Title: An in-silico toxicity study of the phytocompounds found in Pongamia pinnata leaves using the ProTox-II web server

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Abstract:

The aim of the current study was to determine the Insilco toxicity of the phytocompound present in the leaves of *Pongamia pinnata*. The leaves of *Pongamia pinnata* have long been used in traditional medicine for their therapeutic properties. Nowadays, herbal toxicity is a common problem caused by incorrect dosage. The FDA reviews the safety and effectiveness of herbal products only in response to patient or health care provider complaints—problems

with them, such as side effects, interactions, and allergies. ProTox-II is a web server that uses computational methods to predict the toxicity of small molecules. It is a virtual laboratory that uses factors such as most common features, molecular similarity, and fragment propensities to predict a variety of toxicity endpoints including immunotoxicity, carcinogenicity, mutagenicity, acute toxicity, hepatotoxicity, cytotoxicity, adverse signaling pathways (Tox21) and toxicity targets. The server receives a two-dimensional chemical structure as input and returns likely toxicity profiles for the compound. Based on the bioactive compounds we wish to investigate further; We selected bioactive compounds from *Pongamia pinnata* leaves from several published studies. The bioactive compounds rutin, methyl hexadecenoate, isoquercitrin, pongagallon *B, pongagallon* A, bis(2-methylheptyl) phthalate, vitexin, vecinin-2, 2H-1-benzopyran-6-ol, 3,4-dihydro-2, 8-dimethyl -2-[4,8,12-trimethyltridecyl], 12ahydroxy-alpha-toxicarol were selected for *in-silico* toxicity study. According to our knowledge, among the ten bioactive hexadecanoic acid methyl esters 2H-1-benzopyran-6-ol,3,4-dihydro-2,8-dimethyl-2-[4,8,12-trimethyltridecyl] of all toxicity classes are inactive. Based on the prediction of oral LD50 dose value of ten phytocompounds, only 12 α-hydroxy-toxiccarol are toxic. However, other researchers found numerous positive effects *in vitro*.

Keywords:

Insilco toxicity; ProTox-II webserver; *Pongamia pinnata* bioactive compound; Herbal medicine.

1 Introduction

Natural products are simply defined as small organic molecules produced by living things, including microbes, plants, and animals [1]. Metabolites and/or by-products of living things, including bacteria, plants, and animals, are referred to as “natural products”. Human health is at serious risk from bacterial, fungal, and viral infections as they become increasingly common and tend to become resistant to existing treatments. To combat them, discovering and making

new connections is crucial. It was discovered that natural products possess pharmacological or biological properties that could make them valuable as medicines. These substances can be synthesized [using modern methods], used as conventional drugs, or isolated or modified [2, 3]. These compounds must be highly bioavailable, have specific activity and have low toxicity. These include various classes of plant compounds [flavonoids, alkaloids, phenols, and triterpenoids], plant extracts, essential oils and bacteriocins. These also include antimicrobial peptides from small animals [4,2].

Numerous natural products derived from animals have been used for centuries to treat a variety of diseases, such as deer antler velvet, turkesterone, and collagen [2,5,6]. Marine organisms are a rich source of natural products with pharmacological properties. It states that 20 marine-derived drugs are in clinical use, with most approved marine compounds being antineoplastic and marine natural products, with drug discovery success rates up to four times higher than other naturally derived compounds [7,8].

Our study focused on plant-based natural products. Traditional human medical practices traditionally involve the use of a variety of medicinal plants. Due to the enormous therapeutic potential of these plants, newly discovered drugs have proven effective in treating a number of diseases. Karanja or *Pongamia pinnata* is a plant that has antimicrobial and antimetabolic properties in all its plant parts. Secondary metabolites such as sterols, glabrin, pinnatin, pongamol, alkaloids, sterols and flavonoids contribute to these positive properties [9].

Herbal medicines are naturally derived, inexpensive, and have been used extensively for health purposes for generations without the need for a prescription. Additionally, herbal remedies are often more effective at treating specific ailments and typically provide long-term benefits for overall health [10]. However, it is important to note that herbal remedies are not regulated like pharmaceuticals. Regulatory oversight of herbal medicine labels is often minimal, meaning

they may contain undisclosed ingredients or contaminants. Additionally, the effectiveness of herbs can vary, and incorrect dosage can lead to toxicity. They can also cause adverse effects, interactions, and allergic reactions. Herbal products are reviewed by the FDA for safety and effectiveness only when consumers or healthcare professionals report problems with them [11].

Assessing the toxicity of chemical structures is a critical step in drug discovery and regulatory decision-making processes. SAR analyses are essential tools in toxicology and provide a predictive framework for understanding the potential risks associated with chemical substances by studying their structure and biological interactions [12].

EPA's TEST tool, along with agencies such as EMA, FDA, and EEA, uses models to assess chemical toxicity early. As exposure to chemicals increases due to the increasing diversity of chemicals and their mixtures, understanding the potential harm or benefit is critical and requires experimental validation of their toxicity [13,14].

The purpose of *in-silico* toxicity models is to complement current *in vitro* toxicity methods to predict the toxic effects of chemicals while reducing the time, cost, and animal testing required. Knowledge from various fields, including toxicology, biostatistics, and systems biology, is integrated into the *in-silico* toxicity model [15].

The toxicity of phytocompounds is assessed through quantitative measurements such as LD50, which indicates the lethal dose, and qualitative assessments to determine whether they are active or inactive in specific cells, or through tests related to cytotoxicity, immunotoxicity and hepatotoxicity. Phytocompounds can be evaluated for toxicity based on various endpoints such as mutagenicity, carcinogenicity, and other relevant factors [16].

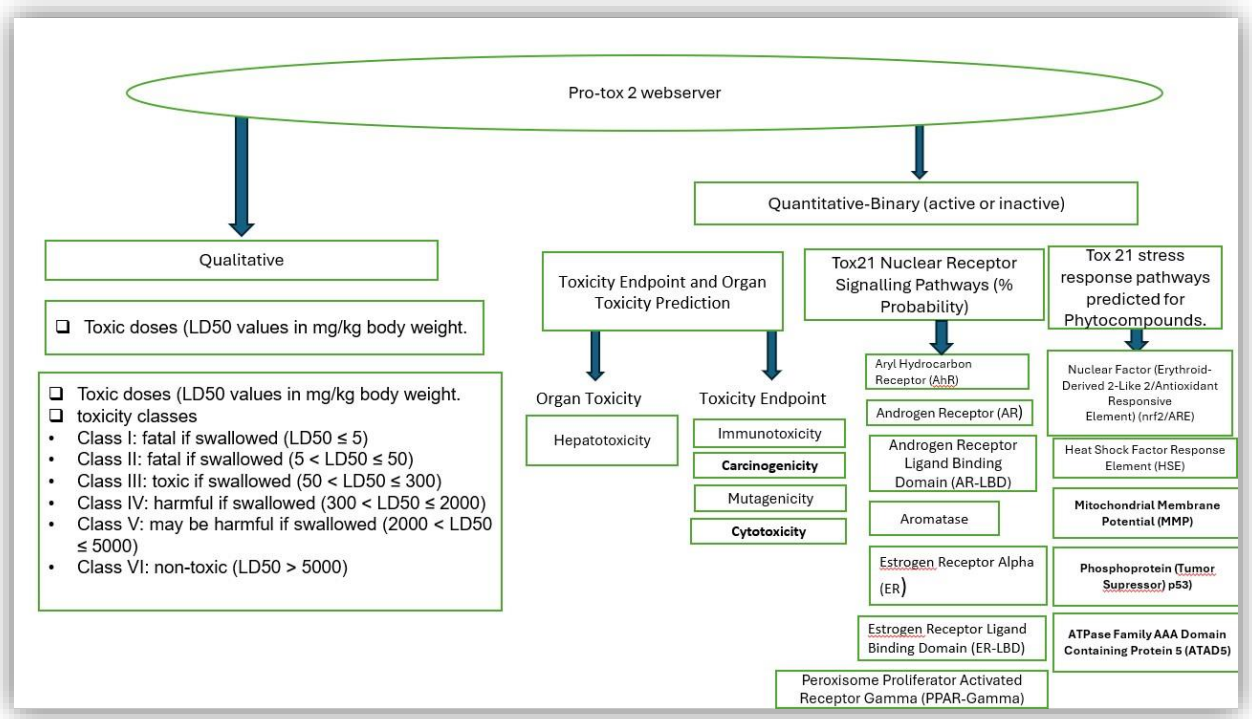

Figure 1: A virtual toxicity prediction lab Protox-II webserver.

To carry out an appropriate risk assessment, the toxicity of phytocompounds must be assessed. In this area, certain *in-silico* approaches can be used to accurately determine the toxicity and toxicokinetics of phytocompounds from *Pongamia pinnata*, which have not yet been extensively studied. Therefore, the current study utilized the in-silico web server ProTox-II to predict oral toxicity along with other identified toxicological endpoints. [17]. The purpose of this study was to determine how *in-silico* methods can be used to accurately predict the

potential toxicity of some phytocompounds from *Pongamia pinnata* so that they can be included in herbal *compositions.*

2 Materials and Methods

2.1 Selection of phytocompounds:

The bioactive components of *Pongamia pinnata* leaves were selected from a body of published research reviewed by several scientists. Ten phytocompounds from the leaf of *Pongamia pinnata* were tested for toxicity using the ProTox-II web server. A detailed list of compounds is shown in Table 1.

Table 1 Ten bioactive compounds from *Pongamia pinnata* leaves. [18,19,20,21]

SN	Phytocompounds Name	SN	Phytocompounds Name
1	Rutin [18]	**6**	Bis[2-methylheptyl] phthalate [20]
2	Pongagallone B [21]	**7**	Vitexin [18]
3	Isoquercitrine [18]	**8**	Vecinin-2 [18]
4	Hexadecenoic acid methyl ester [19]	**9**	2H-1-benzopyran-6-ol,3,4-dihydro-2,8-dimetyl-2-[4,8,12-trimethyltridecyl] [21]
5	Pongagallone A [21]	**10**	12ahydroxy-alpha-toxicarol [18]

2.2 *In-silico* prediction model of the studied compound

The ProTox-II platform uses *in-silico* methods for toxicity prediction. It includes five important classification steps. [1] Acute oral toxicity: This model classifies compounds into six different toxicity categories determined by the degree of their oral toxicity. [2] Organ toxicity: A single model assesses toxicity associated with specific organs. [3] Toxicological endpoints: Four different models are used to predict a range of toxicological endpoints, each designed to assess different aspects of chemical toxicity. [4] Toxicological pathways: Twelve models examine

different toxicity pathways. [2] Toxicity targets: Fifteen models focus on specific toxicity targets [17].

2.3 ProTox-II Platform

2.3.1 Input parameters:

The ProTox-II is intuitive and crystal clear. To predict potential toxicities associated with chemical structures, we enter the compound name or its SMILES notation into prediction models. To do this, we searched for chemical structures by entering the name of the compound into the built-in PubChem search. The user is allowed to opt for any combination of models, including the entire set. By default, the web server calculates acute toxicity prediction and toxicity targets if no additional models are selected [14,17].

2.3.2 Acute Oral Toxicity Prediction

The development of acute toxicity models is based on the identification of toxic fragments and the assessment of chemical similarities between compounds with known toxic effects. These models play a crucial role in predicting the potential toxicity of new compounds. The Super Toxic database is an invaluable source of information about acute toxicity. The latest version of the internal database provides insightful information on acute toxicity data. Substances are classified into different toxicity classes based on their LD50 [mg/kg body weight], which are determined by the Globally Synchronized System of Chemical Labelling [GHS]: [14]

- Class I: fatal if swallowed [LD50 _ 5 mg/kg].
- Class II: fatal if swallowed [5 mg/kg < LD50 _ 50 mg/kg].
- Class III: toxic if swallowed [50 mg/kg < LD50 _ 300 mg/kg].
- Class IV: harmful if swallowed [300 mg/kg < LD50 _ 2000 mg/kg].
- Class V: may be harmful if swallowed [2000 mg/kg < LD50 _ 5000 mg/kg].
- Class VI: non-toxic [LD50 > 5000]

2.3.3 Toxicity Endpoint and Organ Toxicity Prediction

Multiple toxicity endpoints, including hepatotoxicity, cytotoxicity, carcinogenicity, mutagenicity, and immunotoxicity, were predicted using the same *in-silico* prediction tool [ProTox-II]. Based on information from both *in vitro* studies, e.g. HepG2 cytotoxicity assays, Tox21 assays, Ames bacterial mutation assays, and immunotoxicity assays] as well as *in vivo* methods [e.g., as well as hepatotoxicity and carcinogenicity] [17].

Using the same *in-silico* prediction tool [ProTox-II], multiple toxicity endpoints were predicted, including hepatotoxicity, cytotoxicity, carcinogenicity, mutagenicity, and immunotoxicity. Using data from both *in vitro*. e.g. testing for HepG2 cytotoxicity, Tox21, Ames bacterial mutation and immunotoxicity [among others] as well as *in vivo* techniques [e.g. in addition to carcinogenicity and hepatotoxicity] [17].

2.3.4 Toxicological Pathways

ProTox-II has two types of target pathway-based models: the Nuclear Receptor Signaling Pathways with seven tests and the Stress Response Pathways with five tests. [A set of five pathway assays is listed in Table 5.] [17].

ProTox-II runs two target pathway-based model types: the first addresses nuclear receptor signaling pathways, while the second addresses stress response pathways.

This strategy is based on the observation that when a chemical agent interacts, it has the ability to either bind to or inhibit an enzyme or receptor, disrupting various biological signaling pathways and ultimately destroying or killing cells. The main objectives of the initiative are to identify specific mechanisms, such as pathways relevant to disease processes, for further research and to prioritize which substances should undergo more thorough toxicological evaluation. Therefore, the use of this computational prediction tool allows for a quick and

effective determination of whether a particular chemical compound has the potential to impair body functions and harm health [22].

3 Result and discussion

We have highlighted each phytocompound toxicity result predicted using the ProTox-II web server in tabular form.

Table 2: ProTox-II calculated the oral acute toxicity value, class, and percentage of prediction accuracy of ten phytocompounds present in *Pongamia pinnata* leaves.

Sl. No.	Compound name	Oral LD50 value (mg/Kg)	Predicted toxicity class	Prediction Accuracy (%)
1.	Rutin	5000	V	100
2.	Hexadecenoic acid methyl ester	5000	V	100
3.	Isoquercitrin	5000	V	72.9
4.	Pongagallone B	3800	V	68.07
5.	Pongagallone A	3000	V	69.26
6.	Bis[2-methylheptyl] phthalate	1340	IV	100
7.	Vitexin	832	IV	67.38
8.	Vecinin-2	832	IV	67.38
9.	2H-1-benzopyran-6-ol,3,4-dihydro-2,8-dimetyl-2-[4,8,12-trimethyltridecyl]	400	IV	68.07
10.	12ahydroxy-alpha-toxicarol	400	I	69.27

Table 2. Predicted oral acute toxicity, class, and accuracy of *Pongamia pinnata* leaf phytocompound. Based on the prediction of oral LD50 dose value of ten phytocompounds, only 12 α-hydroxy-toxiccarol was found to be toxic. The phytocompounds [bis[2-methylheptyl] phthalate, vitexin, vecinin-2, 2H-1-benzopyran-6-ol,3,4-dihydro-2,8-dimethyl-2-[4,8,12-trimethyltridecyl]] from leaves of class IV can be harmful if swallowed. The compounds such as rutin, hexadecanoic acid methyl ester, isoquercitrin, pongagallone B and pongagallone A underclass V. The LD50 values of this class indicate moderate acute toxicity.

The LD50 values in this class indicate that the substance may be harmful if swallowed but is expected to have a lower level of acute toxicity. This class indicates a milder toxic effect compared to class IV.

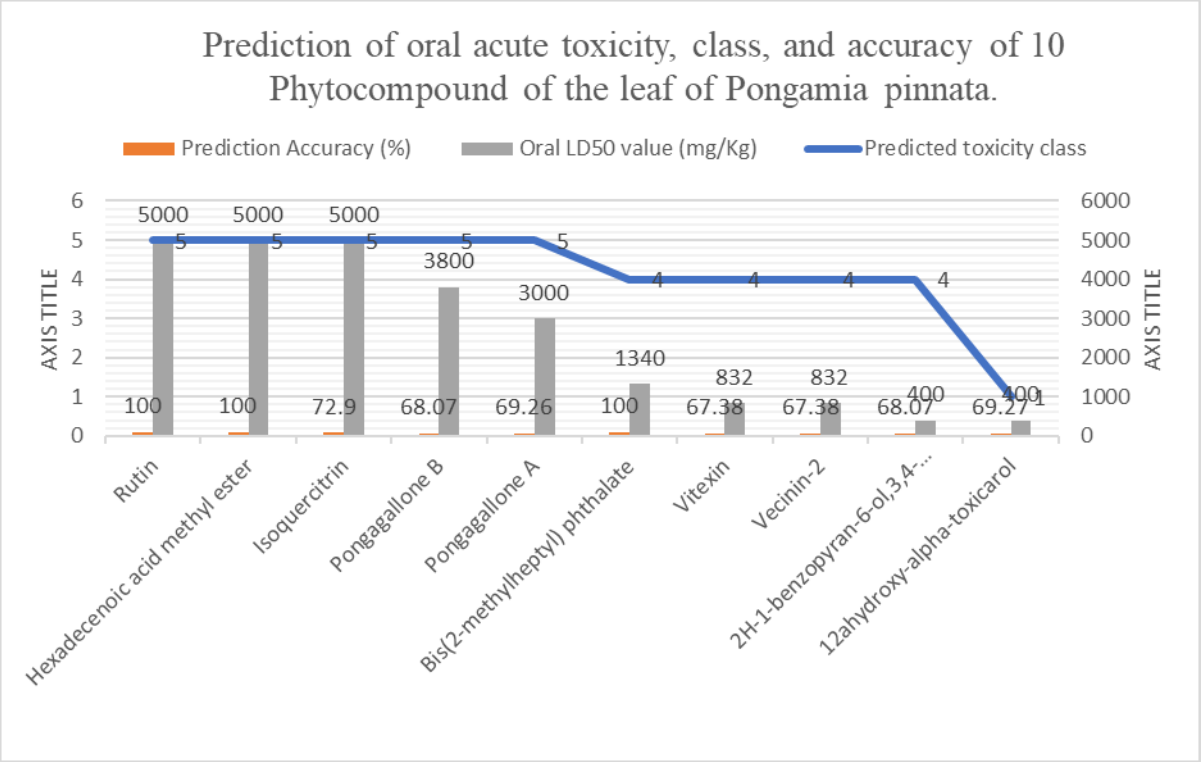

Figure 1: Predicted LD50 dose, Toxicity class and their prediction accuracy of ten compounds of Pongamia pinnata found in leaf by using ProTox-II webserver.

Organ toxicity results and predicted values for various toxicological endpoints calculated using the ProTox-II web server are presented in Table 3. Since the liver is the organ where toxins are metabolized, it is also possible to predict the organ toxicity [hepatotoxicity] of all toxins using phytocompound via the ProTox-II web server. The organ toxicity data showed that none of the compounds were expected to have a hepatotoxic or immunotoxic effect. Immunotoxicity refers to the harmful effects of xenobiotics on immune cells, cytokines, antibodies and other components of the immune system. Xenobiotics are substances that the body does not produce itself [23]. However, based on the various toxicity endpoints studied, certain phytocompounds were found to be cytotoxic, mutagenic and carcinogenic. On the other hand, isoquercitrin, 12a-hydroxy-alpha-toxicarol, rutin, pongagalone A and pongagalone B were predicted to be

carcinogenic. Vitexin and Vecinin-2 only proved to be mutagenic. Mutagens are chemicals that permanently alter DNA, leading to mutations that damage cells and cause certain diseases such as cancer. The substance bis[2-methylheptyl] phthalate is considered cytotoxic.

Tables 4 and 5 show the prediction results for the toxicological pathways, nuclear receptor signaling pathways and stress response pathways, respectively. The Tox21 Consortium states that chemical substances may potentially impair body functions and cause adverse health effects [14]. Seven different signaling pathways were examined with respect to the nuclear receptor signaling pathway. The computational estimates suggest that pongagallone B may interact with the aryl hydrocarbon receptor [AhR]. Vecinin-2, Rutin and Vitexin have been shown to interact with the androgen receptor, while Vecinie-2 may also contact the estrogen receptor [ER].

In Table 5, five different pathways were predicted to be examined for stress response pathways using *in-silico* methods. According to computer predictions, Pongagalone B should be given special attention because it has been shown to actively interact with heat shock response element [HSE], mitochondrial membrane potential [MMP], and nuclear factor [erythroid-derived 2-like 2/antioxidant response element]; nrf2/ARE] and pongagallone A, which functions as a tumor suppressor phosphoprotein p53.

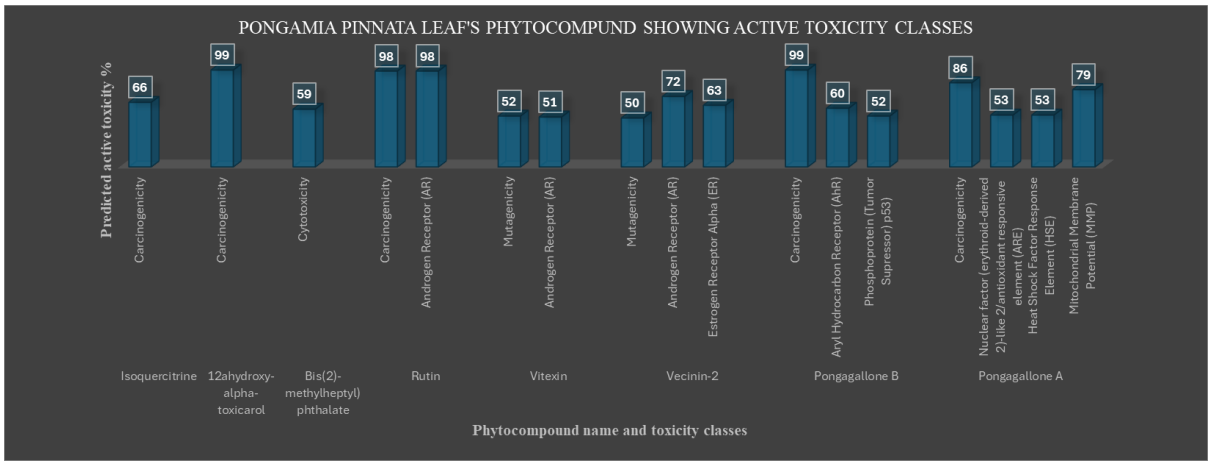

Figure 3 Phytocompound is found to be active for toxicity classes.

Table 1:Organ toxicity and toxicological endpoints predicted activity calculated using the ProTox-II web server.

Compounds Name	Organ Toxicity (% Probability)		Toxicity Endpoint (% Probability)		
	Hepatotoxicity	**Carcinogenicity**	**Immunotoxicity**	**Mutagenicity**	**Cytotoxicity**
Hexadecanoic acid methyl ester	Inactive (58)	Inactive (99)	Inactive (73)	Inactive (99)	Inactive (55)
2H-1-benzopyran-6-ol,3,4-dihydro-2,8-dimethyl-2-[4,8,12-trimethyltridecyl]	Inactive (92)	Inactive (82)	Inactive (88)	Inactive (91)	Inactive (77)
Isoquercitrin	Inactive (82)	Active (66)	Inactive (69)	Inactive (76)	Inactive (85)
12ahydroxy-alpha-toxicarol	Inactive (78)	Active (99)	Inactive (79)	Inactive (52)	Inactive (67)
Bis(2)-methylheptyl) phthalate	Inactive (65)	Inactive (65)	Inactive (76)	Inactive (97)	Active (59)
Rutin,	Inactive (80)	Active (98)	Inactive (64)	Inactive (88)	Inactive (91)
Vitexin	Inactive (81)	Inactive (82)	Inactive (87)	Active (52)	Inactive (72)
Vecinin-2	Inactive (83)	Inactive (85)	Inactive (82)	Active (50)	Inactive (72)
Pongagallone B	Inactive (73)	Active (99)	Inactive (87)	Inactive (54)	Inactive (56)
Pongagallone A	Inactive []	Active (86)	Inactive (59)	Inactive (77)	Inactive (63)

Table 2:Toxicological pathways: nuclear receptor signalling pathways predicted for Phytocompounds.

Tox21 Nuclear Receptor Signalling Pathways (% Probability)

Compound name	Aryl Hydrocarbon Receptor (AhR)	Androgen Receptor (AR)	Androgen Receptor Ligand Binding Domain (AR-LBD)	Aromatase	Estrogen Receptor Alpha (ER)	Estrogen Receptor Ligand Binding Domain (ER-LBD)	Peroxisome Proliferator activated.
Hexadecanoic acid methyl ester	Inactive (100)	Inactive (100)	Inactive (100)	Inactive (100)	Inactive (99)	Inactive (100)	Inactive (100)
2H-1-benzopyran-6-ol,3,4-dihydro-2,8-dimetyl-2-[4,8,12-trimethyltridecyl]	Inactive (87)	Inactive (98)	Inactive (99)	Inactive (74)	Inactive (90)	Inactive (92)	Inactive (99)
Isoquercitrine	Inactive (69)	Inactive (92)	Inactive (90)	Inactive (98)	Inactive (91)	Inactive (99)	Inactive (99)
12ahydroxy-alpha-toxicarol	Inactive (69)	Inactive (99)	Inactive (97)	Inactive (77	Inactive (84)	Inactive (93)	Inactive ((99)
Bis(2)-methylheptyl) phthalate	Inactive (97)	Inactive (98)	Inactive (99)	Inactive (99)	Inactive (90)	Inactive (96)	Inactive (92)
Rutin,	Inactive (83)	Active (98)	Inactive (99)	Inactive (97)	Inactive 63)	Inactive (98)	Inactive (99)
Vitexin	Inactive (83)	Active (51)	Inactive (99)	Inactive (98)	Inactive (53)	Inactive (97)	Inactive (99)
Vecinin-2	Inactive (89)	Active (72)	Inactive (99)	Inactive (97)	Active (63)	Inactive (98)	Inactive (99)
Pongagallone B	Active (60)	Inactive (97)	Inactive (95)	Inactive (87	Inactive (90)	Inactive (98)	Inactive (92)
Pongagallone A	Inactive (73)	Inactive (99)	Inactive (99)	Inactive (80)	Inactive (52)	Inactive (62)	Inactive (84)

Table 3:Toxicological pathways: stress response pathways predicted for Phytocompounds found in Pongamia pinnata leaf by using Protox-II webserver.

Compound name	Nuclear Factor (Erythroid-Derived 2-Like 2/Antioxidant Responsive Element) (nrf2/ARE)	Heat Shock Factor Response Element (HSE)	Mitochondrial Membrane Potential (MMP)	Phosphoprotein (Tumor Suppressor) p53)	ATPase Family AAA Domain Containing Protein 5 (ATAD5)
Hexadecanoic acid methyl ester	Inactive (100)	Inactive (100)	Inactive (100)	Inactive (100)	Inactive (100)
2H-1-benzopyran-6-ol,3,4-dihydro-2,8-dimetyl-2-[4,8,12-trimethyltridecyl]	Inactive (88)	Inactive (88)	Inactive (78)	Inactive (96)	Inactive (98)
Isoquercitrine	Inactive (98)	Inactive (98)	Inactive (98)	Inactive (50)	Inactive (100)
12ahydroxy-alpha-toxicarol	Inactive (82)	Inactive (83)	Inactive (62)	Inactive (57)	Inactive (79)
Bis(2)-methylheptyl) phthalate	Inactive (96)	Inactive (96)	Inactive (86)	Inactive (98)	Inactive (100)
Rutin,	Inactive (99)	Inactive (99)	Inactive (97)	Inactive (90	Inactive (99)
Vitexin	Inactive (97))	Inactive (97)	Inactive (90)	Inactive (72)	Inactive (99)
Vecinin-2	Inactive (96)	Inactive (96)	Inactive (95)	Inactive (86)	Inactive (99)
Pongagallone B	Inactive (79)	Inactive (79)	Inactive (52)	Active (52)	Inactive (92)
Pongagallone A	Active (53)	Active (53)	Active (79)	Inactive (52)	Inactive (87)

In the realm of scientific research, particularly in the field of toxicology, understanding the properties and effects of various chemical substances is of paramount importance. This is especially true for those substances that have the potential to interact with critical receptors and signalling pathways within the human body. These interactions can lead to a range of biological

responses, some of which may be beneficial, while others may result in adverse health effects [24].

One such substance that has been the subject of extensive study is the phytocompound known as 12-α-hydroxy-toxiccarol. This compound, found in the leaves of the *Pongamia pinnata* plant, has been identified as toxic, with a high level of acute oral toxicity. This toxicity is quantified using a measure known as the LD50 value, which represents the dose of the substance that would be lethal to 50% of a test population [25]. In the case of 12-α-hydroxy-toxiccarol, the LD50 value indicates a high level of acute oral toxicity.

However, toxicity is not a simple binary property. Substances can exhibit varying degrees of toxicity, and these degrees are often classified into different categories for ease of understanding and comparison. For instance, compounds of Class IV and V exhibit significant and moderate acute toxicity respectively, implying potential harm if ingested, with Class IV being more toxic than Class V [26].

It's crucial to understand that compounds vary in toxicity levels, and some may present a higher risk. Therefore, caution is advised when handling or consuming these substances. LD50 values provide a measure of their potential acute toxicity [27].

In addition to the LD50 value, other measures are also used to assess the potential effects of a substance. One such measure is the effective dose (ED50), which is the quantity of a substance needed to produce the desired effect in half of the subjects [28]. Another important measure is the Therapeutic Index (TI), which compares the ED50 to the LD50. A higher TI indicates a safer drug, as it means that the drug can produce the desired effect at a dose much lower than the dose that would cause harmful effects. The therapeutic window defines the dosage range where a drug is effective without causing toxicity. This concept is crucial in the field of

pharmacology, as it helps strike a balance between efficacy and safety. It ensures that a drug can produce the desired therapeutic effect without causing undue harm to the patient [29,30].

In our research, we used the ProTox-II web server to determine plant compounds' LD50. Doses below the LD50 provide a safety margin, crucial for regulatory compliance and safety assurance. Healthcare professionals should consider individual variability and administration routes to ensure patient safety and treatment effectiveness [27].

Hexadecenoic acid methyl ester and 2H-1-benzopyran-6-ol,3,4-dihydro-2,8-dimetyl-2-[4,8,12-trimethyltridecyl] do not belong to any toxicity class, while they are categorized under class V and IV respectively. These observations emphasize the need for prudent handling of these substances and indicate the necessity for additional research for validation.

The ProTox-II web server's predictions for phytocompounds in terms of toxicity endpoint, organ toxicity, and toxicological pathways are presented in Tables 3, 4, and 5. The LD50 dose is listed in Table 2. It can be inferred that the compounds are effective for all toxicity classes at this dose.

Paracelsus stated that chemicals are dose-dependent, and even toxic substances can be beneficial in small doses. When it comes to chemicals, dosage is crucial, and even pollutants can have certain advantages at low dosages [31]. Each route should have a specific acute oral toxic dose [32]. It should be noted that doses lower than the predicted LD50 dose for the toxic class should be inactive. Pongagallone A has an 86% probability of being carcinogenic at a dose of 3000 mg/kg LD50. Doses below 3000 mg/kg may not be carcinogenic.

To validate these computational predictions and fully assess the risks associated with these phytocompounds, further experimental validation may be required. These findings highlight the need for careful handling of these substances and suggest further research for validation.

The LD50 values and interactions with receptors indicate varying degrees of toxicity and potential health risks.

In conclusion, the study of toxicology and the effects of various substances on the human body is a complex and nuanced field. It requires a deep understanding of the properties of these substances, as well as the biological systems they interact with. Through careful study and research, we can gain a better understanding of these interactions and develop safer and more effective therapeutic strategies. As we continue to explore and understand the world around us, we must always remember the words of Paracelsus: "All things are poison, and nothing is without poison; the dosage alone makes it non-toxic." This timeless wisdom serves as a guiding principle in our quest to harness the power of nature for the betterment of humanity [31].

4 Conclusion

The present study on the toxicity of phytocompounds found in *Pongamia pinnata* leaves, using the ProTox-II web server. The study reveals that out of ten phytocompounds, only 12 α-hydroxy-toxiccarol was found to be toxic. Some compounds were found to be harmful if swallowed. The organ toxicity data showed that none of the compounds were expected to have a hepatotoxic or immunotoxic effect. However, certain phytocompounds were found to be cytotoxic, mutagenic, and carcinogenic. The study also examined seven different signalling pathways with respect to the nuclear receptor signalling pathway. The computational estimates suggest potential interactions between some compounds and certain receptors. This research provides valuable insights into the toxicity of phytocompounds in *Pongamia pinnata* leaves, contributing to our understanding of their potential effects on human health. However, these in silico findings are preliminary and require validation through empirical bioassays to confirm their implications for human health. This step is crucial to ensure the reliability of the computational predictions and to understand the real-world effects of these phytocompounds.

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6 Authors' contributions

All authors made significant contributions to the conception and design, data acquisition, or analysis and interpretation of data; participated in drafting the article or revising the article critically for important intellectual content; agreed to submit to the current journal; gave final approval for the version to be published; and agreed to be responsible for all aspects of the work. All authors are eligible to be authors in accordance with the requirements/guidelines of the International Committee of Medical Journal Editors (ICMJE).

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8 Conflicts of interest

The authors report no financial or any other conflicts of interest in this work.

9 Ethical approvals

This study does not involve experiments on animals or human subjects.

10 Data availability

All data generated or analyzed during this study are included in this article.

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