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T Senthil Kumar
Assistant Professor, Faculty of Pharmaceutical Technology, Department of Pharmaceutical Technology, Paavai Engineering College, Paacahl, Namakkal, Tamil Nadu, India

JS Ruthra Priya
Assistant Professor, Faculty of Food Technology, Department of Food Technology, Paavai Engineering College, Paacahl, Namakkal, Tamil Nadu, India

Corresponding Author:
T Senthil Kumar
Assistant Professor, Faculty of Pharmaceutical Technology, Department of Pharmaceutical Technology, Paavai Engineering College, Paacahl, Namakkal, Tamil Nadu, India

In silico analysis of oral acute toxicity, organ toxicity, immunotoxicity, genetic toxicity endpoints, nuclear receptor signaling pathways and stress response pathways of phytocopounds from *Cymbopogon flexuosus*

T Senthil Kumar and JS Ruthra Priya

Abstract
The present objective was an *in silico* study to detect oral acute toxicity, organ toxicity, immunotoxicity, genetic toxicity endpoints, nuclear receptor signalling, and stress response pathways of common synthetic pyrethroids by using ProTox-II webserver. The phyto-compounds synthesized from cymbopogon flexuosus such as carbetapentane, 2-propenoic acid, bornyl acetate, Heptadecanoic acid, phytol, 1(3H)-isobenzofuranone,3-ethoxy-, quinhydrone, 9,12,15-octadecatrienoic acid ethyl ester, 15-methylhexadecanoic acid, oxirane, 1-Heptanol-6-methyl, Diacyl phthalate, eicosane. ProTox-II webserver was used for toxicological assessment in organism, organs, cell and gene level along with molecular mechanisms of toxicity. The predictive results for the toxicity of phyto-compounds, 2-propenoic acid showed highly toxic compound among 13 compounds as fatal if swallowed as class III followed by carbetapentane, Heptadecanoic acid, 15-methylhexadecanoic acid, quinhydrone, 1-Heptanol-6-methyl but hepatotoxic potential was only Heptadecanoic acid, quinhydrone, 9,12,15-octadecatrienoic acid ethyl ester while no immunotoxic was obtained. On the other hand, none of the compounds were obtained cytotoxicity and carbetapentane and oxirane shows carcinogenicity and Mutagenecity. In case of NR signalling pathways, 9,12,15-octadecatrienoic acid ethyl ester were obtained nrf2/ARE and HSE active while MMP active compounds were obtained 1(3H)-isobenzofuranone,3-ethoxy-, quinhydrone, respectively. For p53 and ATAD5 parameters, all thirteen compounds were obtained inactive. In conclusion, the present predictive results are suitable for academician, researchers, industries, etc. those who are making drugs and environmental chemicals. This web server helps faster screening of large numbers of compounds within short duration and no animal testing. This present *in silico* study easily detects toxin(s), which can be validated in future through *in vitro* and *in vivo* experimental assay.

Keywords: *In silico* study, phytocompounds, predictive toxicology, molecular mechanism of toxicity, nuclear receptor signalling and stress response pathways

Introduction
Cymbopogon flexuosus has been customarily utilized as a solution for an assortment of wellbeing condition. Ongoing logical investigations have given proof supporting its antimicrobial, cancer prevention agent, antifungal and calming properties in a few infected models (Tsai *et al*., 2011, Boukhatem *et al*., 2014, Amorim *et al*., 2016) [1-3]. In present days, an alteration of experimental toxicity study the predictive study through computational simulation is showing interest in the researchers to prevent cost, less duration and no animal warming. The ProTox II is a webserver (http://tox.charite.de/protox-II/) to predict toxicity and multiple toxicological end points for several chemical compounds developed by Drwal *et al*. (2014) [4] and Banerjee *et al*. (2018) [5]. According to Banerjee et.al the Protox-II platform is classified into five different steps such as acute oral toxicity prediction model as per six different toxicity classess; organ toxicity model especially liver toxicity prediction; toxicological (immunotoxicity model) and genotoxicological (cytotoxicity, mutagenecity and carcinogenicity model) end points; toxicological pathways suchas nuclear receptor signaling pathways is classified seven target pathway based models viz. aryl hydrogen receptor (AhR), androgen receptor (AR), androgen receptor ligand binding domain (AR-LBD), Aromatase (Aro), estrogen recptor alpha (ER), estrogen receptor ligand binding domain (ER-LBD) and peroxisome proliferator activated receptor gamma (PPARGamma) as well as stress response pathways is classified five target pathway based models such as nuclear factor (erythroid-

derived-2) – like 2/ antioxidant responsive element (ARE), heat shock factor response element (HSE), itochondrial membrane potential (MMP), phosprotein tumor su[ressor (p53) and ATPase family AAA domain containing protein 5 (ATAD5) and toxicity target model for 13 Nos.

All the predicitive models for toxicology pathways have been implemented as toxiclogy in the 21st century (TOX21), which is a federal collaboration among united states environmental protection agency (EPA), National institute of health (NIH), including National center for advancing translational sciences, and the National toxicological program at the National instate of environmental sciences and the food and Drug administration (Banerjee *et al* 2016) [6].

Present *in silico* study was to detect oral acute toxicity, organ toxicity, immunotoxicity, genetic toxicity end points, nuclear receptor signaling pathways and stress response pathways of various phtyocompounds synthesized from C.flexuosus by using ProTox-II.

Materials and Methods

Selection of compounds

The various phytocompounds synthesized from cymbopogon flexuosus extract were selected. Detail list of selected compounds is tabulated in table 1.

Table 1: Synthesized compounds from *Cymbopogon flexuosus*

S. No	Compounds Name
1	Carbetapentane
2	2-propenoic acid
3	Bornyl Acetate
4	Heptadecanoic acid
5	Phytol
6	1(3H)-isobenzofuranone,3-ethoxy-
7	Quinhydrone
8	9,12,15-octadecatrienoic acid ethyl ester
9	15-methyl hexadecanoic acid
10	Oxirane
11	1-Heptanol-6-methyl
12	Diacyl phthalate
13	Eicosane

In silico Study of studied compounds

In silico study was done by using ProTox-II webserver developed by Drwal (2014) [4] and the parameters such as rat oral acute toxicity with special reference to median lethal dose (LD_{50}) as mg/kg, organ toxicity endpoints especially hepatotoxicity, immunotoxicity, genetic toxicity end points especially cytotoxicity, mutagenecity and carcinogenicity, nuclear receptor signaling (AhR, AR, AR-LBD, ER, ER-LBD and PPARGamma) and stress response pathways (nrf2/ARE, HSE, MMP, p53 and ATAD5) were predicted for established phytocompounds of 13 Nos as per protocol followed by Banerjee(2016) [6].

Results and Discussion

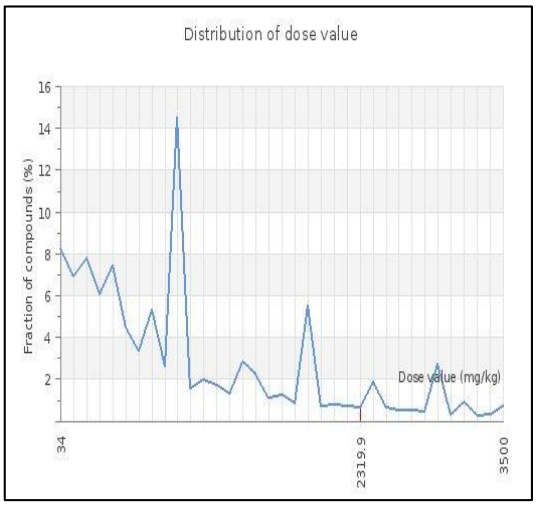
Table-2 indicates the rat oral acute toxicity (LD_{50}) as mg/kg, predicted different toxicity classess (I-VI) and prediction accuracy in % for different phytocompounds. Among 13 phytocompounds, 2-propenoic acid obtained the highest toxicity (LD_{50} =34mg/kg) as class III i.e prescribed as death after swallowing ($5< LD_{50} \leq 50$) with 100% prediction accuracy (Fig1A). Same LD_{50} values were obtained for the compounds carbetapentane, Heptadecanoic Acid, 15-methylhexadecanoic acid and quinhydrone, 1- Heptanol, 6-methyl as 130 mg/kg and 200 mg/kg as class III (i.e)prescribed toxic after swallowing ($50< LD_{50} \leq 300$) with prediction accuracy 100%, only exception the compound quinhydrone has 68.07% (Fig1B-C). The compounds shows LD_{50} value 154 mg/kg with prediction accuracy 100% (Fig1-D). Followed by the compounds phytol, oxirane, Bornyl acetate shows LD_{50} values such as 2610 mg/kg, 2830 mg/kg, 3100 mg/kg as class V (i.e) may be harmful if swallowed ($2000< LD_{50} \leq 5000$) with predicition accuracy 68.26%, 12% and 100% respectively (fig1E-G).

The compounds Diacyl phthalate, 9,12,15-octadecatrienoic acid ethyl ester obtained LD_{50} values 6172 mg/kg, 10000 mg/kg as class VI (i.e) non-toxic if swallowed (LD_{50} >5000) with prediction accuracy 100% respectively (fig1H-I). then the compound 1(3H)-isobenzofuranone, 3-ethoxy- has LD_{50} values 690 mg/kg with prediction accuracy 68.07% respectively (fig1-J).

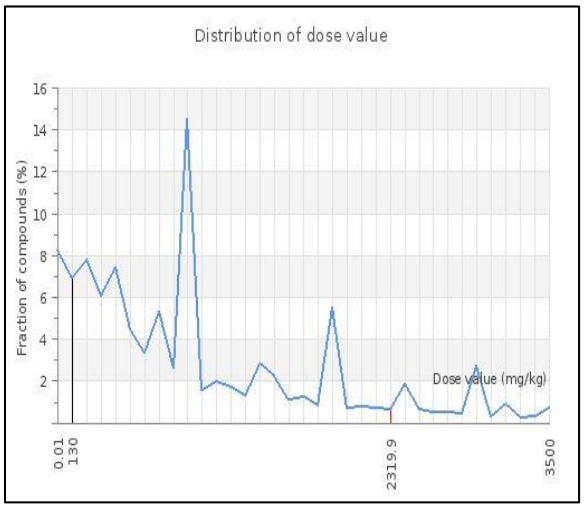
Table 2: Prediction of oral acute toxicity, class and accuracy of studied compounds.

Sl. No.	Compounds name	Oral LD_{50} value (mg/Kg)	Predicted toxicity class	Prediction accuracy (%)
1	Carbetapentane	130	III	100
2	2-propenoic acid	34	II	100
3	Bornyl Acetate	3100	V	100
4	Heptadecanoic acid	130	III	100
5	Phytol	2610	V	69.26
6	1(3H)-isobenzofuranone,3-ethoxy-	690	IV	68.07
7	Quinhydrone	200	III	68.07
8	9,12,15-octadecatrienoic acid ethyl ester	10000	VI	100
9	15-methyl hexadecanoic acid	130	III	100
10	Oxirane	2830	V	12
11	1-Heptanol-6-methyl	200	III	100
12	Diacyl phthalate	6172	VI	100
13	Eicosane	154	III	100

As per Drwal *et al*. (2014) [4], Class I: death after swallowing ($LD_{50} \leq 5$); Class II: death after swallowing ($5< LD_{50} \leq 50$); Class III: toxic after swallowing ($50< LD_{50} \leq 300$); Class IV: harmful after swallowing ($300< LD_{50} \leq 2000$); Class V: may be harmful after swallowing ($2000< LD_{50} \leq 5000$) and Class VI: non-toxic ($LD_{50} > 5000$)


Distribution of dose value
Fraction of compounds (%)
Dose value (mg/kg)
34
2319.9
3500

(A)


Distribution of dose value
Fraction of compounds (%)
Dose value (mg/kg)
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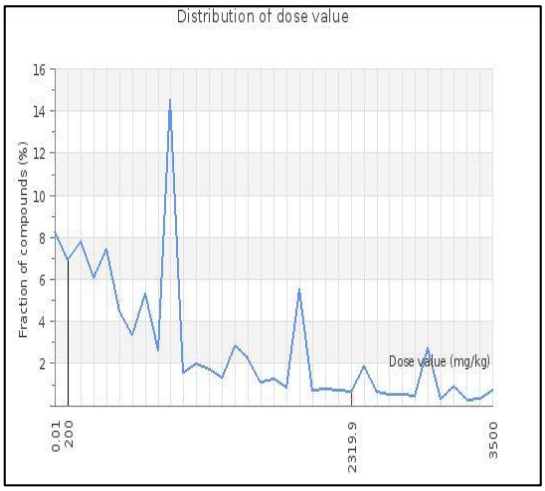
130
2319.9
3500

(B)


Distribution of dose value
Fraction of compounds (%)
Dose value (mg/kg)
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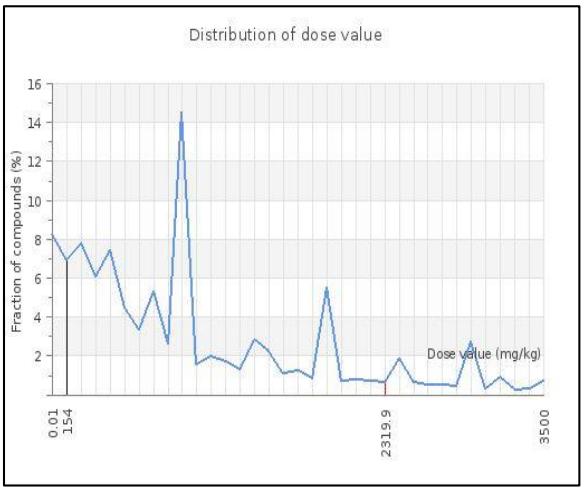
200
2319.9
3500

(C)


Distribution of dose value
Fraction of compounds (%)
Dose value (mg/kg)
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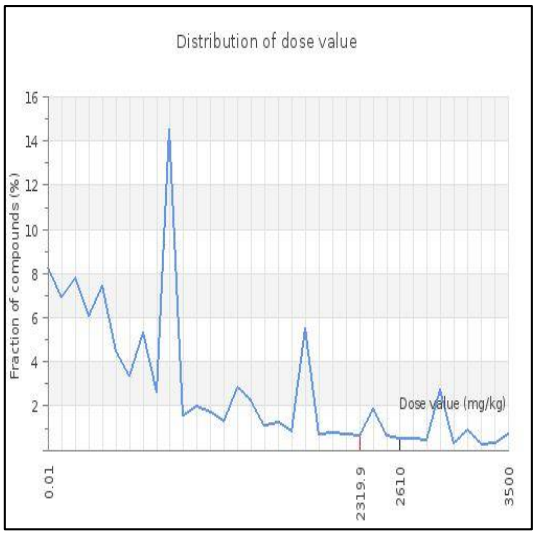
154
2319.9
3500

(D)


Distribution of dose value
Fraction of compounds (%)
Dose value (mg/kg)
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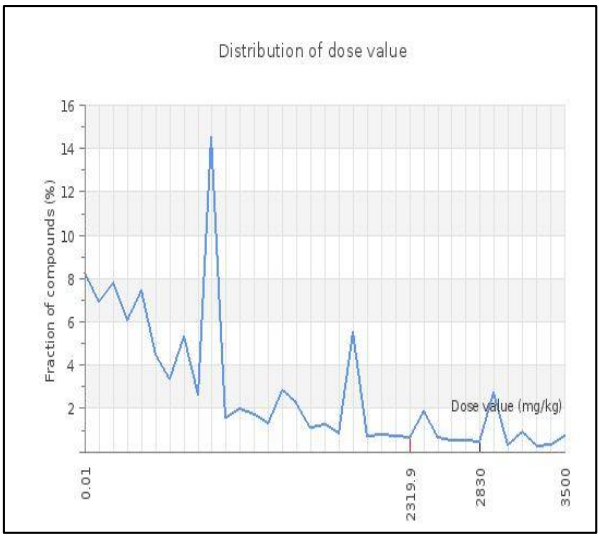
2319.9
2610
3500

(E)


Distribution of dose value
Fraction of compounds (%)
Dose value (mg/kg)
0.01
2319.9
2830
3500

(F)

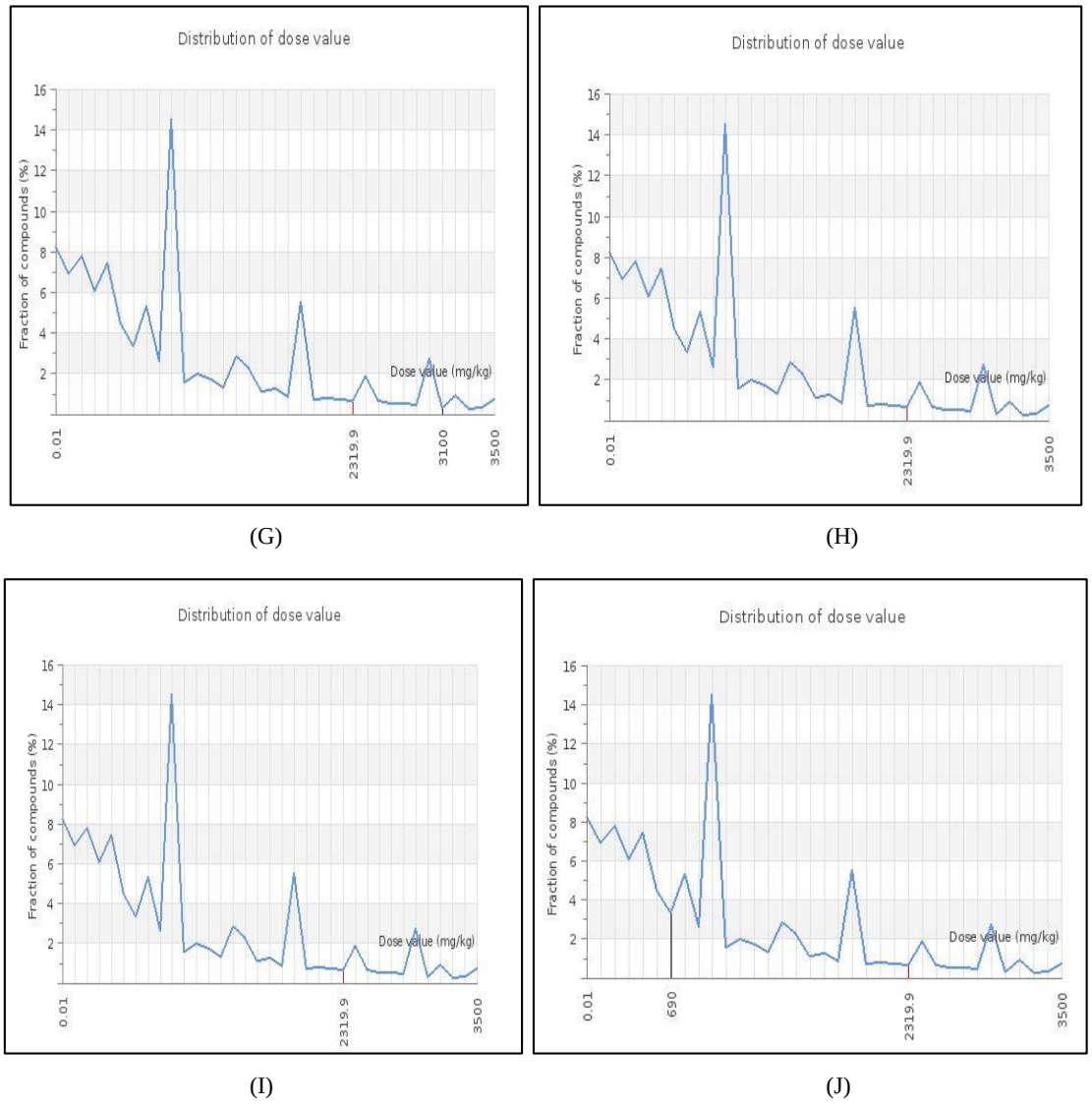


Fig 1: Graphical representation of predicted dose value distribution for studied compounds (A=2-propenoic acid; B=carbetapentane, Heptadecanoic acid, 15-Methylhexadecanoic acid; C=Quinhydrone, 1-Heptanol-6-methyl; D=Eicosane; E=Phytol; F= oxirane; G=Bornyl Acetate; H=Diacyl phthalate; I=9,12,15-octadecatrienoic acid ethyl ester; J= 1(3H) isobenzofurene-3-ethoxy-)

The present predictive result indicated that all synthesized phytocompounds are toxic as per LD_{50} values (34 mg/kg) obtained by the webserver (ProTox-II) but only 2-propenoic acid was obtained fatal as per LD_{50} value.

In table-3, the prediction of organ toxicity with special reference to liver toxicity or hepatotoxicity was observed. Among 13 compounds, three compounds such as Heptadecanoic acid, quinhydrone, 9,12,15-octadecatrienoic acid ethyl ester were showed hepatotoxicity active and probability scores were 0.52, 0.65, 0.54 while all other ten compounds such as carbetapentane, 2-propenoic acid, bornyl acetate, phytol, 1(3H)-isobenzofuranone, 3-ethoxy-,15-methylhexadecanoic acid, oxirane, 1-Heptanol-6-methyl, Diacyl phthalate, eicosane were observed non-hepatotoxic or hepatotoxic inactive with probability scores 0.98, 0.78, 0.58, 0.70, 0.77, 0.52, 0.88, 0.85, 0.77, 0.74 respectively.

The immunotoxicity endpoints of studied 13 phytocompounds such as carbetapentane, 2-propenoic acid, bornyl acetate, Heptadecanoic acid, phytol, 1(3H)-isobenzofuranone, 3-ethoxy-, quinhydrone, 9,12,15-octadecatrienoic acid ethyl ester, 15-methylhexadecanoic acid, oxirane, 1-Heptanol-6-methyl, Diacyl phthalate, eicosane were non-immunotoxic or immunotoxicity inactive with probability scores 0.51, 0.78, 0.62, 0.63, 0.72, 0.56, 0.60, 0.63, 0.70, 0.52, 0.66, 0.65, 0.58 respectively.

In the present predictive results, shows that Heptadecanoic acid, quinhydrone, 9,12,15-octafecatrienoic acid ethyl ester are having hepatotoxic potential and none of the compounds having immunotoxicity potential.

Table 3: Prediction of organ toxicity and immunotoxicity end points of studied compounds

Sl. No.	Compounds name	Hep	P	Imm	P
1.	Carbetapentane	I	0.98	I	0.94
2.	2-propenoic acid	I	0.78	I	0.99
3.	Bornyl Acetate	I	0.58	I	0.94
4.	Heptadecanoic acid	A	0.52	I	0.99
5.	Phytol	I	0.70	I	0.95
6.	1(3H)-isobenzofuranone,3-ethoxy-	I	0.77	I	0.96
7.	Quinhydrone	A	0.65	I	0.98
8.	9,12,15-octadecatrienoic acid ethyl ester	A	0.54	I	0.99
9.	15-methyl hexadecanoic acid	I	0.52	I	0.99
10.	Oxirane	I	0.88	I	0.99
11.	1-Heptanol-6-methyl	I	0.85	I	0.99
12.	Diacyl phthalate	I	0.77	I	0.99
13.	Eicosane	I	0.74	I	0.98

Hep = Hepatotoxicity; Imm = Immunotoxicity; I = Inactive; A = Active and P = Probability

In Table-4, the prediction of genotoxicity with special reference to cytotoxicity, mutagenecity and carcinogenicity were observed. Among 13 compounds such as carbetapentane, 2-propenoic acid, bornyl acetate, Heptadecanoic acid, phytol, 1(3H)-isobenzofuranone,3-ethoxy-, quinhydrone, 9,12,15-octadecatrienoic acid ethyl ester, 15-methylhexadecanoic acid, oxirane, 1-Heptanol-6-methyl, Diacyl phthalate, eicosane were observed non-cytotoxic or cytotoxicity inactive with probability scores 0.78, 0.76, 0.67, 0.74 0.86, 0.79, 0.85, 0.71, 0.77, 0.77, 0.83, 0.92, 0.78 respectively.

Incase of mutagenecity end points, oxirane were observed mutagenic active with probability score 0.69 respectively. While these compounds such as carbetapentane, 2-propenoic acid, bornyl acetate, Heptadecanoic acid, phytol, 1(3H)-isobenzofuranone,3-ethoxy-, quinhydrone, 9,12,15-octadecatrienoic acid ethyl ester, 15-methylhexadecanoic acid, 1-Heptanol-6-methyl, Diacyl phthalate, eicosane were obtained mutagenic inactive with probability score 0.77, 0.93, 0.94, 1.0, 0.95, 0.72, 0.83, 0.95, 0.96, 0.69, 0.97, 0.87, 1.0 respectively (Table-4).

Table 4: Prediction of genetic toxicity end points of studied compounds.

Sl. No.	Compounds name	Cytt	P	Mutg	P	Carcig	P
1.	Carbetapentane	I	0.78	I	0.77	A	0.51
2.	2-propenoic acid	I	0.76	I	0.93	I	0.78
3.	Bornyl Acetate	I	0.67	I	0.94	I	0.62
4.	Heptadecanoic acid	I	0.74	I	1.0	I	0.63
5.	Phytol	I	0.86	I	0.95	I	0.72
6.	1(3H)-isobenzofuranone,3-ethoxy-	I	0.79	I	0.72	I	0.56
7.	Quinhydrone	I	0.85	I	0.83	I	0.60
8.	9,12,15-octadecatrienoic acid ethyl ester	I	0.71	I	0.95	I	0.63
9.	15-methyl hexadecanoic acid	I	0.77	I	0.96	I	0.70
10.	Oxirane	I	0.77	A	0.69	I	0.52
11.	1-Heptanol-6-methyl	I	0.83	I	0.97	I	0.66
12.	Diacyl phthalate	I	0.92	I	0.87	I	0.65
13.	Eicosane	I	0.78	I	1.0	I	0.58

Cytt = Cytotoxicity; Mutg = Mutagenicity; Carcig = Carcinogenicity; I = Inactive; A = Active and P = Probability

Among 13 compounds, carbetapentane showed carcinogenic active with probability score 0.51, while 2-propenoic acid, bornyl acetate, Heptadecanoic acid, phytol, 1(3H)-isobenzofuranone,3-ethoxy-, quinhydrone, 9,12,15-octadecatrienoic acid ethyl ester, 15-methylhexadecanoic acid, oxirane, 1-Heptanol-6-methyl, Diacyl phthalate, eicosane were obtained carcinogenic inactive with probability scores 0.78, 0.62, 0.63, 0.72, 0.56, 0.60, 0.63, 0.70, 0.52, 0.66, 0.65, 0.58 respectively.

For TOX21- nuclear receptor signaling pathways, several parameters such as AhR, AR, AR-LBD, Aro, ER, ER-LBD, PPARGamma, were predicted for carbetapentane, 2-propenoic acid, bornyl acetate, Heptadecanoic acid, phytol, 1(3H)-isobenzofuranone,3-ethoxy-, quinhydrone, 9,12,15-octadecatrienoic acid ethyl ester, 15-methylhexadecanoic acid, oxirane, 1-Heptanol-6-methyl, Diacyl phthalate, eicosane (Table-5).

Table 5: Prediction of Tox21-nuclear receptor signalling pathways of studied compounds

Sl. No.	Compounds name	Tox21-Nuclear receptor signalling pathways							
		Ahr	P	AR	P	AR- LBD	P	Aro	P
1.	Carbetapentane	I	0.99	I	0.99	I	0.99	I	0.99
2.	2-propenoic acid	I	0.99	I	1.0	I	1.0	I	0.95
3.	Bornyl Acetate	I	1.0	I	0.99	I	0.99	I	1.0
4.	Heptadecanoic acid	I	1.0	I	1.0	I	1.0	I	1.0
5.	Phytol	I	0.94	I	0.99	I	1.0	I	0.98
6.	1(3H)-isobenzofuranone,3-ethoxy-	I	0.80	I	0.96	I	0.97	I	0.86
7.	Quinhydrone	I	0.61	I	0.90	I	0.98	I	0.89
8.	9,12,15-octadecatrienoic acid ethyl ester	I	1.0	I	1.0	I	1.0	I	1.0
9.	15-methyl hexadecanoic acid	I	0.99	I	0.99	I	1.0	I	1.0
10.	Oxirane	I	0.99	I	0.99	I	0.99	I	0.99
11.	1-Heptanol-6-methyl	I	1.0	I	1.0	I	1.0	I	1.0
12.	Diacyl phthalate	I	0.99	I	1.0	I	1.0	I	1.0
13.	Eicosane	I	1.0	A	0.77	I	1.0	I	1.0
		ER	P	ER- LBD	P	PPAR-Gamma	P		
1.	Carbetapentane	I	0.97	I	0.98	I	0.98		
2.	2-propenoic acid	I	0.99	I	0.99	I	0.98		
3.	Bornyl Acetate	I	0.99	I	0.99	I	0.99		
4.	Heptadecanoic acid	I	1.0	I	1.0	I	1.0		
5.	Phytol	I	0.94	I	0.99	I	0.93		
6.	1(3H)-isobenzofuranone,3-ethoxy-	I	0.72	I	0.94	I	0.78		
7.	Quinhydrone	I	0.67	I	0.67	I	0.92		
8.	9,12,15-octadecatrienoic acid ethyl ester	I	0.98	I	1.0	A	1.0		
9.	15-methyl hexadecanoic acid	I	0.99	I	1.0	A	1.0		
10.	Oxirane	I	0.94	I	0.98	I	0.98		

11.	1-Heptanol-6-methyl	I	0.97	I	1.0	I	0.98	
12.	Diacyl phthalate	I	0.92	I	0.99	I	0.99	
13.	Eicosane	I	0.56	I	1.0	I	1.0	

AhR = Aryl hydrocarbon Receptor; AR = Androgen receptor; AR-LBD = Androgen Receptor Ligand Binding Domain; Aro = Aromatase; ER = Estrogen Receptor Alpha; ER-LBD = Estrogen Receptor Ligand Binding Domain; PPAR-Gamma = Peroxisome Proliferator Activated Receptor Gamma; I = Inactive; A = Active and P = Probability

All the studied 13 compounds were obtained AhR inactive with probability scores 0.99, 0.99, 1.0, 1.0, 0.94, 0.80, 0.61, 1.0, 0.99, 0.99, 1.0, 0.99, 1.0 respectively. For other parameter, Eicosane shows Ar active with probability score 0.77 while carbetapentane, 2-propenoic acid, bornyl acetate, Heptadecanoic acid, phytol, 1(3H)-isobenzofuranone,3-ethoxy-, quinhydrone, 9,12,15-octadecatrienoic acid ethyl ester, 15-methylhexadecanoic acid, oxirane, 1-Heptanol-6-methyl, Diacyl phthalate were obtained Ar inactive with probability scores 0.99, 1.0, 0.99, 1.0, 0.99, 0.96, 0.90, 1.0, 0.99, 0.99, 1.0, 1.0 respectively. Incase of AR-LBD all the compounds were found AR-LBD inactive and the probability scores0.99, 1.0, 0.99, 1.0, 1.0, 0.97, 0.98, 1.0, 1.0, 0.99, 1.0, 1.0, 1.0 were obtained. The parameter aromatase or aro was found inactive for all the studied compounds and probability scores 0.99, 0.95, 1.0, 1.0, 0.98, 0.86, 0.89, 1.0, 1.0, 0.99, 1.0, 1.0, 1.0 were obtained. In case of parameter ER and ER-LBD it was observed inactive for both cases and probability scores 0.97, 0.99, 0.99, 1.0, 0.94, 0.72, 0.67, 0.98, 0.99, 0.99, 0.94, 0.97, 0.92, 0.56 for ER and 0.98, 0.99, 0.99, 1.0, 0.99, 0.94, 0.67, 1.0, 1.0, 0.98, 1.0, 0.99, 1.0 for ER-LBD were recorded. For the parameter PPARGamma, 9,12,15-octafecatrienoic acid ethyl ester, 15-methylhexadecanoic acid were obtained PPARGamma active with probability scores were recorded as 0.98, 0.98, 0.99, 1.0, 0.93, 0.78, 0.92, 1.0, 1.0, 0.98, 0.98, 0.99, 1.0 respectively.

The present predictive results indicated that eicosane have activity on AR and 9,12,15-octafecatrienoic acid ethyl ester, 15-methylhexadecanoic acid have activity on PPARGamma under nuclear receptor signaling pathways. While other compounds were indicated inactivity for all the parameter such as AhR, AR, AR-LBD, Aro, ER, ER-LBD, PPARGamma. According to kolodkin (2010) [7] Nuclear receptor signaling occurs to maintain development, cellular growth, inflammation and metabolism and ligand distribution appeared dynamic with few nuclear receptor found predominantly in the nucleus (pregnance x receptor and peroxisome proliferator- activated receptor gamma), while some are located either in both compartments (vitamin D receptor and mineralocorticoid receptor) or mostly in the cytoplasm (glucocorticoid receptor and androgen receptor). The present results indicated the inactivity of studied compounds may not lead to carcinogenic with only exception of carbetapentane, which obtained in Table-4.

In Table-6, TOX-21 stress response pathways parameter such as nrf2/ARE, HSE, MMP, p53 and ATAD5 for studied copounds were predicted. Among 13 compounds, 9,12,15-octadecatrienoic acid ethyl ester were obtained ARE and HSE active with probability score 0.51 while rest 12 compounds carbetapentane, 2-propenoic acid, bornyl acetate, Heptadecanoic acid, phytol, 1(3H)-isobenzofuranone,3-ethoxy-, quinhydrone, 15-methylhexadecanoic acid, oxirane, 1-Heptanol-6-methyl, Diacyl phthalate, eicosane were found inactive with probability scores 0.98, 1.0, 1.0, 1.0, 0.88, 0.82, 0.86, 0.99, 0.97, 0.98, 0.99, 1.0 for ARE and 0.98, 1.0, 1.0, 1.0, 0.88, 0.82, 0.86, 0.99, 0.97, 0.98, 0.99, 1.0 for HSE respectively. For MMP parameter, carbetapentane, 2-propenoic acid, bornyl acetate, Heptadecanoic acid, phytol, 9,12,15-octadecatrienoic acid ethyl ester, 15-methylhexadecanoic acid, oxirane, 1-Heptanol-6-methyl, Diacyl phthalate, eicosane were obtained MMP inactive with probability scores 0.95, 0.99, 0.99, 1.0, 0.96, 0.98, 1.0, 0.95, 0.99, 1.0, 1.0 but the compounds viz. 1(3H)-isobenzofuranone,3-ethoxy-, quinhydrone were found MMP active with probability score 0.63 and 0.61 respectively.

Table 6: Prediction of Tox21-stress response pathways of studied compounds.

Sl. No.	Compounds name	Tox21- Stress response pathways									
		nrf2/ARE	P	HSE	P	MMP	P	p53	P	ATAD5	P
1.	Carbetapentane	I	0.98		0.98	I	0.95	I	0.99	I	0.99
2.	2-propenoic acid	I	1.0	I	1.0	I	0.99	I	1.0	I	0.99
3.	Bornyl Acetate	I	1.0	I	1.0	I	0.99	I	1.0	I	1.0
4.	Heptadecanoic acid	I	1.0	I	1.0	I	1.0	I	1.0	I	1.0
5.	Phytol	I	0.88	I	0.88	I	0.96	I	0.99	I	0.99
6.	1(3H)-isobenzofuranone,3-ethoxy-	I	0.82	I	0.82	A	0.63	I	0.82	I	0.94
7.	Quinhydrone	I	0.86	I	0.86	A	0.61	I	0.83	I	0.84
8.	9,12,15-octadecatrienoic acid ethyl ester	A	0.51	A	0.51	I	0.98	I	0.99	I	1.0
9.	15-methyl hexadecanoic acid	I	0.99	I	0.99	I	1.0	I	1.0	I	1.0
10.	Oxirane	I	0.97	I	0.97	I	0.95	I	0.98	I	0.98
11.	1-Heptanol-6-methyl	I	0.98	I	0.98	I	0.99	I	1.0	I	1.0
12.	Diacyl phthalate	I	0.99	I	0.99	I	1.0	I	0.99	I	0.99
13.	Eicosane	I	1.0	I	1.0	I	1.0	I	1.0	I	1.0

nrf2/ARE = Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element; HSE = Heat shock factor response element; MMP = Mitochondrial Membrane Potential; p53 = Phosphoprotein (tumour supressor); ATAD5 = ATPase family AAA domain-containing protein 5; I = Inactive; A = Active and P = Probability

For p53 and ATAD5 parameter all 13 compounds such as carbetapentane, 2-propenoic acid, bornyl acetate, Heptadecanoic acid, phytol, 1(3H)-isobenzofuranone,3-ethoxy-, quinhydrone, 9,12,15-octadecatrienoic acid ethyl ester, 15-methylhexadecanoic acid, oxirane, 1-Heptanol-6-methyl, Diacyl phthalate, eicosane were obtained inactive with probability score 0.99, 1.0, 1.0, 1.0, 0.99, 0.82, 0.83, 0.99, 1.0, 0.98, 1.0, 0.99, 1.0 for p53 parameter as well as 0.99, 0.99, 1.0, 1.0, 0.99, 0.94, 0.84, 1.0, 2.0, 0.98, 1.0, 0.99, 1.0 respectively for ATAD5 parameter.

Several cellular stress in response pathway have been investigated individually through invitro studies and the major signaling components and molecular mechanism have been identified by researchers. Adaptive stress response pathways are signal transduction pathways that ultimately resulted in the transcriptional activation of cytoprotective genes (simons *et al*. 2009).

The compounds such as Pyrethrin I, II, Cinerin I, II, Jasmolin I, II, Allethrin and Resmethrin were obtained nrf2/ARE and HSE active that caused reactive oxygen species (ROS), ultimately oxidative stress in the cell for former case as antioxidant responsive element (ARE) (Kang *et al*., 2005; Kensler *et al*., 2007; Simmons *et al*., 2009) [9, 10, 8] while in the second case, another stress response pathway i.e. heat shock factor response element (HSE), which caused transcriptional upregulation of a family of genes called as heat shock proteins and occurred protein denaturation because chemical insult (Voellmy, 1994; Boellmann *et al*., 2004; Voellmy and Boellmann, 2007; Simmons *et al*., 2009) [11, 12, 13, 8].

In the present prediction, one compound were obtained harmful for cellular stress and may alter molecular mechanism after chronic exposure. Another stress response pathway i.e mitochondrial membrane potential (MMP) the compounds 1(3H)-isobenzofuranone,3-ethoxy-, quinhydrone were obtained active. It is well known that mitochondria consist double membrane which provides the energy to the cell through oxidative phosphorylation and prevent apoptosis (Hill *et al*., 2018) [14]. According to Parikh *et al*. (1987) [15], yeast mitochondria have adapted a mitochondria-to-nucleus signal transduction pathway termed the retrograde response to induce the transcription of nuclear- encoded mitochondrial genes, and alleviate mitochondrial stress. Moreover, mitochondrial stress by toxins may lead to various diseases (Meyer *et al*., 2018) [16]. In recent research by Richter *et al*. (2019) [17] emphasized that toxins inhibit the mitochondrial protein synthesis and block with the stress response.

Other two parameters such as p53 or Phosphoprotein (tumour suppressor) and ATPase family AAA domain-containing protein 5 (ATAD5) observed inactive for all the studied compounds. The p53 gene controls the cell cycle arrest, carcinogenesis, DNA damage, apoptosis, etc. and inactivity in the present prediction showed no incidence of carcinogenesis obtained in Table 4. On the other hand, ATAD5 is involved in DNA damage response. This is also involved in a RAD9A-related damage checkpoint, a pathway which is important in determining whether DNA damage is compatible with cell survival or whether it requires cell elimination by apoptosis (Ishii *et al*., 2005) [18]. The inactivity of studied compounds revealed that DNA damage may repair due to no stress response of ATAD5.

Conclusion

In conclusion, the present predictive results are suitable for academician, researchers, industries, etc. those who are making drugs and environmental chemicals. This web server helps faster screening of large numbers of compounds within short duration as well as without animal testing. These compounds 2-propenoic acid, bornyl acetate, phytol, 15-methylhexadecanoic acid, 1-Heptanol-6-methyl, Diacyl phthalate were indicated inactivity for toxicity screening. This study emphasizes narrow range of compounds, which further easily study in future experimental assay to validate the present prediction.

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