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Research Article

QSAR Modelling for Analysis of Different Medicinaland Toxicological Properties of Different Phytochemicals Extracted from Rare and Endangered Plants in India

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ABSTRACT

India, recognized as a megadiverse country, harbors a vast array of endemic and rare plant species with significant ethnopharmacological potential. This study investigates the medicinal and toxicological properties of phytochemicals derived from four endangered plant species—*Polygala irregularis*, *Psilotum nudum*, *Acacia planifrons*, and *Pterospermum reticulatum*—native to distinct Indian states. About 18 bioactive compounds, previously characterized using NMR spectroscopy, were subjected to *in silico* toxicological profiling via QSAR-based Toxicity Estimation Software Tool (T.E.S.T.), recommended by the U.S. EPA. Toxicity parameters including LC_{50} (*Daphnia magna*), LD_{50} (oral, rat), IGC_{50} (*Tetrahymena pyriformis*), and mutagenicity (*Salmonella typhimurium*) were predicted. Fatty acids such as palmitic and linoleic acid, along with apigenin, demonstrated higher toxicity in aquatic assays (low LC_{50} values), whereas anthraquinone showed potential mutagenicity (score: 0.74). Most other compounds exhibited low toxicity, indicating pharmacological safety. Additionally, molecular docking via PyRx revealed the strong inhibitory potential of amentoflavone against Cathepsin B (binding affinity up to -8.4 kcal/mol). These findings highlight the dual therapeutic and ecotoxicological roles of phytocompounds from endangered Indian flora, emphasizing the importance of conservation and responsible pharmacological exploration.

Keywords: Rare, Floral species, Toxicological analysis, Phytocompounds, Molecular docking

INTRODUCTION

India is a highly diverse country and currently accounts for approximately 7% of the world's biodiversity, covering just 2.5% of the world's land [1]. A significant portion of this biological treasure comprises numerous floral species that are found in various ecological regions of the country, each serving a distinct biochemical function essential for sheltering a large number of species that depend on it [1]. The floral diversity is particularly at a zenith due to the eclectic range of meteorological, geological, and topographical facets. It is estimated that India harbors approximately 18,000 flowering plant species, which is roughly equivalent to 6-7 percent of the total plant species present worldwide [2]. Additionally, a towering level of endemism has played a crucial role in enhancing the floristic diversity of India, which is a sanctuary of approximately 50,000 species of floral species, covering different layers of endemism. The plants are primarily found in a few floristic regions of India, including the Himalayan region, Assam, the Indo-Gangetic Plain, the Central Plateau region, the Malabar coastal plains, and the Andaman Islands [2,3].

Traditional placebos, dating back to the genesis of medicinal practices, have played a crucial role in healing injuries and have consistently formed an effective pharmacophore in addressing a wide range of diseases [4]. Incessant practices like Ayurveda continue to have a profound impact worldwide, where floral species, notably those from the Indian subcontinent, play a significant role in providing bioactive natural products for the preparation of herbal antidotes.[5] Still, when synthetic drugs are dominating the markets for productive and efficacious, time effectiveness and draconian control, the lack of effectiveness has still compelled numerous people to lay dependent on novel chemotypes obtained from natural provenances.[4]At the beginning of the 21st century, 11% of the 252 essential drugs endorsed by WHO derived their radix from flowering plants.[4, 6]In the platitudes of Newman and Cragg (2012), the operational capability of natural products as founts of novel configuration is still active. [7]Nearly half of the approved drugs during the last three decades were obtained from natural products either in active or passive pathways[6, 7]. In oncological research, during the timeline, nearly 48% of their genesis came from natural issues or their derivatives. [8] Pharmaceutical products have long been modeled after the basic structure of herbal remedies.[8]Drugs like Arteetherand semisynthetic natural products developed from Artemisinin got their operation in malarial treatment. Nitisinoneis synthesized from the natural product Leptospermone (*Callistemon citrinus*) and has

found extensive utilization in the treatment of antityrosinaemia, galantamine a natural alkaloid (synthesized from *Galanthus nivalis*) is utilized in the ministrations for Alzhemer's, and various others.[9]

Long before independence, India's forest coverage was facing a gradual decline, and things had become far more diabolical over the past six decades.[10] Though numerous laws have been implemented to protect the floral species, felonious scheme are rampant and inexorable. Events like this frequently pose a significant obstacle to the conservation of faunal resources in India.[11] Due to these issues, various rare and endangered plant species with potential medicinal uses also got lost forever.[12]In this paper, we have analyzed the potential antidote properties of various established phytochemicals present in four rare species in their respective states. Those species are *Polygala irregularis*, *Psilotum nudum, Acacia planifrons,* and *Pterospermum reticulatum,* which are rare species in the states of Gujarat, Karnataka, Tamil Nadu, and Kerala, respectively *(*Figure 1). [13]

Researchers have already found that numerous phytochemicals, such as polyphenols, alkaloids, flavonoids, lignans, tannins, coumarins, and saponins extracted from different anatomical parts of plants, have potential protective effects against diseases and are mainly antimutagenic or anticarcinogenic. However, some of these compounds are still toxic in higher doses, and some have the potential to show mutagenic properties.[14-16]

In 2011, Johann *et al.,* with the utilization of the instrumentnuclear magnetic resonance spectroscopy and using ^{1}H and ^{13}C analysed that *P. irregularis* contains seven important phytochemicals namely Prenyloxycoumarin, Scopoletin, α-spinasterol, 1,2,3,4,5,6-Hexanehexol, Phebalosin, Aurapten and Rutin .[17] Prenyloxycoumarin possesses various anti-microbial, anti-inflammatory and anti-tumoral properties.[18] Scopoletin regulates blood pressure and is also an effective anti-microbial agent.[19] α-spinasterol, 1,2,3,4,5,6-Hexanehexol, Phebalosin shows anti-inflammatory properties with Aurapten acts as a growth inhibitor to leishmania.[17, 20-23] Rutin acts as an auxiliary to Vitamin C and is effective in the treatment of allergies and other inflammatory conditions.[24, 25]

In 2019, Samec *et al.,* with the execution of an NMR spectroscopy machine, found out that *P. nudum* contains five cardinal phytochemicals, of which naringenin is utilized as a paramount agent in weight control, Apigenin and Hinokiflavone are utilized in oncological treatment.[26] Amentoflavone acts as a natural inhibitor of Cathepsin B in human beings.[27] The results of this function are discussed below with the help of PyRx Molecular Docking Software. [28] Robustaflavone is used for the treatment of the hepatitis B virus.[29]

In 2013, Haq *et al.* observed that *A.planifrons* contains cardinal fatty acid derivatives like palmitic acid, palmitoleic acid, stearic acid, and linoleic acid [30], the majority of whom got high anti-diabetic, blood controlling factors including surface reactant properties.[31-33]

In 2014, Rath *et al.* detected the presence of variegated phytochemicals in *P. reticulatum* of which Anthroquinone and saponins deserve special mention for their anti-tumor activity.[34-36]

Acute toxicity and mutagenic factors are major toxicity outcomes that bear in ecotoxicological research time. [37] These factors can be analyzed with the help of quantitative structure-activity relationships (QSAR) modeling software[38] and toxicity estimation software tool (TEST) fabricated on the virtue of 2D molecular descriptor, which can accomplish the task (USEPA, 2012).[39]

Our current study strived to predict the acute toxicity by analyzing LC_{50} in *Daphnia magna,* LD_{50} in rat via oral route, IGC_{50} study in *T. pryreformes*, and mutagenicity study in *T. Typhimurium* of the established photochemical present in the plant's *Polygala irregularis, Psilotum nudum, Acacia planifrons,* and *Pterospermum reticulatum*. The predictive toxicological study was carried out with the help of QSAR software, T.E.S.T.[39-44]

METHODOLOGY

Toxicity Analysis

In the current analysis, the assemblage of stated phytochemicals was executed on the basis of literature anatomization. In Tables 1-4, the 18 established phytochemicals were stated alongside their CAS no., SMILES and molecular structures, which were extracted with

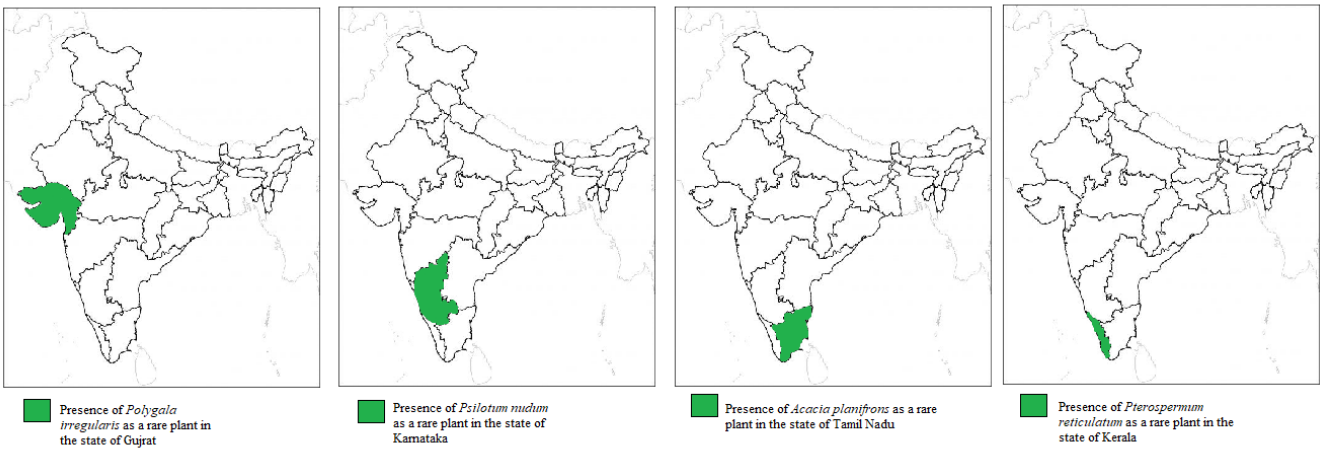

Fig. 1: Map showing the individual extension of four endangered floral species

the succor of the ChemIDPlus database[44]. Individual selections of eighteen phytochemicals were done to predict the toxicity analysis. QSAR modeling software of 2d descriptor, T.E.S.T, Version 4.1 was utilized for our operation (USEPA, 2012).[39] The analysis was effectuated to predict the acute toxicity by analyzing LC_{50} in *Daphnia magna*, LD_{50} in rat via oral route, IGC_{50} study in *T .pyreformes*, and mutagenicity study in *T. Typhimurium* according to the protocol of the software.[45]

The results of toxicity analysis and mutagenicity study were subsequently codified with the securing of predictive data of discrete compounds from T.E.S.T. software. [45]The data was secured with the help of the consensus method, which is actually the mean prognosticated LC_{50}, LD_{50} and mutagenicity positive or negative values and were reckoned from inbuilt QSAR algorithm, which mainly consists of hierarchical clustering, the FDA MDL and nearest neighbor methods (USEPA, 2012). [46] According to the protocol of operation in the software, the structure of our phytocompounds of interest can be obtained by composing the respective CAS registry no. The predicted value is based as stated to the algorithm of the software.[46]

Toxicity prediction in this paper has followed the consensus methodology where empirical toxicity analysis from other QSAR methodologies are obtained and an average calculation is done. [45] This process usually gives the most accurate result cause on error value will be negated by other values, which are all taken into account. [47]

Further analysis of the phytocompounds was done using FDA model of Rat Oral LD_{50}. FDA model is chiefly constructed on the basis of analyzing a particular chemical using a cluster that has got structurally similar compounds segregated from the training set .[47] Martin *et al.,* 2016 created an equation on the training set by using 15 to 20 compounds all of which got a cosine similarity coefficient of 75% with the chemical is testing.[45,48] That cosine similarity coefficient is expressed as $SCi_{,k}$,

$$SC_{i,k}=\frac{\sum_{j=1}^{\#descriptors} x_{ij}\, x_{kj}}{\sqrt{\sum_{j=1}^{\#descriptors} x_{ij}^2}\sqrt{\sum_{j=1}^{\#descriptors} x_{kj}^2}}$$

where,

x_{ij} is the evaluation of the jth normalized descriptor for chemical i (normalized with respect to all the chemicals in the original training set) [45]. x_{kj} is the evaluation of the jth descriptor for chemical k.[45]

Assortment of Ligand and Macromolecule

The crystalline three-dimensional (3-D) structure of Ligand Amentoflavone (PubChem CID:5281600) and Macromolecule Cathepsin B (PDB ID:3AI8) were extracted from the website of pubchem (https://pubchem.ncbi.nlm.nih.gov/) and protein data bank (http://www.rcsb.org). [43, 49] The obtained structure, which was found to be complex with nitroxoline (PDB ID:3AI8) was obtained maintaining the protocol of the wwPDB validation report. This structure was obtained based on the X-ray Diffraction method of 2.0 Å. The structures shown after visualizing in MGL Tool, which The Scripps Research Institute develops.[49]

The molecular docking was executed with the help of PyRx software (Virtual Screening Tool, Ver 0.8) created by Trott and Olson. The sequelae of molecular docking were then subsequently visualized in the form of the output .pdbqt file with the help of the MGL tool, engendered by The Scripps Research Institute and the outcome of the three-dimensional structure was supplied by MGL Tools. The docking was carried out for Ligand Amentoflavone (PubChem CID:5281600) and Macromolecule Cathepsin B (PDB ID:3AI8) to know the result of suitable binding energy value. The macromolecule-ligand interaction of the ligand and macromolecule was identified to detect the effect of the inhibitory action of amentoflavone on cathepsin b. [28]

Equivocal analysis was done with the help of Mcule 1-Click docking. For validation of both data, a t-test was run to detect the level of significance.[50]

A paired t-test was done to detect the difference in binding affinity between the datasets in two different software.[51]

$$t=\frac{\overline{D}}{\frac{SD}{\sqrt{n}}}$$

Where,

t=t value D=differences

SD=standard deviation n=number of samplings

RESULTS AND DISCUSSION

Toxicological analysis

The current interpretation was done on 18 established phytochemicals, which were already deracinated with the help of NMR spectroscopy on the extracts drawn from *Polygala irregularis*, *Psilotum nudum, Acacia planifrons, Pterospermum reticulatum*. These phytocompunds and their therapeutic properties were already obtained from a diversified literature review. They are assorted in the following classes: Coumarines (Prenyloxycoumarin, Scopoletin, Phebalosin, Aurapten), sterol (α-spinasterol), alcohol (1,2,3,4,5,6-Hexanehexol), flavonoids (Rutin, Hinokiflavone, Robustaflavone, Amentoflavone, Apigenin, Naringenin), fatty acid (Palmitic Acid, Palmitoleic acid, Stearic acid, Linoleic acid), quinone (Anthroquinone) and saponin.(Table1)

The Chemical Abstract Services (CAS) no. , SMILES (Simplified Molecular-Input Line-Entry System) and structure were catalogued in for *Polygala irregularis, Psilotum nudum*, *Acacia planifrons* and *Pterospermum reticulatum*. In Table 2, the acute toxicity (LC_{50}) data in *Daphnia magna* along with LD_{50} in rat, IGC_{50} in *T. pyreformes*, and mutagenicity study in *T. Typhimurium* for the above-mentioned 18 phytochemicals were given. Of these results, for about 9 compounds (Scopoletin, 1,2,3,4,5,6-Hexanehexol, Rutin, Apigenin, Palmitic Acid, Palmitoleic acid, Stearic acid, Linoleic acid, Anthroquinone) the predicted data for LC50 (mg/l) for *D. Magna*, LD_{50} (mg/kg) for rat, IGC_{50} for *T. pyreformes* and mutagenicity for *T. Typhimurium* were captured utilizing T.E.S.T. Rest of the 9 compounds were unable due to give appropriate results due to the incognito CAS no. in the current software.

Our research encompasses the toxicity of the said phytochemicals and the consequences when these compounds find their way to the human anatomical system or when they are discharged into the aquatic systems. The consequences of various compounds directly dumped in the aquatic bodies were monitored with the help of tests conducted on *D. magna*. The solid crystalline form of those compounds in the terrestrial system was tested on rat via oral ingestion. Kim et al., 2002 conducted studies which showed that different phytocompounds like sterols, lignans, etc can be toxic in higher doses and may be mutagenic and carcinogenic.[52-54]

The research work on QSAR modeling for the prediction of toxicological parameters and mutagenicity studies were conducted and validated by Arvidson *et al.* back in 2008.[53] In 2001, Norman *et al.* detected that sitosterol and stigmasterol are responsible for colon and rectal cancer.[54]

For *D. magna*, it is seen that the LC_{50} (mg/l) is 73.35 for scopoletin, 7873.53 for 1,2,3,4,5,6-hexanehexol, 72.31 for rutin, 2.37 for apigenin, 1.98 for palmitic acid, 1.79 for palmitoleic acid, 1.41 for stearic acid, 0.87 for linoleic acid, and 2.85 for anthroquinone (Table 2).

In case of rat, oral LD_{50} analysis (mg/kg) is 1004.60 for scopoletin, 479 for α-spinasterol, 13632.65 for 1,2,3,4,5,6-hexanehexol, 3126 for rutin, 1707.99 for apigenin, 13454.34 for palmitic acid, 15307.47 for palmitoleic acid, 13973.13 for stearic acid, 11996.68 for linoleic acid , 4450.09 for anthroquinone, and 250 for saponin (Table 2).

For the mutagenicity test, the results for 9 compounds given below, of which only one, anthroquinone, got positive mutagenicity result. The results for the compounds are as: 0.20 for Scopoletin, 0.18 for 1,2,3,4,5,6-hexanehexol, 0.06 for rutin,

Table 1: Photochemical extracts present in
(1-7) *Polygala irregularis*; **(8-12)** *Psilotum nudum;* **(13-16)** *Acacia planifrons;* **(17-18)***Pterospermumreticulatum*

SL.NO.	*NAME*	*CAS NO.*	*SMILES*	*MOLECLAR FORMULA*
COUMARINS				
1	Prenyloxycoumarin	10387-50-5	CC(=CCOC1=CC2=CC=CC=C2OC1=O)C	$C_{14}H_{14}O_3$
2	Scopoletin	92-61-5	COC1=C(C=C2C(=C1)C=CC(=O)O2)O	$C_{10}H_8O_4$
3	Phebalosin	6545-99-9	CC(=C)C1C(O1)C2=C(C=CC3=C2OC(=O)C=C3)OC	$C_{15}H_{14}O_4$
4	Aurapten	495-02-3	CC(=CCCC(=CCOC1=CC2=C(C=C1)C=CC(=O)O2)C)C	$C_{19}H_{22}O_3$
STEROL				
5	α-spinasterol	481-18-5	CCC(C=CC(C)C1CCC2C1(CCC3C2=CCC4C3(CCC(C4)O)C)C)C(C)C	$C_{29}H_{48}O$
ALCOHOLS				
6	1,2,3,4,5,6-Hexanehexol	69-65-8	C(C(C(C(C(CO)O)O)O)O)O	$C_6H_{14}O_6$
FLAVONOIDS				
7	Rutin	153-18-4	CC1C(C(C(C(O1)OCC2C(C(C(C(O2)OC3=C(OC4=CC(=CC(=C4C3=O)O)O)C5=CC(=C(C=C5)O)O)O)O)O)O)O)O)O	$C_{27}H_{30}O_{16}$
FLAVONOIDS				
8	Hinokiflavone	19202-36-9	C1=CC(=CC=C1C2=CC(=O)C3=C(O2)C=C(C(=C3O)OC4=CC=C(C=C4)C5=CC(=O)C6=C(C=C(C=C6O5)O)O)O)O	$C_{30}H_{18}O_{10}$
9	Robustaflavone	49620-13-5	C1=CC(=CC=C1C2=CC(=O)C3=C(O2)C=C(C(=C3O)C4=C(C=CC(=C4)C5=CC(=O)C6=C(C=C(C=C6O5)O)O)O)O)O	$C_{30}H_{18}O_{10}$
10	Amentoflavone	1617-53-4	C1=CC(=CC=C1C2=CC(=O)C3=C(O2)C(=C(C=C3O)O)C4=C(C=CC(=C4)C5=CC(=O)C6=C(C=C(C=C6O5)O)O)O)O	$C_{30}H_{18}O_{10}$
11	Apigenin	520-36-5	C1=CC(=CC=C1C2=CC(=O)C3=C(C=C(C=C3O2)O)O)O	$C_{15}H_{10}O_5$
12	Naringenin	67604-48-2	C1C(OC2=CC(=CC(=C2C1=O)O)O)C3=CC=C(C=C3)O	$C_{15}H_{12}O_5$
FATTY ACIDS				
13	Palmitic acid	57-10-3	CCCCCCCCCCCCCCCC(=O)O	$C_{16}H_{32}O_2$
14	Palmitoleic acid	373-49-9	CCCCCCC=CCCCCCCCC(=O)O	$C_{16}H_{30}O_2$
15	Stearic acid	57-11-4	CCCCCCCCCCCCCCCCCC(=O)O	$C_{18}H_{36}O_2$
16	Linoleic acid	60-33-3	CCCCCC=CCC=CCCCCCCCC(=O)O	$C_{18}H_{32}O_2$
	QUINONES			
17	Anthroquinone	84-65-1	C1=CC=C2C(=C1)C(=O)C3=CC=CC=C3C2=O	$C_{14}H_8O_2$
	SAPONINS			
18	Saponin	11072-93-8	CC=C(C)C(=O)OC1C(C2(C(CC1(C)C)C3=CCC4C5(CCC(C(C5CCC4(C3(CC2O)C)C)(C)CO)OC6C(C(C(C(O6)C(=O)O)OC7C(C(C(C(O7)CO)O)O)O)O)O)OC8C(C(C(C(O8)CO)O)O)O)C)CO)OC(=O)C	$C_{55}H_{86}O_{24}$

Table 2: Toxicological Analysis of different phytochemicals present in the floral species

Sl. No.	*Phytochemicals*	*Predictive Acute toxicity (LC_{50}) values (mg/l) in D. Magna by T.E.S.T.*	*Predictive mutagenecity values in T. typhimurium T.E.S.T.*	*Predictive Acute toxicity (IGC_{50}) values (mg/l) in T.pryreformes by T.E.S.T.*	*Predictive Acute toxicity rat oral (LD_{50}) values (mg/l) by T.E.S.T.*	*R^2 Value*	*Predictive Acute toxicity rat oral (LD_{50}) values (mg/l) by T.E.S.T.*
1	Prenyloxycoumarin	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
2	Scopoletin	73.35	0.20(-)	15.71	1004.60	0.744	1004.60
3	Phebalosin	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
4	Aurapten	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
5	α-spinasterol	N.A.	N.A.	N.A.	479	N.A.	479
6	1,2,3,4,5,6-Hexanehexol	7873.53	0.18(-)	2859.95	13632.65	0.897	13632.65
7	Rutin	72.31	0.06(-)	N.A.	3126	0.758	3126
8	Hinokiflavone	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
9	Robustaflavone	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
10	Amentoflavone	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
11	Apigenin	2.37	0.29(-)	6.72	1707.99	0.791	1707.99
12	Naringenin	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
13	Palmitic Acid	1.98	-0.04(-)	1.28	13454.34	0.853	13454.34
14	Palmitoleic acid	1.79	-0.04(-)	1.63	15307.47	0.841	15307.47
15	Stearic acid	1.41	-0.06(-)	0.27	13973.13	0.851	13973.13
16	Linoleic acid	0.87	0.01(-)	0.72	11996.68	0.826	11996.68
17	Anthroquinone	2.85	0.74(+)	28.64	4450.09	0.798	4450.09
18	Saponin	N.A.	N.A.	N.A.	250	N.A.	250

N.A.:Not found . (-):Negative activity . (+) :Positive activity

0.29 for apigenin, -0.04 for palmitic acid, -0.04 for palmitoleic acid, -0.06 for stearic acid , 0.01 for linoleic acid , and 0.74 for anthroquinone (Table 2).

For IGC_{50} toxicity analysis in *T.pryreformes*, (mg/l) is 15.71 for scopoletin, 2859.95 for 1,2,3,4,5,6-hexanehexol, 6.72 for apigenin, 1.28 for palmitic acid, 1.63 for palmitoleic acid, 0.27 for stearic acid, 0.72 for linoleic acid, and 28.64 for anthroquinone (Table 2).

FDA analysis of R^2 value on the rat LD_{50} results are: 0.744 for for scopoletin, 0.895 for 1,2,3,4,5,6-hexanehexol, 0.758 for rutin , 0.791 for apigenin , 0.853 for palmitic acid, 0.841 for palmitoleic acid , 0.851 for stearic acid , 0.826 for linoleic acid , and 0.798 for anthroquinone (Table 2).

Molecular docking

As stated by a diverse literature reviews, it is found that amentoflavone can be shackled to Cathepsin B of *Homo sapiens* and can entice its activities by acting as an potential inhibitor. Amentoflavone (Zinc id:tclcactvs000eKwd2b) (Figure 2) binds at Trp122 residue of Cathepsin B .[27] In two different molecular docking softwares, we have analyzed the potential binding energy between them and the value of docking scores at different position of Cathepsin B (PDB id :3ai8). More negative score signifies better binding affinity.[50]**)**

The binding centres are taken as -8.587,-6.437 and -1.829 for X,Y and Z axisses and 4 docking poistures were chosen . The maximum docking score was obtained as -8.4 and the minimum as -7.5. Binding site:TRP221_A3674 (Table 3)

mCULE 1-Click Docking

The Figure 2 interprets the extent to which amentoflavone can hitch to cathepsin B. Probing of literature accorded us a pure insight to the molecular contraption of amentoflavone inhibition. Pan *et al.* 2005 by a thorough scrutinising of different experimental results have dictated that the molecular mechanisms exacerbating inhibitory actions. The pi-pi bond formation of Trp221 residue and the A side chain, in our case we have choose A 3674 residue may get the probability to induce inhibitory activity. (Figure 2).

In Figure 2, for A1 we can see that the binding position coinsides nearly with SER 25,CYS 26 and GLY 121 . For B1 the residues changes a little bit with GLY 123, ASP 124 along with CYS 26 taking the lead

Table 3: Binding sites and docking scores in mCULE molecular docking

Binding site centre		
X-axis	Y-axis	Z-axis
-8.587	-6.437	-1.829

The chart showing the binding affinities of four docking positions or sites of Amentoflavone-Cathepsin B complexes

Docking pose	Docking score
1	-8.4
2	-8.2
3	-8.1
4	-7.5

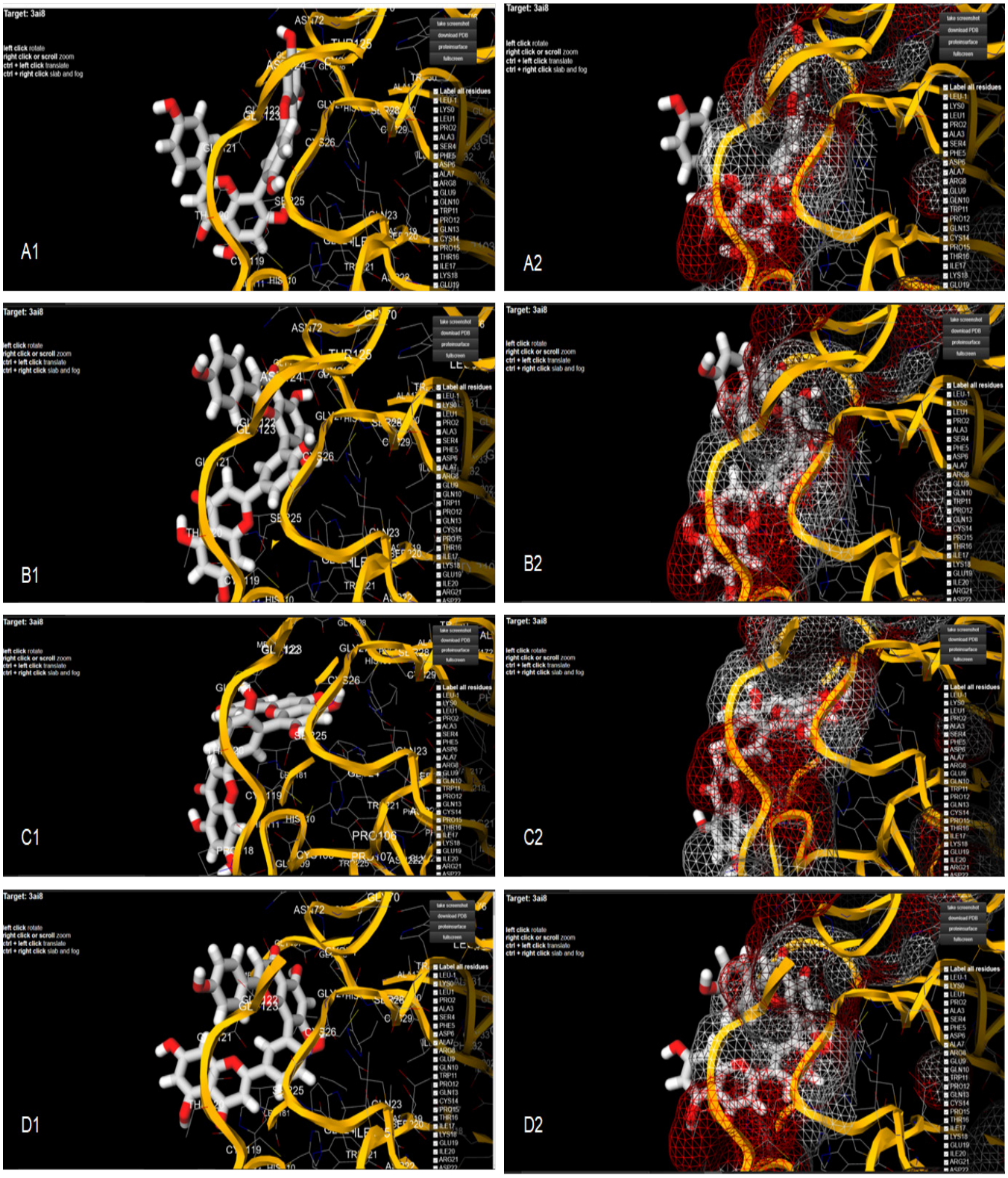

Fig. 2: Different Docking Poses for Amentoflavone-Cathepsin B docking .In the right side the presence of protein residue gave us a red and white latticework (mCULE 1-click docking)

binding role. Considering C1 and D1 it seems that SER 25, GLY 121 ,THR 120 along with GLY120 playing the roles of binding residues .

Another simulation docking was executed on PyRx software (Virtual Screening Tool, Ver 0.8) by taking amentoflavone as ligand

Table 4: Table showing the binding centre, dimensions and the docking results form Amentoflavone-Cathepsin B molecular docking simulation

Binding site centre				
X-axis	Y Axiss		Z-axis	
-20.785	-9.5758		-22.9712	
25.0000	25.0000		25.0000	
LIGAND	Binding affinity(kcal/mol)	Mode	RMSD lower bound	RMSD upper bound
3ai8_tclcactvs000eKwd2b	-4.1	0	0.0	0.0
3ai8_tclcactvs000eKwd2b	-4.1	1	0.017	1.872
3ai8_tclcactvs000eKwd2b	-3.8	2	1.489	2.609
3ai8_tclcactvs000eKwd2b	-3.7	3	0.916	2.337
3ai8_tclcactvs000eKwd2b	-3.7	4	13.778	15.148
3ai8_tclcactvs000eKwd2b	-3.6	5	13.807	15.155
3ai8_tclcactvs000eKwd2b	-3.6	6	13.789	15.06
3ai8_tclcactvs000eKwd2b	-3.5	7	5.573	6.607
3ai8_tclcactvs000eKwd2b	-3.5	8	7.715	8.926

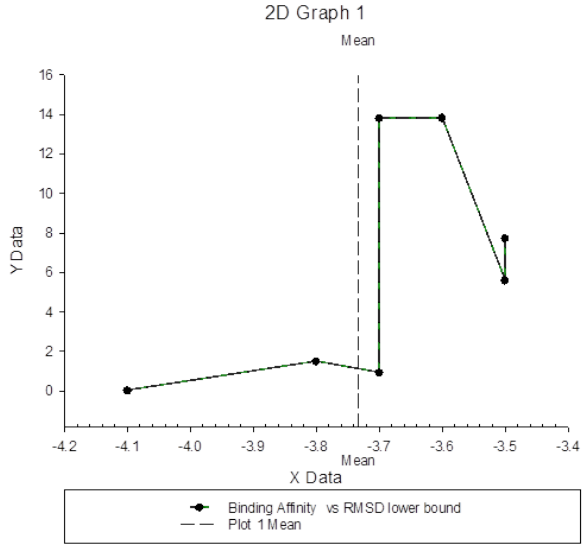

Fig 3: X data represents binding affinity in (kcal/mol)Y data represents RMSD upper bound

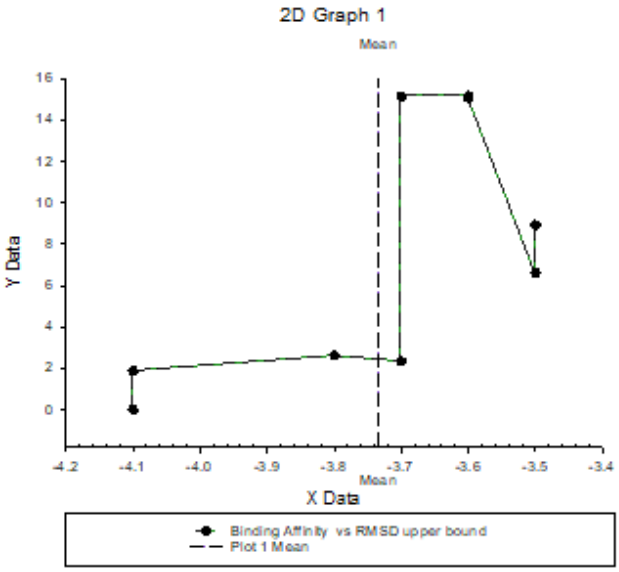

Fig 4: X data represents binding affinity inY data represents RMSD lower bound (kcal/mol) Y data represents RMSD upper bound

and Cathepsin B as macromolecule and the binding axisses were taken as -20.785, -9.5758 and -22.9712 for X, Y and Z axisses. The binding affinity values were a maximum of -4.1 to a minimum of -3.5.(Table 4)

RMSD values are reckoned with respect to the best mode and it utilizes only movable heavy atoms. RMSD upper bound harmonizes each atom in one conformation with itself in the other conformation, neglecting any symmetry. RMSD lower bound equalizes each atom in one conformation with the closest atom of the similar element type in the other conformation. The values were visualized in Table 4 and Figures 3-6.

Root mean square deviation (RMSD) of atomic positions can be expressed as :

$$RMSD=\sqrt{\frac{1}{N}\sum_{i=1}^{N}\delta_i^2}$$

where ,

δi =Denotes the distance between atom i and a reference structure or the mean position of the N equivalent atoms.

To detect the extent of statistical significance between two results, we have concluded a paired t-test. The reults were shown to be extremely statistically significant with a value of less than 0.0001 .

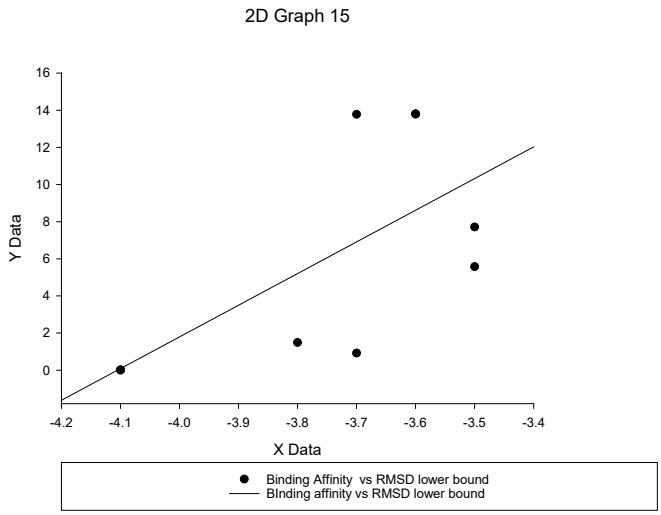

Fig 5: X data represents binding affinity in (kcal/mol) andY data represents RMSD upper bound

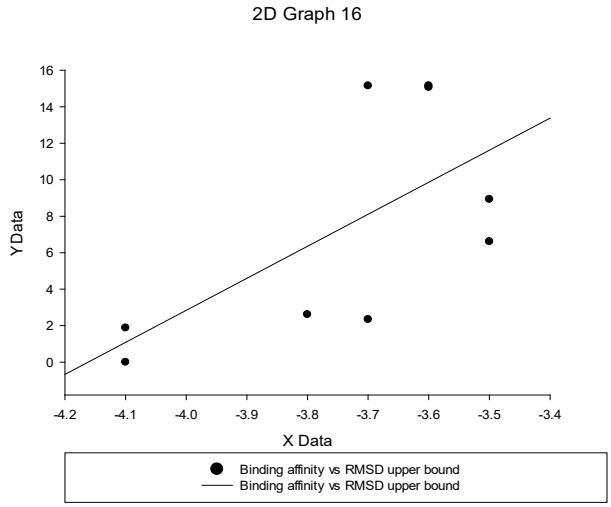

Fig 6: X data represents binding affinity inY data represents RMSD lower bound (kcal/mol) and Y data represents RMSD upper bound

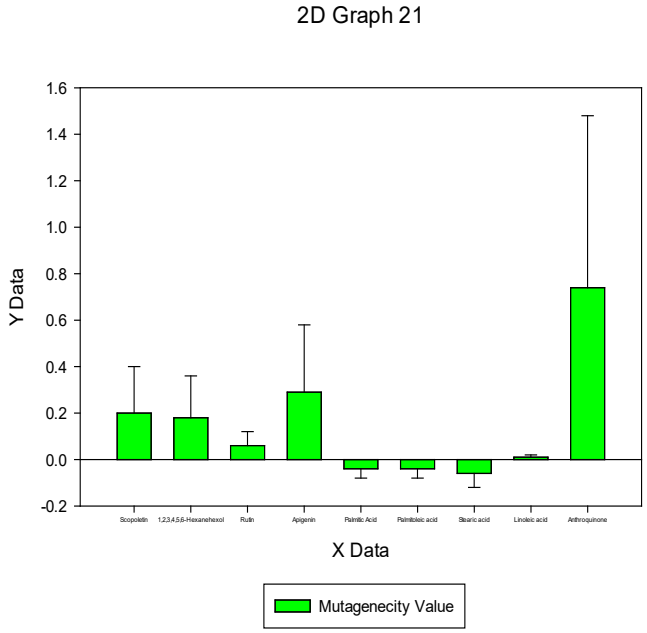

Fig 7 : Depicting the mutagenicity test results of different compounds. X axis denotes compounds under scrutiny and Y axis denotes mutagenicity values.

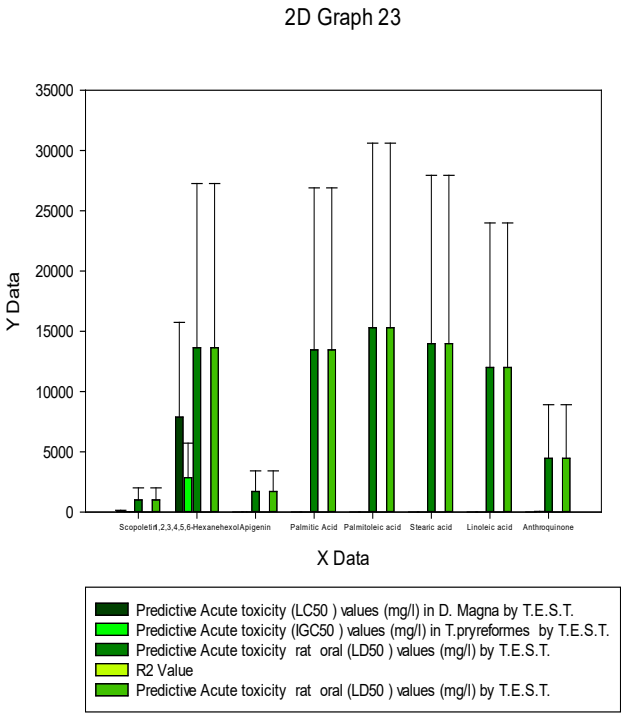

Fig 8 : Predictive toxicity testing on different species .X axis denotes different compounds and whileY axis denotes toxicity values in (mg/l)

DISCUSSION

After analysis of literature works and dry lab experiments, it is concluded that the four plants *Polygala irregularis*, *Psilotum nudum, Acacia planifrons, Pterospermum reticulatum* are endowed with rich pharmacophores, which are present as coumarins, sterols, fatty acids, quinines, etc. The mutagenicity test results of different compounds. X axis denotes compounds under scrutiny and Y axis denotes mutagenicity values is shown in Figure 7. Toxicological analysis of the compounds has resulted in anthroquinone possessing the mutagenic property with a value of 0.74. LC50 test on *Daphnia magna* has revealed that those of fatty acids and apigenin got quite a low value, indicating a high toxicity. Other toxic tests show quite normal values with less toxic outputs, which were done in QSAR modeling software (TEST) recommended by USEPA (2012) (Figure 8).

Now, turning our attention to the molecular binding action of amentoflavone and Cathepsin B in PyRx software revealed the binding affinity, which is responsible for the strong inhibitory effect on Cathepsin B. Interaction between Trp 221 residue and A ring had a high binding value, sometimes as high as -8.4 in one of the scenarios.

CONCLUSION

The biological manoeuvre of natural phytocompound extracts have divulged to be of tremendous efficacy in the pharmaceutical industry. The gradual decline of the floral realm from the Indian subcontinent has shown us the ultimatum and inefficiency we are facing now and it's incontrovertible. Few plants like these contain hordes of compounds that are used in various ailments and a loss of these can't cover nature's depleted resources and will further in future decrease a country's wealth of resources,

This study meticulously investigated the phytochemical constituents extracted from *Polygala irregularis*, *Psilotum nudum*, *Acacia planifrons*, and *Pterospermum reticulatum*, focusing on their classification, toxicity, and molecular interactions. A total of 18 phytocompounds were categorized into various classes such as coumarins, sterols, alcohols, flavonoids, fatty acids, quinones, and saponins. Utilizing NMR spectroscopy and supported by an extensive literature review, these compounds were structurally characterized, and their chemical properties were cataloged using CAS numbers and SMILES notations.

Acute toxicity assessments were conducted through predictive QSAR modeling using the T.E.S.T. software recommended by the USEPA. For nine of the compounds, toxicological endpoints such as LC_{50} in *Daphnia magna*, LD_{50} in rats, IGC_{50} in *Tetrahymena pyriformis*, and mutagenicity in *Salmonella typhimurium* were obtained. Among these, anthraquinone emerged as a potential concern due to its positive mutagenicity result (0.74) and notable toxicity in aquatic assays, indicating potential environmental and health hazards. Fatty acids like palmitic, stearic, and linoleic acid, as well as the flavonoid apigenin, showed low LC_{50} and IGC_{50} values, suggesting higher toxicity to aquatic organisms.

The docking study of amentoflavone with Cathepsin B revealed promising inhibitory interactions, with the most stable binding affinity reaching as low as -4.1 kcal/mol in PyRx simulations. The interaction involved crucial residues such as Trp221, Cys26, and Gly121, affirming the ligand's potential role as a Cathepsin B inhibitor. RMSD analyses confirmed the structural stability of the binding conformations, and the statistical significance of the binding results was strongly supported ($p < 0.0001$) via paired t-tests.

In conclusion, the selected plant species are rich in bioactive phytochemicals with diverse therapeutic potentials. However, several compounds also exhibit varying degrees of toxicity, particularly in aquatic environments, warranting caution regarding their unregulated use or disposal. The molecular docking study further highlights amentoflavone's potential as a lead compound in anti-cancer drug development targeting Cathepsin B. This integrated approach, combining phytochemistry, toxicology, and computational modeling, reinforces the value of traditional medicinal plants while underscoring the need for thorough safety assessments in drug discovery and environmental applications.

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