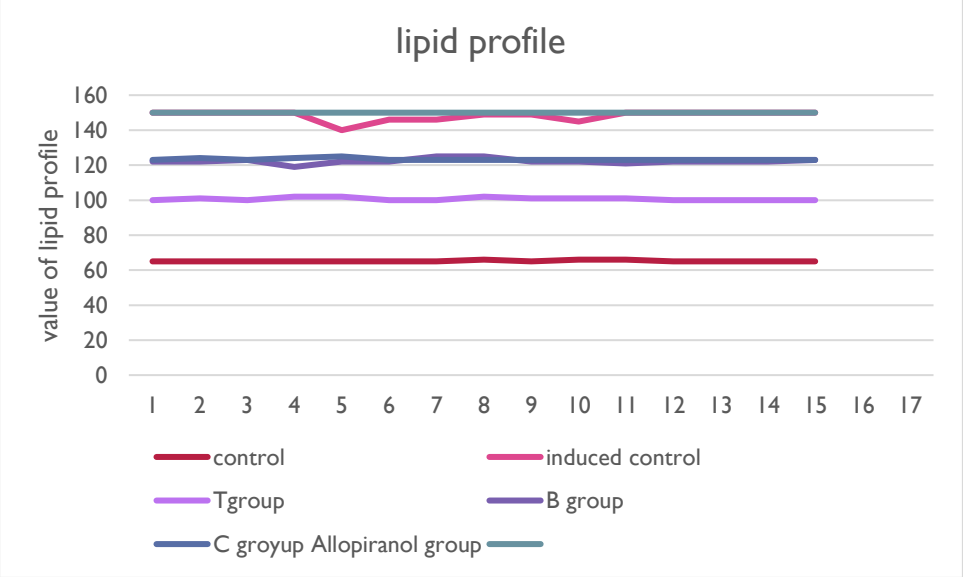
3 DATA ANALYSIS OF STUDY

The statistical analysis of the animal model begins with defining the hypotheses: the **Null Hypothesis (H_0)** states that there is no significant difference in the means of the groups, while the **Alternative Hypothesis (H_1)** posits that there is a significant difference in the means of the groups. Normality is a key assumption and was assessed using the Shapiro-Wilk test. If the data is normally distributed, ANOVA is used to compare more than two groups; otherwise, the Kruskal-Wallis test is applied. The steps involve performing a normality test for each dataset (e.g., Rat Weight, FBS, Lipid Pro), selecting the appropriate test based on normality results, and calculating p-values. If $p<0.05$$p<0.05$, H_0 is rejected, indicating a significant difference; if $p\geq0.05$$p\geq0.05$, H_0 is not rejected, indicating no significant difference. For UAL data, the Shapiro-Wilk test revealed that most groups had pp-values < 0.05 (Control: 0.0009, Inducing Control: 0.0099, T Group: 0.0153, S Group: 0.0024, Allopurinol Group: 0.0119), indicating non-normal distribution, except for "27 Days" ($p=0.2797$$p=0.2797$) and "B Group" ($p=0.0992$$p=0.0992$). Consequently, the Kruskal-Wallis test was used for analysis, yielding a test statistic of 80.63 and a pp-value of $2.65\times10-152.65\times10-15$, leading to rejection of H_0 and concluding significant differences among groups for UAL data. Similarly, Kruskal-Wallis tests for "Rat Weight" (Statistic: 45.09, $p=3.81\times10-9$$p=3.81\times10-9$), "FBS" (Statistic: 45.12, $p=3.76\times10-9$$p=3.76\times10-9$), and lipid (Statistic: 47.94, $p=9.71\times10-10$$p=9.71\times10-10$) all resulted in p$p$-values < 0.05, indicating significant differences among groups for each dataset. Therefore, H_0 was rejected for all tests, confirming significant changes across groups in "Rat Weight," "FBS," and "Lipid Pro


uric acid level
uric acid value
3
2.5
2
1.5
1
0.5
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
control
inducing control
27 days
T group
B group
S group
Allopiranol group


Rat body weight
value of rat weight
350
300
250
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
control
inducing control
T group
B group
S group





6. RELIABILITY PROCEDURES

The reliability procedures that we followed in the research are , first purchasing the seeds of Nigella sativa from reliable and approved sources, and we were careful in this, as we purchased the three types of Turkish, Bangladeshi and Syrian from the sources and stores mentioned.

Second, the animal model, it is necessary to take into account justice in all the conditions surrounding the animal during the experiment and to take into account the equality of food, drink, sleeping hours and the area in which the animal can move is uniform, as well as the method of taking samples and their number.

When starting to take Nigella sativa and allopurinol, the methods of preparing samples must be taken into account if necessary, and any symptoms of illness due to what is being taken must be noted, and this must be recorded accurately. The reliability of the procedures depends on the researcher, the accuracy of the data and results on which the research is based, in bias and the inability to repeat the experiment due to the lack of clarity of the procedures or the inaccuracy of preparing the solutions and chemicals used, which gives weakness to the research and a lack of credibility that threatens the research.

7. DATA INTERPRETATION

Compare of phytochemical GCMS results :

Clearly we can determine which of the three types of NS has the highest percentage through GCMS, we find that Turkish NS has a higher percentage of thymoquinone a (Table 3.3)and p-cymene, and other compounds in varying proportions than the Bangladeshi type (table 3.4) and the Syrian type(Table 3.5), but the pharmacological effect of the Syrian type imposes an important question, how does it reduce the level of uric acid even by a small percentage when it does not contain thymoquinone except 2.2%, which indicates that some of the remaining compounds have an antihyperuricemic property, which is a new gap that must be highlighted.

Animal model of Rats,we will find the statistical results(Tablet 3.2) indicate that there is a significant difference between these groups that underwent and participated in the experiment, and we will notice the presence of a control group, and a control after hyperurecima, and the presence of a group for each type of NS separately, and there are results for the analysis of uric acid using the HLPC method, for each group, and the results were that the group that took the Turkish NS was able to reduce the level of uric acid when compared to the control after hyperurecima, and also in comparison with the Allopurinol group, and we were able to determine the stability of the effect of NS in general and we determined the order of the effect of each type in particular, where the superiority was for the Turkish group, followed by the Bangladeshi, followed by the Syrian, and NS is superior to Allopurinol, in terms of the ability of NS to treat the symptoms of gout and hyperuricemia and treat its causes, including pain relief .

PKCSM OF Thymoquinone :(tablet 3.11)

This compound shows excellent oral absorption, decent distribution in the plasma, and low metabolism-related concerns. However, its low tissue distribution (VDss), moderate clearance, and potential hepatotoxicity require attention. The risks of skin sensitization and environmental toxicity to protozoa (T. Pyriformis) are additional concerns. Its favorable BBB permeability with restricted CNS accumulation indicates limited CNS side effects, which may be beneficial depending on its intended pharmacological use.Further experimental validation, especially on hepatotoxicity and chronic toxicity, is recommended before advancing this compound in the drug development pipeline.

PKCSM OF P-Cymene :(Tablet 3.12)

Strengths,high intestinal absorption (93.54%) and Caco-2 permeability (1.527) suggest excellent oral bioavailability,ability to cross the BBB (log BB = 0.478) and CNS penetration potential (log PS = -1.397) make it suitable for CNS-related applications,non-mutagenic (AMES test negative) and non-hepatotoxic, with a favorable maximum tolerated dose,very poor water solubility (-4.081 log mol/L) may require formulation strategies to enhance bioavailability ,inhibition of CYP1A2 raises the potential for drug-drug interactions.

Skin sensitization risks limit suitability for dermal or transdermal applications.

Moderate protozoan toxicity suggests caution regarding environmental impact.

Overall, p-Cymene exhibits a promising safety and efficacy profile for oral or CNS-targeted applications but requires careful formulation and environmental risk evaluation.

PKCSM OF α Phellandrene :(Tablet 3.13)

This compound exhibits desirable properties, including high intestinal absorption, good BBB permeability, low risk of metabolism-related interactions, and a favorable non-toxic profile (non-mutagenic, non-hepatotoxic). However, its moderate clearance and potential protozoan toxicity warrant further investigation.

PKCSM OF Nigellidine alkaloid (Tablet 3.14)

Strengths:

Absorption: Excellent intestinal absorption (100%) and good Caco-2 permeability suggest high oral bioavailability.

Non-Toxicity: Non-mutagenic (negative AMES test) and a high maximum tolerated dose predict good safety.

Metabolism Advantages: Not a substrate for major CYP enzymes (CYP2D6 and CYP3A4) minimizes variability and drug metabolism interactions.

Limitations:

Distribution: Low tissue distribution (log VDss = -2.287) and high plasma protein binding (Fu = 0.052) may limit target bioavailability.

CNS Activity: Limited BBB permeability (log BB = 0.088) and CNS penetration (log PS = -2.073) make it unsuitable for CNS-targeted therapies.

CYP1A2 Inhibition: Its potential to inhibit CYP1A2 could lead to drug-drug interactions with other medications metabolized by this enzyme.

Skin Permeability: Low skin permeability (log Kp = -2.732) reduces its suitability for transdermal delivery applications.

Nigellidine alkaloid has excellent oral absorption and a good safety profile, with minimal risks of mutagenicity or major CYP metabolism-based variability. However, its low tissue distribution, strong plasma protein binding, and limited CNS penetration may restrict its therapeutic applications. Its moderate clearance and CYP1A2 inhibition should be monitored if co-administered with other drugs. If optimized formulations are developed to overcome solubility issues, it could serve as a promising candidate in non-CNS therapeutic areas.

8. DATA ANALYSIS AND DISCUSSION OF FINDINGS

Anyone who studies this huge number of studies that prove that NS has medical effects and protection for vital organs in the human body or animal models will be biased, which raises astonishment and wonder why the world has not benefited from this miraculous plant. When we discuss this research, we will first address the results of the GCMS analysis, which gives limited results for limited compounds, which became clear when studied with supered and also with the pkCSM toxicity and pharmacokinetics program, which indicated that even compounds with low concentrations have a contribution to the studied medical effect. Rather, there are compounds that have been recently discovered, which are alkaloids, called Nigellidine, which have an antihyperuricemic relationship and may have been important in the studied pharmacological effect, even if it did not appear in the results. GCMS analyses show superiority in reducing uric acid for Turkish NS, followed by Bangladeshi, followed by Syrian, which can be explained by the previous results of GCMS, as they have a direct relationship, but the matter that I would like to point out that Syrian NS can reduce uric acid levels, although thymoquinone was 2.2%, while b-cymene was 10.66%, which indicates that there are other compounds besides thymoquinone that are capable of reducing uric acid levels. We referred to this when we mentioned the results of Superperd, which showed the ability of b-cymene and α phellandrene and Nigellidine alkaloid to reduce uric acid as well.

Thymoquinone though previous study can reduce uric acid level by anti-inflammatory activities and their antioxidant activities but the study through Superperd system (Tablets 3.6 ,3.7, 3.8,3.9) there are other explanation for Thymoquinone Antihyperuricemic activity

dehydrogenase	T40954	Hyperuricaemia [ICD-11: 5C55.Y]	54%	96%

First effect on xanthine dehydrogenase as Allopiranol can Thymoquinone decrease the level of uric acid through inhibition of this enzyme .

resistance-associated protein 1	T11288	Gout [ICD-11: FA25]	52%	96%

Also dehydrogenase inhibition by thymoquinone can effect and treat the Gout

dehydrogenase	T40954	Gout [ICD-11: FA25]	54%	96%

P-Cymene :

Superperd system peridect how P-cymen can treat GOUT that is by effect on multidrug resistance- associated protein 1, which explain why Syrian NS can reduce level of uric acid despite of thymoquinone level is 2.2%

Multidrug resistance-associated protein 1 T11288 Gout [ICD-11: FA25] 55% 96%

Also α phellandrene can treat GOUT that is by effect on multidrug resistance associated protein 1

Multidrug resistance-associated protein 1	T11288	Gout [ICD-11: FA25]	59%	96%

Also this effect could explain why Syrian NS can reduce the uric acid level in Rats blood samples .

based on the analysis of the pkCSM results for Thymoquinone, p-Cymene, α-Phellandrene, and Nigellidine alkaloid: The pharmacokinetic and toxicity profiles of Thymoquinone, p-Cymene, α-Phellandrene, and Nigellidine alkaloid present promising attributes for drug development, while highlighting areas that require optimization. All four compounds exhibit excellent intestinal absorption, as indicated by high human intestinal absorption percentages and sufficient Caco-2 permeability, suggesting strong potential for oral administration. However, solubility concerns are evident, particularly in p-Cymene (log S = -4.081), which may necessitate formulation strategies to enhance bioavailability. BBB permeability varied among the compounds; p-Cymene and α-Phellandrene demonstrated favorable log BB values (>0.3), indicating potential for

CNS-targeted therapies, whereas Nigellidine alkaloid showed limited CNS penetration due to its low log BB (0.088) and log PS (-2.073).Metabolic interaction risks were generally low, with none of the compounds acting as substrates for major CYP enzymes (e.g., CYP2D6 and CYP3A4). However, p-Cymene and Nigellidine alkaloid were identified as CYP1A2 inhibitors, raising concerns about potential drug-drug interactions. In terms of distribution, Nigellidine alkaloid exhibited low tissue distribution (log VDss = -2.287) and high plasma protein binding (Fu = 0.052), which may limit its bioavailability at target sites. Thymoquinone and α-Phellandrene also demonstrated moderate clearance rates, indicating the need for further evaluation of their elimination pathways.Toxicity assessments revealed a generally favorable profile. None of the compounds were mutagenic (negative AMES test), and hepatotoxic risks were minimal, except for Thymoquinone, which showed potential hepatotoxicity. Notable dermatological and environmental toxicity concerns were observed, with p-Cymene and Nigellidine alkaloid being associated with skin sensitization, and p-Cymene and α-Phellandrene showing toxicity to T. Pyriformis.Overall, these compounds exhibit significant potential for therapeutic applications, with specific advantages depending on the desired pharmacological activity. However, limitations such as solubility, CNS barriers, drug interaction risks, and toxicity concerns underscore the need for targeted optimization through formulation strategies and experimental validation, particularly for hepatotoxicity and chronic toxicity profiles. These findings support further investigations into these compounds as candidates for orally bioavailable drug therapies. This paragraph provides a balanced and cohesive discussion of the results, synthesizing the strengths and limitations of the compounds while placing them in the context of their potential for drug development.

ProTox-III: Toxicity Prediction: (Tablet 3.10)

P-cymene has an LD50 of 3 mg/kg, making it the most toxic compound in this list. It is classified as Class 1, indicating the highest hazard level.

Thymoquinone and Nigellidine alkaloid are both moderately toxic but differ in their hazard classifications (Class 5 and Class 4, respectively). Nigellidine alkaloid poses a slightly higher hazard than Thymoquinone.

α-phellandrene has the highest LD50 (5700 mg/kg), meaning it is the least toxic compound. Its classification as Class 6 reflects its very low hazard level.

9. CONCLUSION, RECOMMENDATIONS AND SUGGESSTIONS FOR FURTHER STUDIES

Conclusion

The findings of this study underscore the pharmacological significance of geographical variations in Nigella sativa and their impact on antihyperuricemic potential. Among the three types studied, Although 400mg/kg was used, which is a lower dose than the dose used in previous studies, which was 500mg/kg. the Turkish variety demonstrated the highest efficacy in reducing serum uric acid levels in the hyperuricemic rat model, followed by the Bangladeshi and Syrian varieties. These results align with the phytochemical analysis, which revealed a higher concentration of bioactive compounds, particularly thymoquinone and p-cymene, in the Turkish Nigella sativa seeds. The superior performance of the Turkish variety highlights the pivotal role of environmental factors, such as soil quality, climate, and cultivation practices, in influencing the medicinal properties of Nigella sativa .

Additionally, the study successfully validated the potential of Nigella sativa as a natural alternative to allopurinol, a standard treatment for hyperuricemia, offering comparable efficacy with fewer side effects in controlling serum uric acid levels. This research further contributes to our understanding of Nigella sativa's mechanisms of action, including its antioxidant and anti-inflammatory pathways that mitigate oxidative stress and inflammation in hyperuricemia. The use of predictive tools like Superperd and pkCSM provided deeper insights into the pharmacokinetics and safety profiles of the bioactive compounds, reinforcing their suitability for therapeutic use.

From an economic and pharmaceutical perspective, identifying Turkish Nigella sativa as the most potent variety provides a compelling case for its targeted cultivation and industrial application in the development of natural antihyperuricemic treatments. Furthermore, the study emphasizes the need for additional research to explore the untapped potential of Nigella sativa from other regions and to investigate novel mechanisms of action through advanced predictive modeling. By highlighting the importance of geographical and phytochemical diversity, this research serves as a foundational step towards optimizing the therapeutic use of Nigella sativa and expanding its applications in modern medicine.

Suggestions for Conclusion and Recommendations

Combining Different Varieties of Nigella sativa for Optimal Benefits

Given the significant variability in the active phytochemical compounds of Nigella sativa across different geographical origins, this study suggests that a strategic combination of various types—such as Turkish, Bangladeshi, and Syrian or other geographical origin black seeds—could harness their distinct strengths. Each variety offers unique advantages due to varying concentrations of critical bioactive components like thymoquinone and p-cymene. For instance, while the Turkish variety showed the highest antihyperuricemic potential, the Bangladeshi seeds displayed considerable antioxidant activity. By

blending these varieties, it may be possible to achieve a synergistic effect, optimizing the therapeutic benefits for hyperuricemia and related conditions. Future research could focus on formulating blends that enhance efficacy and explore their pharmacological performance.

Incorporating Nigella sativa into the Dietary Regime of Gout Patients

Given its clear antioxidative and anti-inflammatory properties, as well as its proven ability to lower uric acid levels with a favourable safety profile, this study advocates for considering Nigella sativa as part of the dietary regimen for gout patients. Its inclusion in daily diets—whether as a spice, supplement, or infused oil—could serve as a complementary approach to conventional treatments. This dietary integration may not only assist in managing hyperuricemia but also provide additional health benefits, given the seed's comprehensive pharmacological activities. However, large-scale clinical trials are recommended to determine the appropriate dosage and long-term safety of such dietary applications.

10. ACKNOWLEDGEMENTS

This work is dedicated to the souls of my father and mother and the soul of Profes Mustafa Shahimi. Thanks and appreciation to my wife and the supervisors and distinguished professors.

REFERENCES

[1] M. I.-A.-B. Ibn-Qaiyim al-Ǧauzīya, *Prophetic Medicine: = Aṭ-Ṭibb an-nabawī*, 2nd ed. New Delhi: Islamic Book Service, 2003.

[2] M. Dalli, O. Bekkouch, S.-E. Azizi, A. Azghar, N. Gseyra, and B. Kim, 'Nigella sativa L. Phytochemistry and Pharmacological Activities: A Review (2019-2021)', *Biomolecules*, vol. 12, no. 1, p. 20, Dec. 2021, doi: 10.3390/biom12010020.

[3] Z. Albakry *et al.*, 'A comparative study of black cumin seed (Nigella sativa L.) oils extracted with supercritical fluids and conventional extraction methods', *Journal of Food Measurement and Characterization*, vol. 17, no. 3, pp. 2429–2441, Jun. 2023, doi: 10.1007/s11694-022-01802-7.

[4] N. AlFraj and A. Hamo, 'Evaluation of technical efficiency of some rain-fed cereal and legume crops production in Syria: does crisis matter?', *Agric & Food Secur*, vol. 11, no. 1, p. 49, Oct. 2022, doi: 10.1186/s40066-022-00389-y.

[5] S. Ekren, I. C. Paylan, and A. Gokcol, 'Seed quality improvement applications in black cumin seeds (Nigella sativa L.)', *Front. Sustain. Food Syst.*, vol. 7, p. 1212958, Aug. 2023, doi: 10.3389/fsufs.2023.1212958.

[6] Y. Kabir, Y. Akasaka-Hashimoto, K. Kubota, and M. Komai, 'Volatile compounds of black cumin (Nigella sativa L.) seeds cultivated in Bangladesh and India', *Heliyon*, vol. 6, no. 10, p. e05343, Oct. 2020, doi: 10.1016/j.heliyon.2020.e05343.

[7] S. Hussain *et al.*, 'Phytochemical profile, nutritional and medicinal value of Nigella sativa', *Biocatalysis and Agricultural Biotechnology*, vol. 60, p. 103324, Sep. 2024, doi: 10.1016/j.bcab.2024.103324.

[8] M. Umer *et al.*, '*Nigella sativa* for the treatment of COVID-19 patients: A rapid systematic review and meta-analysis of randomized controlled trials', *Food Science & Nutrition*, vol. 12, no. 3, pp. 2061–2067, Mar. 2024, doi: 10.1002/fsn3.3906.

[9] A. Ahmad *et al.*, 'A review on therapeutic potential of Nigella sativa: A miracle herb', *Asian Pac J Trop Biomed*, vol. 3, no. 5, pp. 337–352, May 2013, doi: 10.1016/S2221-1691(13)60075-1.

[10] A. Tero-Vescan, R. Ștefănescu, T.-I. Istrate, and A. Pușcaș, 'Fructose-induced hyperuricaemia – protection factor or oxidative stress promoter?', *Natural Product Research*, pp. 1–13, Mar. 2024, doi: 10.1080/14786419.2024.2327624.

[11] L. Li, Y. Zhang, and C. Zeng, 'Update on the epidemiology, genetics, and therapeutic options of hyperuricemia', *Am J Transl Res*, vol. 12, no. 7, pp. 3167–3181, 2020.

[12] P. Zhang *et al.*, 'Dietary intake of fructose increases purine de novo synthesis: A crucial mechanism for hyperuricemia', *Front. Nutr.*, vol. 9, p. 1045805, Dec. 2022, doi: 10.3389/fnut.2022.1045805.

[13] M. Halimulati *et al.*, 'Anti-Hyperuricemic Effect of Anserine Based on the Gut–Kidney Axis: Integrated Analysis of Metagenomics and Metabolomics', *Nutrients*, vol. 15, no. 4, p. 969, Feb. 2023, doi: 10.3390/nu15040969.

[14] M. Rashid *et al.*, 'Silver Nanoparticles from Saudi and Syrian Black Cumin Seed Extracts: Green Synthesis, ADME, Toxicity, Comparative Research, and Biological Appraisal', *Journal of Pharmacy and Bioallied Sciences*, vol. 15, no. 4, pp. 190–196, Oct. 2023, doi: 10.4103/jpbs.jpbs_381_23.

[15] *Nutrient Requirements of Laboratory Animals,: Fourth Revised Edition, 1995*. Washington, D.C.: National

Academies Press, 1995, p. 4758. doi: 10.17226/4758.

[16] R. J. Johnson, T. Nakagawa, D. Jalal, L. G. Sanchez-Lozada, D.-H. Kang, and E. Ritz, 'Uric acid and chronic kidney disease: which is chasing which?', *Nephrology Dialysis Transplantation*, vol. 28, no. 9, pp. 2221–2228, Sep. 2013, doi: 10.1093/ndt/gft029.

[17] R. Dangarembizi, K. H. Erlwanger, C. Rummel, J. Roth, M. T. Madziva, and L. M. Harden, 'Brewer's yeast is a potent inducer of fever, sickness behavior and inflammation within the brain', *Brain, Behavior, and Immunity*, vol. 68, pp. 211–223, Feb. 2018, doi: 10.1016/j.bbi.2017.10.019.

[18] Y. Andriana *et al.*, 'Chemometric analysis based on GC-MS chemical profiles of essential oil and extracts of black cumin (Nigella sativa L.) and their antioxidant potentials', *J Appl Pharm Sci*, 2023, doi: 10.7324/JAPS.2023.151774.

[19] S. Wen *et al.*, 'An improved UPLC method for determining uric acid in rat serum and comparison study with commercial colorimetric kits', *Acta Chromatographica*, vol. 31, no. 3, pp. 201–205, Sep. 2019, doi: 10.1556/1326.2018.00449.

[20] C. M. M. R. Barros *et al.*, 'SUBSTITUTION OF DRINKING WATER BY FRUCTOSE SOLUTION INDUCES HYPERINSULINEMIA AND HYPERGLYCEMIA IN HAMSTERS', *Clinics*, vol. 62, no. 3, pp. 327–334, Jun. 2007, doi: 10.1590/S1807-59322007000300019.

[21] C. M. M. R. Barros *et al.*, 'SUBSTITUTION OF DRINKING WATER BY FRUCTOSE SOLUTION INDUCES HYPERINSULINEMIA AND HYPERGLYCEMIA IN HAMSTERS', *Clinics*, vol. 62, no. 3, pp. 327–334, Jun. 2007, doi: 10.1590/S1807-59322007000300019.

[22] H. Zhou *et al.*, 'Hyperuricemia research progress in model construction and traditional Chinese medicine interventions', *Front Pharmacol*, vol. 15, p. 1294755, 2024, doi: 10.3389/fphar.2024.1294755.

[23] P. J. Vento, M. E. Swartz, L. B. Martin, and D. Daniels, 'Food intake in laboratory rats provided standard and fenbendazole-supplemented diets', *J Am Assoc Lab Anim Sci*, vol. 47, no. 6, pp. 46–50, Nov. 2008.

[24] H. Mashayekhi-Sardoo, R. Rezaee, and G. Karimi, '*Nigella sativa* (black seed) safety: an overview', *Asian Biomedicine*, vol. 14, no. 4, pp. 127–137, Aug. 2020, doi: 10.1515/abm-2020-0020.

[25] P. S, R. R, and K. R, 'Blood sample collection in small laboratory animals', *Journal of Pharmacology and Pharmacotherapeutics*, vol. 1, no. 2, pp. 87–93, Dec. 2010, doi: 10.4103/0976-500X.72350.

[26] J. Zhao, 'A simple, rapid and reliable high performance liquid chromatography method for the simultaneous determination of creatinine and uric acid in plasma and urine', *Anal. Methods*, vol. 5, no. 23, p. 6781, 2013, doi: 10.1039/c3ay41061g.

[27] Amtul Hafeez, Abdul Mudabbir Rehan, Zunera Hakim, Attiya Munir, Rabia Naseer Khan, and Aamna Khokhar, 'Nigella sativa Seeds Protective Ability in Pyrazinamide Induced Hyperuricemia in Mice', *Proceedings S.Z.M.C*, vol. 36, no. 1, pp. 44–48, Feb. 2022, doi: 10.47489/PSZMC-825361-44-48.

[28] Y. Tayama, K. Sugihara, S. Sanoh, K. Miyake, S. Kitamura, and S. Ohta, 'Xanthine oxidase and aldehyde oxidase contribute to allopurinol metabolism in rats', *J Pharm Health Care Sci*, vol. 8, no. 1, p. 31, Dec. 2022, doi: 10.1186/s40780-022-00262-x.

[29] M. Martin Fabritius, A. Broillet, S. König, and W. Weinmann, 'Analysis of volatiles in fire debris by combination of activated charcoal strips (ACS) and automated thermal desorption–gas chromatography–mass spectrometry (ATD/GC–MS)', *Forensic Science International*, vol. 289, pp. 232–237, Aug. 2018, doi: 10.1016/j.forsciint.2018.05.048.

[30] X. Wei, I. Koo, S. Kim, and X. Zhang, 'Compound identification in GC-MS by simultaneously evaluating the mass spectrum and retention index', *Analyst*, vol. 139, no. 10, pp. 2507–2514, 2014, doi: 10.1039/C3AN02171H.

[31] J. Nickel *et al.*, 'SuperPred: update on drug classification and target prediction', *Nucleic Acids Research*, vol. 42, no. W1, pp. W26–W31, Jul. 2014, doi: 10.1093/nar/gku477.