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# Toxicity of Aqueous Extracts of the Leaves of *Sonneratia caseolaris* Grown in Ujung Pangkah, Gresik, East Java

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ABSTRACT

Local communities have extensively utilized different components of mangrove (*Sonneratia caseolaris*) plants in traditional medicine. These plants are known for their secondary metabolites, including steroids, triterpenoids, saponins, and flavonoids. *S. caseolaris* grows in Ujung Pangkah waters in Gresik, East Java, Indonesia, and is known for its proximity to environmental waste. It is believed that environmental factors at this site may contribute to the presence of characteristic bioactive compounds in the plant. In this study, the toxicity, bioactive constituents, and pharmacokinetic potentials of the aqueous extracts of the leaves of *S. caseolaris* were investigated. *S. caseolaris* leaves were extracted with methanol, distilled water, and three mineral water products produced and distributed in Indonesia. The toxicity of the aqueous *S. caseolaris* leaf extracts was tested by determining the $LC_{50}$ using *Artemia salina* Leach and a 2,5-diphenyl-2H-tetrazolium bromide (MTT) assay with TIG-1-20 lung fibroblasts. None of the aqueous *S. caseolaris* leaf extracts were categorized as toxic substances based on the $LC_{50}$ and MTT assays. The six compounds detected by liquid chromatography high-resolution mass spectrometry (LC–HRMS) in a previous study were analyzed using quantitative structure-activity relationship (QSAR) and absorption, distribution, metabolism, excretion, and toxicity (ADMET) methods. Bis(3,5,5-trimethylhexyl) phthalate and bis(2-ethylhexyl) phthalate were identified as component from *S. caesolaris* and are known plasticizers. These compounds were suspected to be carcinogenic substances based on QSAR and ADMET analyses, indicating that the environment of *S. caseolaris* may be a site of plastic waste.

***Keywords:*** Ujung Pangkah mangrove, aqueous extract, *Sonneratia caseolaris* leaf

## Introduction

*Sonneratia caseolaris* (L.) is a true mangrove plant that belongs to the *Lythraceae* family. The leaves of this plant are a source of various phytochemical compounds, including sugars, fatty acids, hydrocarbons, pectins, and flavonoids such as luteolin and luteolin-7-O-β-glucoside, as well as sterols, triterpenoids, and their derivatives.[1] Secondary metabolites such as flavonoids, phenolics, steroids, alkaloids, and terpenoids from plant leaves can be obtained by extraction.[2] The extraction of compounds from *S. caseolaris* leaves is commonly performed using ethanol.[3, 4, 5] However, other researchers have utilized solvents such as methanol,[6] carbon tetrachloride, chloroform, ethyl acetate,[7, 8] n-hexane and acetone.[9] Water has also been employed for the extraction of compounds from *S. caseolaris* leaves.[1] Although there are fewer active compounds in aqueous mangrove leaf extracts than in organic solvent extracts, organic solvent residues may still be present in the extract.[10]

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Previous studies have highlighted various beneficial properties of *S. caesolaris* extracts. For instance, the ethanol extract of *S. caseolaris* leaves has been reported to have antidiabetic,[10] antioxidant, antibiofilm,[11-14] antifungal,[15] antibacterial,[16] antiaging,[17] and antiobesity properties[18]. Additionally, the methanol extract of *S. caseolaris* leaves exhibited anti-inflammatory properties.[10] Moreover, *S. caseolaris* extract has been utilized in product applications, such as in the development of edible films[5] and as a component in sunscreen using an ethanol solvent.[20]

Extracts from various mangrove leaves exhibit different toxicities. For instance, the methanol extract of *S. alba* was shown to be nontoxic.[10] In contrast, the ethanol extract of *Avicennia* leaves was reported to be toxic, while the water extract of *Conocarpus erectus* Linnaeus mangrove leaves exhibited low toxicity.[11,12] The ethanol extract of *Aegialitis rotundifolia* mangrove leaves was determined to be nontoxic.[13]

Interestingly, research has revealed that aqueous mangrove leaf extracts are toxic to algae. Dayane *et al*.[12] noted that the aqueous extract of *C. erectis Linn*. shows low acute toxicity and is classified as having category 5 toxicity. However, contrary findings by Jason *et al*.[19] demonstrated that the aqueous *Xylocarpus granatum* leaf extract was nontoxic. Despite the recognized nutraceutical value of *S. caesolaris* mangrove leaf extracts, there is little data regarding the toxicity of aqueous extracts. Hence, the aim of this study was to determine the toxicity profile of the aqueous extract of *S. caseolaris* mangrove leaves, given that water-extracted chemical components can more accurately reflect the inhibitory chemical makeup. Such insights are a crucial initial step in the toxicological evaluation of aqueous *S. caesolaris* mangrove leaf extracts and their application potential in food or medicine.

## Materials and methods

*Plant Collection and Identification*

Mangrove leaves of *S. caseolaris* were collected from trees in April 2023 from the Ujung Pangkah mangrove area in Gresik, East Java, Indonesia, 6°54'16.1"S 112°31'43.4"E. *S. caseolaris* was authenticated by Dr. Rodiyati Azrianingsih, at the Laboratory of Taxonomy, Plant Structure and Development, Department of Biology, University of Brawijaya, Indonesia. Specifically, the third (3rd) to seventh (7th) dark green leaves were selected for the study. Leaf samples were promptly chilled on ice in a cool box at the collection site and then transported to our laboratory, where they were processed within a week. Subsequently, the samples were stored in a laboratory refrigerator at 4°C until further processing.

*Plant Extraction*

The leaves were cleaned to remove dirt, washed with running water, and further cleaned using filter paper. The leaves were subsequently left to dry for a period of seven (7) days at room temperature. The dried leaves were blended and sieved using a 300-mesh size filter. The collected powder was stored in a zip lock bag and placed inside a desiccator until extraction.

Briefly, 10 gr of dried *S. caseolaris* leaves was macerated with 30 mL of each solvent (solvents: *S. caseolaris*, 30:10, v/w). Five solvents were used for the extraction of the samples included methanol (Merck, Cat. 106009), distilled water, and commercial water obtained from mineral sources distributed in Indonesia (A, B, and C). The extraction process was carried out through maceration for three (3) cycles, each lasting 24 hours, at room temperature, each lasting 24 hours, at room temperature. At 24-hour intervals, the extract was filtered using Whatman filter paper no. 42 (Cytiva, Cat. 442-110). The resulting residue from each maceration was subjected to a subsequent maceration process. The filtrates obtained from the first, second and third maceration cycles were combined and then subjected to evaporation using a rotary evaporator (DLab rotary evaporator RG100-S) at 50°C. Each extract obtained was subjected to an $LC_{50}$ test.

*$LC_{50}$ test*

$LC_{50}$ testing was conducted employing *Artemia salina* Leach nauplii, wherein the hatching of *A. salina* eggs occurred through immersion in brine salt water for 48 hours at ambient room temperature. The selection of nauplii involved microscopic observation to identify individuals exhibiting agile movement. Test solutions were prepared at concentrations ranging from 0 ppm to 10 ppm, 100 ppm, 1000 ppm and 10000 ppm. A 10 mL tube was filled with sea water, with each tube containing the designated concentration of the extract. Subsequently, each tube was populated with 10 *A. salina* nauplii, and this procedure was conducted in triplicate to ensure method robustness and reliability. The quantification of deceased nauplii within a 24-hour timeframe served as the basis for the observations. The determination of the $LC_{50}$ value was accomplished through probit analysis, employing the Minitab application, and the results were calculated with a 95% confidence interval.

*Cytotoxicity assay of TIG-1-20 cells (lung fibroblasts)*

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Thermofisher, Cat. M6494) assay serves as a colorimetric technique for determining the population of viable cells through the quantification of mitochondrial dehydrogenase activity, and a previous method was followed, with slight modifications.[20] Briefly, the cell line was obtained from the Laboratory of Physiology, Structure, and Animal Development, Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA), Brawijaya University. TIG-1-20 cells were seeded in a 96-well plate containing MTT medium (Sigma Aldrich, Cat. CT02) and dimethyl sulfoxide (DMSO) (Sigma Aldrich, Cat. D2650), followed by an incubation period of 24 hours at 37°C. The cells were observed under a microscope. Subsequently, varying concentrations (0, 50, 100, 200, 400, 800 ppm) of *S. caseolaris* mangrove leaf extracts were introduced, and the cells were further incubated for 24 hours at 37 °C with 5% $CO_2$. Following the removal of the cell medium, 100 µL of 0.5 ppm MTT was added, and the mixture was incubated for three (3) hours at 37 °C until the formation of purple formazan. After incubation, the media was once again discarded, and the formazan produced within the TIG-1-20 cell lines was solubilized using 100 µL of DMSO, followed by an additional incubation period of 30 minutes at 37 °C. The absorbance of the formazan was measured using a spectrophotometer at a wavelength of 595 nm.

The quantity of formazan is directly proportional to the number of live cells in the culture, which is calculated by the following formula: $\frac{Abs\ of\ sample - absorbance\ of\ media\ control}{Abs\ cell\ control - abs\ media\ control}\ x\ 100\%$

Description:

Abs sample: absorbance of cell samples treated at a wavelength of 595 nm.

Abs control cells : absorbance of the untreated cell sample at a wavelength of 595 nm.

Abs control media : absorbance of the medium culture control sample.

*Prediction of bioactive compound activity*

The subsequent analysis of the mangrove compounds involved an assessment of their potential using the WAY2DRUG PASS prediction web server (http://www.pharmaexpert.ru/ passonline/predict.php). The probability of activity (PA) value served as a descriptor of a compound's likelihood of exhibiting activity under examination. Notably, six (6) compounds derived from a previous liquid chromatography-high resolution mass spectrometry (LC–HRMS) test were predicted to possess toxicological activity based on computational predictions.

*Absorption, distribution, metabolism, excretion and toxicity (ADMET) analysis*

The active constituents within the aqueous extracts were assessed through LC–HRMS. Subsequently, an evaluation of drug likeness and ADMET characteristics was conducted for each compound within the samples. This analysis was facilitated by the application of the Lipinski rule and was executed utilizing ProTox II and ADMETLab 2.0. [21-24] The SMILES notation of each ligand served as the input for both databases. The relevant links for accessing these databases were ADMETLab 2.0 (https://admetmesh.scbdd.com/service/evaluation/index) and ProTox II (https://tox-new.charite.de/protox_II/index.php?site=compound_input).

## Results and discussion

*$LC_{50}$*

The findings from the toxicity assessment of the *S. caseolaris* leaf extracts are presented in Table 1 and Figure 1, revealing that each treatment exhibited an $LC_{50}$ value > 1000 mg/L, indicating that the compounds were nontoxic. Notably, the mineral water extract C yielded the highest $LC_{50}$ value among the treatments. The compound bis(3,5,5-trimethylhexyl) phthalate has an $LC_{50}$ greater than 5000, placing it in Class 6, which indicated that is nontoxic. On the other hand, bis(ethylbenzylidene)sorbitol and monobutyl phthalate have $LD_{50}$ values ranging between 2000 and 5000, placing them in Class 5, which indicates that these compounds may be harmful if swallowed. Other compounds fall into Classes 3 and 4. These results align with those of prior studies, such as the study conducted by Kholis *et al*.,[25] which reported analogous outcomes for *S. caeso*laris fruit extract, characterizing it as a nontoxic substance.[26] Consistent with this, another species of *Sonneratia*, *S. alba*, was also identified as a nontoxic material according to the findings reported by Nancy *et al*. [10]

*Cytotoxicity in TIG-1-20 cells (lung fibroblasts)*

Table 2 shows that, up to a concentration of 800 ppm, all aqueous extracts derived from *S. caseolaris* exhibited nontoxic effects on lung cells, maintaining a cell viability range of 75.8-91.0%. This observation suggested a notable absence of detrimental effects on the TIG-1-20 cell line. In parallel, the research conducted by Thi *et al*.[26] demonstrated the nontoxic nature of *S. ovata* extract toward normal cells. However, contrasting findings were reported by Shi *et al*.,[27] indicating that extracts from *S. caseolaris* and *S. ovata* were moderately toxic. This

discrepancy underscores the nuanced and context-dependent nature of cytotoxicity assessments in botanical extracts, necessitating a comprehensive understanding of the specific characteristics and variations inherent in different plant species.[28]

*Bioactive herbal compounds*

A comprehensive analysis of bioactive herbal compounds was conducted, revealing the detection of 15 active constituents through LC–HRMS, as documented in prior studies. Using quantitative structure-activity relationship (QSAR) bioactivity analysis, the components harboring suspected toxic constituents are shown in Table 3. This integrative approach not only unveils the presence of bioactive constituents but also provides structured insight into their potential activities, thereby contributing to a more nuanced understanding of the herbal composition and its implications.

Shi *et al*. [27] highlighted the presence of tetramethyl and hexamethyl compounds in *Avicena* and *Rhizophora*. Additionally, the mangrove *Avicenia schaueriana* was identified to contain bis(3,5,5-trimethylhexyl) phthalate. [29,30] The ethyl acetate extract of *Bruguera cylindrica*, as described by Sudipta *et al*.,[31] contained 4-hydroxybenzoaldehyde. Moreover, the components found in *S. caseolaris*, as elucidated by Jubaidah *et al*.,[28] included choline and betaine, both of which are recognized for their potential pharmaceutical applications. Specifically, these compounds are known for their antibacterial, anti-inflammatory, antioxidant, and anticancer properties,[18] underlining the diverse therapeutic potential in *S. caseolaris* leaf extracts.

*ADMET analysis*

ADMET analysis of drug candidates plays a crucial role in the drug discovery process. The ADMET lab database (Table 4) was used to predict Lipinski's rule and the ADME/T of a given compound. Additionally, the ProTox II database was utilized for predicting the adherence and toxicity of Lipinski's rule.[22] Lipinski's rule of 5 (Table 5) serves as a key discriminator between molecules with potential as drugs and those without. This rule aids in predicting a compound's success of metabolic failure based on its resemblance to known drugs. Compounds adhering to Lipinski's rule should possess at least two (2) of the five (5) key properties,[32] which include a molecular weight less than 500 Daltons, high lipophilicity (expressed as LogP less than five), fewer than five (5) hydrogen bond donors, fewer than 10 hydrogen bond acceptors, and a molecular refractivity between 30 and 140. Noncompliance with these criteria does not categorically exclude compounds from potential drugs; instead, it implies that these compounds may necessitate additional energy or active transport mechanisms for cellular localization.

**Table 1:** Results of the $LC_{50}$ values of extracts of *S. caesolaris* mangrove leaves

| ***S. caseolaris* leaves solvent extracts** | **$LC_{50}$ (ppm)** | **Category** |
|---|---|---|
| A (Methanol) | 22758.46 ± 0.47[a] | Nontoxic |
| B (Aquadest) | 133352.14 ± 0.45[a] | Nontoxic |
| C (A) | 379269.02 ± 0.40[a] | Nontoxic |
| D (B) | 15332.26 ± 0.34[a] | Nontoxic |
| E (C) | 37849.41 ± 0.33[a] | Nontoxic |

**Table 2:** TIG-1-20 cell viability (%) and cytotoxicity of *S. caesolaris* mangrove leaf extracts

| **Sample** | **Cell viability (%) in concentration (ppm)** | | | | | |
|---|---|---|---|---|---|---|
| | **0** | **50** | **100** | **200** | **400** | **800** |
| A (Methanol) [2] | 100.0 ± 0.6[a] | 98.0 ± 3.5[a] | 95.5 ± 0.8[ab] | 93.8 ± 2.9[ab] | 91.8 ± 1.3[ab] | 89.5 ± 1.0[b] |
| B (Aquadest) [5] | 100.0 ± 6.2[a] | 93.2 ± 10.3[a] | 90.2 ± 13.0[a] | 86.6 ± 9.3[a] | 82.1 ± 8.4[a] | 75.8 ± 11.4[a] |
| C (Axxx) [1] | 100.0 ± 7.4[a] | 99.3 ± 8.6[a] | 96.0 ± 9.2[a] | 94.8 ± 3.6[a] | 92.2 ± 2.9[a] | 91.0 ± 1.5[a] |
| D (Cxxx) [4] | 100.0 ± 5.7[a] | 95.9 ± 5.0[a] | 93.9 ± 3.0[a] | 91.7 ± 1.5[a] | 90.2 ± 2.9[a] | 89.0 ± 3.6[a] |
| E(Kxxxxx) [3] | 100.0 ± 4.6[a] | 97.5 ± 0.4[a] | 96.0 ± 1.8[a] | 95.3 ± 1.0[a] | 94.4 ± 2.2[a] | 90.3 ± 1.4[a] |

*The numbers 1-5 indicate the order of cell viability.
**The numbers provided are mean values ± standard deviation.
***The numbers followed by superscripts [(a, b)] indicate no significant difference between columns at each treatment level with 95% confidence

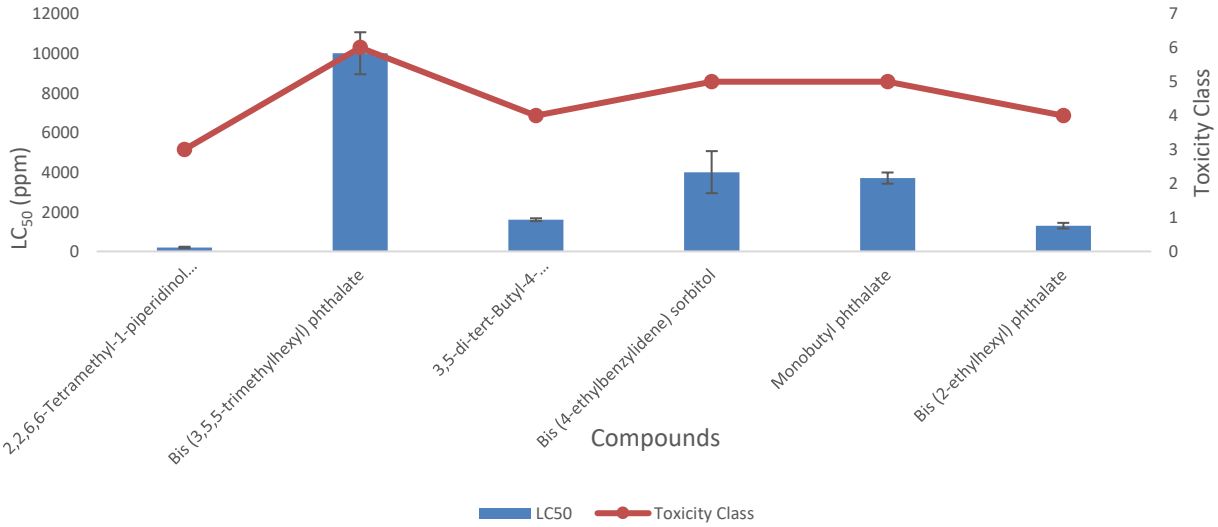


**Figure 1:** Toxicity prediction based on the $LC_{50}$ and toxicity class

Moreover, ADMET analysis revealed that nearly all the compounds exhibited favorable pharmacodynamic and pharmacokinetic profiles. The toxicity of a compound can be classified into six (6) classes: Class I, fatal if swallowed (LD50 ≤ 5); Class II, fatal if swallowed (5 < $LD_{50}$ ≤ 50); Class III, toxic if swallowed (50 < LD50 ≤ 300); Class IV, harmful if swallowed (300 < LD50 ≤ 2000); Class V, may be harmful if swallowed (2000 < LD50 ≤ 5000); and Class VI, nontoxic (LD50 > 5000).[21]

The analysis of all the extracts indicated that the toxicity of the bioactive compounds was generally low, with a few exceptions warranting monitoring for drug-induced liver injury (DILI), maximum recommended daily dose (FDAMDD), carcinogenicity, and immunotoxicity for individual compounds. In addition, the average LD50 and toxicity class of the six potential bioactive compounds ranged from Class IV to Class VI, indicating safety, except for 2,2,6,6-tetramethyl-1-piperidinol (TEMPO), which was in toxicity Class III, indicating toxicity if swallowed.[33] (Figure 1)

Table 5 reveals the identification of bis(3,5,5-trimethylhexyl) phthalate and bis(2-ethylhexyl)phthalate as potential carcinogens. Phthalate, recognized as a plasticizer, is known to be absorbed and accumulated by mangrove plants from their surrounding environment. The isolation of phthalic acid ester from the mangrove *Acrostichum aureum* has been previously documented.[34] Furthermore, findings from Amanda *et al*.[30] indicate that *Avicenna schauriana* contains bis-tridecyl phthalate, bis-isobutyl phthalate and bis-2-ethylhexyl phthalate. This finding aligns with similar results reported by Cristiane *et al*.,[35] who highlighted the presence of dibutyl phthalate and di-n-octyl phthalate in *Avicenna shaueriana* leaves and *Rhizopora mangle* leaves. The *S. caseolaris* mangrove leaves, sourced from the northern waters of Ujung Pangkah, Gresik, East Java, serve as a critical point of protection against the abrasion of the Bengawan Solo River. This area, in close proximity to residential zones, contains mangrove leaves that have adsorbed and accumulated plastic waste,[36] leading to the detection of bis(3,5,5-trimethylhexyl) phthalate and bis(2-ethylhexyl) phthalate.

## Conclusion

The aqueous extract obtained from *S. caseolaris* mangrove leaves in Ujung Pangkah Gresik, East Java, demonstrated low toxicity, suggesting its potential application as a nutraceutical. In this nearby area, which is close to residential areas, mangrove leaves have absorbed and collected plastic waste, resulting in the identification of bis(3,5,5-trimethylhexyl) phthalate and bis(2-ethylhexyl) phthalate. None of these compounds can cause liver damage, genetic mutations, or cell damage. Notably, the active constituents, specifically bis(3,5,5-trimethylhexyl) phthalate and bis(2-ethylhexyl) phthalate, were identified as potential carcinogens based on ADMET analysis. These results indicate the need for careful consideration and further investigation into the safety and potential health implications associated with the consumption of these extracts.

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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**Table 3:** Prediction of secondary metabolites from *S. caesolaris* mangrove leaf extracts using QSAR.

| No. | Compound | CID |
|---|---|---|
| 1 | Monobutylphthalate | 8575 |
| 2 | 2,2,6,6-Tetramethyl-1-piperidinol (TEMPO) | 549976 |
| 3 | Bis-4-ethylbenzyldenesorbitol | 66586233 |
| 4 | Bis-2-ethylhexyl phthalate | 8343 |
| 5 | 3,5 dibutyl 4 hydroxybenzodehyde | 73219 |
| 6 | Bis(3,5,5-trimethylhexyl) phthalate | 34277 |

**Table 4:** Results of drug-likeness and ADMET analysis of potential compounds using AdmetLab 2.0

| No. | Compound of name | Lipinski | Pgp-inh | Pgp-sub | HIA | F (20%) | F (30%) | BBB | H-HT | DILI | FDAMDD |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | 2,2,6,6-Tetramethyl-1-piperidinol (TEMPO) | Accepted | 0.015 | 0.053 | 0.011 | 0,038 | 0.536 | 0.741 | 0.038 | 0.098 | 0.08 |
| **2** | Bis(3,5,5-trimethylhexyl) phthalate | Accepted | 0.999 | 0 | 0.008 | 0.955 | 0.896 | 0.01 | 0.008 | 0.239 | 0.018 |
| **3** | 3,5-di-tert-Butyl-4-hydroxybenzaldehyde | Accepted | 0.825 | 0.003 | 0.153 | 0.971 | 0.89 | 0.784 | 0.026 | 0.018 | 0.73 |
| **4** | Bis(4-ethylbenzylidene)sorbitol | Accepted | 0.262 | 0.028 | 0.883 | 0.014 | 0.075 | 0.132 | 0.153 | 0.942 | 0.017 |
| **5** | Monobutyl phthalate | Accepted | 0.001 | 0.001 | 0.005 | 0.293 | 0.91 | 0.545 | 0.126 | 0.786 | 0.003 |
| **6** | Bis(2-ethylhexyl) phthalate | Accepted | 0.995 | 0.003 | 0.001 | 0.981 | 0.961 | 0.015 | 0.019 | 0.05 | 0.078 |

Note:
Substrate or inhibitor (P-gpinh/P-gpsub); HIA = Human Intestinal Absorption; 20% bioavailability (F20), and 30% bioavailability (F30); BBB = Blood Brain Barrier; H-HT, human hepatotoxicity; DILI, drug-induced liver injury; FDAMDD, FDA maximum daily dose; Red indicates that the compounds have poor drug-likeness and bioavailability and may function as toxins or individual compounds.

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**Table 5:** Results of ADMET analysis of potential compounds using ProTox II.

| | | | Compound of name | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | **2,2,6,6-Tetramethyl-1-piperidinol (TEMPO)** | **Bis(3,5,5-trimethylhexyl) phthalate** | **3,5-di-tert-Butyl-4-hydroxybenzaldehyde** | **Bis (4-ethylbenzylidene) sorbitol** | **Monobutyl phthalate** | **Bis(2-ethylhexyl) phthalate** |
| **Molecular weight** | | | 157.25 | 418.61 | 234.33 | 414.49 | 222.24 | 390.56 |
| **nHA** | | | 21 | 46 | 24 | 36 | 18 | 42 |
| **nHD** | | | 1 | 0 | 1 | 6 | 1 | 0 |
| **nRot** | | | 0 | 14 | 3 | 9 | 6 | 16 |
| **Mol. React** | | | 50.88 | 125.39 | 72.39 | 117.52 | 59.1 | 116.3 |
| **Log P** | | | 2.36 | 6.93 | 3.8 | 2.75 | 2.34 | 6.43 |
| **Toxicity end point predicted by ProTox II** | **Hepatotoxicity** | **Prediction** | Inactive | Inactive | Inactive | Inactive | Inactive | Inactive |
| | | **Probability** | 0.72 | 0.71 | 0.56 | 0.74 | 0.69 | 0.82 |
| | **Carcinogenicity** | **Prediction** | Inactive | Active | Inactive | Inactive | Inactive | Active |
| | | **Probability** | 0.58 | 0.73 | 0.6 | 0.64 | 0.6 | 0.86 |
| | **Immunotoxicity** | **Prediction** | Inactive | Inactive | Inactive | Inactive | Inactive | Inactive |
| | | **Probability** | 0.99 | 0.99 | 0.96 | 0.95 | 0.99 | 0.97 |
| | **Mutagenicity** | **Prediction** | Inactive | Inactive | Inactive | Inactive | Inactive | Inactive |
| | | **Probability** | 0.79 | 0.93 | 0.96 | 0.73 | 0.89 | 0.99 |
| | **Cytotoxicity** | **Prediction** | Inactive | Inactive | Inactive | Inactive | Inactive | Inactive |
| | | **Probability** | 0.72 | 0.87 | 0.84 | 0.84 | 0.89 | 0.87 |

Note: nHA, no. of hydrogen bond acceptors; nHD, no. of hydrogen bond donors; nRot, no. of rotatable bonds; Mol. React, Molecular refractivity; Numerical estimate of confidence in the prediction is 0 to 1. Higher probabilities indicate greater confidence in the prediction. Red indicates that the compounds have poor drug likeness and bioavailability and may function as toxins as individual compounds.

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