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TRITERPENE ACID (3B-HYDROXYURS-12-EN-28-OIC ACID) AGAINST HUMAN LUNG CANCER A-549 CELLS- MULTIPLE LINEAR REGRESSION BASED QSAR MODELING

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ABSTRACT

Here ursolic acidanalogues have been used to correlate the cytotoxic activity with the Eccentric Connectivity index (ECI), Fragment Complexity (FC) and McGowan Volumes (MG) for studying the Quantitative Structure Activity Relationship (QSAR). Correlation may be an adequate predictive model which can help to provide guidance in designing and subsequently yielding greatly specific compounds that may have reduced side effects and improved pharmacological activities. I have used Multiple Linear Regression (MLR), one of the best methods for developing the QSAR model. Results from this QSAR study have suggested that ECI, FC and MG are the important descriptors for cytotoxic activities of ursolic acid analogues against A-549 cells. For the validation of the developed QSAR model, statistical analysis such as data point-descriptor ratio, fraction of variance, cross validation test, standard deviation, quality factor and Fischer's test have been performed and all the tests validated this QSAR model.

Keywords: Ursolic acid; QSAR, Eccentric connectivity index; Fragment complexity; McGowan Volume, Multiple Linear Regression

INTRODUCTION

Ursolic acid (3β-hydroxyurs-12-en-28-oic acid) is a triterpenoid that occurs in numerous plants and is a constituent of several herbal medicines[1]. Ursolic acid is used in cosmetics and is also capable of inhibiting various types of cancer cells by inhibiting the STAT3 activation pathway[2,3] and human fibro sarcoma cells by reducing the expression of matrix metalloproteinase-9 by acting through the gluco-corticoid receptor. It may also decrease proliferation of cancer cells and induce apoptosis.[4] Ursolic acid is present in many plants, including apples, basil, bilberries, cranberries, elder flower, peppermint, rosemary, lavender, oregano, thyme, hawthorn, and prunes. Apple peels contain large quantities of ursolic acid and related compounds.

Ursolic acid can serve as a starting material for synthesis of more potent bioactive derivatives, such as antitumor agents.[5] It has been found to reduce muscle atrophy and to stimulate muscle growth in mice.[6] Ursolic acid has potential use as a cardio-protective compound.[7]

In the present study, we developed a Quantitative Structure Activity Relationship (QSAR) model on a series of ursolic acid with respect to their cytotoxicity against A-549 Cells. The QSAR studies are perfect tool for understanding the drug design process in terms of their chemical-pharmacological activity interaction, along with it is also used in toxicology and pesticide research. QSAR studies can focus on mechanism of action of ligands with human, bacteria, virus, membranes, enzymes etc. It can also be used for the evaluation of the metabolism, absorption, distribution and excretion phenomena. The QSAR methodology comprises of computationally derived descriptors to correlate with pharmacological activities. These descriptors are principally of four types such as electronic, stearic, hydrophobic and topological indices[8]. The descriptors used by us for developing the QSAR model are Eccentric Connectivity Index (ECI)[9], Fragment complexity (FC)[10] and McGowan's volume (MG)[11].

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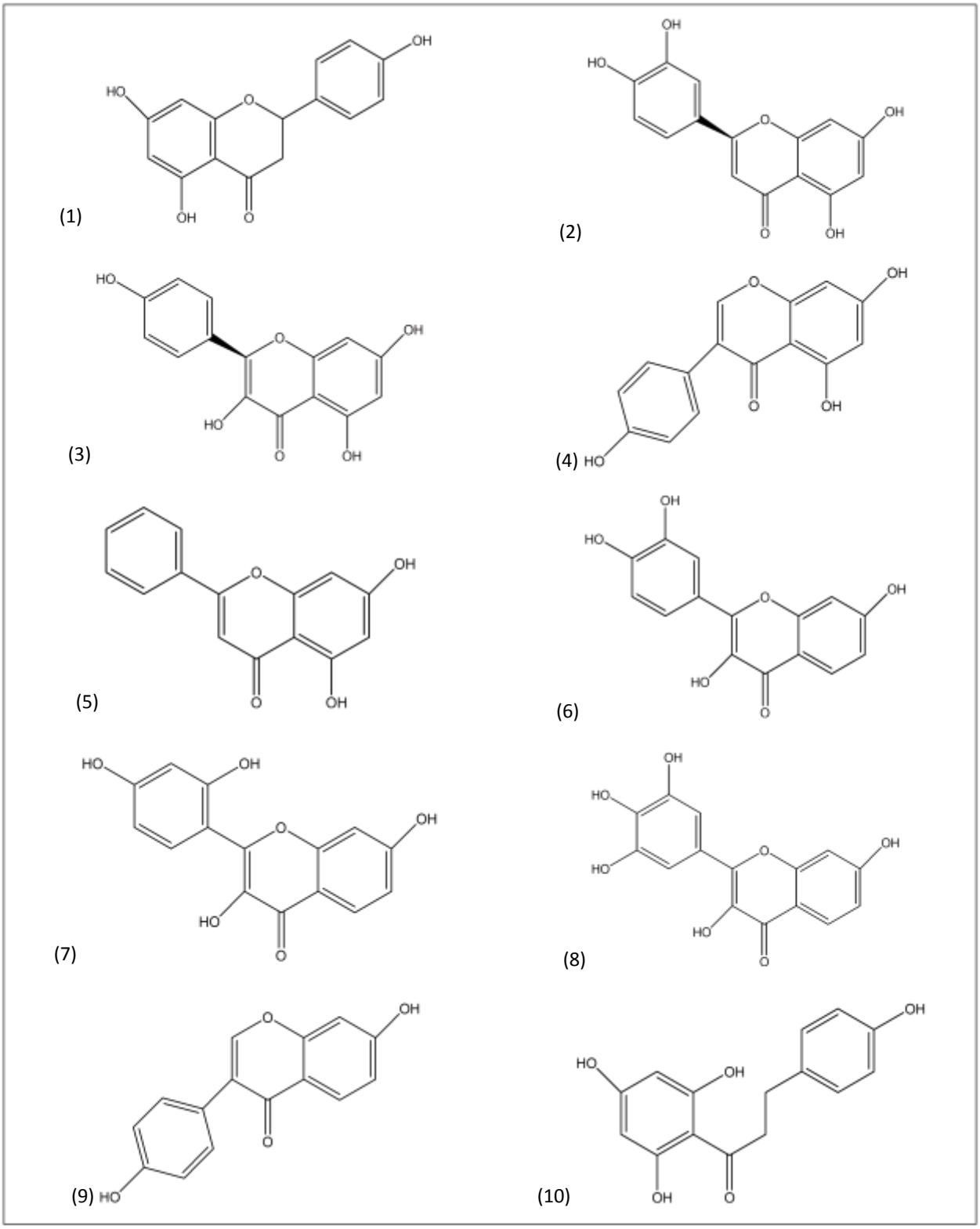
Table1: 2D structure of Ursolic acid derivatives for which the QSAR model has been developed.



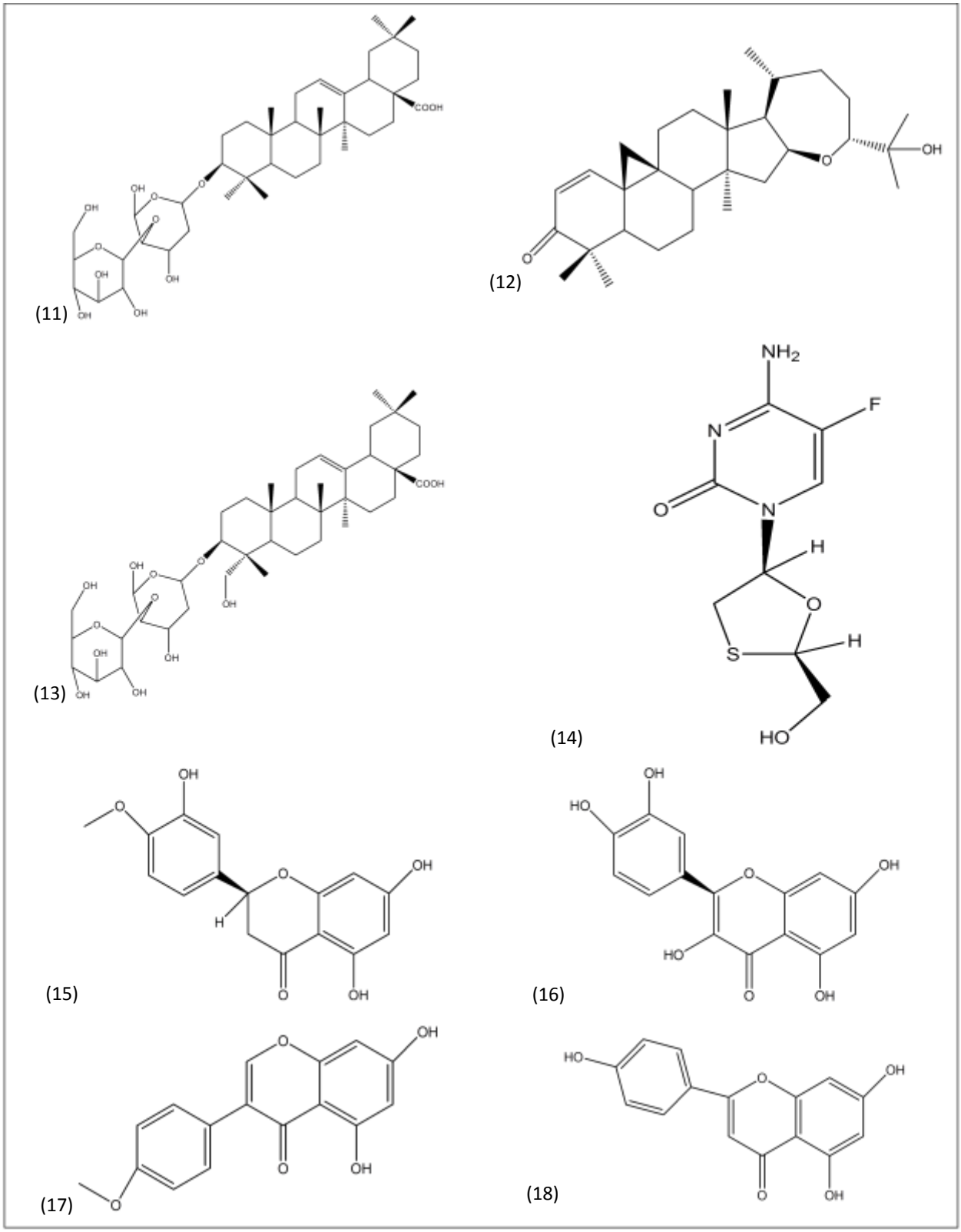

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Table 2: Descriptors used to derive QSAR equation along with bioactivities of Ursolic acid analogues.

	$logIC_{50}$			ECI	FC	MG
	Obs.	Pred.	Resid.			
1	2.009	1.931071	0.077929	338	776.05	1.8888
2	1.804	1.922213	-0.11821	355	669.06	1.9045
3	1.906	1.920731	-0.01473	351	669.06	1.9045
4	2.021	1.954412	0.066588	354	644.05	1.8458
5	1.989	1.96512	0.02388	295	619.04	1.7871
6	1.894	1.919989	-0.02599	349	669.06	1.9045
7	1.661	1.919248	-0.25825	347	669.06	1.9045
8	1.716	1.893719	-0.17772	366	694.07	1.9632
9	1.972	1.980683	-0.00868	337	619.04	1.7871
10	1.982	1.888777	0.093223	383	845.05	1.9974
11	0.999	1.004671	-0.00567	2011	12869.12	5.7437
12	1.29	1.282412	0.007588	734	6000.03	3.7741
13	0.984	0.98891	-0.00491	2038	13014.13	5.8024
14	2.093	2.07198	0.02102	227	489.08	1.5463
15	2.004	1.852008	0.151992	405	982.06	2.0884
16	1.855	1.89446	-0.03946	368	694.07	1.9632
17	2.111	1.900115	0.210885	403	805.05	1.9867
18	1.949	1.948483	0.000517	338	644.05	1.8458

Table 3: Results of statistical validation

n/p >=4	r^2	q^2	s	$r^2 - q^2 < 0.3$	Q	F	RMSD	variance
6	0.9013	0.9013	0.3339	0	2.843	42.6146	0.025011	0.014477

MATERIALS AND METHOD

All the bioactivity values and information about 2D structure of **Ursolic acid** derivatives were taken from literature[12]. IC_{50} is referred as the molar concentration of a compound that inhibits 50% growth of bacteria[8, 14]; $logIC_{50}$ is subsequent variable that comprises the bioactivity parameter for the QSAR model. In order to calculate the 2D molecular descriptors, PaDEL descriptor software[13] which incorporate CDK library for descriptor calculation have been used. For the development of QSAR model, Multiple Linear Regression has been employed[8].

Modeling parameters and structure optimization

The 2D structure construction, energy minimization and geometry optimization of the selected Ursolic acid derivatives were carried out by using ChemDraw Ultra 7.0 and Chem3D Pro 7.0 (CambridgeSoft Corporation, 100 CambridgePark Drive, Cambridge MA, 02140 USA) on an Intel(R) Core(TM)2 Duo Central Processing Unit T6670 @ 2.20 GHz and 4.00 GB of RAM, running the Windows 7 Home Basic, 64-bit compatible operating system. The energy minimization was carried out to minimum RMS Gradient of 0.100, with step interval of 2.0 Fs and frame interval of 10 Fs.

Statistical Parameters

In the QSAR model, number of data points is denoted as n, number of descriptors as p, squared correlation coefficient as r^2 (fraction of variance), cross-validated r^2 is denoted as q^2, s is standard deviation, RMSD is root mean square deviation, variance. Q is quality factor, where Q= r/s (here r is correlation coefficient and s is standard deviation). Fischer statistics is denoted by F.

Model Validation

The QSAR model validation was carried with statistical analysis.

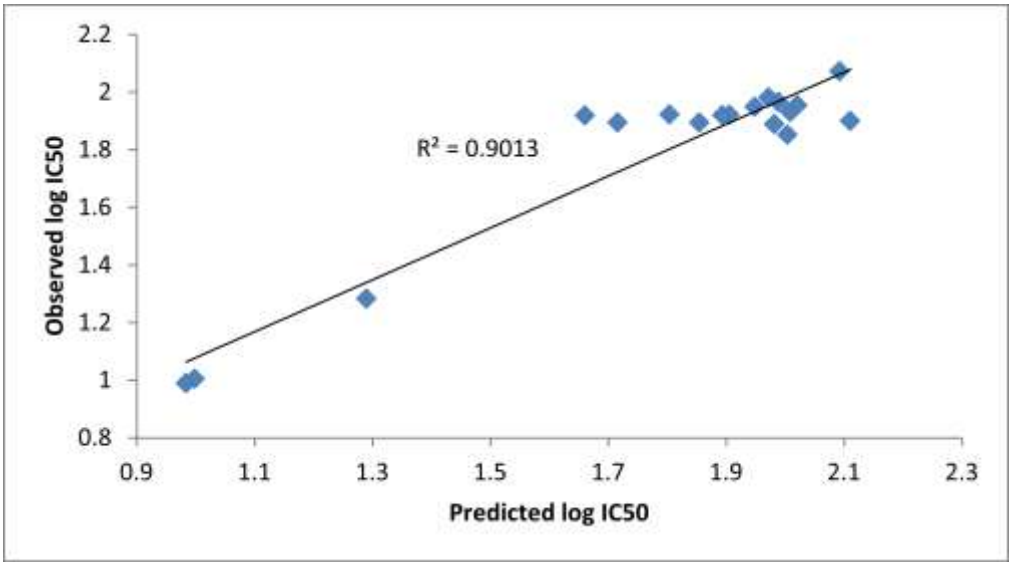

Fig. 1: Multiple linear regression plot for QSAR study

RESULTS

The 2D structures of Ursolic acid analogues for which the QSAR model has been developed are shown in Table 1.

From the data in Table 2, QSAR equation have been developed where number of data point (n) is 18, is given below, here 95% confidence intervals are given in parentheses.

$\log IC_{50}$ = 2.855459 (± 1.140871) + 0.0003705 (0.000763) (ECI) + 5.67E-05 (± 0.0002483) (FC) - 0.5790109 (± 0.6538785) (MG)

Validation of QSAR Model

A quantitative assessment of model robustness has been performed through model validation. All

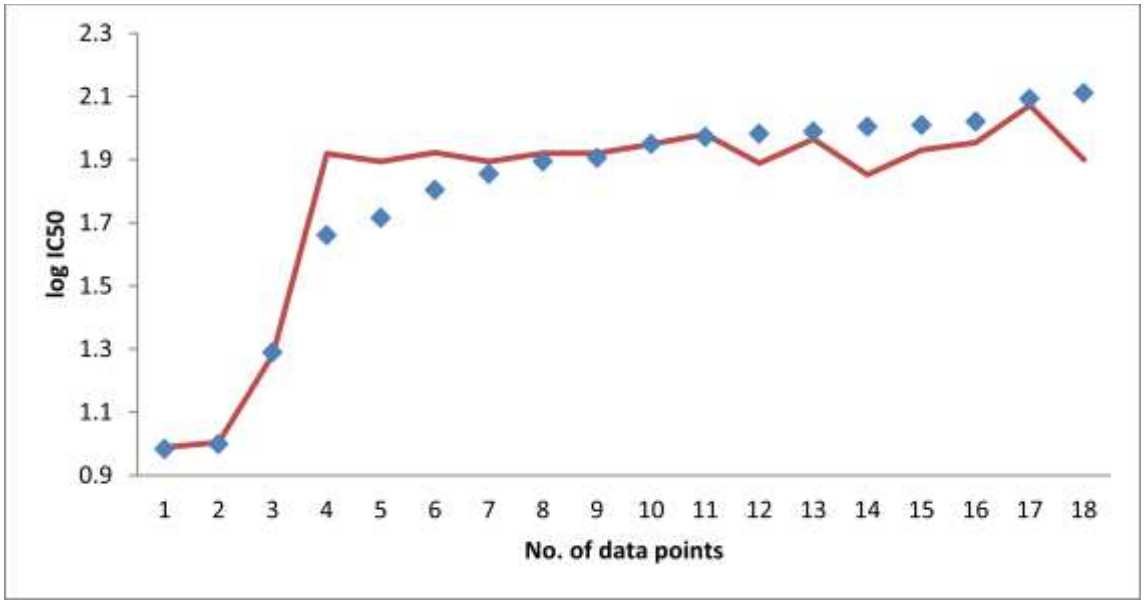

Fig. 2: Multiple linear graph between No. of data points and bioactivities

the statistical results of model validation have been given in Table 3.

Statistical Analysis

1. **Fraction of variance (r^2):** The value of fraction of variance may vary between 0 (means model without explanatory power) and 1 (means perfect model). QSAR model having $r^2 > 0.6$ will only be considered for validation[8, 15]. The value for this QSAR model is 0.9013.
2. **Cross-Validation Test (q^2):** A QSAR model must have $q^2 > 0.5$ for the predictive ability[8,16]. The value of q^2 for this QSAR model is 0.9013.
3. **Standard deviation (s):** The smaller s value is always required for the predictive QSAR model. The value of s for this QSAR model is 0.3339.
4. **r^2-q^2 < 0.3:** The difference between r^2 and q^2 should never be exceed by 0.3. A large difference suggests the following: presence of outliers, over-fitted model, and presence of irrelevant variables in data[8]. The value of r^2-q^2 for this QSAR model is 0.
5. **Quality Factor (Q):** Over fitting and chance correlation, due to excess number of descriptors, can be detected by Q value. Positive value for this QSAR model suggests its high predictive power and lack of overfitting[8].
6. **Fischer Statistics (F):** The F value of QSAR model was compared with their literature value at 95% level. The F value of this QSAR model is 42.6146 (where $F > F_{lit}$) suggests that the QSAR model is statistically significant at 95% level[8].

DISCUSSION

According to the developed QSAR model, the Ursolic acidsmust have positive Eccentric Connectivity Index for enhanced cytotoxic action against A-549 cells. A positive coefficient of Fragment Complexity also elevates the activity of described triterpenes towards its cytotoxic action against lung cancer. Moving towards the effects of the McGowan Volume on the bioactivity of derivatives of Ursolic acid, the developed QSAR model suggest that a negative elevation in MG will definitely be favorable to the activity, as discussed by R. P. Verma and Corwin Hansch[8] in 2010, Ajeet et. al.[17] in 2013, Ajeet[18] in 2013 and Ajeet[19-21] in 2012. A comparison (multiple linear regression plots) of observed values and predicted values of $\log IC_{50}$ for Ursolic acidderivatives used for development of QSAR equation is shown in Figure 1 and multiple linear graph is shown in Figure 2.

CONCLUSION

An analysis of developed QSAR model reflects a number of important points. Firstly it reveals that ECI, FC and MG are essential descriptors for the development of Ursolic acid derivatives. The developed QSAR model equation suggest that cytotoxic activity in terms of inhibition concentration might be improved by increasing the eccentric connectivity index and fragment complexity by making modifications to Ursolic acid pharmacophore along with ensuring that McGowan Volume should be reduce simultaneously.

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