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Targeting TLRs with the Derivatives of *Mimosa Pudica*: An *In Silico* Approach

Muniyandi Vijayalakshmi [1], Venkatesh Dhanapradeeba [1], Selvaraj Kunjiappan [1], Krishnan Sundar [1], Sureshbabu Ram Kumar Pandian [1,*]

[1] Department of Biotechnology, Kalasalingam Academy of Research and Education, Krishnankoil-626126, Tamilnadu, India
* Correspondence: srkpandian@gmail.com (S.R.K.P.);

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Abstract: Inflammation is a set of proteins and immune cell interactions that can arise in any part of the human body in response to an infection and an autoimmune reaction. Although this process aims for recovery from infection, dysregulated inflammation leads to persistent tissue damage by myeloid and lymphoid cells. Toll-like receptors (TLRs) are one of the key molecules for the activation of innate immune cells and pro-inflammatory reactions. Therefore, they are recognized as potential targets for treating inflammatory conditions. TLRs are type I transmembrane proteins, playing a crucial role in the pathogenesis of autoimmune diseases. TLRs comes under the family of pattern recognition receptors (PRR) which identify and recognize a wide range of pathogen-associated molecular patterns (PAMPs). In the present study, an Indian medicinal plant, *Mimosa pudica*, widely used in the traditional 'Siddha' and 'Ayurvedha' formulations, has been chosen to target TLRs *in silico*. *M. pudica* was studied for its various activities, including anti-inflammatory. However, their mechanism of activity is not established yet. The metabolites of *M. pudica* were retrieved from the IMPPAT database, and their binding affinity against the targets was studied. Further, the ADMET studies revealed good gastrointestinal absorption for seven- and blood-brain barrier crossing ability for two compounds. The two compounds, β-carotene, and turgorin, could be investigated as candidate drugs, further using *in vitro* and *in vivo* analysis.

Keywords: inflammation; TLR; medicinal plant; *Mimosa pudica*; *in silico*.

1. Introduction

Inflammation is a combinatorial outcome of innate and adaptive immune systems against response to an infection. However, inflammation seldom results in autoimmune- [1], neurodegenerative disorders [2], or cancer [3]. Inflammation is a highly regulated process involving pro-inflammatory cytokines [4], such as tumor necrosis factor (TNF) -α [5], interleukin (IL)-1β [6], and vascular endothelial growth factor (VEGF) [7]. Immune cells such as monocyte [8], macrophages [9], dendritic cells [10], and neutrophils [11] are considered crucial factors of inflammation [12]. Over the years, various synthetic and biological molecules have been developed to treat inflammation, and these anti-inflammatory therapies target immune cells, co-receptors, and cytokines. Macrophages are a heterogeneous population of innate immune cells playing a critical role in homeostasis and disease. M1 and M2 are phenotypical differentiation of macrophages involved in pro-inflammatory and anti-inflammatory responses, respectively [13]. Toll-like receptors (TLRs) play a crucial role in inflammatory reactions. Hence targeting TLR is comprehended as a potential tool for anti-

inflammatory therapy. Down-regulating inflammation by plant metabolites has been demonstrated by various studies. The anti-inflammatory effect of royal Poinciana was demonstrated by inhibiting the TLR 4 pathway [14]. Although diverse plant species were reported in ethnomedicine against inflammation, only a few were demonstrated for anti-inflammatory studies [15].

Mimosa pudica Linn, a medicinal plant that consists of pharmaceutical and nutraceutical potentials, is popular among folk healers to treat several diseases. This plant is admired for its thigmotactic and seismonastic movements [16]. *M. pudica* is recognized for its analgesic, anti-inflammatory [17], hypoglycemic, blood-purifying, diuretic activities [18], diarrhea, insomnia, alopecia, urogenital infections, and wounds [19]. Microwave-mediated extraction of bioactive metabolites from *M. pudica* has been demonstrated earlier using response surface methodology (RSM) and adaptive network-based fuzzy inference system (ANFIS) modeling [20]. The extracts of *M. pudica* were demonstrated for their anti-inflammatory activity *in vitro* using RAW 264.7 and J774A.1 cells. The study extracted fourteen compounds from *M. pudica* and proved the reduction of nitric oxide (NO), tumor necrosis factor (TNF)-α, and interleukin (IL)-β expression in macrophage cells [21]. The present study investigated the potential effect of *M. pudica* metabolites on TLRs using *in silico* methods. The binding efficacy of the screened compounds from the *M. pudica* was analyzed against the TLR. Further, the high binding affinity compounds were scrutinized for ADMET (absorption, distribution, metabolism, excretion, and toxicity) characteristics.

2. Materials and Methods

2.1. Prediction of the target.

The protein sequences of human TLR 4, 7, and 9 were retrieved from the Uniprot database (https://www.uniprot.org/). Since the structures of target proteins are not yet elucidated, they were modeled using the Swiss model server (https://swissmodel.expasy.org), a database that predicts protein 3D structures. The protein structures of TLR 4, 7, and 9 were predicted with appropriate templates that have the highest sequence identity.

2.2. Preparation of ligands.

The bioactive compounds of *M. pudica* were obtained from The Indian Medicinal Plants, Phytochemistry, and Therapeutics (IMPPAT) database, and the standard antagonist (fumaric acid and chloroquinone) of TLR 4, 7, and 9 were identified from research reports [22, 23]. IMPPAT database provides information on 1742 Indian medicinal plants on their phytochemical composition and therapeutic uses. The identified molecule structures of the plant were sketched using Chem draw software 20.1.1.

2.3. Molecular docking.

Molecular docking of *M. pudica* compounds with the targets (TLR 4, 7, and 9) was carried out using the Autodock vina tool incorporated in the PyRx software. PyRx software was used to add polar hydrogen bonds and Kollman partial charges to the 3D structure of the protein. The active binding sites of the proteins were identified using the Prankweb tool (https://prankweb.cz). Based on the scoring functions in PyRx, the binding affinities between the target and ligand were determined. The binding interactions of the target proteins with

bioactive compounds /standard drugs were analyzed by visualization in Discovery studio visualizer 2020 (BIOVIA).

2.4. In silico target prediction.

In computer-aided drug design (CADD), targets are predicted based on structure- or ligand-based using the 3D structure of a protein. Swiss target prediction (http://www.swisstargetprediction.ch) is a web-based tool that predicts the protein targets of bioactive molecules through reverse screening. This is done to ensure that the compounds are not binding to unwanted human targets.

2.5. In silico prediction of ADME properties.

The Swiss ADME (https://www.swissadme.ch) online tool was used to estimate the selected compounds' pharmacokinetics (absorption, distribution, metabolism, excretion) and physicochemical properties together with the standard drugs of TLR 4, 7, and 9. This method is widely used in drug discovery to optimize the properties that convert the lead molecules into a drug. The boiled-egg model generated provides information on brain penetration and human gastrointestinal absorption of the bioactive compounds.

2.6. Toxicity prediction.

The toxicity of the bioactive compounds and standard drugs was analyzed using pkCSM-pharmacokinetic (http://biosig.unimelb.edu.au/pkcsm/prediction), a web-based server. The toxicology prediction of the identified compounds determines the physiological characteristics of the compound to be used as a drug.

3. Results and Discussion

3.1. Retrieval of bioactive compounds & target prediction.

The metabolites of *M. pudica* were analyzed for their anti-inflammatory activity by targeting TLRs 4, 7, and 9 *in silico*. A total of eleven bioactive compounds from *M. pudica* were retrieved from the IMPPAT database (Table 1). The IMPPAT database was utilized by various researchers for the retrieval of plant metabolites [24, 25]. Successful interaction between the identified plant metabolites and target proteins was observed and compared with standard drugs.

Table 1. Phytochemicals name retrieved from IMPPAT database.

S. No	Phytochemical names
1.	β-carotene
2.	β-D-xylopyranose
3.	Crocetin
4.	D-Glucuronic Acid
5.	L-Norepinephrine
6.	Gallic acid
7.	L-ascorbic acid
8.	Linolenic acid
9.	Mimosine
10.	Octadeca-9, 12-dienoic acid
11.	Turgorin

For *in silico* analysis, the structure of the metabolites was sketched by Chem Draw. Fumaric acid [22] was taken as a standard antagonist for TLR4, and chloroquinone [23] was taken as a standard antagonist for TLR 7 and 9. The protein sequences of TLR 4 (839 AA), TLR 7 (1,049 AA), TLR 9 (1032 AA) were retrieved from the Uniprot database, and the Swiss model was employed for homology modeling. The structures were modeled with the templates of 4g8a.1.B (sequence identity of 99.67%), 7cyn.1.A (sequence identity of 100.00%) and 3wpb.1.A (sequence identity of 83.69%), respectively. The modeled proteins were retrieved in PDB format and visualized using Discovery studio visualizer 2020 (BIOVIA). The use of homology modeling for the development of protein structure has been reported earlier [26].

3.2. Molecular docking.

The structure-based molecular docking method was utilized to evaluate the bioactive compounds [27] of *M. pudica*, and the active site of the proteins was identified from the PrankWeb software. Eleven bioactive metabolites of *M. pudica* and the standard antagonists were docked against TLR 4, 7, and 9 using the Autodock vina tool in PyRx software. Out of the eleven docked compounds, β-carotene and turgorin have a greater binding affinity to TLRs when compared with the standard drugs (Table 2). The bioactive compound, β-carotene, showed a higher affinity against TLR 4 (-8.9 kcal.mol-1) and TLR 7 (-8 kcal.mol-1), whereas turgorin showed a better affinity for TLR 9 (-7.6 kcal.mol-1). While the standard drug fumaric acid exerted a binding affinity of -4.3 against TLR 4 and chloroquinone exhibited a binding affinity of -7.1 and -6.3 kcal.mol-1 against TLR 7 and 9. Discovery studio visualizer 2020 (BIOVIA) was used to visualize interactions between the ligands and target proteins. The compounds with higher binding affinity exhibited hydrophobic interaction with the target as a result of interaction between the non-polar surfaces of the molecules. However, turgorin exerts a conventional hydrogen bonding with the targets (TLR 4, 7, and 9), but incidentally, in the case of TLR 4 and 7, β-carotene exerted lower binding energy than turgorin. Both the antagonists, fumaric acid and chloroquinone, exhibited conventional hydrogen bonding with their target proteins.

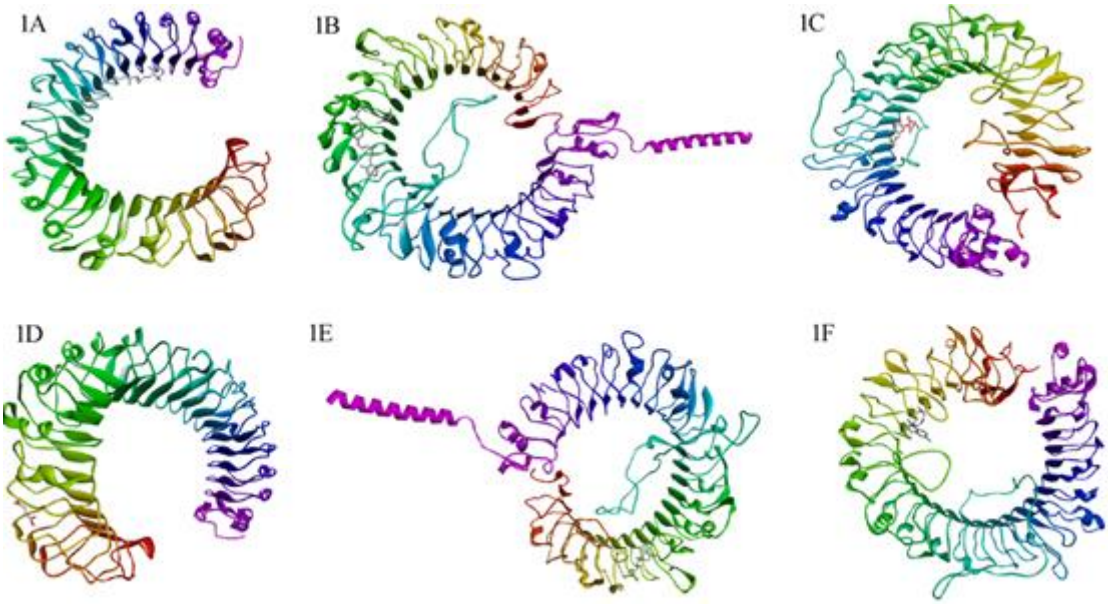

Figure 1. Interactions of bioactive compounds of *M. pudica* with TLR proteins. (**a**) TLR 4 protein with β-carotene, (**b**) TLR 7 protein with β-carotene, (**c**) TLR 9 protein with turgorin, (**d**) TLR 4 protein with fumaric acid, and (**e**) TLR 7 protein with chloroquinone, and (**f**) TLR 9 protein with chloroquinone.

Hydrophobic interactions brought conformational changes to the target proteins more than other conventional interactions. β-carotene created hydrophobic interactions with the amino acids PHE500, VAL524, VAL475, TYR451, ILE450, HIS426, PHE377 with TLR 4 and through VAL381, PHE408, TYR356, LYS432 with TLR 7. Turgorin formed a conventional hydrogen bonding with SER480, ASP478, TYR419, SER529, SER462, and SER504 against TLR 9. The intermolecular interactions between TLR proteins and potential bioactive phytocompounds (higher binding affinity molecules) and standard drugs or antagonists are presented in Figure 1.

Patel *et al*. demonstrated the anti-inflammatory effect of *M. pudica in vitro* and *in vivo*, by inhibiting the expression of pro-inflammatory cytokines. The inflammatory effect was induced by lipopolysaccharide, and the role of *M. pudica* in reducing the expression of nitric oxide (NO), tumor necrosis factor (TNF)-α, and interleukin (IL)-1β expression was demonstrated [21]. The importance of TLR in regulating inflammation was also reported *in vitro*. The extracts of *Delonix regia* were prepared, and its role in reducing LPS induced inflammation was attested by reducing the expression of TLR 4, MyD88, c-Jun N-terminal kinases (JNK), nuclear factor kappa-B cells (NF-kB), and NF-kB inhibitor alpha (IK-Ba) in macrophage cells [14]. Other earlier investigations also assured that TLRs are significant targets for handling inflammatory reactions [15, 16].

Table 2. Binding affinities of *M. pudica* derivatives and the standard antagonists against TLR 4, 7, and 9 identified by PyRx software.

Ligands	Binding affinities (kcal.mol^{-1})		
	TLR 4	TLR 7	TLR 9
β-carotene	-8.9	-8	-7.4
β -D-xylopyranose	-5.3	-5.4	-5.3
Crocetin	-5.9	-6.8	-7.4
D-Glucuronic Acid	-5.4	-6	-6.2
L-Norepinephrine	-5.2	-5.9	-5.6
Gallic acid	-5.3	-6.5	-6.3
L-ascorbic acid	-5.4	-5.9	-5.7
Linolenic acid	-5.3	-5.6	-6
Mimosine	-5.3	-5.7	-6
Octadeca-9-12-dienoic acid	-4.4	-5.7	-6.2
Turgorin	-6.3	-7.3	-7.6
Fumaric acid	-4.3	-	-
Chloroquinone	-	-7.1	-6.3

3.3. In silico target prediction.

The higher binding affinity of β-carotene and turgorin molecules was predicted for its target using the Swiss target prediction (Figure 2). From the results observed β-carotene and turgorin exhibit possible interactions with plasma retinol-binding protein and tyrosyl-DNA phosphodiesterase 1 enzyme, respectively. The values illustrated in Table 3 represent the probability of bioactive molecules binding with the respective targets [30].

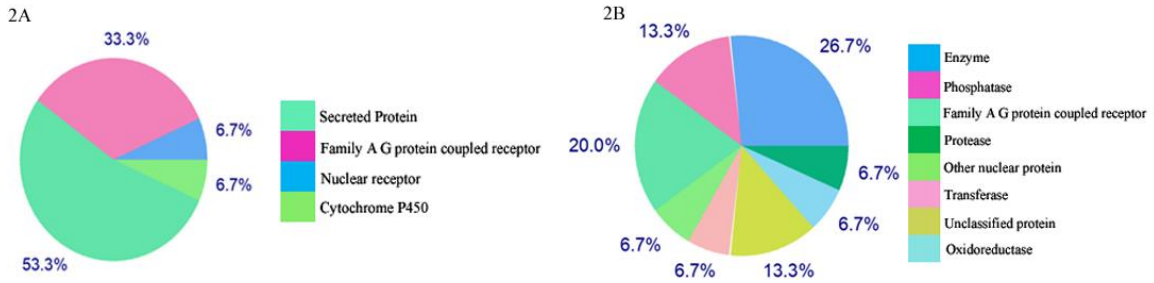

Figure 2. Target classes of high-affinity bioactive molecules (**a**) β-carotene, and (**b**) Turgorin.

3.4. In silico prediction of ADME properties.

Pharmacokinetic and physicochemical characteristics of the *M. pudica* compounds were predicted using Swiss ADME and the standard drugs (Table 4). The pharmacokinetic study focuses dynamic movement of the small molecules or compounds into the body and observing ADME properties [31]. Molecules such as β-carotene, turgorin, octadeca-9-12-dienoic acid, and linolenic acid were observed to contradict the Lipinski's rule of five due to the higher molecular weight (>536.87), higher lipophilicity (>11.11) of β-carotene, higher rate of hydrogen bond donors (>6) and hydrogen bond acceptors (>13) of turgorin, higher lipophilicity (>5.45) of octadeca-9-12-dienoic acid and higher lipophilicity (>5.09) of linolenic acid.

Table 3. Target prediction of high-affinity molecules.

Compounds	Target	Target class	Probability
β-carotene	Plasma retinol-binding protein	Secreted protein	0.087
Turgorin	Tyrosyl-DNA phosphodiesterase 1	Enzyme	0.113

The compound's permeability across the membrane is influenced by the molecular weight and topological polar surface area (TPSA). Compounds with low molecular weight are proved to have enhanced permeability than high molecular weight molecules [32]. Lipophilicity influences the molecule absorption in the body, which suggests that a lower absorption rate is related to a higher logP value [33].

Table 4. ADME analysis of *M. pudica* phytocompounds and standard drugs identified by Swiss ADME tool.

S. No	Compounds	Molecular weight	TPSA	GI absorption	BBB permeation	Pgp substrate	H-bond acceptors	H-bond donors	Consensus logP	Bio-availability	Synthetic accessibility
1.	β-carotene	536.87	0	Low	No	Yes	0	0	11.11	0.17	6.19
2.	β-D-xylopyranose	150.13	90.15	Low	No	No	5	4	-2	0.55	3.8
3.	Crocetin	328.4	74.6	High	No	No	4	2	4.21	0.85	3.99
4.	D-Glucuronic Acid	194.14	127.45	Low	No	Yes	7	5	-2.25	0.56	3.94
5.	L-Norepinephrine	169.18	86.71	High	No	No	4	4	-0.17	0.55	1.69
6.	Gallic acid	170.12	97.99	High	No	No	5	4	0.21	0.56	1.22
7.	L-ascorbic acid	176.12	107.22	High	No	No	6	4	-1.28	0.56	3.47
8.	Linolenic acid	278.43	37.3	High	Yes	No	2	1	5.09	0.85	3.03
9.	Mimosine	198.18	105.55	High	No	No	5	3	-1.96	0.55	2.35
10.	Octadeca-9, 12-dienoic acid	280.45	37.3	High	Yes	No	2	1	5.45	0.85	3.1
11.	Turgorin	411.32	231.72	Low	No	No	13	6	-2.21	0.11	4.57
12.	Fumaric acid	116.07	74.6	High	No	No	4	2	-0.35	0.85	1.8
13.	Chloroquinone	319.87	28.16	High	Yes	No	2	1	4.15	0.55	2.76

The ADME analysis highlights that the compounds β-carotene, β-D-xylopyranose, calcium, D-glucuronic acid, and turgorin exhibit a lower absorption rate in humans. Fumaric acid and chloroquninone adhere to the Lipinski rule and demonstrate higher absorption in the human intestine, but chloroquinone crosses the blood-brain barrier. The synthetic accessibility of β-carotene and turgorin was 6.19 and 4.57, respectively, which is important in developing a lead molecule. However, the bioavailability of β-carotene (0.17) and turgorin (0.11) is lower than other compounds and standard drugs. The bioavailability of drugs Fumaric acid and Chloroquinone was found to be 0.85 and 0.55, while their synthetic accessibility is 1.8 and 2.76, respectively. Therefore, the compounds β-carotene and turgorin have the potential ability to become drug equivalent for the inflammatory process.

The two important pharmacokinetic characteristics, namely passive gastrointestinal absorption (HIA) and blood-brain barrier (BBB) penetration, were predicted using a boiled egg model. The presence of these molecules in yolk and albumin indicates the BBB penetration and gastrointestinal absorption of the compounds, respectively. The Swiss ADME boiled egg analysis was employed in previous studies to analyze drug likeliness properties [34],[35]. β-carotene and turgorin are not BBB permeant due to the TPSA of β-carotene and turgorin (0 A^0 and 231.72 A^0) which is comparable to fumaric acid (74.6 A^o). D-glucuronic acid and β-carotene were found to be Pgp^+ substrates. Differential absorption of other bioactive molecules is depicted in Figure 3. Earlier investigations revealed that β-carotene [36] and turgorin play a significant role in inflammation. The drug likeliness score of norepinephrine of *M. pudica* extract was 1.41 [37]. In rats, hexane, ethyl acetate, and methanol extracts of M. pudica roots were lethal at 100kg/mg concentration [38].

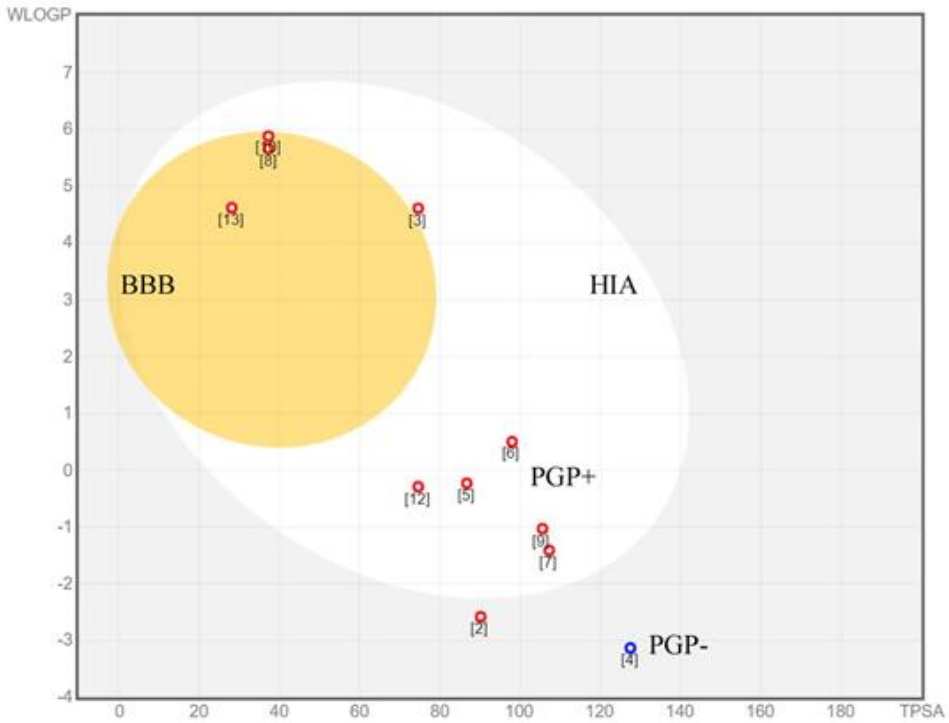

Figure 3. Boiled egg model predicted by Swiss ADME for the bioactive compounds of *M. pudica* and standard drug of TLR-4,7, and 9 (fumaric acid and chloroquinone).

3.5. Toxicity prediction.

Toxicity data was generated using Swiss ADME concerning the adverse effects of a drug. It was identified that 20% of drug fails in the development process due to a positive range of toxicity. *In silico* analysis of toxicity avoids animal trials which are expensive and time-consuming [39]. *In silico* toxicity prediction using pkCSM-pharmacokinetic for the active biomolecules and elements of *M. pudica* was carried out. The parameters include AMES toxicity, drug-induced hERG toxicity, which mediates the cardiac action potential by potassium ion channels, LD50, hepatotoxicity, and skin sensitization. Most of the compounds of *M. pudica* were observed not to cause hepatotoxicity and skin sensitization except dl-Norepinephrine and octadeca-9-12-dienoicacid. Meanwhile, linolenic acid caused skin sensitization, and octadeca-9-12-dienoic acid, linolenic acid, turgorin, and chloroquinone exhibited hepatotoxicity. Various toxicity profiles predicted are indicated in Table 5.

Table 5. Toxicity prediction of bioactive compounds of *M. pudica* and standard drug of TLR-4,7 (Fumaric acid, Chloroquinone).

Compounds	AMES toxicity	Max. tolerated dose (human)	hERG I inhibitor	hERG II inhibitor	Oral rat acute toxicity (LD50)	Oral rat chronic toxicity (LOAEL)	Hepatotoxicity	Skin sensitisation	*T. pyriformis* toxicity	Minnow toxicity
β-carotene	Yes	-0.379	No	Yes	2.073	0.65	No	No	0.326	-4.028
β-D-xylopyranose	No	1.852	No	No	0.892	3.307	No	No	0.283	4.229
Crocetin	No	-0.558	No	No	2.094	2.53	No	No	0.298	-0.581
D-Glucuronic Acid	No	1.838	No	No	1.314	4.037	No	No	0.285	4.57
L-Norepinephrine	No	0.815	No	No	2.221	1.465	No	Yes	0.037	3.313
Gallic acid	No	0.7	No	No	2.218	3.06	No	No	0.285	3.188
L-ascorbic acid	No	1.598	No	No	1.063	3.186	No	No	0.285	4.386
Linolenic acid	No	-0.84	No	No	1.441	3.115	Yes	Yes	0.722	-1.183
Mimosine	No	1.012	No	No	2.166	1.771	No	No	0.27	3.558
Octadeca-9, 12-dienoic acid	No	-0.827	No	No	1.429	3.187	Yes	Yes	0.701	-1.31
Turgorin	No	0.664	No	No	2.408	3.655	Yes	No	0.285	8.164
Fumaric acid	No	0.69	No	No	1.626	3.029	No	No	-0.065	2.956
Chloroquninone	Yes	-0.167	No	Yes	2.85	1.026	Yes	No	1.558	0.747

4. Conclusions

Over many centuries plant extracts have been used as traditional medicine in treating various ailments. Research on discovering bioactive compounds in plant extracts has its applications in the food, cosmetics, and pharmaceutical industries. TLRs play a crucial role in activating immune cells leading to pro-inflammatory reactions. The present study deals with the *in silico* analysis of *M. pudica* metabolites on TLRs. The standard drugs of TLR 4, 7, and 9 were taken for comparison. The bioactive compounds, β-carotene exhibited greater binding affinity towards TLR 4 (-8.9 kcal.mol^{-1}) and TLR 7 (-8 kcal.mol^{-1}), whereas turgorin showed a higher binding affinity towards TLR 9 (-7.6 kcal.mol^{-1}) which was higher than the known antagonists fumaric acid (-4.3 kcal.mol^{-1} for TLR4) and chloroquinone (-6.3 & -7.1 kcal.mol^{-1} for TLR 7 and 9). Therefore, the bioactive compounds with higher binding affinity could effectively regulate immune cells. Compared to the standard drug, the physicochemical and pharmacokinetic property of identified bioactive compounds (β-carotene and turgorin) predicts that the bioactive compounds are non-toxic to humans and could be used as a potential lead molecule in drug discovery. Though the identified compounds face some restrictions in ADME analysis, these metabolites can be evaluated further *in vitro* and *in vivo to* be used as a potential candidate drug for targeting TLRs.

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Conflicts of Interest

The authors declare no conflict of interest.

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