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In-silico evaluation of relative compound toxicity of *Pedilanthus tithymaloides* against *Pimephales promelas*

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ABSTRACT

Pedilanthus tityhymaloides, a common inhabitant of tropical countries, is known for its ethnomedicinal values. Despite its uses, its milky latex is toxic to sensitive parts and its usage is restricted. Thus, to study the extent of toxicity of the plant, in-silico toxicity evaluation of its compounds is performed. The methanolic extract of the plant was analyzed by Gas Chromatography and the compounds detected are used in this study. Toxicity Estimation Software Tool (TEST), an in-silico QSAR model, was used to assess the toxicity of the compounds against Pimephales promelas. The toxicity of the plant compounds 10-Octadecenoic acid, methyl ester; Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentyl cyclo propyl) methyl] cyclopropyl] methyl] cyclo propyl] methyl]-, methyl ester; Pentadecanoic acid, 14-methyl-, methyl ester; (4,4-Diphenyl-butyl)-(3-phenyl-piperidin-4-yl-)-amine and Rescinnamine were evaluated and their LC_{50} recorded were 0.57, 1.87 E^{-02}, 0.85, 4.30 E^{-02} and 5.36 E^{-04} mg/mL respectively, concluding that these compounds are super toxic in nature.

Keywords: *Pedilanthus tithymaloides*, Toxicity evaluation, QSAR model, TEST, *Pimephales promelas*.

INTRODUCTION

Pedilanthus tithymaloides (L.) Poit. (Euphorbiaceae), a common inhabitant of tropical countries, is reported with a wide range of healing properties, namely antimicrobial, antiviral, antioxidant, wound healing [1], anti-tuberculosis [2], antitumoral [3] and anti-inflammatory [4]. On the other hand, the milky latex is found to contain diterpene esters (fatty acids) [5], a primary irritant and a co-carcinogen which causes irritation of the mouth and throat, vomiting and diarrhea when ingested; skin irritation, rash, blistering and eye irritation, swelling and lacrimation upon contact [6], thus enlisting the plant as poisonous [7].

Taking into account the above mentioned rationale, there arises a need to analyze the toxicity profile of the plant. The compounds of the plant detected by Gas Chromatography-Mass Spectrometry [8] lacked experimental data and were thus evaluated for their toxicity using *in-silico* QSAR model, TEST. Since *in-silico* predictions may be advantageous with respect to time and cost, Toxicity Evaluation Software Tool (TEST), a highly reliable QSAR model [9], was used in this study.

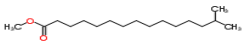
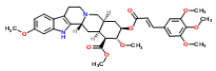
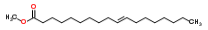
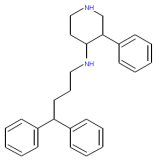
Previous studies reported the toxicity of *Pedilanthus tithymaloides* as super toxic on *Daphnia magna* [10], specifying the need for studies on higher organisms. The present study was conducted to evaluate the toxicity (LD_{50})

of the compounds of *Pedilanthus tithymaloides*, detected by Gas Chromatography against *Pimephales promelas* (Fat head minnow), extensively used for regulatory testing and research, using QSAR modeling tool TEST.

MATERIALS AND METHODS

The Gas Chromatography-Mass Spectroscopy analysis of the methanolic extract of the plant has been reported **[8]** and the nature of compounds detected through GC-MS is presented in Table 1.

Table 1: List of compounds detected through GC-MS from the methanolic leaf extract of *Pedilanthus tithymaloides*

S.No	Chemical name	Representation	Structure	Molecular weight (Da)
1	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$		296.49
2	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl] cyclopropyl] methyl] cyclo propyl] methyl]-, methyl ester	$C_{25}H_{42}O_2$		374.60
3	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$		270.45
4	Rescinnamine	$C_{35}H_{42}N_2O_9$		634.72
5	(4,4-Diphenyl-butyl)-(3-phenyl-piperidin-4-yl-)-amine	$C_{27}H_{32}N_2$		384.61

The detected compounds were subjected to toxicity prediction using QSAR modeling tool Toxicity Estimation Software Tool (TEST US EPA) [11, 12]. The structure of the compounds as reported was utilized for the study on *Pimephales promelas* (fat head minnow) and their LD_{50} values at 96 hours were predicted using TEST. The model predicts the toxicity using various QSAR methods namely, Hierarchical clustering, Food and Drug Administration (FDA) MDL and Nearest neighbor [13]. The predicted toxicity is estimated by Consensus model, the average of the predicted toxicities of the above QSAR methodologies. The data reliability is tested by plotting a graph between experimental and predicted values of similar compounds (compounds whose similarity coefficient with test comnpound is greater than 0.5). The confidence on the predicted value is high, if the plot between predicted and experimental values of similar compounds gives an ideal line.

RESULTS

The compounds 10-Octadecenoic acid, methyl ester; Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentyl cyclo propyl) methyl] cyclopropyl] methyl] cyclo propyl] methyl]-, methyl ester; Pentadecanoic acid, 14-methyl-, methyl ester; Rescinnamine and (4,4-Diphenyl-butyl)-(3-phenyl-piperidin-4-yl-)-amine detected by GC-MS analysis of the plant lacked experimental toxicity data at 96 hours against the test organism *Pimephales promelas* (fat head minnow), while the predicted values were 0.57, 1.87 E^{-02}, 0.85, 4.30 E^{-02} and 5.36 E^{-04} mg/L. The predicted results using consensus method are tabulated in table 2 while table 3 shows the toxicity of compounds predicted by QSAR models, Hierarchical, Single model, Group contribution, FDA and Nearest neighbor method expressed in terms of $-Log_{10}$ (mol/L).

Table 2: Experimental value and predicted value of the compounds predicted by Consensus method

S.no	Compound	Experimental value(48 hr) $-Log_{10}$ (mol/L)	Experimental value(48 hr) (mg/L)	Predicted value(48hr) $-Log_{10}$ (mol/L)	Predicted value(48hr) (mg/L)
1	$C_{19}H_{36}O_2$	N/A	N/A	5.72	0.57
2	$C_{25}H_{42}O_2$	N/A	N/A	7.30	$1.87*E^{-02}$
3	$C_{17}H_{34}O_2$	N/A	N/A	5.50	0.85
4	$C_{27}H_{32}N_2$	N/A	N/A	6.95	$4.30*E^{-02}$
5	$C_{35}H_{42}N_2O_9$	N/A	N/A	9.07	$5.36*E^{-04}$

N/A- Not Applicable

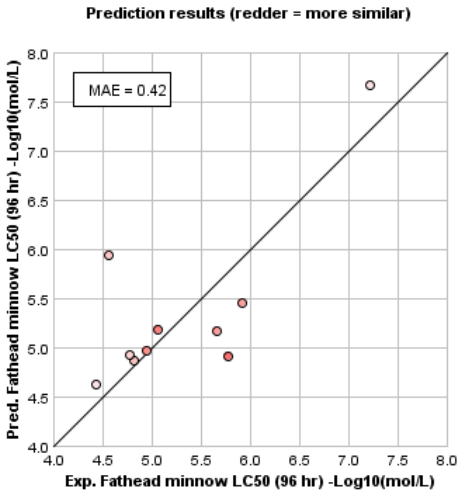
Table 3: Toxicity of compounds predicted by Hierarchical, Single model, Group contribution, FDA and Nearest neighbor method in terms of $-Log_{10}$ (mol/L)

S.no	Compound	Hierarchical clustering	Single model	Group contribution	FDA	Nearest neighbor
1	$C_{19}H_{36}O_2$	4.10	7.94	6.72	5.21	4.62
2	$C_{25}H_{42}O_2$	N/A	N/A	8.26	6.35	7.30
3	$C_{17}H_{34}O_2$	6.24	6.89	6.08	3.69	4.62
4	$C_{27}H_{32}N_2$	5.91	6.35	6.68	7.53	8.28
5	$C_{35}H_{42}N_2O_9$	N/A	N/A	9.46	N/A	8.68

N/A- Not Applicable

The prediction value for Mean Absolute Error (MAE) for different compounds studied for their toxicity and the other similar compounds are provided in Fig. 1-5 respectively.

Fig. 1: Prediction of MAE for the test chemical ($C_{19}H_{36}O_2$) and the most similar chemicals



Test set chemicals	MAE*
Entire set	0.73
Similarity coefficient ≥ 0.5	0.42

*Mean absolute error in $-Log_{10}$ (mol/L)

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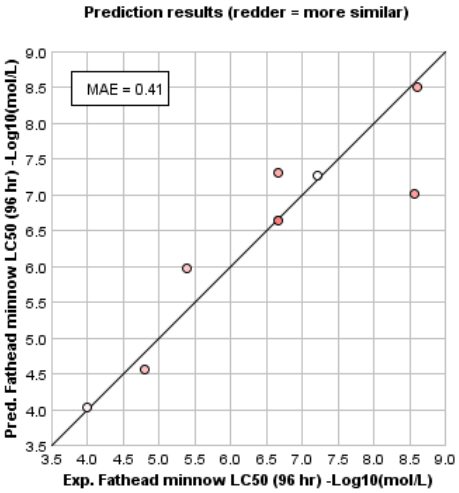
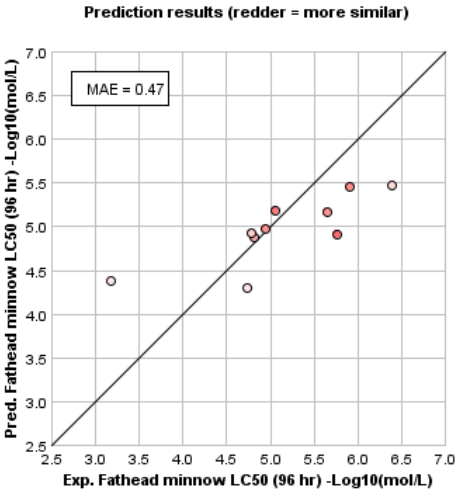
Fig. 2: Prediction of MAE for the test chemical ($C_{25}H_{42}O_2$) and the most similar chemicals



Test set chemicals	MAE*
Entire set	0.50
Similarity coefficient ≥ 0.5	0.41

*Mean absolute error in -Log_{10} (mol/L)

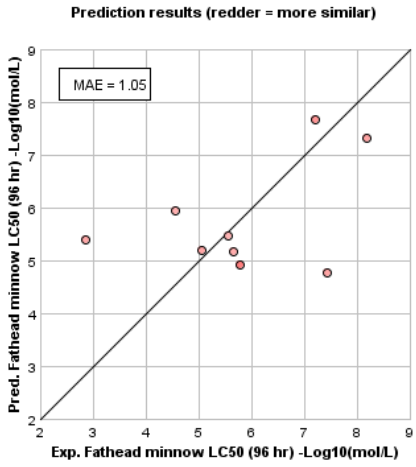
Fig. 3: Prediction of MAE for the test chemical ($C_{17}H_{34}O_2$) and the most similar chemicals



Test set chemicals	MAE*
Entire set	0.73
Similarity coefficient ≥ 0.5	0.47

*Mean absolute error in -Log_{10} (mol/L)

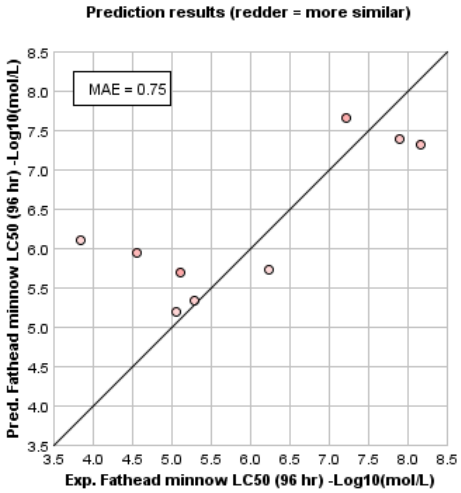
Fig. 4: Prediction of MAE for the test chemical ($C_{27}H_{32}N_2$) and the most similar chemicals



Test set chemicals	MAE*
Entire set	0.50
Similarity coefficient ≥ 0.5	1.05

*Mean absolute error in -Log_{10} (mol/L)

Fig. 5: Prediction of MAE for the test chemical ($C_{35}H_{42}N_2O_9$) and the most similar chemicals



Test set chemicals	MAE*
Entire set	0.50
Similarity coefficient ≥ 0.5	0.75

**Mean absolute error in -Log$_{10}$ (mol/L)*

Though the mean absolute error value falls within the acceptable corridor for the first three graphs, it exceeds the acceptable range for last two compounds.

DISCUSSION

Researchers, nowadays, are relying a lot on QSAR models for Toxicity predictions [14, 15] since they reduce the time consumed, cost and also minimize animal testing. These models are also highly reliable and used widely. Fat head minnow, an experimental fish for determining toxicity, was tested **with the plant compounds using** QSAR model TEST and the LD_{50} values estimated for the compounds **ranged** from $5.36*E^{-04}$ mg/L to 0.85 mg/L. These LD_{50} values of the compounds are assigned to various toxic levels according to aquatic toxicity scale [16]. Though unacceptable values were recorded in mean absolute error of last two predictions (1.05 and 0.75),

the compounds are placed under super toxic level. The reliability of the data is solely based on user confidence [17-20].

The plant compounds evaluated for their toxicity towards the fat head minnow, which has the capability to survive in harsh environments, reveals that they are highly toxic. The plant latex was already reported as toxic to sensitive parts of eye and skin **[7]** in humans which may also be the reason for high toxicity in this test organism. However, similar study on higher animal models may define its toxicity and refine its use as ethnomedicine. The data raised supports the fact that *Pedilanthus tithymaloides* has a toxic nature to certain extent and the data may also serve as a base for researchers as they lack experimental data on the toxicity of compounds of *Pedilanthus tithymaloides*.

CONCLUSION

The study concludes that the compounds of *Pedilanthus tithymaloides* are super toxic. However, further studies on the same are recommended.

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