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Research Article

IN SILICO APPROACH FOR ACUTE TOXICITY PREDICTION OF PHYTOCOMPOUNDS PRESENT IN THE FRUIT OF *MUSA* SP. LINN. AND TO DETECT GASTRIC ULCER PROTECTIVE ABILITIES THROUGH RECEPTOR (MMP-9)-LIGAND (FLAVONOIDS) BINDING

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ABSTRACT

Musa sp. Linn. is commonly known asBanana andthis species is used traditionally for the prevention of different diseases and the present *in silico*study concerns on the gastric ulcer protective properties of phytocompounds (flavonoids) of banana fruit. The study was attempted for acute toxicity especially rat oral LD_{50} values of phytocompoundscompared to synthetic medicine Ranitidine through quantitative structure activity relationship (QSAR) modelling and receptor-ligand binding energy and interaction through molecular docking for phytoligandsofbanana compared to Ranitidine on matrix metalloproteinase-9 or MMP-9 (PDB ID: 2OW0)receptor. Thetoxicity prediction was done by using T.E.S.T. (Version 4.1) and molecular docking was performed by usingPyRxtool (Version 0.8).The present toxicity prediction with special reference to rat oral LD_{50} value (mg/Kg) indicated that Vanillic acid and Salicylic acid were obtained lower median lethal dose (LD_{50}) values (747.56 and 815.58) as moderately toxic while higher value was found in β-carotene (8032.73 mg/Kg) as non-toxic compared to Ranitidine (1608.80). Rests of the phytocompounds were obtained moderately toxic. The data of favourable binding energy values (Kcal/mol), three phytoligands such as Quercetin (-8.2), Myricetin (-8.2) and Kaempferol (-8.0) were observed favourable binding energy in comparison with Ranitidine (-5.6). In conclusion, Quercetin and Myricetin of *Musa* sp. showed binding near mouth of the active site at Zn^{2+} ion, which can be lead molecules for the MMP-9 inhibition and may prevent gastric ulcer. It is suggested that these two phytocompounds should be validated through experimental assay to confirming the present prediction.

Keywords: Musa sp., *In silico* study, Toxicity prediction, QSAR modelling, Molecular docking, Gastric ulcer prevention, Phytocompounds

1. INTRODUCTION

A medicinal and nutritional fruit is commonly known as banana (*Musa* sp.) belonging to family Musaceaeand having high nutritive value due to the presence of several bioactive compounds [1]. It is found all over the world as well in India. Among other fruits it is low cost fruit crop. The production of different varieties of banana crops are obtained from India and China.

The ulceration in the gastro-intestinal tract is a common disease in India and the causative factors such as smoking, consumption of alcohol, continuous use of steroidal and nonsteroidal anti-inflammatory drugs (NSAIDs), infection by *Helicobacter pylori*, malnutrition, stress, etc.are reported by several researchers [2-6].

It was already known that the gastric ulcer may lead to cancer due to the upregulation of matrix metalloproteinases (MMPs) [7].

Among several MMPs, MMP-2 observedto participate in the physiological turnover of the gastric extracellular matrix degradationwhile MMP-9 expression found in the early phase of indomethacin-induced chronic gastric ulcers [4, 8].

Interestingly, Swarnakar et al. [4] studied that down-regulation of MMP-9 activity and up-regulation of MMP-2 activity during the prevention of gastric ulcer by using curcumin. Moreover, de Lira Mota et al. [5] reviewed that flavonoids have capacity to prevent gastric ulcer. The well-known fruit as banana is having anti-ulcer activity documented by Rao et al. [9].

Earlier research works revealed mainly isolation of different phytochemicals in banana fruit and peel by solvent extraction [9-13] as well as experimentation by crude extract for the prevention of gastric ulcer [9].

It is unclear that single compound or multiple compounds are protecting gastric ulcer and it is not possible to do experiment on isolation of each compound and study of the prevention of gastric ulcer. In this context, *in silico*study with special reference to toxicity prediction and molecular docking for each phytochemical to know efficacy of the above-mentioned therapy that cannot need long duration, huge laboratory expanses, animal harming, etc. [14-15].Generally, phytocompounds are safe but few compounds are known as allelochemicals [16-17]. Thus, toxicity screening is an important part prior to new drug design.

Present *in silico* study was to predict rat oral acute toxicity through quantitative structure activity relationship (QSAR) modelling and suitable receptor-ligand binding energy and molecular interaction through molecular docking for common bioactive compounds of *Musa* sp. on MMP9 receptor (PDB ID: 2OW0) to prevent gastric ulcer.

2. MATERIALS AND METHODS

2.1. Selection of phytochemicals

The selection of phytochemicals in *Musa* sp. was done based on available literature study [1, 9]. In Table 1, all 19 types of phytochemicals and 1 synthetic drug were listed along with CAS no. and SMILES and these were taken from PubChem database (Table 1).

Table 1: Different flavonoids in banana fruit

Ligands	CAS no.*	SMILES*
α-carotene	7488-99-5	CC1=C(C(CCC1)(C)C)C=CC(=CC=CC(=CC=CC=C(C)C=CC=C(C)C=CC2C(=CCCC2(C)C)C)C)C
β-carotene	7235-40-7	CC1=C(C(CCC1)(C)C)C=CC(=CC=CC(=CC=CC=C(C)C=CC=C(C)C=CC2=C(CCCC2(C)C)C)C)C
β-cryptoxanthin	472-70-8	CC1=C(C(CCC1)(C)C)C=CC(=CC=CC(=CC=CC=C(C)C=CC=C(C)C=CC2=C(CC(CC2(C)C)O)C)C)C
Gallic acid	149-91-7	C1=C(C=C(C(=C1O)O)O)C(=O)O
Catechin	154-23-4	C1C(C(OC2=CC(=CC(=C21)O)O)C3=CC(=C(C=C3)O)O)O
Epicatechin	490-46-0	C1C(C(OC2=CC(=CC(=C21)O)O)C3=CC(=C(C=C3)O)O)O
Anthocyanin	11029-12-2	C1=CC=C(C=C1)C2=[O+]C3=CC=CC=C3C=C2
Ferulic acid	537-98-4	COC1=C(C=CC(=C1)C=CC(=O)O)O
Sinapic acid	7362-37-0	COC1=CC(=CC(=C1O)OC)C=CC(=O)O
Salicylic acid	69-72-7	C1=CC=C(C(=C1)C(=O)O)O
p-hydroxybenzoic acid	99-96-7	C1=CC(=CC=C1C(=O)O)O
Vanillic acid	121-34-6	COC1=C(C=CC(=C1)C(=O)O)O
Syringic acid	530-57-4	COC1=CC(=CC(=C1O)OC)C(=O)O
Gentisic acid	490-79-9	C1=CC(=C(C=C1O)C(=O)O)O
p-coumaric acid	501-98-4	C1=CC(=CC=C1C=CC(=O)O)O
Quercetin	117-39-5	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O)O
Myricetin	529-44-2	C1=C(C=C(C(=C1O)O)O)C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O
Kaempferol	520-18-3	C1=CC(=CC=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O
Cyanidin	13306-05-3	C1=CC(=C(C=C1C2=C(C=C3C(=CC(=CC3=[O+]2)O)O)O)O)O
Ranitidine	66357-35-5	CNC(=C[N+](=O)[O])NCCSCC1=CC=C(O1)CN(C)C

**Obtained from PubChem database (https://pubchem.ncbi.nlm.nih.gov/)*

Table 2: Grid size for studied receptor (in Å)

Receptor	Size			Position from center		
	X	Y	Z	X	Y	Z
PDB ID: 2OW0	80.3179	55.8085	49.4903	41.1978	12.1622	49.0970

2.2. Acute toxicity (rat oral) prediction of phytochemicals by QSAR modelling

The prediction of toxicity of different compounds was done by using software T.E.S.T, Version 4.1 [18]. The predictive values for acute toxicity with special reference to rat oral LD_{50} (median lethal dose) for 19 phytochemicals and 1 synthetic drug was obtained in T.E.S.T. software [18]. The prediction through QSAR modelling was done for each compound after inserting CAS no. in the particular place of the software. All the data were predicted by consensus method, which is basically the average predicted LD_{50} values, which were calculated from average inbuilt QSAR methodologies such as hierarchical clustering method, the FDA-MDL method and nearest neighbor methods [18].

2.3. Selection of receptor

The crystal three-dimensional (3-D) structure of receptorMMP-9(PDB ID: 2OW0) were downloaded from the website of protein data bank (http://www.rcsb.org). The crystal structure was found complexed with five inhibitor molecules (PDB ID: 2OW0) was selected according to the wwPDB validation report [19].This structure was obtained based on X-ray Diffraction method of 2.0 Å. The 3-D ribbon structuresare exhibited in Fig 1 (A-B) after visualizing in MGL Tool developed by The Scripps Research Institute [20].

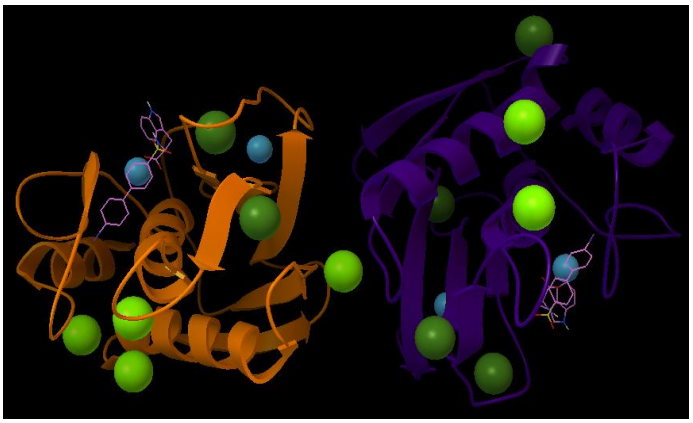

Fig. 1: 3-D ribbon structure of MMP-9 receptor (PDB ID: 2OW0) attached with Zn = blue at 444-445, Ca = green at 446-449, Cl = yellow ball at 450-453 and 6MR = line structure at 501 positions and Zn = blue at 444-445, Ca = green at 446-449, Cl = yellow ball at 450-451 and 6MR (N-[(4'-Iodobiphenyl-4-Yl)Sulfonyl]-D-Tryptophan) = line structure at 502 in chain A and B

2.4. Molecular docking and interaction study

The docking was done by usingPyRx software (Virtual Screening Tool, Ver 0.8) developed by Trott and Olson [21]. The molecular docking was visualized the output .pdbqt file by using MGL tool, developed by The Scripps Research Institute [20] and the results of three-dimensional structure were rendered by using MGL Tools. The docking was carried out for 19 phytochemicals and 1 synthetic chemical on MMP-9 receptor (PDB ID: 2OW0) were studied to know suitable binding energy value. The receptor-ligand interaction of the receptor and phytoconstituents (ligands) were identified to detect the residues involved in each case for the therapeutic efficacy of gastric ulcer.

Table 2 describes the 3-D grid box size values and central position values for docking site on the studied target protein with a grid spacing of 0.375 Å. This tool predicts energy value for each ligand through virtual screening.

3. RESULTS

The predictive study was done on 19 types of common flavonoids of fruit of *Musa* sp. and 1 no. of known anti-ulcer synthetic medicine (Ranitidine).

Table 3: Acute toxicity (rat oral) predictive value of different flavonoids present in banana fruit compared to synthetic medicine through QSAR modeling

Ligands	Rat oral LD_{50} value (mg/Kg)	R^2 value (%)
α-Carotene	3798.80	94
β-Carotene	8032.73	90
β-Cryptoxanthin	3792.95	95
Gallic acid	3912.42	84
Catechin	1367.58	72
Epicatechin	1367.58	72
Anthocyanin	NF	---
Ferulic acid	NF	---
Sinapic acid	NF	---
Salicylic acid	815.58	73
p-Hydroxybenzoic acid	2433.72	74
Vanillic acid	747.56	84
Syringic acid	1336.01	67
Gentisic acid	1521.88	71
p-Coumaric acid	NF	---
Quercetin	2782.81	78
Myricetin	1251.16	81
Kaempferol	2452.85	76
Cyanidin	NF	---
Ranitidine	1608.80	79

NF = CAS no. not found in software

The present toxicity prediction with special reference to rat oral LD_{50} value (mg/Kg) indicated that Vanillic acid and Salicylic acidwere obtained lower LD_{50} values (747.56 and 815.58 mg/Kg) while higher value was found in β-carotene (8032.73 mg/Kg). Rest of the phytocompounds viz. Myricetin, Syringic acid, Catechin and Epicatechin, Gentisic acid, p-Hydroxybenzoic acid, Kaempferol, Quercetin, β-Cryptoxanthin, α-Carotene, Gallic acid, and synthetic drug as Ranitidine were obtained LD_{50} values(mg/Kg) 1251.16, 1336.01, 1367.58, 1521.88, 2433.72, 2452.85, 2782.81, 3792.95,3798.80, 3912.42 and 1608.80 respectively. Five phytocompounds such as Anthocyanin, Ferulic acid, Sinapic acid, p-Coumaric acid and Cyanidin did not obtain the LD_{50} value due to unavailability of CAS no. in the database of the software (Table 3).

Table 4: Binding energy value of different flavonoids present in banana fruit compared to synthetic medicine against MMP9 (PDB ID: 2OW0)

Ligands	Binding energy (Kcal/mol)
Phytoligands	
Quercetin	-8.2
Myricetin	-8.2
Kaempferol	-8.0
Cyanidin	-7.8
α-Carotene	-7.7
Catechin	-7.6
Epicatechin	-7.6
β-Carotene	-7.5
β-Cryptoxanthin	-7.5
Anthocyanin	-7.1
Gallic acid	-6.4
Ferulic acid	-6.4
Sinapic acid	-6.0
Vanillic acid	-6.0
Syringic acid	-5.9
Salicylic acid	-5.8
p-Hydroxybenzoic acid	-5.8
Gentisic acid	-5.8
p-Coumaric acid	-5.8
Synthetic ligand	
Ranitidine	-5.6

In Table 4, the data of favourable binding energy values (Kcal/mol), three phytoligands such as Quercetin (-8.2), Myricetin (-8.2) and Kaempferol (-8.0) were observed suitable in comparison with synthetic medicine Ranitidine (-5.6).

In case of binding pose and interaction for receptor-ligand binding study on MMP-9 (PDB ID: 2OW0), the contact residues viz. GLY176, GLN169, ASP201, ILE198, GLY197, ARG162 at chain A along with CA446 while ARG162 in chain B without hydrogen bonding for Quercetin, the contact residues viz. TYR160, GLY176, ARG162, ASP201, ILE198, GLY197at chain A along with CA446 without hydrogen bonding for Myricetin and the contact residues viz. TYR160, HIS160, GLY176, ARG162, ASP201, ILE198 at chain A without hydrogen bonding for Kaempferol (Fig 3A-C and Fig 3 a-c). But the contact residues SER161, ILE198, TYR160 and GLY176 along with two hydrogen bonding attached with GLN126 and HIS203 were observed (Fig 3D and 3d).

4. DISCUSSION

According to Drwal et al. [22], the toxicity doses and classes such as death if swallowed ($LD_{50} \leq 5$) as Class I, death if swallowed ($5 < LD_{50} \leq 50$) as Class II, toxic if swallowed ($50 < LD_{50} \leq 300$) as Class III, harmful if swallowed ($300 < LD50 \leq 2000$) as Class IV, may be harmful if swallowed ($2000 < LD_{50} \leq 5000$) as Class V and non-toxic ($LD_{50} > 5000$) as Class VI described in ProTox-II webserver. In the present rat oral acute toxicity (LD_{50}) prediction, eight compounds such as Vanillic acid, Salicylic acid, Myricetin, Syringic acid, Catechin, Epicatechin, Gentisic acid and Ranitidine were found as Class IV and p-Hydroxybenzoic acid, Kaempferol, Quercetin, β-Cryptoxanthin, α-Carotene, Gallic acid as Class V while β-carotene as Class VI (Table 3). Some similarities were found in other studies that polyphenols are least toxic [23] and some flavonoids have low acute toxicity effect on mice [24]. In T.E.S.T. software, the statistical interpretation in relation to R^2 value were easily obtained, which are an indication of highly significant data, supported by previous QSAR model [25]. In the T.E.S.T. manual, it was reported that more than 60% R^2 values in QSAR modelling may lead to suitable prediction ability of studied compounds [25].

It was observed that there is binding of ligands near mouth of the active site of MMP-9 with a Zn^{2+} ion. As per Jacobsen et al. [26], the phytochemicals have tendency as zinc binding groups of inhibition for MMP-9. In another experiment, kaempferol-3-O-βrutinoside

(flavonoid) of neem leaf was observed suitable inhibitor for MMP-9 during docking [27]. Likewise, flavonoid containing extract of *Musa* sp. was prevent gastric ulcer in mice reported by Rao et al. [9] and flavonoids as natural products are also suitable for gastroprotective effect reviewed by de Lira Mota et al. [5]. The present study is found a similarity that Quercetin and Myricetin can be used as lead molecules due to active site binding as competitive inhibition of MMP-9. Ultimately, MMP-9 downregulation may lead to the prevention of gastric ulcer, which induced by several factors.

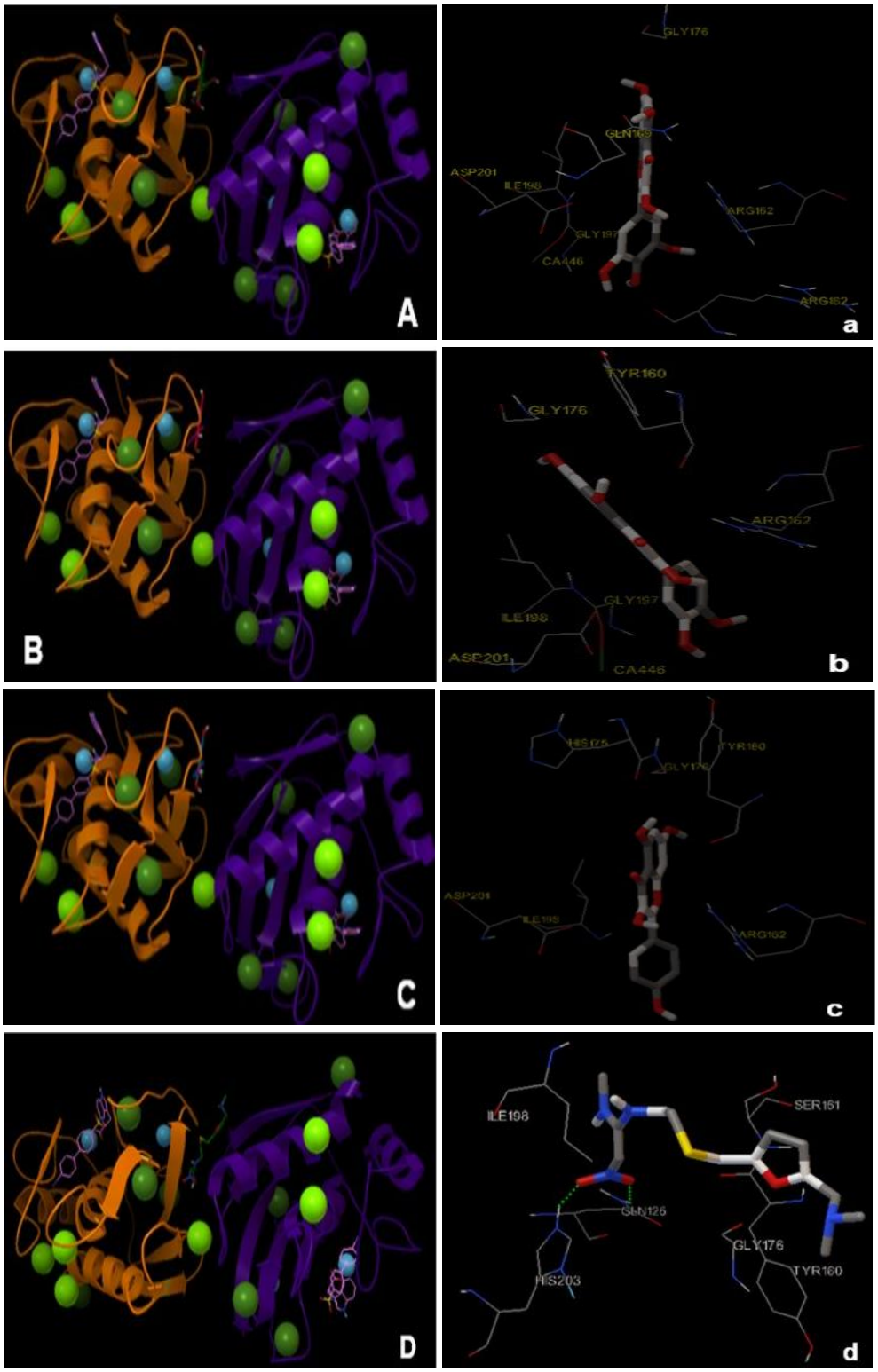

Fig. 2: Binding pose and interaction study of favourable energy based phytoligands on MMP9 (A & a = Quercetin; B &b = Myricetin; C & c = Kaempferol and D & d = Ranitidine)

5. CONCLUSION

It is concluded from *in silico* study that Quercetin and Myricetin of *Musa* sp. can be lead molecules for the MMP-9 inhibition for gastric ulcer prevention. Although, these two phytocompounds are of toxicity Class III and IV and the dose should be experimented prior to drug design. It is suggested that these two phytocompounds should be validated through experimental assay to confirming the present prediction.

6. ACKNOWLEDGEMENT

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Conflict of interest

Authors declare no conflict of interest.

7. REFERENCES

1. Sidhu JS, Zafar TA. *Food Quality and Safety*, 2018; **2:**183-188.
2. Mayty P, Biswas K, Roy S, Barnergee RK, Bandyopadhyay U. *Mol Cell Biochem.*, 2003; **253:**329-338.
3. Prados CMA, Miquel DB. *Rev EspEnferm Dig.*, 2004; **96:**81-82.
4. Swarnakar S, Ganguly K, Kundu P, Banerjee A, et al. *The Journal of Biological Chemistry*, 2005; **280(10):**9409-9415.
5. de Lira Mota KS, Dias GEN, Pinto MEF, Luiz-Ferreira A, et al. *Molecules*, 2009; **14(3):**1420-3049.
6. Amandeep K, Robin S, Ramica S, Sunil K. *International Research of Journal of Pharmacy*, 2012; **3(6):**34-38.
7. Egeblad M, Werb Z. *Nat Rev Cancer*, 2002; **2:**161-174.
8. Lempinen M, Inkinen K, Wolff H, Ahonen J. *EuSurg Res.*, 2000; **32:**169-176.
9. Rao USM, Ahmad BA, Mohd KS, Zin T. *Malaysian Journal of Analytical Sciences*, 2016; **20(5):**1203-1216.
10. Lewis DA, Fields WN, Shaw GP. *Journal of Ethnopharmacology*, 1999; **65(3):**283-288.
11. Mohan Kumar M, Joshi MC, Prabha T, Dorababu M,et al. *Indian Journal of Experimental Biology*, 2006; **44:**292-299.
12. Koffuor GA, Ainoonson GK, Amponsah KI, Addotey JN, et al. *Journal of Medical and Biomedical Science*, (2013); **2(2):**30-39.
13. Bashir AA, Khamsah MM, Abdurrazak M, Mahadeva Rao US et al. *International Journal of Pharmacy and Pharmaceutical Science*, 2015; **7(5):**242-246.
14. Sullivana KM, Manuppellob JR, Willett CE. *SAR and QSAR in Environmental Research*, 2014. doi.org/10.1080/1062936X.2014.907203
15. Gupta M, Sharma R, Kumar A. *Computational Biology and Chemistry*, 2018; **76:**10-217.
16. Cheng F, Cheng Z. *Front Plant Sci.*, 2015; **6:**1020. doi: 10.3389/fpls.2015.01020.
17. War AR, Taggar GK, Hussain B, Taggar MS, et al. *AoB Plants*, 2018; **10(4):** ply037. https://doi.org/10.1093/aobpla/ply037
18. USEPA (United States Environmental Protection Agency), T. E. S. T. Tool, User's Guide for T.E.S.T, Version 4.1.; A Program to Estimate Toxicity from Molecular Structure, Cincinatti, OH, USA; 2012.
19. Tochowicz A, Maskos K, Huber R, O ltenfreiter R, et al. *The Journal of Molecular Biology*, 2007; **371:**989-1006.
20. Morris GM, Goodsell DS, Halliday RS, Huey R, et al. *Journal of Computational Chemistry*, 1998; **19:**1639-1662.
21. Trott O, Olson AJ. *J Comput Chem.*, 2010; **31:**455-461.
22. Drwal MN, Banerjee P, Dunkel M, Wettig MR, et al. *Nucleic Acids Research*, 2014; **42:**W53-W58.
23. Almeida J, Modig T, Petersson A, Hähn-Hägerdal B, et al. *J ChemTechnol Biotechnol.*, 2007; **4:**340-349.
24. Akroum S, Bendjeddou D, Satta D, Lalaoui K. *International Journal of Green Pharmacy*, 2010; 165-169.doi: 10.4103/0973-8258.69174.
25. Golbraikh A, Shen M, Xiao Z, Xiao Y-D, et al. *Journal of Computer-Aided Molecular Design*, 2003; **17(2-4):**241-253.
26. Jacobsen JA, Major Jourden JL, Miller MT, Cohen SM. *Biochimicaet Biophysica Acta*. 2013; **1803:**72-94.
27. Yadav DK, Talapatra SN, Gupta K, Swarnakar S. *Int J Pharm Sci Rev Res.*, 2017; **44(2):**103-111.