International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.6, No.6, pp 1908-1913, Oct-Nov 2014

In-silico prediction of relative compound toxicity of *Pedilanthus tithymaloides* against *Daphnia magna*

Udaya Prakash N.K*., Balamurugan A, Sripriya N, AshwinKarthick N., Pujitha Lakshmi, R and S. Bhuvaneswari

Research and Development, MARINA LABS
40, Anna Nedum Pathai, Choolaimedu, Chennai 600094

***Corres.author: marinalabs@gmail.com**

Abstract: *Pedilanthus tityhymaloides,* though not used in allopathic drug preparations, is a widely used ethnomedicine. Their use as ethnomedicine is however restricted due to toxic nature of its latex. Hence, the study was conducted to evaluate the toxicity of its constituents through *in-silico* QSAR model. The compounds detected using Gas chromatography from the methanolic extract of the plant, through previous studies, were assessed for their toxicity, *in-silico*, using Toxicity Estimation Software Tool (T.E.S.T.) against *Daphnia magna* (LC-50). The relative compound toxicity of the following compounds, i.e. 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-piperdin-4-yl-)-amine and Rescinnamine were studied. The LC_{50} recorded were 0.72, 6.99 E^{-02}, 1.43, 0.15 and 2.06 E^{-03} respectively. The study inferred that the compounds were toxic against the test organism.
Keywords: *Pedilanthus tithymaloides*, Relative Compound Toxicity, QSAR, TEST, *Daphnia magna*, LC_{50}

Introduction

Pedilanthus tithymaloides, a member of Euphorbiaceae, is native to tropical countries and is widely used in ethnomedicine. Indian medicine uses its leaves to heal wounds, burns, and mouth ulcers while in other folk systems, leaf tea is used for laryngitis, mouth ulcers, veneral disease, asthma, cough, abortifacient and as substitute for Ipecacuanha, a drug for purgative. *Pedilanthus tithymaloides* has also been reported to possess anti-inflammatory,antioxidant[1], antimalarial,anti-tuberculosis[2], antifungal[3], mosquitocontrol[4], larvicidal[5] and wound healing[6] properties.

Despite its medicinal properties, diterpene ester (fatty acid), a toxic compound, is reported in the milky white latex[7] which causes oral irritations, vomiting, diarrhea[8,9], conjunctivitis, swelling and lacrimation of eye[8,10] and also intense irritation, rashes and blistering of the skin[8]. Hence, there is a need to evaluate the toxicity of other compounds present in the plant to determine its usage as medicine. The Gas chromatography-Mass spectroscopy analysis of the plant has been reported[11].

Determination of toxicity *in-silico* may be advantageous since, it does not involve much time and is inexpensive. A QSAR model is a mathematical relationship between the chemical's quantitative molecular descriptors and its toxicological, biological, and physicochemical activities[12-15]. Among various QSAR models Toxicity Evaluation Software Tool (T.E.S.T) has been reported to give better prediction results[16] and is highly reliable.

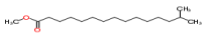
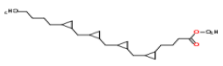
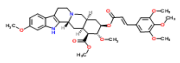
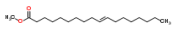
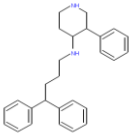
Thus the study aims at evaluating the toxicity (LC_{50}) of relative compounds of *Pedilanthus tithymaloides,* detected by Gas Chromatography against *Daphnia magna* at 48 hours, a standard toxicity indicator, using QSAR modeling tools.

Materials and Methods

In the present study QSAR modeling tool Toxicity Estimation Software Tool (T.E.S.T. US EPA)[17,18] was used to estimate the toxicity of the relative compounds of the plant analyzed by Gas Chromatography[11]. These compounds were subjected to toxicity prediction. T.E.S.T. uses consensus method which predicts the toxicity by taking an average of the predicted toxicities from the QSAR methodologies- Hierarchical clustering, the Food and Drug Administration (FDA) MDL and nearest neighbor[19]. The required descriptors are calculated without requiring any external programs. And the toxicity of the compounds was estimated along with a set of similar chemicals, whose experimental value is available to plot similarity toxicity graphs.

The composition of the plant as analyzed by Gas Chromatography-Mass Spectroscopy is tabulated in Table 1 and their structures are given in Figure 1-4. The data shows that the plant contains three esters, an amine and an alkaloid.

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

S.No	Chemical name	Structure	Representation	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

Result

The result for the toxicity studies for the compounds showed that they lack experimental value and the predicted LC_{50} values using Consensus method for 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 at 48 hours against the test organism *Daphnia magna* were 0.72, 6.99 E^{-02}, 1.43, 2.06 E^{-03} and 0.15 mg/L. The predicted values for toxicity showed that the alkaloid, Rescinnamine exhibited the highest toxicity followed by the amine and the esters. The toxicity results are tabulated in table 2.

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	0.72
2	$C_{25}H_{42}O_2$	N/A	N/A	6.73	$6.99*E^{-02}$
3	$C_{17}H_{34}O_2$	N/A	N/A	5.28	1.43
4	$C_{35}H_{42}N_2O_9$	N/A	N/A	8.49	$2.06*E^{-03}$
5	$C_{27}H_{32}N_2$	N/A	N/A	6.41	0.15

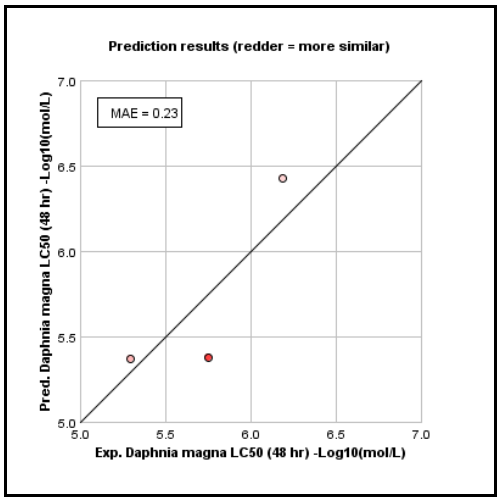
Table 3 shows the toxicity of compounds predicted by Hierarchical, single model, Group contribution, FDA and Nearest Neighbor method in terms of $-Log_{10}$ (mol/L). The clustering of all data in table 3 accounts for the data obtained using Consensus method.

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	$C_{19}H_{36}O_2$	5.35	5.71	5.55	5.95	5.52
2	$C_{25}H_{42}O_2$	5.86	5.86	N/A	6.81	8.38
3	$C_{17}H_{34}O_2$	5.50	4.99	5.00	5.38	5.52
4	$C_{35}H_{42}N_2O_9$	9.40	9.40	N/A	N/A	6.66
5	$C_{27}H_{32}N_2$	7.00	6.00	N/A	5.47	7.16

The experimental values were plotted against predicted values along with data of similar chemicals available in the T.E.S.T database. If the predicted value matches the experimental values for similar chemicals in the training set (and the similar chemicals were predicted well), one has greater confidence in the predicted value. The chemicals whose similarity ≥ 0.5 were selected for comparing and the mean absolute error of the Entire set and similar chemicals are tabulated.

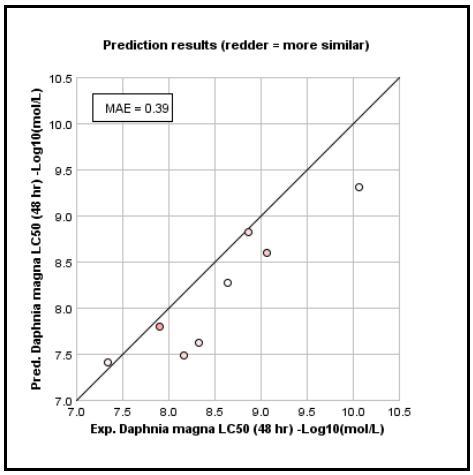
Graph 1 shows the Predictions for the test chemical ($C_{19}H_{36}O_2$) and for the most similar chemicals in the training set**.**



Test set chemicals	MAE*
Entire set	0.73
Similarity coefficient ≥ 0.5	0.23

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

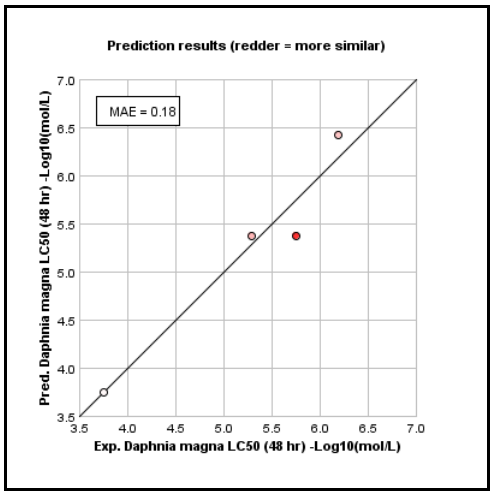
Graph 2 shows the Predictions for the test chemical ($C_{25}H_{42}O_2$) and for the most similar chemicals in the training set.



Test set chemicals	MAE*
Entire set	0.50
Similarity coefficient ≥ 0.5	0.39

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

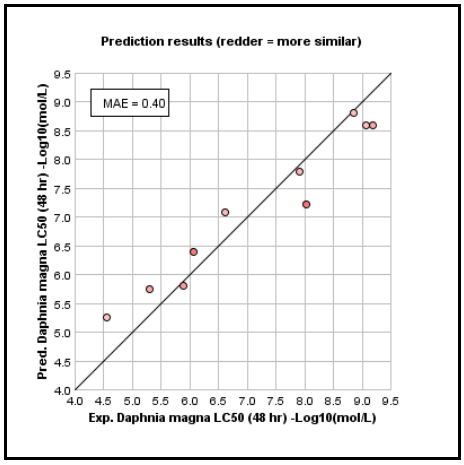
Graph 3 shows the Predictions for the test chemical ($C_{17}H_{34}O_2$) and for the most similar chemicals in the training set**.**



Test set chemicals	MAE*
Entire set	0.73
Similarity coefficient ≥ 0.5	0.18

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

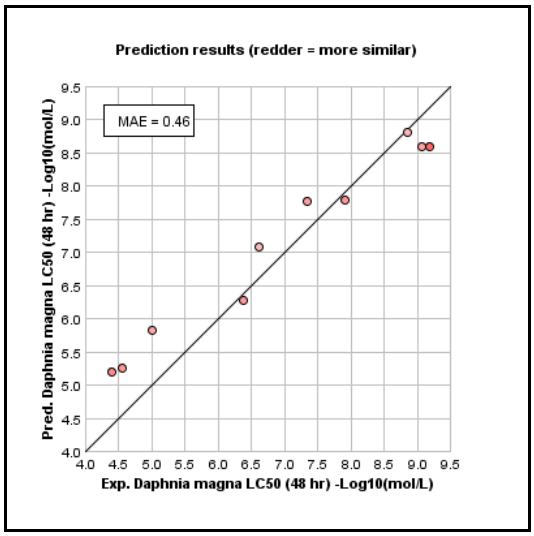
Graph 4 shows the Predictions for the test chemical ($C_{35}H_{42}N_2O_9$) and for the most similar chemicals in the training set**.**



Test set chemicals	MAE*
Entire set	0.50
Similarity coefficient ≥ 0.5	0.40

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

Graph 5 shows the Predictions for the test chemical ($C_{27}H_{32}N_2$) and for the most similar chemicals in the training set**.**



Test set chemicals	MAE*
Entire set	0.50
Similarity coefficient ≥ 0.5	0.46

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

Discussion

QSAR modeling was used for the estimation of LD_{50} values of the compounds. The application and transparency of increasing QSAR models will depend on the user confidence and the transparency of the model[20-22]. Using acceptable toxicity scales, the compounds of the plant are assigned to various groups. The toxicity levels are categorized according to Aquatic toxicity scale[23].

The QSAR estimated LD_{50} values for the breakdown products ranged from $6.99E^{-02}$ mg/L to 1.43 mg/L. This implies that the toxicity of all these compounds fall within super toxic levels (<5mg/L). These compound which lack experimental data, are predicted to be toxic though the plant is commonly used.

Though the study terms the plant to be toxic, the toxicity may be selective and in other terms the compounds may be toxic in sensitive parts such as eye and skin[8]. Hence the compounds must be studied in higher animal models to know their toxicity on higher level of ecosystem.

These QSAR models helps in estimating the toxicity of chemicals that lack experimental data, thus, reducing the time-consumed, cost and minimizing animal testing. Since, several ethical issues restrict animal studies, *in-silico* studies may serve as a vital tool for assessing the pharmaceutical toxicity for those compounds especially derived from nature.

Conclusion:

The study concludes that the compounds detected from the methanolic extract of the leaves of *Pedilanthus tithymaloides* against *Daphnia magna* are super toxic in nature.

References:

1. Pedro Abreu, Susan Matthew, Tania Gonza´lez, David Costa, Marcela A. Segundo and Eduarda Fernandes., Anti-inflammatory and antioxidant activity of a medicinal tincture from *Pedilanthus tithymaloides*, Life Sciences, 2006, 78(14), 1578 – 1585.
2. Wantana Mongkolvisut and Somyote Sutthivaiyakit., Antimalarial and antituberculous poly-O-acylate jatrophane diterpenoids from *Pedilanthus tithymaloides*, J. Nat. Prod., 2007, 70 (9), 1434–1438.
3. Suhaila Mohamed, Suzana Saka, Saleh H. El-Sharkawy, Abdul Manaf Ali and Sepiah Muid, Antimycotic Screening of 58 Malaysian Plants against Plant Pathogens, Pesticide Science, 1996, 47(3), 259-264.
4. Abdul Rahuman A., Geetha Gopalakrishnan P., Venkatesan and Kannappan Geetha., Larvicidal activity of some Euphorbiaceae plant extracts against *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae), Parasitology Research, 2008, 102(5), 867-873.

5. Raveen R., Dhayanidhi P., Dhinamala K., Arivoli S, and Samuel Tennyson., Larvicidal activity of *Pedilanthus tithymaloides* Poit (Euphorbiaceae) leaf against the dengue vector *Aedes aegypti* (Diptera: Culicidae), Int. J. of Env. Bio., 2012; 2(2), 36-40.
6. Ghosh S, Samanta A, Mandal N. B, Bannerjee S, Chattopadhyay D., Evaluation of the wound healing activity of methanol extract of *Pedilanthus tithymaloides* (L.)Poit leaf and its isolated active constituents in topical formulation, 2012, 142(3), 714-722.
7. Kinghorn, A.D, and Evans, F.J., A Biological screen of selected species of the genus Euphorbia for skin irritant effect, Planta Med., 1975, 28(4), 326-335.
8. Morton, J.F., Plants Poisonous to people in Florida and other warm areas, Hurricane Press, Miami, 1971, 112.
9. Lampe, K.F, and McCann, M.A., AMA Handbook of Poisonous and Injurious Plants, American Medical Association, Chicago, 1985,128.
10. Lim T.K., and Engkik Soepadmo, Eye Injury from Plant Sap of *Pedilanthus tithymaloides* Poit., Case Report, Singapore Medical Journal, 1984, 25(6), 412-419.
11. Udayaprakash NK, Ranjithkumar M, Sripriya N, Pujitha Lakshmi, Deepa S, and Bhuvaneswari S., Antioxidant, free radical scavenging activity and GC-MS studies on *Pedilanthus tithymaloides* (L.) Poit. Int. Journ. of Pharma and Pharmaceutical Sci. 2014, (Accepted).
12. Demchuk, E, Ruiz, P, Chou, S, Fowler, B.A, SAR/QSAR methods in public health practice, Toxicol. Appl. Pharmacol. 2011, 254, 192–197.
13. Ruiz, P, Mumtaz, M, and Gombar, V., Assessing the toxic effects of ethylene glycol ethers using Quantitative Structure Toxicity Relationship models, Toxicol. Appl. Pharmacol. 2011, 254, 198–205.
14. Enslein K., The future of toxicity prediction with QSAR., In Vitro Toxicol., 1993, 6, 163–169.
15. Ruiz P., Faroon O., Moudgal C.J., Hansen H., De Rosa C.T., Mumtaz M., Prediction of the health effects of polychlorinated biphenyls (PCBs) and their metabolites using quantitative structure-activity relationship (QSAR). Toxicol.Lett. 2008, 181(1), 53–65.
16. Patricia Ruiz, Gino Begluitti, Terry Tincher, John Wheeler and Moiz Mumtaz., Prediction of Acute Mammalian Toxicity Using QSAR Methods: A Case Study of Sulfur Mustard and Its Breakdown Products. Molecules, 2012, 17(8), 8982-9001.
17. User Manual Version 5.5; Simulation Plus Inc, S. P., Lancaster, CA, USA, 2011.
18. T. E. S.T Tool, User's Guide for T.E.S.T, Version 4.0., US EPA: Cincinatti, OH, USA, 2011.
19. Zhu, H, Martin, T. M, Ye, L, Sedykh, A, Young D.M and Tropsha A., Quantitative structure activity relationship modeling of rat acute toxicity by oral exposure. Chem. Res. Toxicol. 2009, 22(12), 1913–1921.
20. Devillers J and Mombelli E., Evaluation of the OECD QSAR Application Toolbox and Toxtree for estimating the mutagenicity of chemicals. Part 2. alpha-beta unsaturated aliphatic aldehydes. SAR QSAR Environ. Res. 2010, 21(7-8), 771–783.
21. Fjodorova N., Novich M., Vrachko M., Smirnov V., Kharchevnikova N., Zholdakova Z., Novikov S., Skvortsova N., Filimonov D., Poroikov V., and Benfenati E. Directions in QSAR modeling for regulatory uses in OECD member countries, EU and in Russia. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev. 2008, 26(2), 201-236.
22. Liu H., Papa E., Gramatica P., QSAR prediction of estrogen activity for a large set of diverse chemicals under the guidance of OECD principles. Chem. Res. Toxicol. 2006, 19(11), 1540–1548.
23. Agency for Toxic Substances and Disease Registry (ATSDR), EPA Reportable Quantity Methodology Used to Establish Toxicity/Environmental Scores for the Substance Priority List, 2011.

*****