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In silico Approach for Acute Toxicity Prediction of Selected Phytochemicals from Marigold Flower (*Tagetes erecta* Linn.) Compared to Antibiotic

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Abstract: ***The present study was attempted an in silico method to screen acute toxicity especially median lethal dose (LD_{50}) values of established phytochemicals marigold flower (Tagetes erecta Linn.) prior to analyze drug compounds. All established phytochemicals especially flavonoids of marigold flower (Tagetes errecta Linn.) and synthetic medicine viz. Ciprofloxacin were taken from available literature The ProTox-II webserver (version, 3.0) was used in this study. The acute toxicity especially median lethal dose (LD_{50}) values of the phytochemicals of this flower and synthetic medicine viz. Ciprofloxacin were predicted. The acute toxicity was predicted as per rat oral LD_{50} (mg/Kg) values. The prediction of rat oral LD_{50} value (mg/Kg) in which five phytocompounds such as Kaempferol, Kaempferol-3-O-glucoside, Kaempferitrin, Patuletin and Patulitrin were predicted Class V as may be harmful if swallowed, two phytocompounds viz. Quercetin-7-O-glucoside and Myrecetin-3-O-glucoside and one antibiotic Ciprofloxacin were predicted Class IV as harmful if swallowed, two phytocompounds namely Quercetin and Quercetagetin were predicted Class III as toxic if swallowed while three phytochemicals viz. Lutein, Zeaxanthin and Lutein-5,6-epoxide were predicted Class II as fatal if swallowed. These flavonoids could be suitable phytomedicines as toxicity class of V in the near future. It is suggested that experimental bioassay should be conducted with these flavonoids individually***.

Keywords: Acute toxicity, Antibiotic, Flavonoids, *In silico*, Marigold flower, Phytochemicals, *Tagetes errecta*, Toxicity prediction

1. Introduction

From traditional knowledge, many phytocompounds are well-known bioactive compounds from natural origin especially from plant products, called as phytomedicines to prevent various diseases. [1-4]

According to the WHO (World Health Organization), it has been known that 80% of the developing countries used traditional medicines originated from medicinal plants. [5-7]

Among many plant species, an ornamental plant called as marigold (*Tagetes erecta* Linnaeus) under Asteraceae family, is cultivated in many parts of India. The phytocompounds of the flower of this plant is well-established anti-bacterial agents reported through experimental studies in many articles. [8-12]

In this regard, *in silico* study can be suitable to identify non-toxic phytocompounds for new drug design. The toxicity and toxicological mechanisms prediction can easily be done through QSAR modelling by using ProTox webserver developed by Drwal et al. [13] and further updated ProTox II online tool as per Banerjee et al., [14], [15] which utilized many investigators for toxicity studies. [16-19]

The present study was attempted an *in silico* method to screen acute toxicity especially median lethal dose (LD_{50}) values of established phytochemicals marigold flower (*Tagetes erecta* Linn.) prior to analyze drug compounds.

2. Materials and Methods

All established phytochemicals especially flavonoids of marigold flower (*Tagetes errecta* Linn.) and synthetic medicine viz. Ciprofloxacin were taken from available literature. [20-23] As per Banerjee et al., [14], [15] the ProTox-II webserver (version, 3.0) was used in this study. The acute toxicity especially median lethal dose (LD_{50}) values of the phytochemicals of this flower and synthetic medicine viz. Ciprofloxacin were predicted. The acute toxicity was predicted as per rat oral LD_{50} (mg/Kg) values. Table 1 describes Simplified Molecular Input Line Entry System (SMILES) notation for phytocompounds from *Tagetes errecta* and synthetic antibiotic.

Table 1: SMILES notation for phytocompounds from *Tagetes errecta* and synthetic antibiotic

Sl. No.	Compounds	SMILES
Phytocompounds		
1.	Lutein	CC1=C(C(C[C@@H](C1)O)(C)C)/C=C/C(=C/C=C/C(=C/C=C/C=C(\C)/C=C/C=C(\C)/C=C/[C@H]2C(=C[C@@H](CC2(C)C)O)C)/C)/C
2.	Zeaxanthin	CC1=C(C(C[C@@H](C1)O)(C)C)/C=C/C(=C/C=C/C(=C/C=C/C=C(/C=C/C=C(/C=C/C2=C(C[C@H](CC2(C)C)O)C)\C)\C)/C)/C
3.	Lutein-5,6-epoxide	CC1=C[C@@H](CC([C@H]1/C=C/C(=C/C=C/C(=C/C=C/C=C(\C)/C=C/C=C(\C)/C=C/[C@]23[C@](O2)(C[C@H](CC3(C)C)O)C)/C)/C)(C)C)O

4.	Quercetin	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O)O
5.	Quercetin-7-O-glucoside	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)OC4C(C(C(C(O4)CO)O)O)O)O)O)O)O
6.	Quercetagetin	C1=CC(=C(C=C1C2=C(C(=O)C3=C(O2)C=C(C(=C3O)O)O)O)O)O
7.	Kaempferol	C1=CC(=CC=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O
8.	Kaempferol-3-O-glucoside	C1=CC(=CC=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O[C@H]4[C@@H]([C@H]([C@@H]([C@H](O4)CO)O)O)O)O
9.	Kaempferitrin	C[C@H]1[C@@H]([C@H]([C@H]([C@@H](O1)OC2=CC(=C3C(=C2)OC(=C(C3=O)O[C@H]4[C@@H]([C@@H]([C@H]([C@@H](O4)C)O)O)O)C5=CC=C(C=C5)O)O)O)O)O
10.	Patuletin	COC1=C(C2=C(C=C1O)OC(=C(C2=O)O)C3=CC(=C(C=C3)O)O)O
11.	Patulitrin	COC1=C(C=C2C(=C1O)C(=O)C(=C(O2)C3=CC(=C(C=C3)O)O)O)O[C@H]4[C@@H]([C@H]([C@@H]([C@H](O4)CO)O)O)O
12.	Myricetin-3-O-glucoside	C1=C(C=C(C(=C1O)O)O)C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O[C@H]4[C@@H]([C@H]([C@@H]([C@H](O4)CO)O)O)O
Synthetic antibiotic		
13.	Ciprofloxacin	C1CC1N2C=C(C(=O)C3=CC(=C(C=C32)N4CCNCC4)F)C(=O)O

3. Results

The prediction of rat oral LD_{50} value (mg/Kg) in which five phytocompounds such as Kaempferol, Kaempferol-3-O-glucoside, Kaempferitrin, Patuletin and Patulitrin were predicted Class V as may be harmful if swallowed, two phytocompounds viz. Quercetin-7-O-glucoside and Myrecetin-3-O-glucoside and one antibiotic Ciprofloxacin were predicted Class IV as harmful if swallowed, two phytocompounds namely Quercetin and Quercetagetin were predicted Class III as toxic if swallowed while three phytochemicals viz. Lutein, Zeaxanthin and Lutein-5,6-epoxide were predicted Class II as fatal if swallowed (Table 2). The dose-response curves of studied compounds are depicted in Fig 1-13.

Table 2: Prediction of rat oral acute toxicity, class and accuracy of studied flavonoids and synthetic antibiotic

Sl. No.	Compounds	Oral LD_{50} value (mg/Kg)	Predicted toxicity class	Prediction accuracy (%)
Phytocompounds				
1.	Lutein	10	II	69.26
2.	Zeaxanthin	10	II	82.54
3.	Lutein-5,6-epoxide	37	II	61.15
4.	Quercetin	159	III	100.0
5.	Quercetin-7-O-glucoside	5000	IV	83.49
6.	Quercetagetin	159	III	99.02
7.	Kaempferol	3919	V	70.97
8.	Kaempferol-3-O-glucoside	5000	V	72.90
9.	Kaempferitrin	5000	V	70.97
10.	Patuletin	5000	V	70.97
11.	Patulitrin	5000	V	70.97
12.	Myricetin-3-O-glucoside	1190	IV	100.0
Synthetic antibiotic				
13.	Ciprofloxacin	2000	IV	100.0

Class III: toxic if swallowed ($50 < LD50 \leq 300$); Class IV: harmful if swallowed ($300 < LD_{50} \leq 2000$) and Class V: may be harmful if swallowed ($2000 < LD_{50} \leq 5000$)

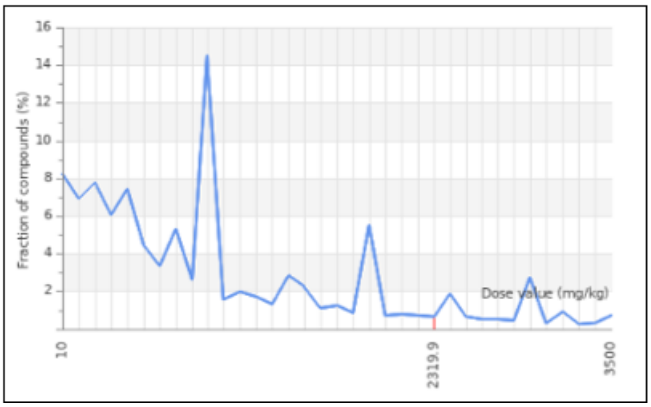

Figure 1: Distribution of dose value for Lutein

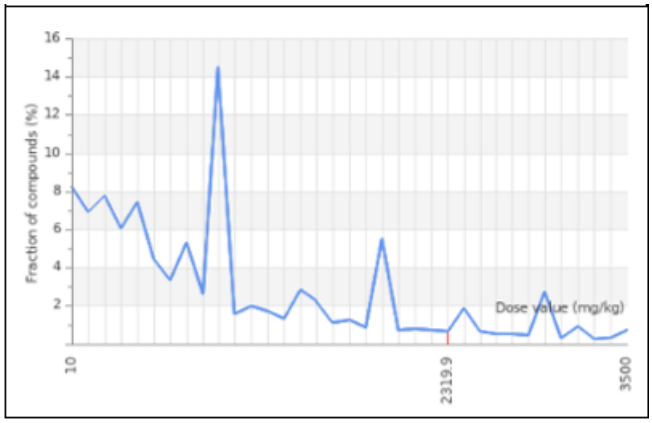

Figure 2: Distribution of dose value for Zeaxanthin

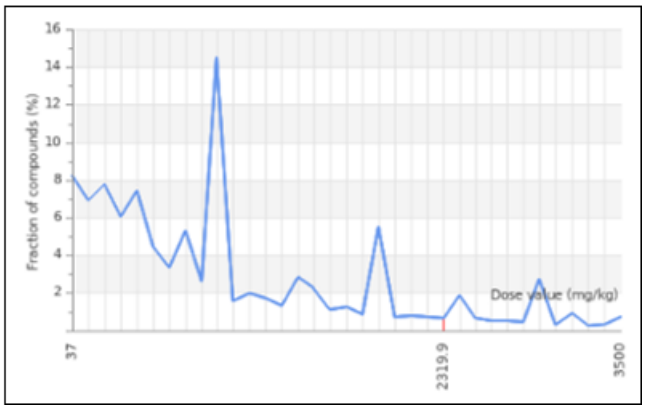

Figure 3: Distribution of dose value for Lutein-5,6-epoxide

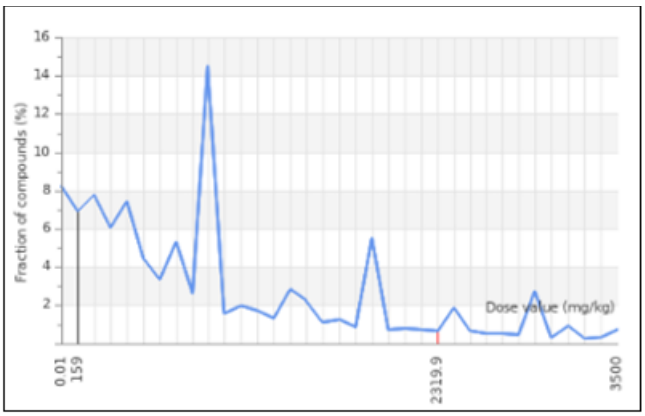

Figure 4: Distribution of dose value for Quercetin

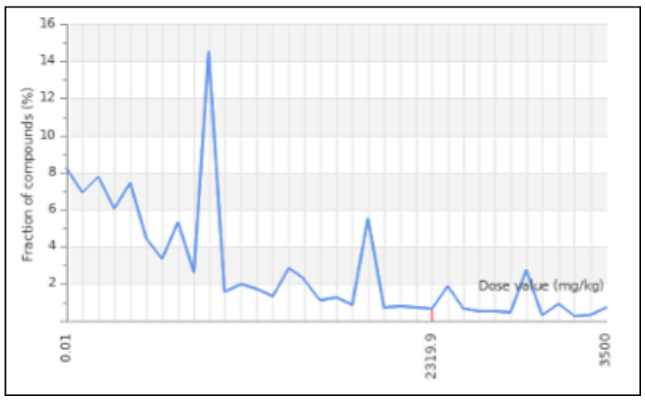

Figure 5: Distribution of dose value for Quercetin-7-O-glucoside

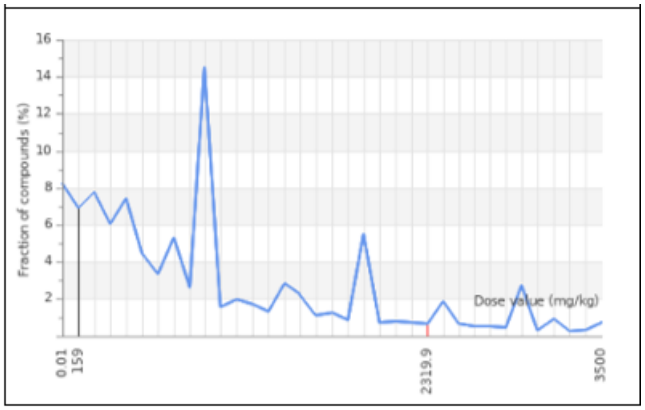

Figure 6: Distribution of dose value for Quercetagetin

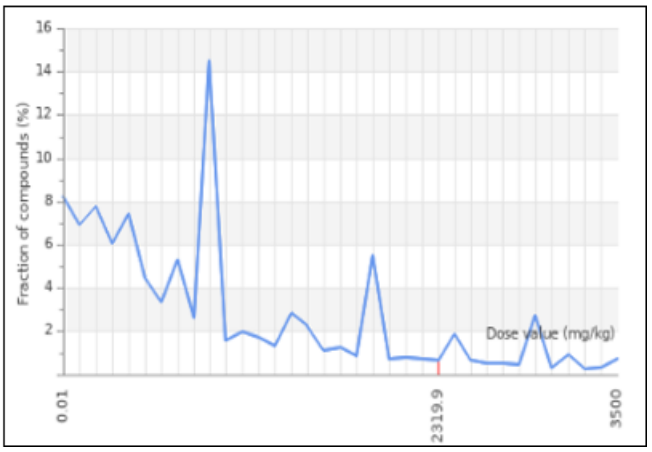

Figure 7: Distribution of dose value for Kaempferol

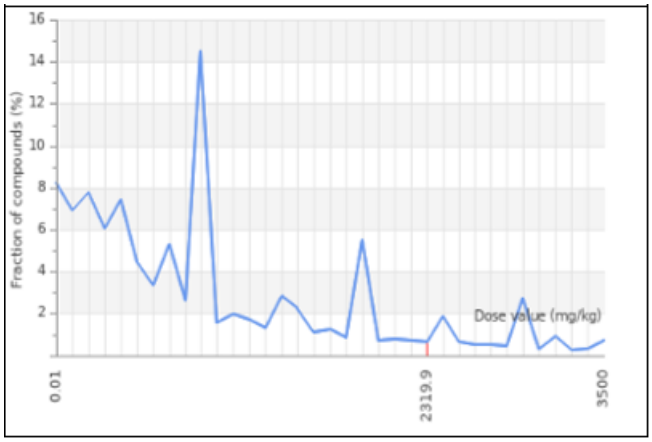

Figure 8: Distribution of dose value for Kaempferol-3-O-glucoside

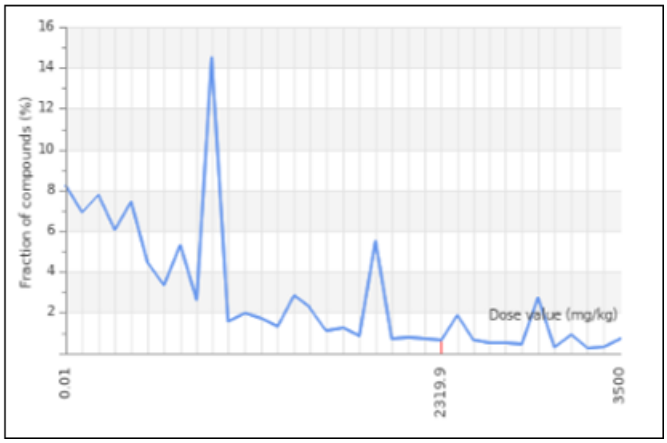

Figure 9: Distribution of dose value for Kaempferitrin

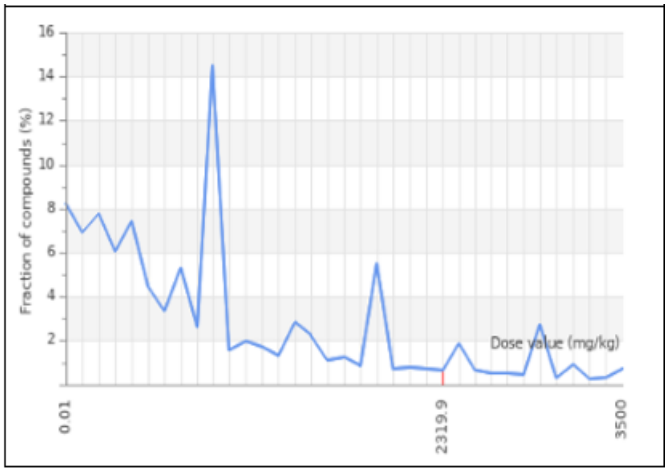

Figure 10: Distribution of dose value for Patuletin

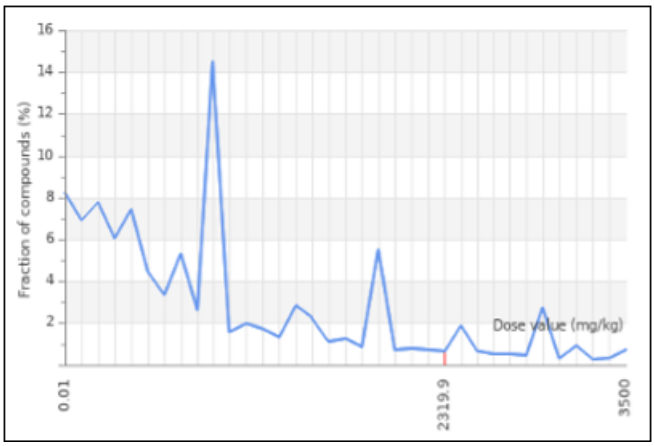

Figure 11: Distribution of dose value for Patulitrin

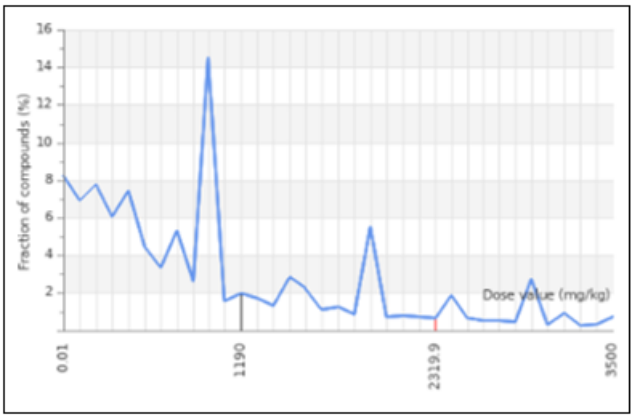

Figure 12: Distribution of dose value for Myricetin-3-O-glucoside

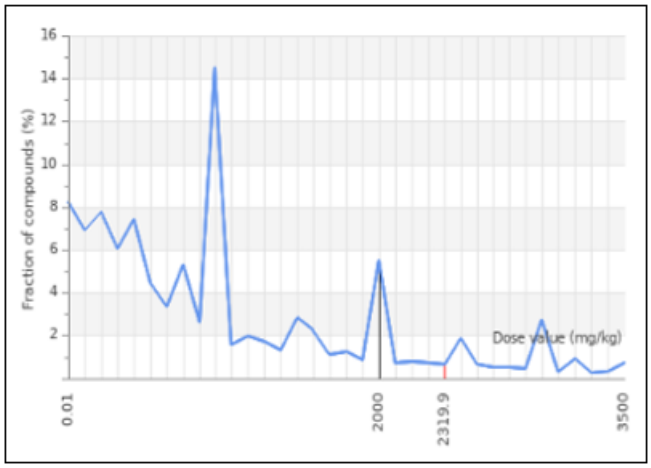

Figure 13: Distribution of dose value for Ciprofloxacin

4. Discussion

The present study has close similarities with other wet lab studies. In the acute oral toxicity test, flavonoids of marigold inflorescence received at the dose of 5000 mg/kg body weight for 14 days, which didn't show any abnormal clinical symptoms or mortality in Sprague-Dawley rats and mice bred in Institute for Cancer Research (both sex, n = 5). [24]

According to Chaniad et al., [25] the ICR mice were treated with a single dose of 2000 mg/kg *T. erecta* aqueous extract on the first day of the experiment following the physical and behavioral alterations were observed daily after long-term treatment for 14 days. It was observed that there were no notable symptoms, such as erection of hair, feeding patterns, vomiting, diarrhoea, abnormal secretion and sleep, or excitement as non-toxic phytocompounds during the experiment. No mortality was observed in any of the ICR mice within the first 24 hrs or for the following 14 days. The lethal doses of the *T. erecta* extract seem to be >2000 mg/kg body weight.

Moreover, the prediction of five phytocompounds (flavonoids) such as Kaempferol, Kaempferol-3-O-glucoside, Kaempferitrin, Patuletin and Patulitrin were confirmed as safe where LD_{50} values obtained 5000 mg/Kg, which may be utilized for future drug compound(s) as compared to synthetic antibiotic namely Ciprofloxacin (2000 mg/Kg).

5. Conclusion

These flavonoids could be suitable phytomedicines as toxicity class of V, i.e., may be harmful if swallowed in the near future. It is suggested that experimental bioassay should be conducted with these flavonoids individually.

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Conflict of interest
None during this study and manuscript preparation.

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