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ADMET & CYTOTOXICITY PREDICTION OF RED SEAWEED *GRACILLARIA DURA*: AN IN SILICO APPROACH

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ABSTRACT

In the present study, marine red algae *Gracillaria dura* was selected from the study performed by Biji Cyriac and K. Eswaran (2015). The screening of phycocompounds was carried out by using GCMS analysis from which a total of 10 compounds selected for this study. Chemical structure details (such as molecular weight, CAS, EC number, SMILE file etc.) of selected phycocompounds obtained from PubChem database. Prime requisite ADMET properties were predicted by using Danish (Q)SAR. Then, toxicity prediction was done by using ProTox-II whereas cytotoxicity (for both normal and cancerous cell line) prediction was carried out by using online webtool CLC Pred. The above study showed that the Ethyl cholate and Squalene compound can be applied as a drug candidate as well as further pharmacological evaluation.

KEYWORDS: *Gracillaria dura*, Toxicity, ProTox-II, In-silico, Phycocomopund.

INTRODUCTION

Marine algae similarly known as seaweeds, considered as a potential source of primary as well as secondary metabolites that might useful in the designing of new medicinal agents.[1] It can be classified into three different types of Chlorophyta (green algae), Phaeophyta (brown algae) and Rhodophyta (Red algae) based on the presence of its pigments.[2]

Algae is well famous for the richness of its bioactive constituents.[3] It contains a huge amount of primary and secondary metabolites such as polysaccharides, proteins, amino acids,

MAAs, fatty acids, pigments[3] etc. This type of phycocompounds exhibits a different range of biological activities such as Antioxidant, Antimicrobial, Skin itching, Anti-aging, Photoprotection, Cosmeceutical activities, Food and Dairy industry, Health nourishments, etc. Due to the beneficial effects of this marine resource, it can be used in a wide range of applications.

As suggested above, marine algae exhibit an antimicrobial activity due to the presence of bioactive components such as phenol, flavonoid, fatty acids, polysaccharides etc. responsible for the above applications.[4][5] The rate of drug designing and discovery become potential with the means of virtual screening. It is similarly known as In-Silico analysis.[6] Advantageous point of view, fast prediction for a large set of the query in a high throughput mode possible only in this approach.[7] Various methods include databases, QSAR (Quantitative Structure-Activity Relationship), homology modeling, pharmacophore, ADMET prediction, data mining, data analysis, Toxicity prediction, Drug-Target interaction study possible by means of computational tools and technique within this computational approach. Among all screening, toxicity testing of new compounds is essential for the drug development process. This is a part of a preclinical study. This prediction helps to calculate the "No Adverse Effect level" that helps to initiate a clinical investigation.[8] Nowadays there are many in silico tools available for toxicity prediction such as OpenTox, Danish (Q)SAR, VEGA QSAR, OECD-QSAR, Nexus, METEOR etc.[9]

ADMET – Absorption, Distribution, Metabolism, Excretion and Toxicity study play a pivotal role in drug discovery and development. Appropriate and adequate ADMET properties are the primary requisite of a therapeutic agent.[10] Nowadays, there are many freely available software like DSSTox, CPDB, PK Tutor etc. useful to predict ADMET properties whereas many online web servers such as preADMET prediction, VEGA QSAR, Danish (Q)SAR, Molinspiration available to predict compounds.

ProTox-II is a virtual web tool for the prediction of toxicities of small molecules of phytochemicals.[11] It is helpful to predict acute Toxicity, Hepatotoxicity, LD50, Cytotoxicity, Carcinogenicity, Mutagenicity, Toxicity class, Toxicity pathway as well as toxicity targets.[12] [13] LD50 - lethal dose, 50% or medial lethal dose is the amount of substance required to kill 50% test population. LD 50 values are given in (mg/kg).[14][15] Toxicity class can be predicted as following:[16][17] Toxicity class prediction revealed total six different toxicity groups in which five classes representing different grades as well as non-toxicity class. In addition, a

radar chart is the illustration of the confidence of positive toxicity results compared to the average of its class.

The aim of the present study is to check the applicability of red algae *Gracillaria dura* as a drug candidate in pharmacological practices. The phycocompounds of selected algae were screened by GCMS analysis that would be further checked for the ADMET properties by using Danish (Q)SAR. The toxicity of selected phycocompounds was predicted by using ProTox-II whereas the drug bioavailability (Lipinski's Rule of 5) screened out by using software DruLito. In last, cytotoxicity or effects of thy compounds on normal or cancer cell lines predicted by using CLCPred.

MATERIALS AND METHODS

SELECTION OF PHYCOCOMPOUNDS

In this study, marine red alga *Gracillaria dura* was selected from the study performed by Biji Cyriac and K. Eswaran (2015). The selected research study is GC - MS determination of bioactive components of *Gracilaria dura* (C.Agardh) J. Agardh.[18]

GCMS ANALYSIS

The phycocompounds screening was carried out by Gas chromatography-mass spectrometry (GC-MS) technique. This is a combination of of gas-chromatography as well as mass spectrometry to identify different chemical substances within a test sample. GCMS analysis revealed 11 phycocompounds as listed in table no.1 Out of 11, a total of 10 compounds were selected for this study due to the unavailability of EC number of E2-tetracene-1OL for further study.

DATA RETRIEVAL FROM PUBCHEM DATABASE

PubChem is a chemical database that provides a platform for a chemical information such as search chemicals by name, molecular formula, structure, and other properties include chemical and physical properties, biological activities, safety and toxicity information etc. (https://pubchem.ncbi.nlm.nih.gov/).[19] This is the platform that contains data for more than 6,00,000 chemical substances. The simplified molecular-input line-entry system (SMILES) is a specification in the form of a line notation for describing the structure of chemical compounds. This file was downloaded from the same which used as an input for further toxicity as well as ADMET prediction.[20]

TOXICITY PREDICTION

Danish (Q)SAR

The Danish (Q)SAR Database is a platform that provides information related to physicochemical properties, ecotoxicity, environmental Fate, ADME and toxicity. In this study, toxicity for different targets selected as follow: Gastrointestinal absorption(1 mg dose in %), Dermal absorption, Brain barrier penetration, MRDD in humans, severe skin irritation in rabbit, allergic contact dermatitis in human, respiratory sensitization in humans and Mutagenicity AMES test.[21,22,23] It required a smile format as an input to predict toxicity. (http://qsar.food.dtu.dk/)

LIPINSKI'S RULE OF FIVE

Pfizer's rule of five, which is helpful to check drug-likeness(oral availability) or determine if a chemical compound with a certain pharmacological properties that would market a likely orally active drug. The violation of 2 or more of these conditions predicts a molecule as a non-orally available drug.[24]

DruLiTo – Drug Likeness Tool, is an open-source virtual screening tool for calculation of drug-likeness such as Lipinski is the rule, MRDD like a rule, verbal rules, BBB ruler etc.[25] (http://www.niper.gov.in/pi_dev_tools/DruLiToWeb/DruLiTo_index.html)
Bioavailable compound (orally active) must possess No. of HBD<=5, No. of HBA<=10, Molecular weight<500 and CLogP<=5.

ProTox-II

Toxicity class and LD50 prediction for different phycocompounds carry out by using ProTox-II. And the radar chart prepared for a significant compound that shows a suitable outcome.[26, 27] The Radar chart is the illustration of the confidence of positive toxicity results compared to the average of its class. (http://tox.charite.de/protox_II/)

CYTOTOXICITY PREDICTION BY CLC PRED

It is a cell line cytotoxicity predictor, browsing tool for in silico prediction of cytotoxic effects of phycocomponents.[28] It can be check on non-transformed and cancers cell lines base on structural formula. it is used to check the suitability of phycocompounds for experimental screening. There are many bioinformatics tools widely used for the prediction of cytotoxicity such as flax CTTX, CLCpred, QSAR, Bio –tools etc.[29]

Prediction of phycocompounds carried out by using an input SMILE structure in searching tool.(http://www.way2drug.com/Cell-line/) In output, Pa (probability "to be active") estimates the chance that the studied compound is belonging to the subclass of active compounds. Pi (Probability "to be inactive") estimates the chance that the studied compound is belonging to the subclass of inactive compounds. Only activities with Pa>Pi are considered as possible for a particular compound.[30, 31]

RESULTS AND DISCUSSION

RESULTS

Table 1: Phycochemicals obtained in GC-MS output.

NO	Name of compound
1	3,7,11,15-tetrametyl 2-hexadecen-1OL
2	E2-tetracen-1OL
3	2-Tridecen1-OL
4	Ethyl cholate
5	Pentadecanoic acid, 14-methyl-methyl ester
6	n-Hexadecanoic acid
7	Oleic acid
8	Palmitoleic acid
9	9,12-octadecanoic Acid
10	9-octadecanoic Acid
11	Squalene

Table 2: Chemical structural details retrieved from the PubChem database.

NO	Name of compound	Pub Chem ID	Molecular formula	Molecular weight g/mol	CAS	EC
1	3,7,11,15-tetrametyl 2-hexadecen-1OL	5366244	C20H40O	296.5	7541-49-3	205-776-6
2	E2-tetracen-1OL	5353006	C14H28O	212.37	51534-36-2	-----
3	2-Tridecen1-OL	5364949	C13H26O	198.34	68480-25-1	270-902-9
4	Ethyl cholate	6452096	C26H44O5	436.6	47676-48-2	256-328-1
5	Pentadecanoic acid, 14-methyl-methylester	23518	C16H32O2	270	7132-64-1	273-095-1
6	n-Hexadecanoic acid	985	C16H32O2	256.43	57-10-3	200-312-9
7	Oleic acid	445639	C18H34O2	282.5	112-80-1	270-164-8
8	Palmitoleic acid	445638	C16H30O2	254.41	373-49-9	206-765-9
9	9,12-octadecanoic Acid	5280450	C18H32O2	280.4	60-33-3	200-470-9
10	9-octadecanoic Acid	637517	C18H34O2	282.5	112-79-8	204-006-6
11	Squalene	638072	C30H50	410.7	112-02-4	203-826-1

Table 3: Smile structure obtained from PubChem.

NO	Name of compound	SMILE ID
1	3,7,11,15-tetrametyl 2-hexadecen-1OL	CC(C)CCCC(C)CCCC(C)CCCC(=CCO)C
2	E2-tetracen-1OL	CCCCCCCCCCCC=CCO
3	2-Tridecen1-OL	CCCCCCCCCCC=CCO
4	Ethyl cholate	CCOC(=O)CCC(C)C1CCC2C1(C(CC3C2C(CC4C3(CCC(C4)O)C)O)O)C
5	Pentadecanoic acid, 14-methyl-methylester	CCCCCCCCCCCCCCC(=O)OC
6	n-Hexadecanoic acid	CCCCCCCCCCCCCCCC(=O)O
7	Oleic acid	CCCCCCCCC=CCCCCCCCC(=O)O
8	Palmitoleic acid	CCCCCC/C=C\CCCCCCCC(=O)O
9	9,12-octadecanoic Acid	CCCCCC=CCC=CCCCCCCCC(=O)O
10	9-octadecanoic Acid	CCCCCCCCC=CCCCCCCCC(=O)O
11	Squalene	CC(=CCCC(=CCCC(=CCCC=C(C)CCC=C(C)CCC=C(C)C)C)C)C

Table 4: Toxicity prediction for each phycocompounds by using Danish (Q)SAR.

No.	Name of Molecules	Severe Skin Irritation in Rabit	Allergic Contact Dermatitis in Human	Respiratory Sensitization in Human	Mutagenicity Ames Test	Gastrointestinal Absorption 1 Mg Dose(%)	MRDD In Humans
1	3,7,11,15-tetrametyl 2-hexadecen-1OL	POS_IN	NEG_IN	INC_OUT	NEG_IN	100	INC_OUT
2	2-Tridecen1-OL	POS_IN	NEG_IN	NEG_IN	NEG_IN	100	NEG_OUT
3	Ethyl cholate	NEG_OUT	NEG_IN	NEG_IN	NEG_IN	100	NEG_IN
4	Pentadecanoic acid, 14-methyl-methylester	INC_OUT	NEG_IN	NEG_IN	NEG_IN	100	NEG_OUT
5	n-Hexadecanoic acid	INC_OUT	NEG_IN	INC_OUT	NEG_IN	100	NEG_IN
6	Oleic acid	POS_OUT	NEG_IN	INC_OUT	NEG_IN	100	NEG_OUT
7	Palmitoleic acid	POS_OUT	NEG_IN	INC_OUT	NEG_IN	100	NEG_OUT
8	9,12-octadecanoic Acid	NEG_OUT	NEG_IN	INC_OUT	NEG_IN	100	NEG_OUT
9	9-octadecanoic acid	POS_OUT	NEG_IN	INC_OUT	NEG_IN	100	NEG_OUT
10	Squalene	NEG_IN	NEG_IN	NEG_IN	NEG_IN	100	INC_OUT

Table 5: Toxicity prediction details by ProTox-II.

No.	Name of compound	Predicted LD 50(mg/kg)	Predicted Toxicity Class
1	3,7,11,15-tetrametyl 2-hexadecen-1OL	N.A.	N.A.
2	2-Tridecen1-OL	5000	5
3	Ethyl cholate	N.A.	N.A.
4	Pentadecanoic acid, 14-methyl-methyl ester	N.A.	N.A.
5	n-Hexadecanoic acid	130	3
6	Oleic acid	48	2
7	Palmitoleic acid	48	2
8	9,12-octadecanoic Acid	N.A.	N.A.
9	9-octadecanoic Acid	48	2
10	Squalene	5000	5

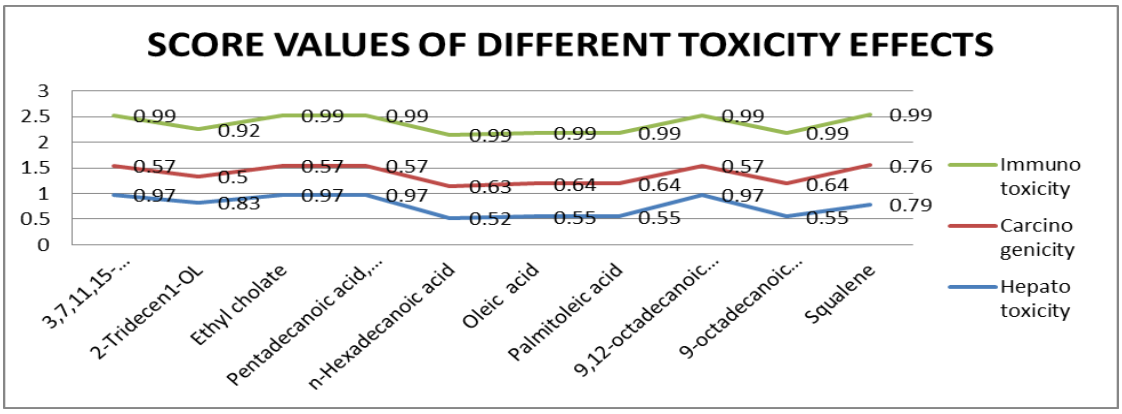

Fig. 1: Effects of phycocompounds on Immunotoxicity, Carcinogenicity & Hepatotoxicity.

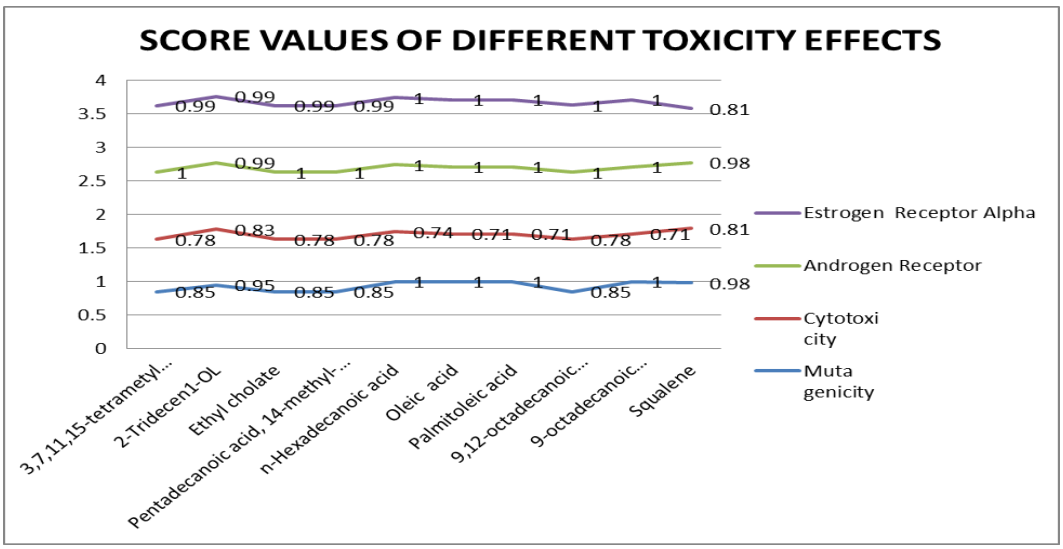

Fig. 2: Effects of phycocompounds on Cytotoxicity, Mutagenicity, Estrogen, and Androgen.

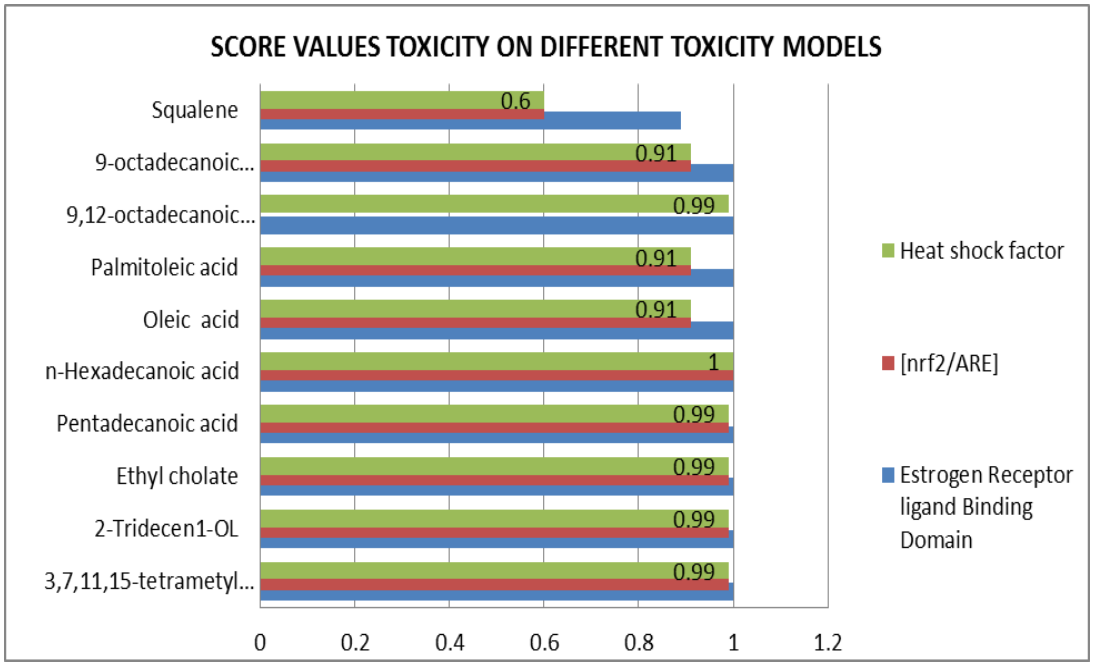

Fig. 3: Score values of phycocompounds on Heat Shock Factor, Nrf2/Are & Estrogen Receptor Binding domain.

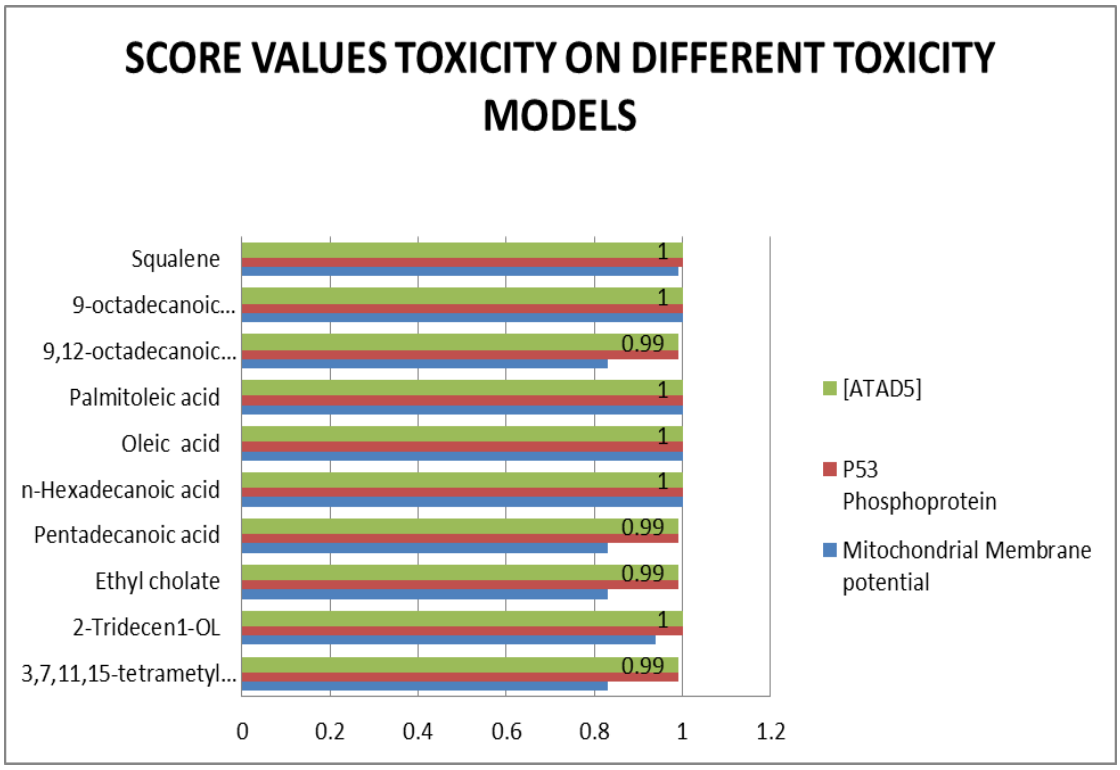

Fig. 4: Score values of phycocompounds on Atad5, P53 & Mitochondrial Membrane Protein Complex.

Table 6: Lipinski's Rule of 5 study for all phycocompounds by using DruLito.

No.	TITLE	MW	Log p	HBA	HBD	Total No Of molecules Violated The Rule
1	5366244	296.31	8.949	1	1	1
2	5364949	198.2	5.656	1	1	1
3	6452096	436.32	4.837	5	3	0
4	23518	256.24	7.322	2	0	1
5	958	256.24	7.57	2	1	1
6	445639	282.26	8.192	2	1	1
7	445638	254.22	7.054	2	1	1
8	5280450	280.24	7.865	2	1	1
9	637517	282.26	8.192	2	1	1
10	638072	410.39	11.482	0	0	1

Table 7: Cytotoxicity prediction of Ethyl cholate by using CLCPred.

EFFECT OF ETHYL CHOLATE ON VARIOUS CANCER CELL LINES					
Pa	**Pi**	**Cell line**	**Cell line full name**	**Tissue**	**Tumor type**
0.649	0.008	UO-31	Renal carcinoma	Kidney	Carcinoma
0.583	0.012	EKVX	Non-small cell lung carcinoma	Lung	Carcinoma
0.575	0.015	HepG2	Hepatoblastoma	Liver	Hepatoblastoma
0.546	0.012	LOX IMVI	Melanoma	Skin	Melanoma
0.463	0.021	HOP-92	Non-small cell lung carcinoma	Lung	Carcinoma
0.490	0.056	NCI-H838	Non-small cell lung cancer. 3 stage; Lung	Lung	-------
EFFECT OF SQUALENE ON VARIOUS CANCER CELL LINES					
0.756	0.005	RXF 393	Renal carcinoma	Kidney	Carcinoma
0.717	0.008	HOP-92	Non-small cell lung carcinoma	Lung	Carcinoma
0.683	0.008	U-251	Giloma	Brain	-----
0.669	0.007	SNB-75	Giloblastoma	Nervous system	-----
0.680	0.022	MCF7	Breast carcinoma	Breast	Carcinoma
0.665	0.007	UO-31	Renal carcinoma	Kidney	Carcinoma
0.666	0.009	786-0	Renal carcinoma	Kidney	Carcinoma
0.634	0.005	RPMI-8226	Multiple myelomas	Hematopoietic and lymphoid tissue	Myeloma
0.609	0.010	KM12	Colon adenocarcinoma	Colon	Adenocarcinoma
0.603	0.012	OVCAR-4	Ovarian adenocarcinoma	Ovarium	Adenocarcinoma
0.594	0.010	LOX IMVI	Melanoma	Skin	Melanoma
0.593	0.012	A498	Renal carcinoma	Kidney	Carcinoma
0.589	0.012	TK-10	Renal carcinoma	Kidney	Carcinoma
0.583	0.011	SF-295	Giloblastoma	Brain	-----
0.579	0.011	SR	Adult immunoblastic lymphoma	Hematopoietic and lymphoid tissue	Lymphoma
0.561	0.014	Malme-3M	Melanoma	Skin	Melanoma
0.553	0.014	IGROV-1	Ovarian adenocarcinoma	Ovarium	Adenocarcinoma
0.539	0.022	NCI-H226	Non-small cell lung	Lung	Carcinoma

			carcinoma		
0.538	0.04	NCIH522	Non small cell lung carcinoma	Lung	Carcinoma
0.512	0.019	MDA-MB-231	Melanoma	Skin	Melanoma

PubChem STUDY

Table 1 showed the name of compounds that selected for this present study whereas the compound's name with its with PubChem ID and molecular formula, contains the molecular weight, CAS and EC id of all listed in table no. 2. SMILE structure used as an input for prediction of ADMET properties as well as toxicity in Danish (Q)SAR and ProTox-II, respectively. All selected compounds with its smile structure listed in table no. 3.

ADMET PREDICTION

According to Danish (Q)SAR model, out of total ten phycocompounds – only two compounds named Ethyl cholate and Squalene reported no toxicity Whereas other compounds such as 3, 7, 11, 15-tetramethyl 2-hexadecane-1OL, Oleic acid, Palmitoleic acid and 9-octadecanoic Acid revealed positive toxicity ef fects on selected targets such as on Severe Skin Irritation in Rabit. In addition 3, 7, 11, 15-tetramethyl 2-hexadecane-1OL, n-Hexadecanoic acid, Oleic acid, Palmitoleic acid and 9-octadecanoic Acid reported inconclusive result that may be toxic or nontoxic. Toxicity prediction for each phycocompounds by using Danish (Q)SAR tabulated in table no. 4.

LIPINSKI'S RULE OF FIVE

RO5 or Lipinski's rule of five, used to check drug bioavailability or oral activeness. The violation of 2 or more of these conditions predicts a molecule as a non-orally available drug. But in this study all the compounds reported 0 or 1 violation that suggested these are bioavailable or orally available drug.

TOXICITY STUDY

In ProTox-II, all the compounds predicted with different toxicity models such as the Hepatotoxicity model, Carcinogenicity model, Immunotoxicity model, Mutagenicity model, Cytotoxicity model, Androgen receptor and Estrogen receptor model. Predicted values found significant variation in result with variable scoring value. Another prediction study is to check the effects of these compounds on the different binding domains such as the Estrogen receptor ligand-binding domain, Heat shock factor response element, mitochondrial membrane, P53 and ATPase domain Protein family. In this study, All compounds reported

notifiable less toxicity with high scoring value. Different compounds with its predicted toxicity class and LD50 value expressed in table no. 5.

Effects of phycocompounds on Immunotoxicity, Carcinogenicity & Hepatotoxicity expressed in Fig. 1 whereas the effect of Cytotoxicity, Mutagenicity, Estrogen and Androgen Receptor revealed in Fig. 2. Score values of phycocompounds on Heat Shock Factor, Nrf2/Are & Estrogen Receptor Binding domain represented in Fig.3 whereas Score values of phycocompounds on Atad5, P53 & Mitochondrial Membrane Protein Complex showed in Fig. 4.

Out of selected 10 compounds, compound no. 1, 2, 3, 4, 8 and 10 reported highest LD in 50 mg/kg with toxicity class 5. This toxicity class suggested given compounds may be harmful if swallowed. Whereas 5, 6, 7 and 9 showed less LD value with toxicity class 2 that indicate given compounds fatal if swallowed. Table no. 6 showed compounds with LD50 value with its toxicity class.

CYTOTOXICITY STUDY

Table 7 showed prediction about the effect of Ethyl cholate on different cancer cell lines such as UO-31, EKVX, HepG2, LOX IMVI, HOP-92, NCI-H838, SF-539, 786-0, SN12C, SK-MEL-1, IGROV-1, HCC 2998, U-251 and TK-10 whereas squalene showed effect on RXF 393,HOP-92,U-251,SNB-75,MCF7,UO-31,786-0,RPMI-8226,KM12,OVCAR-4,LOXIMVI, A498,TK-10,SF-295,SR,Malme-3M,IGROV-1,NCI-H226,NCIH522,MDA-MB-231. It also contains Pa and Pi value for each phycocompounds including its target tissue and tumor type. Among all, ethyl cholate showed maximum Pa value with UO-31whereas Squalene showed maximum Pa value with RXF 393. Both compounds showed a better effect on the cell lines of renal carcinoma that causes carcinoma tumors in the kidney. This is a. Pa (Probability “to be active”) estimates the chance that the studied compound is belonging to the subclass of active compounds.

DISCUSSION

This obtained results suggested that two compounds can be applied as a medication molecule. The compounds namely Ethyl cholate and squalene showed better predictability with less toxicity value and lesser effect. These potential compounds are further applicable to study docking interaction between ligand and proteins.

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