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Computational toxicology and food safety assessment of *Parkia timoriana* phytoconstituents using quantitative structure-activity relationship (QSAR) modeling approaches

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As a lead compound, natural compounds have undergone extensive research in different enterprises. Since they might also have other adverse effects, determining their toxicity is crucial. Computational methods can circumvent the main challenges associated with assessing the toxicity of substances using *in vivo* and *in vitro* techniques, including time, money, labor, and the use of animal models. Although *Parkia timoriana* (PT) has a significant economic potential, its exploitation has yet to be thoroughly explored in terms of its toxicity and food safety. In PT seed pod extracts, 61 phytocompounds with a predominance of alkaloids, flavonoids, and terpenoids were identified using GC-MS and LC-MS/MS analysis. Utilizing the TEST, OECD QSAR toolkit, VEGA-HUB, Toxtree, and PASS tools, phytocompounds from PT were assessed for toxicity, food safety risk assessment, and biological activity. The phytochemicals were tested on multiple species, including *Daphnia magna*, *Pimephales promelas*, *Tetrahymena pyriformis*, and rats, to determine their toxicity using the QSAR-TEST tool. For aquatic and mammalian organisms, the phytocompounds from PT were shown to be hazardous in the following four hierarchical orders: i) *P. promelas>T. pyriformis>D. magna>R. norvegicus*, ii) *P. promelas>D. magna>T. pyriformis>R. norvegicus*, iii) *D. magna> P. promelas>T. pyriformis>R. norvegicus*, and *T. pyriformis>P. promelas>D. magna>R. norvegicus*. Despite being non-bioaccumulative, non-mutagenic, and non-carcinogenic in nature, the majority of phytocompounds were developmental toxins. More than half of the phytochemicals derived from PT were highly toxic (Cramer oral toxicity) and manifested negative side effects (with a lower NOAEL value).Most of the substances did not exhibit organ toxicity in the repeated dose toxicity test, were bioavailable, metabolized by cytochrome-P450 pathway, and were excreted from the body. PASS predicted that the examined phytoconstituents from PT were to demonstrate a wide range of anti-oxidant, free radical scavenger, anti-inflammatory, antiviral, anti-fungal, anti-neoplastic, antibacterial, and anti-protozoal activities. For the purpose of exploring drug discovery, additional research of the phytocompounds on *in vivo* models is advised.

Keywords: Computational toxicology, Food safety, GC-MS, LC-MS/MS, *Parkia timoriana*, Quantitative structure-activity relationship modeling, Risk assessment

Parkia timoriana (PT, Fabaceae), also known as the tree bean, is a nutritionally rich, underutilized leguminous tree that grows in northeastern India and various Southeast Asian countries. From an ethnobotanical standpoint, tree beans are significant to ethnic groups in a number of provinces of Northeast India, Burma, Bangladesh, Pakistan, China, Thailand, Malaysia, and the Gambia. Numerous ailments are treated with concoctions of bark, fruit, and leaf portions. All edible parts of this plant, from the flowers and young pods through the mature seeds, are in seasonally high demand and offer a good supply of nutrients[1]. If utilized properly, tree beans could serve as an additional source of vegetable proteins. The protein level of pods ranged from 12.1% in tender to 18.8% in mature pods, but kernels had substantially greater protein content (28.8%) than pods[1] it has been found that the pods of PT contain significant amounts of tannins, flavonoids, saponins, anthocyanins, and leuco-anthocyanins[2]. Tree bean has been found to have antioxidant, α-glucosidase and α-amylase inhibitory, antibacterial, antidiabetic, antiproliferative, antiviral, immune boosting, and insecticidal effects. It has also been used to treat liver and skin diseases, ulcer, colon cancer, leprosy, hypertension, and painful eyes[3]. In the case of PT pods, leaves, and other plant parts are eaten

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either raw or boiled with additional ingredients to treat various disorders, and pods are eaten raw as salads which contribute to health advantages among the ethnic people. PT seeds have been shown to contain a number of hazardous compounds, including lectins, alkaloids, and non-protein amino acids. Despite having a considerable commercial potential, PT's exploitation has not yet been sufficiently investigated in terms of its toxicity and food safety[1].

Aside from that, pharmaceutical industries use medicinal plants as a component in the manufacturing of drugs. But the two main issues that prevent the use of plants before they are commercialized are safety and efficacy[4]. Preclinical toxicity and adverse drug responses account for about one-third of product failures[5]. Time, money, and labor are the three biggest challenges in determining the toxicity of substances using *in vivo* and *in vitro* approaches. Toxicology evaluation using computational approaches aids in early drug development by allowing for the early identification of compounds for which there are no experimental data[6]. These techniques also provide an alternative to toxicity research using animal models. Based on the compound's physiochemical characteristics, *in silico* prediction techniques estimate a chemical's activity in a specific biological system. Mathematical models known as structure activity relationship (SAR) and quantitative structure activity relationship (QSAR) are used to forecast the relative activities of a compound's structure[7]. SAR and quantitative structure activity relationship (QSAR) strategies have been primarily used by a number of regulatory agencies, such as authorities in the agrochemical, educational, food, and health care sectors, to handle the toxicological evaluation of a major substance that exists in nature with comprehensible annotation. As a result, SAR/QSAR programs are regarded as effective, substantial, and improved core prediction tools in systems biology, and they may offer useful methods for deepening our understanding of the possibly harmful impacts of phytochemicals. There are numerous academic (Virtual models for property Evaluation of chemicals within a Global Architecture−HUB (VEGA−HUB), Toxicity Estimation Software Tool (T.E.S.T), Toxic Hazard Estimation by decision tree approach (Toxtree), the OECD QSAR Toolbox, and the Online Chemical Modeling Environment (OCHEM)) and commercial (CoMFA/CoMSIA, ADMET−Predictor™ and MetaDrug™) SAR/QSAR modeling programs and packages that have been described for use in defining a wide range of toxicological endpoints for environmental contaminants and phytochemicals in plants[3,8-10]. In comparison to the mentioned QSAR models, the read across strategy-based tools VEGA-HUB, TEST, and QSAR toolbox provide realistic, repeatable, transparent, and verifiable results[11].

Despite the fact that PT seed pods offer enormous nutritional and therapeutic benefits, there are still numerous activities and interactions that have not yet been fully understood in terms of toxicity and food safety. Only limited amounts of research on the effects of specific chemicals of PT are now accessible in the literature. To increase our understanding and appreciation of the usage of PT in daily diet as functional food and nutraceuticals, further research must be done to grasp its potential for health promotion and possible drug discovery. The purpose of this study was to identify the phytoconstituents in PT (seed pods) by GC-MS and LC-MS/MS analyses, and their toxicity and risk were predicted using *in silico* toxicity prediction as a preliminary assessment of PT phytocompounds to be employed as a functional food or a therapeutic agent. Reliable, open-source, and user-friendly tools like TEST, Toxtree, VEGA HUB, OECD QSAR toolbox, and PASS were used to predict the (i) acute toxicity [*Pimephales promelas* (Fathead minnow, LC_{50} 96 h), *Daphnia magna* (Giant water flea, LC_{50} 48 h), *Tetrahymena pyriformis* (Free living ciliate, IGC_{50} 48 h), and *Rattus norvegicus* (rat, LD_{50} oral)], (ii) bioconcentration factor (BCF), (iii) developmental toxicity, (iv) mutagenicity (Ames test), (v) oral toxicity (Cramer), (vi) carcinogenicity,(vii) reproduction toxicity, (viii) chronic toxicity (NOAEL, no-observed-adverse-effect-level), and acceptable daily intake (ADI) value, (ix) repeated dose toxicity (HESS), (x) Lipinski rule oasis, (xi) Cyt-P450 metabolism, and (xii) biological activities of the phytocompounds from PT.

Materials and Methods

Collection and identification of plant material

Fresh leaves and seed pods of PT were collected from the Tanhril forest in Aizawl, Mizoram, India. The botanical survey of India, Eastern Regional Centre, Shillong, identified and verified the PT plant materials. The plant was submitted under the voucher number MZU/ZOO/22/08 into the Department of Zoology's herbarium at Mizoram University in Aizawl, Mizoram, India (Fig. 1).

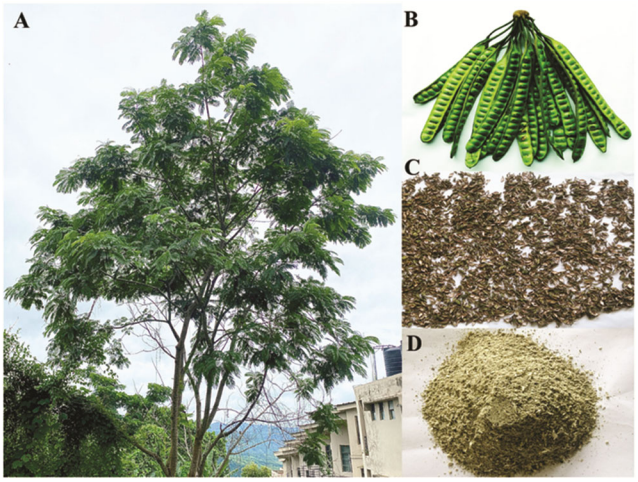

Fig. 1 — Wild bean tree, green tender and processed pods. (A) *Parkia timoriana* tree from the Tanhril forest in Aizawl, Mizoram, India; (B) Seed pods of *Parkia timoriana* consumed as vegetable, salad and chutney; (C) Cleaned, sliced, and air-dried seed pods of *Parkia timoriana*; (D) Processed forms of *Parkia timoriana* seed pods for extraction process

Preparation and processing of seed pods

PT seed pods were collected, cleaned, diced, and dried by air at room temperature (22°C), after which they were ground into a powder for extraction. To extract the phytoconstituents from the seed pod extract, the cold percolation method was used. The powder (100 g) was macerated in 400 mL of 100% methanol and allow keep at room temperature for 72 h. A rotary evaporator was used to condense the filtrate into a dark brownish-green semi-solid extract after the mixture was filtered using Whatman No. 1 filter paper. A 35 g yield of the dry extract was obtained, which was then stored for later use at 4°C.

Gas chromatography-mass spectrometry (GC–MS) analysis

Analytical conditions and analysis

PT seed pod extracts were analyzed using the GC-MS-2010 Shimadzu system (Shimadzu, Japan) in EI mode at 70 eV with a Restek-5MS column (30 0.25 mm film thickness 0.25 m). The carrier gas was pure helium gas, 99.99 percent pure, flow at a constant rate of 1 mL/min. An electron ionization energy approach with high ionization energy of 70 eV (electron Volts), 0.2 s of scan time, and fragments spanning from 40 to 650 (*m/z*) was selected for GC-MS spectrum detection. The injector temperature was kept constant at 260°C, and a 2 µL injection volume was used (split ratio: 10:1). The flow control mode was linear with a pressure of 99.3 kPa. The total flow and column flow were 16.3 mL/min and 1.21 mL/min, respectively. The column oven's temperature was first set at 120°C for two min, subsequently raised by 10°C per min up to 280°C, and then raised to 300°C for 20 min. The chromatogram and mass spectra were evaluated using the Xcalibur™ software embedded in the GC–MS/LC–MS system. Based on a comparison of the retention time (min), peak area, peak height, and mass spectral patterns of the test samples with those of authentic compounds stored in the National Institute of Standards and Technology (NIST) library, which has more than 62,000 patterns, and Dr. Duke's ethnobotanical phytochemical databases, the phytochemical contents of the test samples were determined[12]. A comparison was made between the spectra of the unknown component and the spectrum of the known components kept in the NIST collection. The phyto-compounds names, molecular weights, and structures were determined.

Liquid chromatography-mass spectrometry (LC-MS/MS) characterization

Using an auto sampler and a binary pump (Waters, USA) with a 10L loop, the Acquity Ultra Performance Liquid Chromatography (ACQUITY UPLC H-Class) system was employed. The separation of phyto-compounds from PT seed pods was investigated under a variety of chromatographic circumstances, including mobile phase composition, flow rate, and injection volume at different gradient programs. Several mobile phase mixtures, including acetonitrile-water, methanol-water, and acetonitrile-0.1% (*v/v*) formic acid aqueous solution, were evaluated in the gradient program at 1.5 mL/min flow rate. a mobile phase with a 1.5 mL/min flow rate, consisting of 0.1% (*v/v*) formic acid aqueous solution (A) and acetonitrile (B). The optimal circumstances for separation were found to be 0-300 bar column pressure and 30°C column temperature. The compounds were separated at 30°C using an Acquity CSH C18 column (2.1 mm 100 mm, 1.7 m). Two solvents—0.1% (*v/v*) formic acid in water (A) and methanol (B)—were employed to achieve a gradient elution at a flow rate of 1.5 ml/min. The gradient program consisted of 5% (B) at initial to 1 min then increased to 30% (B) at 6-12 min, 60% (B) at 12-16 min, 80% (B) at 16-20 min and 5% (B) at 26-30 min, while 95% (D) at 0-1 min, 70% (D) at 6-12 min, 40% (D) at 12-16 min, 20% (D) at 16-20 min and 95% (D) at 26-30 min, with a sample injection volume of 10 µL. The data-dependent automatic switching between MS and MS/MS acquisition modes was employed to conduct the MS analysis on the Water UPLC-TQD Mass Spectrometer (XEVO-TQD#QCA1232). Positive and negative ionization

modes were used to record the spectra, and the mass acquisition range was 150–2000 *m/z*.

Endpoints for toxicity prediction (LC_{50}, IGC_{50}, LD_{50}) by QSAR-TEST

Using QSAR-TEST (Version 5.1.2, http://www.epa.gov/nrmrl/std/cppb/qsar/index.html#TEST), an open-source application developed by the US EPA[13], the toxicity of the phytoconstituents identified through GC-MS and LC-MS/MS studies was assessed. To estimate the toxicity of a compound, this program integrates a number of QSAR strategies, including hierarchical, single-model, group contribution, closest neighbor, consensus, and mode of action methods as appropriate for each of the endpoint. The weighted average of the predictions from many models is used in the hierarchical method to estimate the toxicity of a drug after utilizing Ward's methodology to divide the training set into numerous structurally similar clusters[14]. A genetic algorithm is used to create models for each cluster. A genetic algorithm-based multilinear regression model that is fitted to the training set to make predictions employs a single-model strategy to predict the toxicity of a chemical substance based on molecular descriptors as independent variables. The group contribution approach uses molecular fragment counts as independent variables to fit a multilinear regression model to the training set and predict the toxicity of a substance. By averaging the three compounds in the training set that are most similar to the test chemical, the nearest neighbor technique determines the predicted toxicity. By averaging the expected toxicities from each of the aforementioned QSAR techniques, the predicted toxicity is calculated using the consensus method. A two-step procedure is utilized to determine the predicted toxicity using the mode of action method: first, the aquatic toxicity mode of action is predicted using linear discriminant models, and then the quantitative toxicity is predicted using a multiple linear regression model created for that mode of action[15].

The TEST software includes models for the following endpoints: 96-h fathead minnow 50 percent lethal concentration (LC_{50})[16,17], 48-h *Daphnia magna* 50% lethal concentration (LC_{50})[17], *Tetrahymena pyriformis* 50 percent growth inhibition concentration (IGC_{50})[18], oral rat 50 percent lethal dose (LD_{50})[18], bioaccumulation factor, developmental toxicity (DevTox[19]), and Ames mutagenicity[20]. The LC_{50} endpoint for fathead minnows (*P. promelas*) denotes the concentration in water that, after 4 days (96 h, mg/L), causes death in half of the exposed organisms. By accessing the ECOTOX aquatic toxicity database, the data set for this endpoint was retrieved[17]. The *D. magna* LC_{50} endpoint denotes the amount of water that, after 48 h, causes the death of half of any exposed *D. magna* (a water flea). The ECOTOX aquatic toxicity database offered the data set for this endpoint[17]. Using the same standards as for the 96-h fathead minnow LC_{50}, the database was filtered. The total number of compounds in the *D. magna* LC_{50} data set was 541. The endpoint for the model was -Log10 (LC_{50} mg/L). After 40 h, the *T. pyriformis* IGC_{50} endpoint represents the protozoan ciliate's 50% growth inhibitory concentration. The IGC_{50} training set was retrieved and compounds were included in the final *T. pyriformis* IGC_{50} data set. The endpoint that was modeled was -Log10 (IGC_{50} mg/L).The amount of the chemical (measured as the mass of the chemical in mg per kilogram of rat body weight) that, when consumed orally, is lethal to 50% of the rats is referred to as the oral rat LD_{50} endpoint. Downloading data from the ChemIDplus database, from which 13548 entries were acquired, generated the dataset for this endpoint. The total number of compounds in the oral rat LD_{50} data set was 7413. The -Log10 (LD_{50} mol/kg) endpoint was the modeled endpoint.

The agency for toxic substances and disease registry's (ATSDR) criteria for aquatic and mammalian toxicity metrics were linked with the estimated degrees of toxicity for the chemical compounds. The toxicity metrics categorized as extreme toxicity (X), very high toxicity (A), high toxicity (B), moderate toxicity (C), and low toxicity (D). Additionally, the phytoconstituents mutagenicity, developmental toxicity, and bioconcentration factor (BCF) were also evaluated. The definition of the BCF is the ratio of the chemical concentration in biota as a result of absorption via the respiratory surface to that in water at steady state[21]. Multiple databases were used to compile the data. After salts, mixes, and unclear compounds are filtered out, the final dataset contains 676 substances. The Log_{10} (BCF) was the modeled endpoint. The EURAS BCF Gold Standard Database was employed to predict the bio-accumulation factor using QSAR-TEST. Based on the Teratogen Information System (TERIS), CAESAR project, and FDA regulations, the developmental toxicity was predicted. The mutagenicity of the compounds was predicted using the data set based on the process by which the compounds responded to the Ames test. To estimate the bioaccumulation factor and the toxicity against *D. magna* and *P. promelas*, various modeling techniques including nearest neighbor, single model,

group contribution, and the food and drug administration (FDA) were applied. Hierarchical clustering, group contribution, FDA, and closest neighbor models were utilized to predict the toxicity against *T. pyriformis*. The mutagenicity and toxicity of the chemicals against rat (oral) were predicted using hierarchical clustering, FDA, and closest neighbor models. Hierarchical clustering, a single model, the FDA, and nearest neighbor models were used to predict the chemical substances developmental toxicity. The consensus method was adopted for current research because it takes into consideration all of the models mentioned above for the prediction of toxicity.

Toxicological endpoints prediction by OECD QSAR, VEGA, and Toxtree tools

The toxicological endpoints/outcomes that were computed using OECD QSAR toolbox (https://qsartoolbox.org/download/) include oral toxicity, repeated dosage toxicity (HESS), and Lipinski rule oasis[24]. NOAEL, ADI, carcinogenicity, and developmental toxicity prediction was made using VEGAHUB (www.vegahub.eu) online tool. Cytochrome P450-mediated drug metabolism was predicted by the Toxtree (https://toxtree.sourceforge.net/) user-friendly open-source platform.

PASS biological activity prediction

Using the PASS bioinformatics tool, anti-oxidant, free radical scavenger, anti-inflammatory, anti-viral, anti-fungal, anti-neoplastic, anti-bacterial, and anti-protozoal activities of PT was predicted. The predicted activity spectrum of a phytocompound is computed by PASS program as probable activity (Pa) and probable inactivity (Pi). Pa and Pi have values ranging from 0.000 to 1.000. For a given phytocompound, only biological actions with Pa > Pi are taken into consideration. Pa values more than or equal to 0.7 indicate a high likelihood of experimental pharmacological activity, while values between 0.5 and 0.7 indicate a lower likelihood. If Pa is less than 0.5, there is a lower likelihood that the activity will be identified by experiments, but it could additionally suggest that an entirely novel compound may be detected[25].

Results

GC-MS and LC-MS/MS

Using the peak retention time, peak area (%), peak height (%), and mass spectral fragmentation patterns of the known compounds (Fig. S1) listed in the NIST library, the bioactive compounds present in the methanol seed pod extracts of PT were identified as 9 distinct peaks on the GC-MS chromatogram (Fig. 2). The phytoconstituents in the methanol seed pod extracts of PT were found to be 3,7,11-trimethyl-2,4-dodecadiene, 5-ethyl-3-methyl-3,4-nonadien-6-yne, 4-isopropyl-3,4-dimethylcyclohexa-2,5-dienone, 1-(4-isopropylphenyl)-2-methylpropyl acetate, patch-oulialcohol, tricycle[4.3.0.0(7,9)]nonane,2,2,5,5,8,8-hexamethyl-,(1.alpha.,6.beta.,7.alpha.,9.alpha.), 1H-Purin-6-amine, N-((3-fluorophenyl)methyl)-bisabolene, and 1,1,3,3,4-Pentamethyl-6-t-butyl-2,3-dihydroindene (Table 1).

Based on the results of the LC-MS/MS analysis, alkaloids, flavonoids, and terpenoids constituted the majority of the phytocompounds that were extracted

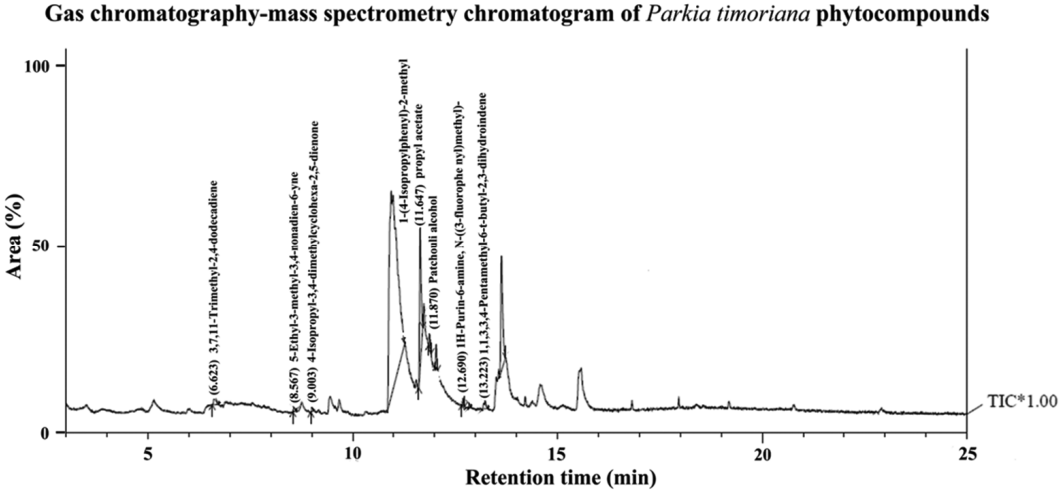

Fig. 2 — Gas chromatogram of methanol seed pod extract of *Parkia timoriana* using gas chromatography-mass spectrometry (GC-MS) analysis

Table 1 — Phytocompounds detected from methanol seed pod extract of *Parkia timoriana* using gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) analyses

Phytocompounds identified using gas chromatography-mass spectrometry (GC-MS) analysis†

Sl. No.	Phytocompound	Formula	Retention time	Mass spectrum (m/z)	Area (%)	Molecular weight
1.	3,7,11-Trimethyl-2,4-dodecadiene	$C_{15}H_{28}$	6.623	95.05	0.80	208.38
2.	5-Ethyl-3-methyl-3,4-nonadien-6-yne	$C_{12}H_{18}$	8.567	133.20	0.30	162.27
3.	4-Isopropyl-3,4-dimethylcyclohexa-2,5-dienone	$C_{11}H_{16}O$	9.003	122.10	0.21	164.24
4.	1-(4-Isopropylphenyl)-2-methylpro pyl acetate	$C_{15}H_{22}O_2$	11.647	191.15	10.13	234.33
5.	Patchouli alcohol	$C_{15}H_{26}O$	11.870	83.05	0.77	222.37
6.	Tricyclo[4.3.0.0(7,9)]nonane, 2,2,5, 5,8,8-hexamethyl-, (1.alpha.,6.beta., 7.alpha.,9.alpha.)	$C_{15}H_{26}$	12.037	135.10	1.08	206.37
7.	1H-Purin-6-amine, N-((3-fluorophe nyl)methyl)-	$C_{12}H_{10}FN_5$	12.690	73.05	0.28	243.24
8.	Bisabolene (sesquiterpene)	$C_{15}H_{24}$	12.847	119.10	0.15	204.35
9.	1,1,3,3,4-Pentamethyl-6-t-butyl-2,3-dihydroindene	$C_{18}H_{28}$	13.223	229.15	0.80	244.42
Phytocompounds identified using liquid chromatography-mass spectrometry (LC-MS) analysis††						
10.	Scopolamine-N-butyl (tropane alkaloid)	$C_{21}H_{30}NO_4^+$		1.12	360.2690	360.5
11.	1-dodecanoyl-2-(11Z-eicosenoyl)-glycero-3-phospho-(1'-sn-glycerol)(fatty acid)	$C_{38}H_{73}O_{10}P$		1.13	491.1929	721
12.	Napelline(C20-diterpenoid alkaloid)	$C_{22}H_{33}NO_3$		1.19	360.3060	359.5
13.	Taurodeoxycholate (oxidosqualene)	$C_{26}H_{45}NO_6S$		5.15	459.2112	499.7
14.	Voacamine (alkaloid)	$C_{43}H_{52}N_4O_5$		5.19	457.2131	704.9
15.	Ginsenoside F3 (steroid glycosides, and triterpene saponins)	$C_{41}H_{70}O_{13}$		5.22	459.2112	771.0
16.	Isoschaftoside (flavone)	$C_{26}H_{28}O_{14}$		5.28	457.1761	564.5
17.	Azadirachtin (tetra-triterpenoid)	$C_{35}H_{44}O_{16}$		8.72	479.0069	720.7
18.	E-Resveratrol trimethyl ether (polyphenolic phytoalexin)	$C_{17}H_{18}O_3$		11.01	225.1370	270.32
19.	Ginkgolide C (terpenic lactones)	$C_{20}H_{24}O_{11}$		14.57	325.3393	440.4
20.	Quinine (cinchona alkaloid)	$C_{20}H_{24}N_2O_2$		14.60	325.1912	324.4
21.	Ergocristine (ergot alkaloid)	$C_{35}H_{39}N_5O_5$		15.14	223.1758	609.7
22.	Demethoxycurcumin (phenol - curcuminoid)	$C_{20}H_{18}O_5$		15.18	223.1388	338.4
23.	Guan-fu base Y (alkaloid)	$C_{22}H_{29}NO_5$		15.25	264.3597	387.5
24.	Cycloheximide (piperidone alkaloid)	$C_{15}H_{23}NO_4$		15.37	264.3597	281.35
25.	Anabasamine (alkaloid)	$C_{16}H_{19}N_3$		15.42	264.3597	253.34
26.	Vincamine (alkaloid ester)	$C_{21}H_{26}N_2O_3$		15.47	264.3967	354.4
27.	Artocarpin (flavonoid)	$C_{26}H_{28}O_6$		16.04	325.4133	436.5
28.	Rauwolscine (alkaloid)	$C_{21}H_{26}N_2O_3$		16.12	293.2585	354.4
29.	Lithocholenic Acid (bile acid)	$C_{24}H_{38}O_3$		16.22	241.2701	374.6
30.	Rotenone (isoflavone)	$C_{23}H_{22}O_6$		16.25	241.3441	394.4
31.	Silychrystin (flavonolignan)	$C_{25}H_{22}O_{10}$		16.71	299.5488	482.4
32.	Speciosine (tropolone alkaloid)	$C_{28}H_{31}NO_6$		17.00	297.5137	477.5
33.	Apiin (flavonoid)	$C_{26}H_{28}O_{14}$		17.04	299.5118	564.5
34.	Gardnerine (alkaloid)	$C_{20}H_{24}N_2O_2$		20.23	325.2652	324.4
35.	Loganin (iridoid glycoside)	$C_{17}H_{26}O_{10}$		20.27	227.2831	390.4
36.	Apigenin glucoside	$C_{26}H_{28}O_{14}$		20.34	227.0981	564.5
37.	Linolenic acid (fatty acid)	$C_{18}H_{30}O_2$		20.40	279.5306	278.4
38.	Isocorydine (quinoline alkaloid)	$C_{20}H_{23}NO_4$		20.59	279.3087	341.4
39.	Nantenine (alkaloid)	$C_{20}H_{21}NO_4$		20.64	279.2716	339.4
40.	Phosphatidylcholine 15:0/18:1(11Z) (phospholipids)	$C_{41}H_{80}NO_8P$		20.76	205.3775	746.0
41.	Luteolin-8-C-glucoside (flavone)	$C_{21}H_{20}O_{11}$		21.52	253.3330	448.4
42.	Nicotiflorin (flavonoid)	$C_{27}H_{30}O_{15}$		21.56	253.4070	594.5
43.	Hydroxygardnutine (alkaloid)	$C_{20}H_{22}N_2O_3$		22.37	279.3826	338.4
44.	Phophatidylethanolamine(22:1/20:1) (phospholipid)	$C_{47}H_{90}NO_8P$		22.42	279.4566	828.2
45.	Phosphatidylethanolamine 18:0-22:6 (phospholipid)	$C_{45}H_{78}NO_8P$		22.51	279.4566	792.1
46.	Maritimetin-6-O-glucoside (phenol)	$C_{21}H_{20}O_{11}$		22.70	241.3071	448.4
47.	Ouabain (cardiac glycoside)	$C_{29}H_{44}O_{12}$		23.25	403.3759	584.7
48.	4,4'-Diaponeurosporene (carotenoid triterpenoid)	$C_{30}H_{42}$		23.39	403.3389	402.7

(*Contd.*)

Table 1 — Phytocompounds detected from methanol seed pod extract of *Parkia timoriana* using gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) analyses — (*Contd.*)

Phytocompounds identified using gas chromatography-mass spectrometry (GC-MS) analysis†

Sl. No.	Phytocompound	Formula	Retention time	Mass spectrum (m/z)	Area (%)	Molecular weight
49.	1-(5Z,8Z,11Z,14Z,17Z-eicosapentaenoyl)-2-(9Z-nonadecenoyl)-glycero-3-phosphoserine	$C_{45}H_{76}NO_{10}P$		25.43	255.4051	822.1
50.	Isorhamnetin-3-O-rutinoside (flavonoid)	$C_{28}H_{32}O_{16}$		25.57	255.2571	624.5
51.	Paeoniflorin (monoterpene glycoside)	$C_{23}H_{28}O_{11}$		25.62	255.3311	480.5
52.	Procyanidin B1 (flavonoid)	$C_{30}H_{26}O_{12}$		25.98	281.3438	578.5
53.	1,2-diarachidonoyl-sn-glycero-3 phosphoethanolamine (phospholipid)	$C_{45}H_{74}NO_8P$		26.02	281.3807	788.0
54.	Phosphatidylcholine(14:0/18:3n6) (phospholipid)	$C_{40}H_{74}NO_8P$		26.10	281.4547	728.0
55.	Hirsutine (indole alkaloid)	$C_{22}H_{28}N_2O_3$		27.15	369.2234	368.5
56.	Taurochenodeoxycholate (oxidosqualene)	$C_{26}H_{45}NO_6S$		27.43	465.5754	499.7
57.	[3-hexadecoxy-2-[(9Z,11E)-13-hydroxyoctadeca-9,11-dienoyl]oxypropyl] 2-(trimethylazaniumyl)ethyl phosphate	$C_{42}H_{82}NO_8P$		27.77	610.8422	760.1
58.	Alpha-Hederin (pentacyclic triterpene saponins)	$C_{41}H_{66}O_{12}$		28.00	391.4244	751.0
59.	Soyasapogenol B base + O-DDMP, O-HexA-HexA (pentacyclic triterpenoid)	$C_{48}H_{72}O_{18}$		28.21	391.4244	937.1
60.	Taurocholic acid (bile acid)	$C_{26}H_{45}NO_7S$		36.54	463.3923	515.7
61.	3-oxo-C8-homoserine lactone	$C_{12}H_{19}NO_4$		36.92	214.3692	214.28

†Oven: Initial temperature 120°C for 2 min, ramp 10°C/min to 300°C, hold for 20 min; Injection temperature: 260°C; Split ratio: 10:1; Ion Source Temp: 220°C; Interface temperature: 270.00°C; Scan: 40 – 650 m/z.

††Flow: 1.500 mL/min; Stop Time: 5.0 min; Column Temperature: 30°C; Min Pressure: 0.0 Bar; Max Pressure: 300.0 Bar; Acquisition mode: spectra were recorded in positive and negative ionization mode between *m/z* 150 and 2000.

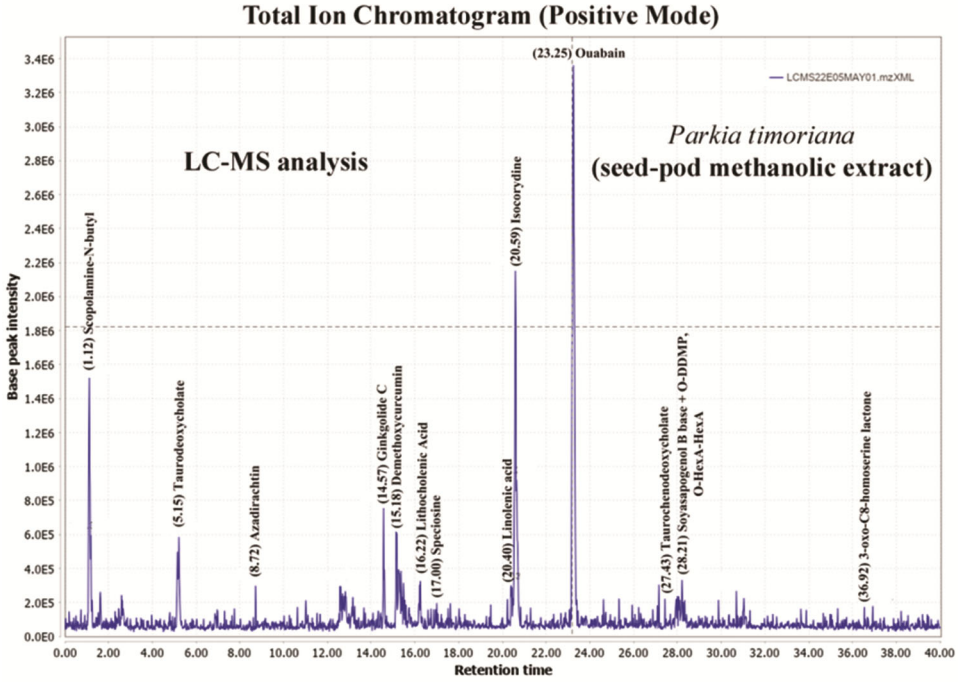

Fig. 3 — Liquid chromatography-mass spectrometry (LC-MS/MS) analysis data-positive ion mode for compounds identified methanol seed pod extracts of *Parkia timoriana*

from PT seed pods. In the positive (Fig. 3) and negative (Fig. 4) ion modes, 52 peaks were found in the LC-MS/MS chromatogram of the methanol extract from PT seed pod extracts. Compounds identified from PT (seed-pod methanolic extract) in the positive (29 compounds) and negative

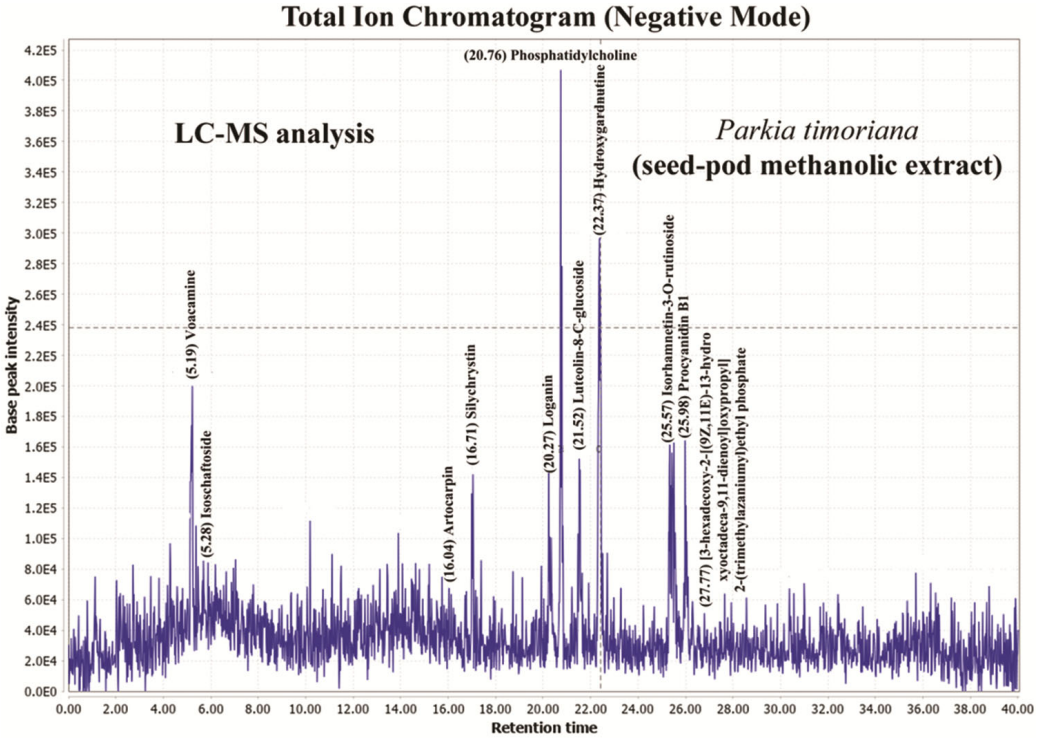

Fig. 4 — Total ion chromatogram (negative mode) of methanol seed pod extracts of *Parkia timoriana* using liquid chromatography-mass spectrometry (LC-MS/MS) analysis

(24 compounds) ion modes using MS/MS parent ion fragmentation and the resulting mass spectrum (*m/z*) are presented in (Figs S2 and S3), respectively. Sixteen alkaloids from PT were identified using LC-MS/MS, including scopolamine, napelline, voacamine, quinine, ergocristine, guan-fu base Y, cycloheximide, anabasamine, vincamine, rauwolscine, speciosine, gardnerine, isocorydine, nantenine, hydroxygardnutine, hirsutine. Ten flavonoids, including artocarpin, isoschaftoside, rotenone, silychrystin, apiin, apigenin, luteolin-8-C-glucoside, nicotiflorin, isorhamnetin-3-o-rutinoside, procyanidin B1, were found in the PT seed pod extracts, along with six terpenoids, including azadirachtin, ginkgolide C, loganin, 4,4'-diaponeurosporene, paeoniflorin, soyasapogenol B base. Moreover, the presence of phenols (demethoxycurcumin, maritimetin-6-O-glucoside), polyphenols (E-resveratrol trimethyl ether), saponins (ginsenoside F3, alpha-hederin), fatty acids [linolenic acid, 1-dodecanoyl-2-(11Z-eicosenoyl)-glycero-3-phospho-(1'-sn-glycerol)], bile acids (taurodeoxycholate, lithocholenic acid, taurocholic acid), phospholipids (phosphatidylcholine, phophatidylethanolamine, 1,2-diarachidonoyl-sn-glycero-3-phosphoethanolamine), cardiac glycoside (ouabain), and 3-oxo-c8-homoserine lactone was also detected (Table 1).

Environmental toxicity prediction of phytocompounds of *P. timoriana* seed pods by QSAR-TEST tool

Fathead minnow – *P. promelas* (LC_{50}-96 h)

Voacamine, ginsenoside F3, isoschaftoside, ergocristine, demethoxycurcumin, artocarpin, rotenone, silychrystin, speciosine, apigenin glucoside arabinoside, nicotiflorin, 4,4'-diaponeurosporene, isorhamnetin-3-O-rutinoside, procyanidin B1, 1-dodecanoyl-2-(11Z-eicosenoyl)-glycero-3-phospho-(1'-sn-glycerol), and soyasapogenol B base + O-DDMP, O-HexA-HexA are all classified to be extremely toxic compounds due to their extremely low LC_{50} values (96 h: 0.0007 - 0.053 mg/L) against *P. promelas*. The phytocompounds bisabolene, napelline, azadirachtin, E-resveratrol trimethyl ether, quinine, rauwolscine, lithocholenic acid, apiin, gardnerine, linolenic acid, isocorydine, nantenine, luteolin-8-C-glucoside, hydroxygardnutine, phophatidylethanolamine (22:1/20:1), maritimetin-6-O-glucoside, ouabain, hirsutine, alpha-hederin, and taurocholic acid, 3,7,11-Trimethyl-2,4-dodecadiene, tricyclononane 2,2,5,5,8,8-hexamethyl (1.alpha., 6.beta.,7.al pha.,9. alpha.), and 1,1,3,3,4-pentamethyl-6-t-butyl-2,3-dihydroindene were all found to be under category A as very high toxicity to *P. promelas* (LC_{50} – 96 h: 0.16 - 4.61 mg/L) (Table 2).

Table 2 — Predicted toxicity values (LC_{50}, IGC_{50}, LD_{50}) and categorization of phytocompounds identified from seed pods of *Parkia timoriana* against the giant water flea, free living ciliate, the fathead minnow, and rat using Toxicity Estimation Software Tool (TEST, https://epa.figshare.com/articles/ software/Toxicity_Estimation_Software_Tool_TEST_/21379365) based on quantitative structure–activity relationship (QSAR) model

Sl. No.	Phytocompound (*Parkia timoriana*)	*Pimephales promelas* (Fathead minnow) (96 h)†		*Daphnia magna* (Giant water flea) (48 h)††		*Tetrahymena pyriformis* (Free living ciliate) (48 h)†††		Rat (*Rattus norvegicus*) Oral††††	
		LC_{50} (mg/L)	Category	LC_{50} (mg/L)	Category	IGC_{50} (mg/L)	Category	LD_{50} (mg/kg)	Category
1.	3,7,11-Trimethyl-2,4-dodecadiene	0.19	A	0.25	A	0.33	A	5463.81	NT
2.	5-Ethyl-3-methyl-3,4-nonadien-6-yne	N/A	N/A	N/A	N/A	3.47	B	4365.01	NT
3.	4-Isopropyl-3,4-dimethylcyclohexa-2,5-dienone	10.15	C	4.65	B	9.32	B	4232.12	NT
4.	1-(4-Isopropylphenyl)-2-methylpropyl acetate	4.50	B	1.12	B	9.64	B	637.06	NT
5.	Patchouli alcohol	7.27	B	3.19	B	42.00	C	1368.51	NT
6.	Tricyclo[4.3.0.0(7,9)]nonane, 2,2,5,5,8,8-hexamethyl-, (1.alpha.,6.beta.,7.alpha.,9. alpha.)	0.68	A	0.049	X	6.52	B	4193.31	NT
7.	1H-Purin-6-amine,N-((3-fluorophenyl)methyl)	12.39	C	3.14	B	48.51	C	2001.30	NT
8.	Bisabolene	0.23	A	0.13	A	0.54	A	4720.54	NT
9.	1,1,3,3,4-Pentamethyl-6-t-butyl-2,3-dihydroindene	0.87	A	0.11	A	2.20	B	1068.10	NT
10.	Scopolamine-N-butyl	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
11.	1-dodecanoyl-2-(11Z-eicosenoyl)-glyce-ro-3phospho-(1'-sn-glycerol)	0.0029	X	0.011	X	7.55	B	21377.17	NT
12.	Napelline	0.62	A	15.86	C	4.91	B	233.91	D
13.	Taurodeoxycholate	1.94	B	4.26	B	2.37	B	1988.17	NT
14.	Voacamine	0.039	X	0.13	A	10.31	C	210.12	D
15.	Ginsenoside F3	0.043	X	46.74	C	3.24	B	36.52	C
16.	Isoschaftoside	0.035	X	122.66	D	18.09	C	1209.68	NT
17.	Azadirachtin	0.16	A	2.62	B	10.54	C	32.63	C
18.	E-Resveratrol trimethyl ether	0.17	A	2.53	B	5.05	B	1289.40	NT
19.	Ginkgolide C	N/A	N/A	19.95	C	11.28	C	30.32	C
20.	Quinine	0.61	A	6.64	B	7.58	B	430.63	D
21.	Ergocristine	0.0013	X	3.08	B	6.53	B	94.37	C
22.	Demethoxycurcumin	0.053	X	17.97	C	5.13	B	1747.94	NT
23.	Guan-fu base Y	2.37	B	4.49	B	5.29	B	36.06	C
24.	Cycloheximide	10.48	C	276.37	D	119.50	D	8.88	B
25.	Anabasamine	5.20	B	2.68	B	33.28	C	45.29	C
26.	Vincamine	1.42	B	3.98	B	7.96	C	996.51	NT
27.	Artocarpin	0.0003	X	0.62	A	2.73	B	2767.18	NT
28.	Rauwolscine	0.42	A	5.91	B	3.85	B	275.55	D
29.	Lithocholenic Acid	0.20	A	6.76	B	2.43	B	1936.85	NT
30.	Rotenone	0.0213	X	0.44	A	5.77	B	197.20	D
31.	Silychrystin	0.011	X	0.57	A	10.94	C	784.25	NT
32.	Speciosine	0.014	X	0.84	A	5.12	B	571.76	NT
33.	Apiin	0.23	A	177.95	D	37.59	C	1217.88	NT
34.	Gardnerine	0.44	A	3.71	B	4.41	B	457.91	D
35.	Loganin	143.94	D	1420.01	NT	25.40	C	3863.40	NT
36.	Apigenin glucoside arabinoside	0.035	X	87.89	C	18.09	C	712.93	NT
37.	Linolenic acid	0.35	A	2.01	B	0.43	A	10529.89	NT
38.	Isocorydine	0.57	A	0.92	A	5.77	B	6.91	B
39.	Nantenine	0.66	A	0.73	A	4.96	B	1849.47	NT
40.	Phosphatidylcholine 15:0/18:1(11Z)	124.19	D	0.62	A	N/A	N/A	2326.86	NT
41.	Luteolin-8-C-glucoside	0.26	A	31.54	C	24.64	C	1463.62	NT
42.	Nicotiflorin	0.0012	X	144.24	D	55.92	C	2154.77	NT
43.	Hydroxygardnutine	0.19	A	4.10	B	4.62	B	55.68	C

(*Contd.*)

Table 2 — Predicted toxicity values (LC_{50}, IGC_{50}, LD_{50}) and categorization of phytocompounds identified from seed pods of *Parkia timoriana* against the giant water flea, free living ciliate, the fathead minnow, and rat using Toxicity Estimation Software Tool (TEST, https://epa.figshare.com/articles/ software/Toxicity_Estimation_Software_Tool_TEST_/21379365) based on quantitative structure–activity relationship (QSAR) model — (*Contd*.)

Sl. No.	Phytocompound (*Parkia timoriana*)	*Pimephales promelas* (Fathead minnow) (96 h)†		*Daphnia magna* (Giant water flea) (48 h)††		*Tetrahymena pyriformis* (Free living ciliate) (48 h)†††		Rat (*Rattus norvegicus*) Oral††††	
		LC_{50} (mg/L)	Category	LC_{50} (mg/L)	Category	IGC_{50} (mg/L)	Category	LD_{50} (mg/kg)	Category
44.	Phophatidylethanolamine(22:1/20:1)	4.61	A	0.0012	X	8.67	B	28810.26	NT
45.	Phosphatidylethanolamine 18:0-22:6	10.26	C	0.0008	X	8.30	B	20068.13	NT
46.	Maritimetin-6-O-glucoside	0.31	A	24.94	C	22.13	C	2110.06	NT
47.	Ouabain	0.45	A	352.41	D	6.27	B	26.91	C
48.	4,4'-Diaponeurosporene	0.0007	X	0.0001	X	4.22	B	8948.19	NT
49.	1-(5Z,8Z,11Z,14Z,17Z-eicosapentaenoyl) -2-(9Z-nonadecenoyl)-glycero-3-phospho serine	0.87	A	0.0013	X	8.61	B	20827.58	NT
50.	Isorhamnetin-3-O-rutinoside	0.0013	X	143.13	D	58.74	C	1610.91	NT
51.	Paeoniflorin	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
52.	Procyanidin B1	0.0012	X	0.45	A	5.11	B	2248.22	NT
53.	1,2-diarachidonoyl-sn-glycero-3-phosphoethanolamine	0.83	A	0.0008	X	8.25	B	19965.79	NT
54.	Phosphatidylcholine(14:0/18:3n6)	3.20	B	0.60	A	7.63	B	530.65	NT
55.	Hirsutine	0.40	A	3.64	B	2.88	B	180.63	D
56.	Taurochenodeoxycholate	2.00	B	4.26	B	2.37	B	1988.17	NT
57.	3-hexadecoxy-2-[(9Z,11E)-13-hydroxy octadic a-9,11-dienoyl]oxypropyl] 2-(trimethylazaniumyl)ethyl phosphate	3.34	B	0.63	A	7.97	B	554.01	NT
58.	Alpha-hederin	0.58	A	14.70	C	8.05	B	41.93	C
59.	Soyasapogenol B base + O-DDMP, O-HexA-HexA	0.0019	X	20.32	C	3.94	B	61.21	C
60.	Taurocholic acid	3.74	A	5.82	B	2.45	B	801.94	NT
61.	3-oxo-C8-homoserine lactone	267.39	D	488.36	D	189.82	D	995.41	NT

X: Extreme toxicity; A: very high toxicity; B: high toxicity; C: moderate toxicity; D: low toxicity; NT:nontoxic; T: Toxic; N/A: not applicable.

† LC_{50}-96 h: the amount of the test substance in water that, after 96 hours, is fatal to 50% of fathead minnows exposed in mg/L.

†† LC_{50}-48 h: the amount of the test substance in water that, after 96 hours, is fatal to 50% of *Daphnia magna*exposed in mg/L.

†††IGC_{50}-48 h: concentration of the test substance in water that, after 48 hours, inhibits *Tetrahymena pyriformis* development by 50%.

†††† LD_{50} - chemical dose in mg/kg body weight that, when consumed orally, will cause death in 50% of rats.

Giant water flea – *D. magna* (LC_{50} – 48 h)

The compounds tricyclononane 2,2,5,5,8,8-hexamethyl(1.alpha.,6.beta.,7.alpha.,9. alpha.),1-dodecanoyl-2-(11Z-eicosenoyl)-glycero-3-phospho-(1'-sn-glycerol)], Phophatidylethanolamine (22:1/20:1 and 18:0-22:6),4,4'-diaponeurosporene1-(5Z,8Z,11Z,14Z, 17Z-eicosapentaenoyl)-2-(9 Z-nonadecenoyl)-glycero-3-phosphoserine, and 1,2-diarachidonoyl-sn-glycero-3-phosphoethanolamine were exhibited extremely high levels of toxicity (category X) based on the LC_{50} 48 h data (0.0001 – 0.049 mg/L) against *D. magna*. The compounds 3,7,11-trimethyl-2,4-dodecadiene, bisabolene, 1,1,3,3,4-Pentamethyl-6-t-butyl-2,3-dihydroindene, voacamine, artocarpin, rotenone, silychrystin, speciosine, isocorydine, nantenine, phosphatedylcholine (15:0/18:1(11Z) and (14:0/ 18:3n6)), procyanidin B1, and 3-hexadecoxy-2-[(9Z,11E)-13-hydroxy octadica-9,11-dienoyl]oxy propyl] 2-(trimethylazaniumyl) ethylphosphate were categorized as very high toxicity under category A (LC_{50} 48 h:0.11 – 0.92 mg/L), whereas the substances 4-isopropyl-3,4-dimethyl cyclohexa-2,5-dienone,1-(4-Isopropylphenyl)-2-methylpropyl acetate, patchouli alcohol, 1H-purin-6-amine,N-((3-fluoropheny l)methyl), taurodeoxycholate, azadirachtin, E-resveratrol trimethyl ether, quinine, ergocristine, guan-fu base Y, anabasamine, vincamine, rauwolscine, lithocholenic acid, gardnerine, linolenic acid, hydroxygardnutine, hirsutine, taurochenodeoxycholate, and taurocholic acid recorded as highly toxic under category B against *D. magna* (LC_{50} 48 h: 1.12 – 6.76 mg/L) (Table 2).

Free living ciliate – *T. pyriformis* (IGC_{50} – 48 h)

It was predicted that none of the PT chemicals would be extremely toxic (category X) to *T. pyriformis*. 3,7,11-Trimethyl-2,4-dodecadiene, bisabolene, and linolenic acid all showed veryhigh toxicityagainst *T. pyriformis* (category A), with IGC_{50} values 0.33, 0.54, and 0.43 mg/L, respectively. While the IGC50 of5-ethyl-3-methyl-3,4-nonadien-6-yne, 4-isopropyl-3,4-dimethyl-cyclohexa-2,5-dienone1-(4-Isopropylphenyl)-2-methy-lpropyl acetate, tricyclo[4.3.0.0(7,9)]nonane2,2,5,5,8,8-hexamethyl-,(1.alpha.,6.beta.,7.alpha.,9.alpha.), 1,1,3,3,4-pentamethyl-6-t-butyl-2,3-dihydroindene, 1-dodecanoyl-2-(11Z-eicosenoyl)-glycero-3 phospho-(1'-sn-glycerol), napelline, taurodeoxycholate, ginsenoside F3, E-resveratrol trimethyl ether, quinine, ergocristine, demethoxycurcumin, guan-fu base Y, artocarpin, rauwolscine, lithocholenic acid, rotenone, speciosine, gardnerine, isocorydine, nantenine, hydroxygardnutine, phophatidylethanolamine (22:1/20:1 and 18:0/22:6), ouabain, 4,4'-diaponeurosporene, 1-(5Z,8Z,11Z,-14Z,17Z-eicosapentaenoyl) -2-(9 Z-nonadecenoyl)-glycero-3-phosphoserine, procyanidin B1, 1,2-diarachidonoyl-sn-glycero-3-phosphoethanolamine, phosphatidylcholine (14:0/18:3n6), hirsutine, taurochenodeoxycholate, 3-hexadecoxy-2-[(9Z,11E)-13-hydroxy octadic a-9,11-dienoyl]oxy propyl] 2-(trimethylazaniumyl) ethyl phosphate, alpha-hederin, soyasapogenol B base + O-DDMP, O-HexA-HexA, and taurocholic acid was estimated to be between 2.37 and 9.64 mg/L. Consequently, it falls under the toxicity scale's Category B (high toxicity) (Table 2).

Rat – *R. norvegicus* (LD_{50} – oral)

Compounds detected from the seed pods of PT exhibited neither extreme toxicity (category X) nor very high toxicity (category A) against the rat model. Isocorydine, and cycloheximide from PT exhibited very high toxicity (category B) against the rat model, with the respective LD_{50} values of 6.91 and 8.88 mg/kg. According to the mammalian toxicity scale of ATSDR, EPA, the majority of the chemicals (40 out of 61) found in the methanol seed pod extract of PT possessed LD_{50} values above 500 mg/kg and were therefore categorized as non-toxic against rats. Napelline, voacamine, quinine, rauwolscine, rotenone, gardnerine, and hirsutine comprise chemical compounds from PT that were predicted to demonstrate low toxicity (category D, LD_{50}: 180.63 - 457.91 mg/kg). In the rat model, the PT compounds ginsenoside F3, azadirachtin, ginkgolide C, ergocristine, guan-fu base Y, anabasamine, hydroxygardnutine, ouabain, alpha-hederin, and soyasapogenol B base + O-DDMP, O-HexA-HexA showed moderate toxicity (category C) with LD_{50} values of 26.91 and 61.21 mg/kg, respectively (Table 2).

Hierarchical order of aquatic (LC_{50}, and IGC_{50}) and mammalian (LD_{50}) organism toxicity

Twenty phytocompounds from PT including napelline, taurodeoxycholate, ginsenoside F3, isoschaftoside, demethoxycurcumin, cycloheximide, lithocholenic acid, apiin, loganin, apigenin glucoside arabinoside, nantenine, luteolin-8-C-glucoside, nicotiflorin, maritimetin-6-O-glucoside, ouabain, isorhamnetin-3-O-rutinoside, hirsutine, taurochen-odeoxycholate, alpha-hederin, and soyasapogenol B base + O-DDMP, O-HexA-HexAshowed the organism toxicity in the order of *P. promelas*> *T. pyriformis*>*D. magna*>*R. norvegicus* (oral).

Nineteen phytochemicals from PT comprising 3,7,11-trimethyl-2,4-dodecadiene, 1-dodecanoyl-2-(11Z-eicosenoyl)-glycero-3 phospho-(1'-sn-glycerol), voacamine, azadirachtin, E-resveratrol trimethyl ether, quinine, ergocristine, guan-fu base Y, vincamine, artocarpin, rauwolscine, rotenone, silychrystin, speciosine, gardnerine, linolenic acid, isocorydine, hydroxygardnutine, and procyanidin B1 demonstrated aquatic and mammalian toxicity in the order listed below: *P. promelas*>*D. magna*> *T. pyriformis*>*R. norvegicus* (oral).

In the following order: *D. magna*>*P. promelas*> *T. pyriformis*>*R. norvegicus* (oral), fifteen phytocompounds from PT, including 4-isopropyl-3,4-dimethylcyclohexa-2,5-dienone, 1-(4-isopropyl-phenyl)-2-methylpropyl acetate, patchouli alcohol, tricyclo[4.3.0.0(7,9)] nonane, 2,2,5,5, 8,8-hexa methyl-,(1.alpha.,6.beta.,7.al pha.,9.alpha.), 1H-purin-6-amine,N-((3-fluorophenyl)methyl), bisabolene, 1,1,3,3,4-pentamethyl-6-t-butyl-2,3-dihydroindene, anabasamine, phophatidylethanolamine(22:1/20:1and 18:0-22:6), 4,4'-diaponeurosporene, 1-(5Z,8Z,11Z, 14Z,17Z-eicosapentaenoyl)-2-(9 Z-nonadecenoyl)-glyce ro-3-phosphoserine, 1,2-diarachidonoyl-sn-glycero-3-phosphoethanolamine, phosphatidylcholine (14:0/18:3n6), and 3-hexadecoxy-2-[(9Z,11E)-13-hydroxyoctadica-9,11-dienoyl]oxy propyl] 2-(trimethylazaniumyl) ethyl phosphate, demonstrated the organism's toxicity.Taurocholic acid and 3-oxo-C8-homoserine lactone, two PT phytocompounds, demonstrated the toxicity of the organisms in the following order: *T. pyriformis*>*P. promelas*> *D. magna*>*R. norvegicus* (oral).

Bioconcentration factor, developmental toxicity and mutagenicity prediction analyses by QSAR-TEST tool

Tricyclo[4.3.0.0(7,9)]nonane,2,2,5,5,8,8-hexa-methyl-,(1.alpha.,6.beta.,7.alpha.,9.alpha.) recorded very high bioconcentration factor with a value of 16515.36, followedby 1,1,3,3,4-pentamethyl-6-t-butyl-2,3-dihydroindene(5726.79), showing their potent bio-accumulative nature. The BCF values for all the other compounds were less than 2000, indicating non-bioaccumulative properties of the PT compounds (Table 3). Isoschaftoside, apiin, apigenin glucoside arabinoside, maritimetin-6-O-glucoside, ouabain, and 3-oxo-C8-homoserine lactone were among the compounds identified from PT that were developmental non-toxicants with a score of 0.37 to 0.48, while the remaining compounds (46 out of 61) were found to be developmental toxicants with a score of 0.53 to 1.38. Procyanidin B1, lithocholenic acid (1.00), hydroxygardnutine (1.06), and ergocristine (1.38), all of which registered very high scores, were predicted to be developmental toxic substances (Table 3). The substances 1H-purin-6-amine,N-((3-fluorophenyl)methyl), taurodeoxy cholate, E-resveratrol trimethyl ether, anabasamine, gardnerine, nantenine, luteolin-8-C-glucoside, procyanidin B1, taurochenodeoxycholate, and taurocholic acid were projected to be mutagens and have scores greater than 0.5, whilst all other

Table 3 — Bioaccumulation factor, developmental toxicity and mutagenicity (Ames test) prediction analyses of *Parkia timoriana* phytocompounds (seed pods) using Toxicity Estimation Software Tool (TEST, https://epa.figshare.com/articles/ software/ Toxicity_Estimation_Software_Tool_TEST_/21379365) based on quantitative structure–activity relationship (QSAR) model

Sl. No.	Phytocompound (*Parkia timoriana* seed pods)	Bioconcentration factor (BCF)†	Developmental toxicity††		Mutagenicity (Ames test)†††	
			Value	Result	Value	Result
1.	3,7,11-Trimethyl-2,4-dodecadiene	1170.35	0.79	T	-0.02	-
2.	5-Ethyl-3-methyl-3,4-nonadien-6-yne	1905.46	0.81	T	0.19	-
3.	4-Isopropyl-3,4-dimethylcyclohexa-2,5-dienone	16.03	0.65	T	0.19	-
4.	1-(4-Isopropylphenyl)-2-methylpropyl acetate	187.91	0.64	T	0.21	-
5.	Patchouli alcohol	729.56	0.63	T	-0.09	-
6.	Tricyclo[4.3.0.0(7,9)]nonane, 2,2,5,5, 8,8-hexa methyl-, (1.alpha.,6.beta.,7.al pha.,9. alpha.)-	16515.36	0.79	T	-0.06	-
7.	1H-Purin-6-amine,N-((3-fluoropheny l)methyl)	7.31	0.69	T	0.90	+
8.	Bisabolene	937.61	0.92	T	0.00	-
9.	1,1,3,3,4-Pentamethyl-6-t-butyl-2,3-dihy-droin dene	5726.79	0.96	T	-0.16	-
10.	Scopolamine-N-butyl	N/A	N/A	N/A	N/A	N/A
11.	1-dodecanoyl-2-(11Z-eicosenoyl)-gly ce-ro-3 ph ospho-(1'-sn-glycerol)	2.37	N/A	N/A	0.22	-
12.	Napelline	81.13	0.88	T	0.05	-
13.	Taurodeoxycholate	3.91	0.87	T	0.60	+
14.	Voacamine	22.22	0.67	T	0.31	-
15.	Ginsenoside F3	48.49	0.67	T	-0.02	-
16.	Isoschaftoside	6.34	0.37	NT	0.37	-
17.	Azadirachtin	263.94	0.67	T	0.27	-
18.	E-Resveratrol trimethyl ether	87.43	0.67	T	0.82	+
19.	Ginkgolide C	N/A	0.64	T	-0.07	-
20.	Quinine	71.55	0.88	T	0.39	-
21.	Ergocristine	52.71	1.38	T	-0.03	-
22.	Demethoxycurcumin	10.12	0.97	T	0.10	-
23.	Guan-fu base Y	N/A	0.67	T	0.07	-
24.	Cycloheximide	1.39	0.86	T	-0.12	-
25.	Anabasamine	142.98	0.57	T	0.60	+
26.	Vincamine	67.55	0.81	T	0.27	-
27.	Artocarpin	60.14	0.76	T	0.22	-
28.	Rauwolscine	17.31	0.97	T	0.42	-
29.	Lithocholenic Acid	42.03	1.00	T	0.26	-
30.	Rotenone	34.77	0.86	T	0.33	-
31.	Silychrystin	1.50	0.65	T	0.50	-
32.	Speciosine	N/A	0.96	T	-0.09	-
33.	Apiin	10.39	0.48	NT	0.09	-
34.	Gardnerine	44.17	0.92	T	0.55	+

(*Contd*.)

Table 3 — Bioaccumulation factor, developmental toxicity and mutagenicity (Ames test) prediction analyses of *Parkia timoriana* phytocompounds (seed pods) using Toxicity Estimation Software Tool (TEST, https://epa.figshare.com/articles/ software/ Toxicity_Estimation_Software_Tool_TEST_/21379365) based on quantitative structure–activity relationship (QSAR) model (*contd.*)

Sl. No.	Phytocompound (*Parkia timoriana* seed pods)	Bioconcentration factor (BCF)†	Developmental toxicity††		Mutagenicity (Ames test)†††	
			Value	Result	Value	Result
35.	Loganin	5.90	0.57	T	0.24	-
36.	Apigenin glucoside arabinoside	6.34	0.38	NT	0.38	-
37.	Linolenic acid	9.93	0.72	T	0.24	-
38.	Isocorydine	103.38	0.92	T	0.42	-
39.	Nantenine	123.77	0.84	T	0.57	+
40.	Phosphatidylcholine 15:0/18:1(11Z)	10.52	N/A	N/A	0.19	-
41.	Luteolin-8-C-glucoside	7.46	0.57	T	0.53	+
42.	Nicotiflorin	16.32	0.53	T	0.02	-
43.	Hydroxygardnutine	30.15	1.06	T	0.34	-
44.	Phophatidylethanolamine(22:1/20:1)	10.52	N/A	N/A	0.06	-
45.	Phosphatidylethanolamine 18:0-22:6	10.52	N/A	N/A	0.27	-
46.	Maritimetin-6-O-glucoside	0.30	0.46	NT	0.47	-
47.	Ouabain	27.93	0.37	NT	0.02	-
48.	4,4'-Diaponeurosporene	214.88	0.93	T	0.29	-
49.	1-(5Z,8Z,11Z,14Z,17Z-eicosapentaen oyl) -2-(9 Z-nonadecenoyl)-glyce ro-3-phospho serine	10.52	N/A	N/A	0.28	-
50.	Isorhamnetin-3-O-rutinoside	3.86	0.54	T	0.02	-
51.	Paeoniflorin	N/A	0.70	T	0.13	-
52.	Procyanidin B1	23.17	1.00	T	0.64	+
53.	1,2-diarachidonoyl-sn-glycero-3-phosphoethano lamine	10.52	N/A	N/A	0.29	-
54.	Phosphatidylcholine(14:0/18:3n6)	10.52	N/A	N/A	0.39	-
55.	Hirsutine	355.88	0.91	T	0.43	-
56.	Taurochenodeoxycholate	4.00	0.89	T	0.60	+
57.	[3-hexadecoxy-2-[(9Z,11E)-13-hydroxy octadic a-9,11-dienoyl]oxy propyl] 2-(trimethylazanium yl) ethyl phosphate	10.52	N/A	N/A	0.37	-
58.	Alpha-hederin	48.49	0.67	T	-0.01	-
59.	Soyasapogenol B base + O-DDMP, O-HexA-HexA	6.79	0.67	T	-0.03	-
60.	Taurocholic acid	1.35	0.67	T	0.60	+
61.	3-oxo-C8-homoserine lactone	N/A	0.45	NT	0.02	-

T: toxicant; NT: nontoxicant; N/A: not applicable; -: negative – non-mutagen; +: positive - mutagen.
†A compoundis termed bioaccummulative, if its BCF value is greaterthan 2000 and very bioaccummulative if its value is greaterthan 5000.
†† The substance is regarded as not being hazardous to development if the calculated value is less than 0.5, and it is regarded as toxic to development if the calculated value is greater than 0.5.
†††If the computed score is less than 0.5, the activity is considered to be negative and non-mutagenic, and if the calculated value is greater than 0.5, the activity is considered to be positive and mutagenous.

compounds possessed scores below 0.5 designating them as non-mutagens (Table 3).

Prediction of oral, repeated dose and reproductive toxicity, carcinogenicity, NOAEL, Lipinski rule oasis, and Cyt-P450 metabolism by Toxtree, VEGA HUB, and OECD QSAR tools

When a chemical is administered orally, its toxicological profile is evaluated using the Cramer categorization methodology, which assigns the substance to one of three classes: Class I (Low toxicity), Class II (Intermediate toxicity), and Class III (High toxicity)[26]. A significant portion of the compounds found in PT (46 out of 61) were categorized as class III high toxicity substances using the Cramer oral toxicity classification system (Table 4). 3,7,11-trimethyl-2,4-dodecadiene, 1-(4-Isopropyl-phenyl)-2-methylpropyl acetate, patchouli alcohol, tricyclo[4.3.0.0(7,9)]nonane,2,2,5,5,8,8-hexamethyl-,(1.alpha.,6.beta.,7.alpha.,9.alpha.),1-methyl-4-(1,5-dimethyl-4-hexenylidene)-1-cyclohexene, 1,1,3,3,4-penta methyl-6-t-butyl-2,3-dihydroindene, 1-dodecanoyl-2-(11Z-eicosenoyl)-glycero-3-phospho-(1'-sn-glycerol), linolenic acid, phophatidyl ethanolamine (22:1/20:1and18:0-22:6), 4,4'-diaponeurosporene, 1-(5Z,8Z,11Z,14Z,17Z-eicosapentaenoyl)-2-(9Z-nonad-ecenoyl)-glycero-3-phosphoserine, 1,2-diarachidon-oyl-sn-glycero-3-phosphoethanolamine, and phos-

Table 4 — Prediction of toxicity of *Parkia timoriana* (seed pods) phytoconstituents in relation to oral toxicity, carcinogenicity, reproductive toxicity, NOAEL, repeated dose toxicity, Lipinski rule of five and cytochrome-P450 Metabolism using Toxtree, VEGA HUB, and OECD QSAR tools based on quantitative structure–activity relationship (QSAR) model

Sl. No.	Phytocompound (*Parkia timoriana*)	Oral Toxicity (Cramer)	Carcinogenicity	Reproductive toxicity	NOAEL		Repeated dose toxicity (HESS)	Lipinski Rule Oasis†	Cyt-P450 Metabolism	Conclusion
					mg/kg	ADI				
1.	3,7,11-Trimethyl-2,4-dodecadiene	Low	-	NT	24.99	0.7	Renal toxicity	Less bioavailable	Yes	+
2.	5-Ethyl-3-methyl-3,4-nonadien-6-yne	High	-	NT	11.22	0.438	Renal toxicity	Less bioavailable	Yes	+
3.	4-Isopropyl-3,4-dimethylcyclohexa-2,5-dienone	Intermediate	+	NT	49.18	0.85	-	Bioavailable	Yes	+
4.	1-(4-Isopropylphenyl)-2-methylpro- pyl acetate	Low	-	NT	160.36	0.85	-	Bioavailable	Yes	-
5.	Patchouli alcohol	Low	-	T	15.86	0.85	-	Bioavailable	Yes	+
6.	Tricyclo[4.3.0.0(7,9)]nonane, 2,2,5,5,8,8-hexamethyl-, (1.alpha.,6.beta.,7.alpha.,9.alpha.)-	Low	-	NT	1161.72	0.7	Renal toxicity	Less bioavailable	Yes	+
7.	1H-Purin-6-amine, N-((3-fluorophe- nyl)methyl)-	High	+	NT	24.9	0.85	-	Bioavailable	Yes	+
8.	1-Methyl-4-(1,5-dimethyl-4-hexenylidene)-1-cyclohexene	Low	-	NT	10.65	0.85	Renal toxicity	Less bioavailable	Yes	+
9.	1,1,3,3,4-Pentamethyl-6-t-butyl-2,3-dihydroindene	Low	-	T	19.84	0.85	Liver enzyme induction, Renal toxicity	Less bioavailable	Yes	+
10.	Scopolamine-N-butyl	High	+	T	28.67	0.85	-	Bioavailable	Yes	+
11.	1-dodecanoyl-2-(11Z-eicosenoyl)-glycero-3-phospho-(1'-sn-glycerol)	Low	-	T	66.74	0.85	-	Less bioavailable	Yes	+
12.	Napelline	High	-	NT	1097.23	0.7	-	Bioavailable	Yes	-
13.	Taurodeoxycholate	High	-	NT	171.95	0.625	-	Bioavailable	Yes	-
14.	Voacamine	High	-	NT	7346.83	0.585	-	Less bioavailable	Yes	-
15.	Ginsenoside F3	High	-	NT	2663790	0.7	-	Less bioavailable	Yes	-
16.	Isoschaftoside	High	-	NT	1734203	0.434	-	Less bioavailable	Yes	-
17.	Azadirachtin	High	+	NT	610379	0.476	-	Less bioavailable	Yes	+
18.	E-Resveratrol trimethyl ether	High	-	NT	9.67	0.85	Renal toxicity	Bioavailable	Yes	+
19.	Ginkgolide C	High	-	NT	8733.73	0.7	-	Bioavailable	Yes	-
20.	Quinine	High	-	NT	302.06	0.85	Hepatotoxicit, Renal toxicity	Bioavailable	Yes	+
21.	Ergocristine	High	-	NT	9.87	0.248	-	Less bioavailable	Yes	-
22.	Demethoxycurcumin	High	-	NT	74.44	0.85	Hepatotoxi-city, Renal toxicity	Bioavailable	Yes	+
23.	Guan-fu base Y	High	-	NT	59.14	0.7	-	Bioavailable	Yes	-
24.	Cycloheximide	High	-	T	259.84	0.85	Hepatotoxicity	Bioavailable	Yes	+

(*Contd.*)

Table 4 — Prediction of toxicity of *Parkia timoriana* (seed pods) phytoconstituents in relation to oral toxicity, carcinogenicity, reproductive toxicity, NOAEL, repeated dose toxicity, Lipinski rule of five and cytochrome-P450 Metabolism using Toxtree, VEGA HUB, and OECD QSAR tools based on quantitative structure–activity relationship (QSAR) model (*Contd.*)

Sl. No.	Phytocompound (*Parkia timoriana*)	Oral Toxicity (Cramer)	Carcinogenicity	Reproductive toxicity	NOAEL		Repeated dose toxicity (HESS)	Lipinski Rule Oasis†	Cyt-P450 Metabolism	Conclusion
					mg/kg	ADI				
25.	Anabasamine	High	-	T	25.62	0.85	Renal toxicity	Bioavailable	Yes	+
26.	Vincamine	High	-	NT	31.94	0.468	-	Bioavailable	Yes	-
27.	Artocarpin	High	-	T	2766.3	0.482	-	Less bioavailable	Yes	+
28.	Rauwolscine	High	-	T	714.99	0.85	Renal toxicity	Bioavailable	Yes	+
29.	Lithocholenic Acid	High	-	NT	396.83	0.7	-	Less bioavailable	Yes	-
30.	Rotenone	High	-	NT	381.59	0.85	-	Bioavailable	Yes	-
31.	Silychrystin	High	-	NT	26791	0.7	-	Less bioavailable	Yes	-
32.	Speciosine	High	+	T	301.23	0.642	-	Bioavailable	Yes	+
33.	Apiin	High	-	NT	3437.95	0.442	-	Less bioavailable	Yes	-
34.	Gardnerine	High	-	T	4298.33	0.7	-	Bioavailable	Yes	+
35.	Loganin	High	-	NT	5989.63	0.7	Renal toxicity	Bioavailable	Yes	+
36.	Apigenin glucoside arabinoside	High	-	NT	1029200	0.434	-	Less bioavailable	Yes	-
37.	Linolenic acid	Low	-	NT	268.29	0.7	-	Less bioavailable	Yes	-
38.	Isocorydine	High	+	T	3187.13	0.85	-	Bioavailable	Yes	+
39.	Nantenine	High	+	T	59.89	0.651	-	Bioavailable	Yes	+
40.	Phosphatidylcholine 15:0/18:1(11Z)	High	-	T	20.81	0.85	-	Less bioavailable	Yes	+
41.	Luteolin-8-C-glucoside	High	-	T	187154	0.466	-	Less bioavailable	Yes	+
42.	Nicotiflorin	High	-	NT	17607.6	0.433	-	Less bioavailable	Yes	-
43.	Hydroxygardnutine	High	+	T	187.72	0.85	-	Bioavailable	Yes	+
44.	Phophatidylethanolamine (22:1/20:1)	Low	-	T	10.42	0.85	-	Less bioavailable	Yes	+
45.	Phosphatidylethanolamin e 18:0-22:6	Low	-	T	70.79	0.85	-	Less bioavailable	Yes	+
46.	Maritimetin-6-O-glucoside	High	-	NT	35563	0.7	-	Less bioavailable	Yes	-
47.	Ouabain	High	-	NT	3180.53	0.7	-	Less bioavailable	Yes	-
48.	4,4'-Diaponeurosporene	Low	-	NT	14.02	0.699	Renal toxicity	Less bioavailable	Yes	+
49.	1-(5Z,8Z,11Z,14Z,17Z-eicosapentaenoyl)-2-(9Z-nonadecenoyl)-glycero-3-phosphoserine	Low	-	T	338.69	0.85	-	Less bioavailable	Yes	+
50.	Isorhamnetin-3-O-rutinoside	High	+	NT	50886.2	0.428	-	Less bioavailable	Yes	+
51.	Paeoniflorin	High	-	NT	3445.09	0.48	-	Bioavailable	Yes	-

(*Contd.*)

Table 4 — Prediction of toxicity of *Parkia timoriana* (seed pods) phytoconstituentsin relation to oral toxicity, carcinogenicity, reproductive toxicity, NOAEL, repeated dose toxicity, Lipinski rule of five and cytochrome-P450 Metabolismusing Toxtree, VEGA HUB, and OECD QSAR toolsbased on quantitative structure–activity relationship (QSAR) model (*Contd.*)

Sl. No.	Phytocompound (*Parkia timoriana*)	Oral Toxicity (Cramer)	Carcinogenicity	Reproductive toxicity	NOAEL		Repeated dose toxicity (HESS)	Lipinski Rule Oasis†	Cyt-P450 Metabolism	Conclusion
					mg/kg	ADI				
52.	Procyanidin B1	High	-	NT	347456	0.625	-	Less bioavailable	Yes	-
53.	1,2-diarachidonoyl-sn-glycero-3-phosphoethanolamine	Low	-	T	172.19	0.85	-	Less bioavailable	Yes	+
54.	Phosphatidylcholine(14:0/18:3n6)	Low	-	T	54.24	0.85	-	Less bioavailable	Yes	+
55.	Hirsutine	High	-	T	854.28	0.85	-	Bioavailable	Yes	+
56.	Taurochenodeoxycholate	High	-	NT	127.15	0.625	-	Bioavailable	Yes	-
57.	[3-hexadecoxy-2-[(9Z,11E)-13-hydroxyoctadeca-9,11-dienoyl]oxypropyl]2-(trimethylazaniumyl)ethyl phosphate	High	-	T	92.62	0.85	-	Less bioavailable	Yes	+
58.	Alpha-Hederin	High	-	NT	15881.81	0.7	-	Less bioavailable	Yes	-
59.	Soyasapogenol B base + O-DDMP, O-HexA-HexA	High	+	NT	376530	0.7	-	Less bioavailable	Yes	+
60.	Taurocholic acid	High	-	NT	358.59	0.629	-	Less bioavailable	Yes	-
61.	3-oxo-C8-homoserine lactone	High	-	NT	31.52	0.85	-	Bioavailable	Yes	-

ADI: Acceptable daily intake; NOAEL: No-observed-adverse-effect-level; HESS: Hazard evaluation support system; NT: nontoxic (developmental);T: Toxic (developmental); -: negative; +: positive; Oral Toxicity (Cramer): the Cramer classification scheme is used for assessing the toxicological profile of the chemicals, when administered orally, which places the compound in one of the three classes-Class I (Low toxicity), Class II (Intermediate toxicity), and Class III (High toxicity); Carcinogenicity: carcinogenicity is predicted based on a list of 55 structural alerts (SAs).The SAs for carcinogenicity are molecular functional groups or substructures known to be linked to the carcinogenic activity of chemicals. As one or more SAs embedded in a molecular structure are recognised, the system flags the potential carcinogenicity of the chemical; Reproductive toxicity: predicts the possible harm that a specific chemical agent will have on both male and female fertility as well as the development of the children; NOAEL: The highest concentration or amount of a chemical at which a population exposed to it experiences no discernible negative effects; Repeated dose (HESS): Predicts the possible organ toxicity for oral repeated dose base on test data in the database of Hazard Evaluation Support System (HESS); Lipinski Rule Oasis: Lipinski's Rule of Five is a rule of thumb to evaluate drug likeness. An orally active drug should have hydrogen bond donors < 5, hydrogen bond acceptors < 10, molecular weight < 500 Da, and octanol-water partition coefficient (log P) < 5; Cyt-P450 Metabolism: the enzymes of cytochrome P450 function to metabolize compounds that are possibly toxic, including drugs. The tool predicts the oral toxicity, carcinogenicity, reproductive toxicity, NOAEL, repeated dose toxicity, Lipinski rule of five and cytochrome-P450 Metabolism: Toxtree: https://toxtree.sourceforge.net/; VEGA HUB: https://www.vegahub.eu/; OECD QSAR: https://qsartoolbox.org/download/

phatidylcholine (14:0/18:3n6), which were all found in *P. timoriana*, were all predicted to have low oral toxicity under class I category. 4-isopropyl-3,4-dimethylcyclohexa-2,5-dienone showed intermediate toxicity under Class II type (Table 4).

The majority of the phyto-compounds in the PT methanol extract were predicted to be non-carcinogenic and to not be potentially dangerous to reproduction. The identified PT phytoconstituents 4-isopropyl-3,4-dimethylcyclohexa-2,5-dienone, 1H-purin-6-amine, N-((3-fluorophenyl)methyl), scopolamine-N-butyl, azadirachtin, speciosine, isocorydine, nantenine, hydroxygardnutine, isorhamnetin-3-O-rutinoside, and soyasapogenol B base + O-DDMP, O-HexA-HexA were found to be potent carcinogens (Table 4).

PT was found to contain reproductively harmful chemicals such as patchouli alcohol, 1,1,3,3,4-pentamethyl-6-t-butyl-2,3-dihydroindene, scopolamine-N-butyl, 1-dodecanoyl -2-(11Z-eicosenoyl)-glycero-3-phospho-(1'-sn-glycerol), cycloheximide, anabasamine, artocarpin, rauwolscine, speciosine, gardnerine, isocorydine, nantenine, hydroxygardnutine,

phophatidylethanolamine (22:1/20:1and 18:0-22:6), 1-(5Z,8Z,11Z,14Z,17Z-eicosapentae noyl)-2-(9Z-nonadecenoyl)-glycero-3-phosphoserine, 1,2-diarachidonoyl-sn-glycero-3-phosphoetha nolamine, phosphatidylcholine (14:0/18:3n6), and [3-hexadecoxy-2-[(9Z,11E)-13-hydroxyoctadeca-9,11-dienoyl]oxy-propyl]2-(trimethylazaniumyl)ethylphosphate (Table 4).

The measure of the repeated dose toxicity is the no observed adverse effect level (NOAEL) that is the dose at which no effects are observed, i.e., this endpoint indicates the safety level for a substance. The NOEL number indicated long-term toxicity. The estimated value of the ADI was used to construct the NOEL predictive values .The majority of the phytocompounds from PT demonstrated higher NOAEL values, indicating that these chemicals are safe and have not been linked to any negative side effects. For instance, ginsenoside F3, isoschaftoside, apigenin glucoside arabinoside, Luteolin-8-C-glucoside, nicotiflorin, isorhamnetin-3-O-rutinoside, procyanidin B, and Soyasapogenol B base + O-DDMP, O-HexA-HexA (Table 4). However, the phytocompounds ergocristine (9.67 mg/kg), E-resveratrol trimethyl ether (9.67 mg/kg), phophatidylethanolamine (22:1/20:1) (10.42 mg/kg), 1-methyl-4-(1,5-dimethyl-4-hexenyli dene)-1-cyclohexene (10.65 mg/kg), 5-ethyl-3-methyl-3,4-nonadien-6-yne (11.22 mg/kg), patchouli alcohol (15.86 mg/kg), were shown to have low NOAELs, indicating their potential for deleterious effects (Table 4).

According to the HESS database on repeated dosage toxicity utilizing OECD QSAR software, significant parts of the phytocompounds (48 out of 61) were found to be safe and not causing any organ toxicity. There is a likelihood that the phytocompounds 1,1,3,3,4-pentamethyl-6-t-butyl-2,3-dihydroindene, quinine, and demethoxycurcumin may cause nephrotoxicity and hepatotoxicity. The OECD QSAR tool predicts that cycloheximide results in hepatotoxicity, whilst 3,7,11-trimethyl-2,4-dodecadiene, 5-ethyl-3-methyl-3,4-nonadien-6-yne, tricyclo[4.3.0.0(7,9)] nonane, 2,2,5,5,8,8-hexamethyl-,(1.alpha.,6.beta.,7.alpha., 9.alpha.), 1-methyl-4-(1,5-dimethyl-4-hexenyli dene)-1-cyclohexene, E-resveratrol trimethyl ether, rauwolscine, loganin, 4,4'-diaponeurosporene may be responsible for renal toxicity (Table 4).

In accordance with the Lipinski rule, 27 out of the 61 phytocompounds identified in PT are bioavailable, which refers to the quantity and rate at which the active moiety (metabolite) enters the bloodstream and thus reaches the site of action (Table 4). The following are some examples: 4-isopropyl-3,4-dimethylcyclohexa-2,5-dienone, 1-(4-Isopropylphenyl)-2-methylpropyl acetate, patchouli alcohol, 1H-purin-6-amine, N-((3-fluorophenyl)methyl), scopolamine-N-butyl, napelline, taurodeoxycholate, E-resveratrol trimethyl ether, ginkgolide C, quinine, demethoxycurcumin, guan-fu base Y, cycloheximide, anabasamine, vincamine, rotenone, speciosine, gardnerine, loganin, isocorydine, nantenine, paeoniflorin, and 3-oxo-C8-homoserine lactone (Table 4). All spotted phytocompounds of PT (methanol seed pod extract) are metabolized by the cytochrome P450 metabolic enzymes and systems into metabolites that that can be excreted more easily (Table 4).

PASS biological activity prediction

The phytoconstituents of PT identified by GC-MS and LC-MS/MS were analyzed by the PASS for their different types of biological activity and results were used in a flexible manner. All the phytocompounds showed greater probable activity (Pa) than probable inactivity (Pi) (Table 5). In response to the PASS prediction, the phytocompounds from PT, such as 3,7,11-trimethyl-2,4-dodecadiene, 4-isopropyl-3,4-dimethylcyclohexa-2,5 dienone, tricyclo [4.3.0.0(7,9)] nonane,2,2,5,5,8,8-hexamethyl-(1.alpha.,6.beta.,7. alpha.,9.alpha.), bisabolene, ginsenoside F3, isoschaftoside, artocarpin, silychrystin, loganin, apigenin glucoside arabinoside, linolenic acid, luteolin-8-C-glucoside, nicotiflorin, maritimetin-6-O-glucoside, 4,4'-diaponeurosporene, isorhamnetin-3-O-rutinoside, paeoniflorin, procyanidin B1, alpha-hederin, demonstrated nearly eight biological activities, including antioxidant, free radical scavenger, anti-inflammatory, anti-viral, anti-fungal, anti-neoplastic, anti-bacterial, and anti-protozoal.

The PT phytocompounds' (61 metabolites) probable activity (Pa) values for antioxidant, free radical scavenger, anti-inflammatory, anti-viral, anti-fungal, anti-neoplastic, anti-bacterial, and anti-protozoal properties ranged from 0.138 to 0.924, 0.147 to 0.993, 0.246 to 0.891, 0.091 to 0.922, 0.130 to 0.828, 0.104 to 0.939, 0.141 to 0.678, and 0.188 to 0.953, respectively (Table 5).Only the anti-protozoal (Pa: 0.379) and antineoplastic (Pa: 0.383) activities were demonstrated by hirsutine. The antiviral (Pa: 0.294), antineoplastic (Pa: 0.419), and antiprotozoal (Pa: 0.202) actions of hydroxygardnutine proved to be observed.Nantenine showed four biological activities: free radical scavenger (Pa: 0.206), antiviral (Pa: 0.443), antineoplastic (Pa: 0.671), and antiprotozoal

Table 5 - Biological activities predicted for *Parkia timoriana* (seed pods) bioactive molecules by PASS (prediction of activity spectra for biologically active substances) prediction tool

Sl. No.	Phytocompounds (*Parkia timoriana*)	Anti-oxidant		Free radical scavenger		Anti-inflammatory		Anti-viral		Anti-fungal		Anti-neoplastic		Anti-bacterial		Anti-protozoal	
		Pa	Pi	Pa	Pi	Pa	Pi	Pa	Pi	Pa	Pi	Pa	Pi	Pa	Pi	Pa	Pi
1.	3,7,11-Trimethyl-2,4 -do decadiene	0.608	0.004	0.403	0.017	0.597	0.032	0.520	0.018	0.637	0.015	0.514	0.032	0.428	0.024	0.331	0.045
2.	5-Ethyl-3-methyl-3,4-no nadien-6-yne	-	-	0.158	0.119	0.460	0.011	0.489	0.029	0.316	0.074	0.104	0.071	0.166	0.149	0.261	0.096
3.	4-Isopropyl-3,4-dimethylcyclohexa-2,5dienone	0.173	0.076	0.221	0.061	0.704	0.015	0.185	0.183	0.395	0.051	0.387	0.109	0.241	0.088	0.226	0.089
4.	1-(4-Isopropylphenyl) -2-methylpropylacetate	-	-	0.147	0.131	0.663	0.021	0.457	0.044	0.571	0.021	0.229	0.015	0.367	0.039	0.289	0.078
5.	Patchouli alcohol	-	-	-	-	0.314	0.060	0.668	0.008	0.428	0.044	0.376	0.027	0.193	0.124	0.464	0.030
6.	Tricyclo[4.3.0.0(7,9)]nonane,2,2,5,5,8,8-hexamet hyl-, (1.alpha.,6.beta.,7.alpha.,9. alpha.)	0.165	0.085	0.212	0.067	0.347	0.030	0.532	0.038	0.314	0.075	0.351	0.031	0.284	0.066	0.365	0.064
7.	1H-Purin-6-amine, N-((3-fluorophe nyl)methyl)-	-	-	-	-	-	-	0.182	0.117	-	-	0.251	0.027	0.141	0.072	0.366	0.053
8.	Bisabolene	0.269	0.030	0.206	0.071	0.505	0.055	0.649	0.004	0.361	0.060	0.903	0.005	0.368	0.038	0.337	0.078
9.	1,1,3,3,4-Pentamethyl-6-t-butyl-2,3dihyd roindene	-	-	-	-	-	-	0.268	0.119	-	-	-	-	-	-	0.330	0.082
10.	Scopolamine-N-butyl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11.	1-dodecanoyl-2-(11Z-eicosenoyl)glycero-3-phos pho-(1'-sn-glycerol)	0.235	0.040	-	-	0.550	0.043	0.688	0.003	0.592	0.019	0.460	0.013	0.199	0.118	0.826	0.004
12.	Napelline	-	-	-	-	0.302	0.158	0.222	0.159	-	-	0.855	0.006	-	-	-	-
13.	Taurodeoxycholate	0.167	0.082	-	-	0.493	0.059	0.598	0.013	0.532	0.026	0.696	0.002	0.454	0.021	-	-
14.	Voacamine	-	-	-	-	-	-	0.246	0.136	-	-	0.556	0.004	-	-	0.319	0.088
15.	Ginsenoside F3	0.862	0.003	0.253	0.045	0.794	0.007	0.549	0.017	0.701	0.010	0.869	0.005	0.466	0.020	0.279	0.055
16.	Isoschaftoside	0.829	0.003	0.873	0.002	0.530	0.048	0.842	0.002	0.722	0.009	0.852	0.007	0.526	0.014	0.626	0.013
17.	Azadirachtin	0.198	0.056	-	-	0.891	0.004	0.399	0.036	0.741	0.008	0.939	0.004	0.522	0.014	0.518	0.009
18.	E-Resveratrol trimethyl ether	0.388	0.013	-	-	0.494	0.059	0.400	0.035	0.357	0.061	0.612	0.042	0.172	0.031	0.562	0.017
19.	Ginkgolide C	0.971	0.002	-	-	-	-	0.088	0.058	0.130	0.013	0.163	0.090	-	-	0.563	0.007
20.	Quinine	-	-	-	-	-	-	0.435	0.036	0.261	0.101	0.478	0.005	0.417	0.002	0.873	0.002
21.	Ergocristine	-	-	-	-	-	-	-	-	-	-	0.351	0.023	-	-	-	-
22.	Demethoxycurcumin	0.624	0.004	-	-	0.667	0.020	0.462	0.030	0.503	0.030	0.678	0.030	0.270	0.072	0.574	0.016
23.	Guan-fu base Y	-	-	-	-	-	-	0.408	0.082	0.261	0.101	0.719	0.005	0.263	0.076	-	-
24.	Cycloheximide	-	-	-	-	0.246	0.186	0.922	0.002	0.623	0.016	0.537	0.025	0.541	0.013	0.222	0.043
25.	Anabasamine	-	-	-	-	-	-	0.359	0.048	-	-	0.461	0.009	0.176	0.028	0.302	0.066
26.	Vincamine	-	-	-	-	-	-	-	-	-	-	0.435	0.011	-	-	-	-
27.	Artocarpin	0.608	0.004	0.775	0.003	0.613	0.029	0.429	0.024	0.596	0.019	0.802	0.011	0.477	0.019	0.536	0.020
28.	Rauwolscine	-	-	-	-	-	-	0.302	0.088	0.175	0.156	0.455	0.009	-	-	0.281	0.115
29.	Lithocholenic Acid	0.170	0.079	-	-	0.459	0.070	0.658	0.009	0.533	0.026	0.525	0.064	0.233	0.093	0.394	0.051
30.	Rotenone	0.278	0.028	0.474	0.012	0.368	0.113	0.399	0.092	0.329	0.070	0.823	0.009	-	-	-	-
31.	Silychrystin	0.906	0.003	0.929	0.002	0.759	0.009	0.464	0.015	0.503	0.030	0.690	0.028	0.369	0.038	0.369	0.025
32.	Speciosine	0.182	0.067	0.242	0.049	0.293	0.089	0.091	0.053	-	-	0.439	0.090	-	-	0.221	0.187
33.	Apiin	0.765	0.004	0.896	0.002	0.743	0.011	0.513	0.008	0.770	0.006	0.899	0.005	0.670	0.005	0.872	0.003
34.	Gardnerine	-	-	-	-	-	-	0.311	0.082	-	-	0.361	0.022	-	-	0.216	0.045
35.	Loganin	0.647	0.004	0.535	0.008	0.738	0.012	0.591	0.014	0.828	0.004	0.829	0.009	0.647	0.006	0.591	0.006
36.	Apigenin glucoside arabi noside	0.837	0.003	0.877	0.002	0.510	0.054	0.759	0.002	0.686	0.010	0.833	0.008	0.515	0.015	0.548	0.019
37.	Linolenic acid	0.364	0.015	0.281	0.035	0.804	0.006	0.603	0.006	0.509	0.029	0.386	0.122	0.348	0.044	0.458	0.026
38.	Isocorydine	0.182	0.067	0.348	0.023	0.356	0.119	0.362	0.047	-	-	0.606	0.003	-	-	0.246	0.136
39.	Nantenine	-	-	0.206	0.071	-	-	0.443	0.015	-	-	0.671	0.003	-	-	0.277	0.117
40.	Phosphatidylcholine 15:0 /18:1(11Z)	0.138	0.118	-	-	0.766	0.009	0.574	0.009	0.683	0.011	0.528	0.063	-	-	0.953	0.002
41.	Luteolin-8-C-glucoside	0.828	0.003	0.955	0.001	0.626	0.027	0.750	0.002	0.714	0.009	0.844	0.007	0.541	0.013	0.658	0.011
42.	Nicotiflorin	0.924	0.003	0.984	0.001	0.743	0.011	0.742	0.004	0.786	0.006	0.851	0.007	0.678	0.005	0.898	0.003
43.	Hydroxygardnutine	-	-	-	-	-	-	0.294	0.266	-	-	0.419	0.090	-	-	0.202	0.194
44.	Phophatidylethanolamine(22:1/20:1)	0.164	0.086	-	-	0.493	0.059	0.561	0.010	0.567	0.022	0.466	0.012	0.183	0.134	0.887	0.003
45.	Phosphatidylethanolamine 18:0-22:6	0.189	0.062	-	-	0.637	0.025	0.546	0.013	0.590	0.019	0.429	0.015	0.198	0.119	0.830	0.004
46.	Maritimetin-6-O-glucoside	0.750	0.004	0.940	0.001	0.566	0.039	0.434	0.023	0.618	0.017	0.821	0.009	0.552	0.012	0.367	0.025
47.	Ouabain	0.302	0.023	-	-	0.642	0.024	0.462	0.030	0.605	0.018	0.878	0.005	0.397	0.031	0.429	0.038
48.	4,4'-Diaponeurosporene	0.812	0.003	0.446	0.014	0.754	0.010	0.783	0.001	0.609	0.018	0.903	0.005	0.439	0.023	0.458	0.026
49.	1-(5Z,8Z,11Z,14Z,17Z-eicosapentaenoyl)-2-(9Z-nonadecenoyl)-glycero-3-phospho serine	0.217	0.047	-	-	0.457	0.070	0.540	0.014	0.531	0.026	0.468	0.012	0.205	0.112	0.590	0.015
50.	Isorhamnetin-3-O-rutinoside	0.896	0.003	0.993	0.000	0.721	0.013	0.728	0.004	0.774	0.006	0.849	0.007	0.663	0.006	0.918	0.003
51.	Paeoniflorin	0.302	0.023	0.203	0.074	0.578	0.036	0.408	0.032	0.634	0.015	0.643	0.036	0.617	0.008	0.690	0.009
52.	Procyanidin B1	0.803	0.003	0.798	0.003	0.430	0.081	0.631	0.011	0.534	0.025	0.629	0.039	0.319	0.053	0.352	0.060
53.	1,2-diarachidonoyl-sn-glycero-3-phospho ethanolamine	0.176	0.073	-	-	0.570	0.038	0.553	0.012	0.569	0.022	0.448	0.013	0.186	0.132	0.858	0.004
54.	Phosphatidylcholine(14:0/18:3n6)	0.155	0.097	-	-	0.802	0.007	0.566	0.010	0.684	0.011	0.511	0.068	-	-	-	0.002
55.	Hirsutine	-	-	-	-	-	-	-	-	-	-	0.383	0.018	-	-	0.379	0.023
56.	Taurochenodeoxycholate	0.160	0.091	-	-	0.540	0.046	0.541	0.018	0.555	0.023	0.693	0.002	0.466	0.020	0.190	0.116
57.	[3-hexadecoxy-2-[(9Z,11 E)-13-hydroxy octadeca-9,11-dienoyl]oxypropyl] 2-(tri methylazaniumyl) ethyl phosphate	0.210	0.050	-	-	0.794	0.007	0.593	0.007	0.774	0.006	0.479	0.046	0.190	0.127	0.944	0.002
58.	Alpha-Hederin	0.575	0.005	0.265	0.040	0.839	0.005	0.789	0.003	0.826	0.004	0.914	0.005	0.611	0.008	0.948	0.002
59.	Soyasapogenol B base + O-DDMP, O-Hex A-HexA	0.567	0.005	-	-	0.686	0.018	0.724	0.004	0.668	0.012	0.911	0.005	0.661	0.006	0.508	0.023
60.	Taurocholic acid	0.161	0.090	-	-	0.540	0.046	0.600	0.013	0.564	0.022	0.729	0.002	0.490	0.017	0.188	0.118
61.	3-oxo-C8-homoserine lac tone	-	-	0.205	0.072	0.281	0.110	0.385	0.122	0.381	0.054	0.255	0.039	0.383	0.034	0.255	0.138

Pa: Probable activity; Pi: Probable inactivity.

Pa>Pi: experimentally active compound.

Pa>0.7: reflects the probability of experimental pharmacological action is high.

0.5<Pa<0.7: indicates the probability ofexperimental pharmacological action is less.

Pa<0.5: the chance of finding the activity experimentally is less, but it may indicate a chance of finding a new compound.

(Pa: 0.277). Gardnerine and voacamine demonstrated biological activity that were antiviral (Pa: 0.311 and 0.246), antineoplastic (Pa: 0.361 and 0.556), and antiprotozoal (Pa: 0.216 and 0.319). Antineoplastic (Pa: 0.455), antiviral (Pa: 0.302), antifungal (Pa: 0.175), and antiprotozoal (Pa: 0.281) activities were demonstrated by rauwolcine. Only the antineoplastic activity was demonstrated by ergocristine (Pa: 0.351) and vincamine (Pa: 0.435). Napelline exhibited anti-inflammatory, antiviral, and antineoplastic (Pa: 0.302, 0.222, and 0.855, respectively) actions (Table 5).

Discussion

Every day, humans are exposed to phytochemicals, primarily through dietary sources. The information is required to comprehend potential toxicological concerns because there haven't been many investigations on the long-term toxicity of this class of compounds. As a result, reliable methodologies for evaluating the risks associated with these compounds would be helpful in determining their safety. The current study's objective was to evaluate the applicability of *in silico* predictive global QSAR-based tools used by the US EPA and FDA for predicting the toxicity of natural compounds found in the methanolic extract of PT (seed pods) by GC-MS and LC-MS/MS analyses, against a variety of end points. The most frequently computationally modeled endpoints involve parameters related to aquatic (LC_{50} and IGC_{50}) and mammalian toxicity (LD_{50}), bioconcentration factor, developmental toxicity, mutagenicity, oral toxicity (Cramer), carcinogenicity, reproductive toxicity, NOAEL, repeated dose toxicity (HESS), Lipinski rule oasis, Cyt-P450 metabolism, and biological activities (PASS). These endpoints are the most frequently computationally modeled for a number of reasons, including their cost- and time-efficiency compared to a conventional 5-year laborious bioassay and their importance as key evidence for regulatory risk assessors and safety evaluators. Five different *in silico* QSAR-based tools (TEST, Toxtree, VEGA HUB, OECD QSAR, and PASS) were used in this study to screen an external validation set of natural compounds from PT (seed pods) in order to evaluate the predictive performance of the software programs and ascertain their applicability as an alternative to *in vivo* testing. PT's traditional applications, phytochemistry, pharmacological, and biological activity have been described[1,9,27-29]. Their toxicity has, however, received little research. Therefore, determining their toxicity may provide insight into the potential usage of the natural compounds and possible future applications in a number of novel domains.

Utilizing pertinent descriptors known as molecular descriptors, it is possible to connect the physiochemical characteristics to biological activity. The importance of molecular descriptors in the prediction of toxicity depends on the endpoints, the involved biological system, and the chemical class[30]. *In silico* predictions have identified a variety of toxic end points. Some of the most often researched end points include acute oral toxicity, hepatotoxicity, cardiotoxicity, endocrine disruption, mutagenicity, carcinogenicity, general toxicity, and the 12 Tox21 data challenge end points. In comparison to using only one prediction method, combining many different prediction methods will result in more accurate and reliable predictions. Thus, the platforms TEST, VEGA HUB, OECD QSAR, Toxtree, and PASS integrate and forecast the toxicity based on the toxic mechanism of the molecule and important structural similarities. As a result, these open-source and user-friendly methods were used in this work to assess the toxicity of PT's natural phytoconstituents.

Prediction of multispecies acute toxicity

The accuracy of assessing the toxicity of natural substances can be greatly enhanced with the development of QSAR prediction tools. In a QSAR model, the applicability domain identifies the physicochemical, structural, or biological details of phytocompounds[31].The model ellipsoid, R_{max}, and fragment constraints serve as the foundation for the application domain of the hierarchical clustering, single model, and group contribution models. Different components of the toxicological effects are modeled by several models using various descriptors and statistical techniques. The toxicity of PT phytocompounds was observed in four different hierarchical orders for aquatic (LC_{50}, IGC_{50}) and mammalian (LD_{50}) organisms, as follows: i) *P. promelas>T. pyriformis>D. magna>R. norvegicus* (oral) (20 compounds), ii) *P. promelas>D. magna> T. pyriformis>R. norvegicus* (oral) (19 compounds), iii) *D. magna>P. promelas>T. pyriformis>R. norvegicus* (oral) (15 compounds), and *T. pyriformis>P. promelas>D. magna>R. norvegicus* (oral) (2 compounds) (Fig. 5).

Prediction of BCF

The ability of phytomolecules to accumulate in life forms when chemical compounds exist in the surrounding environment is referred to as the

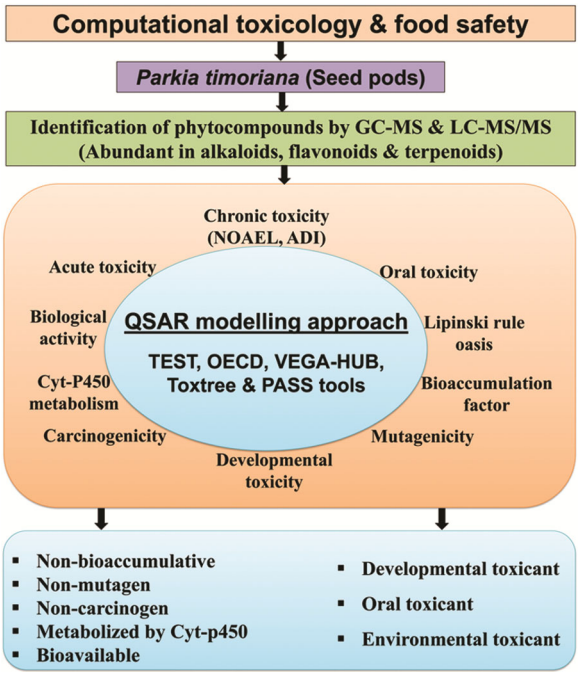

Fig. 5 — Toxicity and food safety risk assessment of *Parkia timoriana* (seed pods) phytocompounds by quantitative structure-activity relationship (QSAR) modeling approaches

bioconcentration factor (BCF). The bioaccumulation factor measures the concentration of a chemical in fish as compared to water at steady state after it has been absorbed through the respiratory surface. A chemical may be considered to be bio-accumulative in general if its BCF value is greater than 2000 and very bio-accumulative if its value is greater than 5000[32]. With the exception of tricyclo[4.3.0.0(7,9)] nonane,2,2,5,5,8,8-hexamethyl-,(1.alpha.,6.beta., 7.alpha.,9.alpha.) and 1,1,3,3,4-pentamethyl-6-t-butyl-2,3-dihydroindene), all of the phytochemicals from PT used in this study had a bioaccumulation factor below 2000 and thus were all considered to be non-bio-accumulative (Fig. 5).

Prediction of developmental toxicity

The limited success of *in silico* methods can be partly attributed to the numerous and diverse mechanisms of action that mediate developmental toxicity, which are unavoidably poorly understood[33]. A significant proportion (46 out of 61 and score: 0.53 to 1.38) of the phytocompounds from PT were unsafe for development. Procyanidin B1, lithocholenic acid, hydroxygardnutine, and ergocristine were the only phytocompounds from PT that scored higher than 1 out of 46 developmental toxicants in the study. Developmentally safe substances included isoschaftoside, apiin, apigenin glucoside arabinoside, maritimetin-6-O-glucoside, ouabain, and 3-oxo-C8-homoserine lactone (Fig. 5).

Prediction of mutagenicity and carcinogenicity

Preclinical drug discovery phases could help in the development of safe therapeutic agents by detecting mutagenicity early on and preventing the production of potentially dangerous medications. In order to accurately predict the mutagenicity of substances, expert prediction systems DEREK for Windows, OECD QSAR, VEGA HUB, and Toxtree use structural alerts[34]. A significant portion of the phytochemicals from PT were found to be non-mutagens in mutagenicity and carcinogenicity testing (51 out of 61 compounds), suggesting that the phytocompounds can be investigated in different fields.On the other hand, it was projected that 1H-purin-6-amine,N-((3-fluorophenyl)methyl), taurodeoxycholate, E-resveratrol trimethyl ether, anabasamine, gardnerine, nantenine, luteolin-8-C-glucoside, procyanidin B1, taurochenodeoxycholate, and taurocholic acid were mutagens (score: >0.5). The phytocompounds 4-isopropyl-3,4-dimethylcyclohexa-2,5-dienone, 1H-purin-6-amine, N-((3-fluorophenyl) methyl), scopolamine-N-butyl, azadirachtin, speciosine, isocorydine, nantenine, hydroxyl gardnutine, isorhamnetin-3-O-rutinoside, and soyasapogenol B base + O-DDMP,O-HexA-HexA were considered to be strong carcinogens based on carcinogenicity studies (Fig. 5).

Prediction of oral and chronic toxicity

The oral toxicity test revealed that 75% of the PT compounds detected (46 out of 61) were highly toxic under category class III and were confirmed to be phytochemicals with a significant risk for toxicity. According to the results of the reproductive toxicity test, sixty-four percent of the phytoconstituents from PT (39 out of 61 compounds) had no negative effects on either the male and female fertility or the growth of the offspring. The NOAEL is an indicator of the toxicity of repeated doses, and the estimated value from the ADI was used to produce the NOEL projected values. The safe level of the chemical in respect to long-term toxicity is thus determined by this endpoint[35]. The twenty-three phytocompounds from PT out of sixty-one (37.70%) showed higher NOAEL values, showing that these chemicals are safe and have not been associated with any negative side effects, while the remaining substances (62.29%)

were found to be toxic and have positive side effects (Fig. 5).

Prediction of repeated dose organ toxicity, Lipinski rule of oasis, Cyt-P450 metabolism

By giving details on the toxicity, metabolism, and potential processes of analog drugs, the Hazard Evaluation assist System (HESS) Integrated Platform was created as a system to assist repeated-dose toxicity predictions using a category method. The NOAELs, administration period, chemical information, purity of the tested materials, and more than 400 test results are all included in the toxicity database[36]. The repeated dosage toxicity study of the phytochemicals from PT (78.68%, and 48 out of 61 compounds) demonstrated their safety and causes no organ toxicity. A significant portion of the phytochemicals from PT were exhibited to be bioavailable, transformed by cytochrome-P450 metabolism, and eliminated from the body, according to Lipinski rule oasis and Cyt-P450 analysis (Fig. 5).

Prediction of biological activity

The computer-aided drug discovery application PASS was employed to predict the biological activity in order to speed up the search for effective natural compounds. Utilizing 20,000 main chemicals, PASS methods for prediction were developed. The prediction's outcome is displayed as a list of activities with the proper Pa and Pi ratios[37]. The broad spectrum of anti-oxidant, free radical scavenger, anti-inflammatory, antiviral, anti-fungal, anti-neoplastic, antibacterial, and anti-protozoal activity of the investigated phytoconstituents from PT (seed pod) was predicted by PASS (Fig. 5).

The fundamental objective of predictive toxicology is to develop computational models to quickly evaluate chemicals according to their potential toxicity and choose those that appear to have a higher harmful potential for in-depth biological investigation. These *in silico* methods[38-39] would make it possible to focus research efforts on chemicals that may be more dangerous as early as possible and propose legislative measures to stop or reduce human exposure to these toxic substances[40-41]. Environmental chemical combinations are a constant threat to all living things, but there are few data on their toxicity, raising severe questions. Because of the interactions (synergism/antagonism) among the mixture's constituent compounds, the toxicity of mixtures is far more complex and varied than that of individual substances. There are a few methodologies and guidelines developed by various regulatory agencies and the scientific community for assessing the negative effects of multicomponent mixtures, but no significant, standardized, or reliable method exists for assessing the toxicity of chemical mixtures and their management across various fields. Laboratory animal toxicity tests are problematic, expensive, time-consuming, and unethical[41-45]. Therefore, in order to decrease the need of animal testing, the scientific community, government organizations, and business sectors are now relying on already validated machine learning alternatives. The use of computational methods[46-49] can prioritize compounds, forecast toxicities, and assess risk. In addition to these advantages, *in silico* procedures are affordable, quick, and simple. The cost of time, money, and labor associated with screening drug targets during preclinical investigations is reduced through toxicity assessment using *in silico* models[41]. *In silico* research can also help to decrease the usage of animals in toxicity testing. Through the QSAR-TEST, the toxicity of each component found in the plant PT's seed pods was assessed in the current study (Fig. 5). Prior to doing *in vivo* experiments, it is advised to conduct more research on the interactions between the chemicals of the PT plant using *in silico* technologies like docking and modeling.

Conclusion

According to GC-MS and LC-MS/MS investigations, the majority of the compounds found in PT seed pods are alkaloids, flavonoids, and terpenoids. PT phytochemicals are not bioaccumulative, mutagenic, or carcinogenic, bioavailable, are metabolized by Cyt-P450, and eliminated from the body. A wide range of biological effects including anti-oxidant, free radical scavenger, anti-inflammatory, antiviral, anti-fungal, anti-neoplastic, antibacterial, and anti-protozoal activities are being demonstrated by *P. timoriana* phytochemicals without causing organ damage. Potent developmental, oral (low NOAEL), and aquatic toxicants are found in *P. timoriana* phytocompounds (Fig. 5). It is advised to continue studying the phytocompounds after *in silico* analysis utilizing docking, simulation, and *in vivo* models for drug development research.

Conflict of interest

All authors declare no conflict of interest.

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