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The novel antioxidant activity method for phenolic compounds

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ABSTRACT

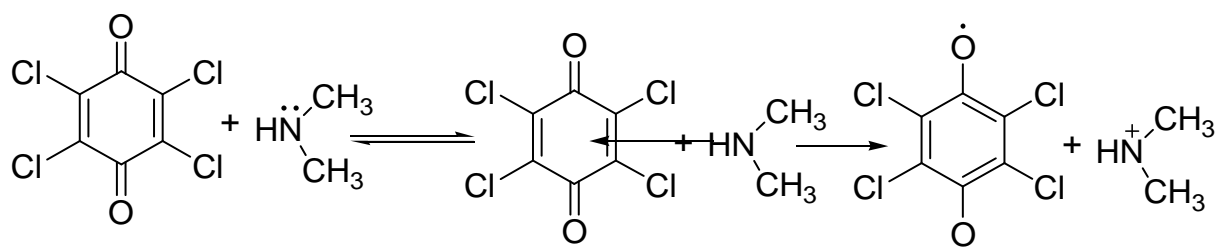
A simple sensitive spectrophotometric method was developed using Phenolic compounds. The method is based on the reaction of the secondary amine as n -electron donor with the π -acceptor 2,3,5,6-tetra chloro 1,4-benzo quinone. The coloured charge-transfer complex was measured at 412nm. The Free radical generation was confirmed with the polymerization of acrylonitrile with charge transfer complex as initiator. The procedure was applied successfully to the determination of the Free radical Scavenging activity of the title compounds. Among the Sixteen analogs, V_{16} exhibited highest antioxidant activity and very low IC_{50} value, V_6 exhibited least antioxidant activity with highest IC_{50} value. In the present study, Quantitative Structure-activity relationship modeling was performed on a series of Phenolic derivatives. IP, EN, Soft and EI are found to be the explainable variables for the semi empirical methods. The statistical parameters from the models indicate that the data well fitted and have high predictive ability. The external predictive capability of the established model was evaluated by test set of 16 compounds. It is recommended that the bulky electron-donation groups at the R^1 and R^3 position can increase the biological activities of the inhibitors. It is expected that the developed model could provide some useful information for the future synthesis of highly potent Transferase inhibitor.

Key words: Free Radical scavenging, chloranil, antioxidant activity, QSAR and Docking etc.

INTRODUCTION

The Intermolecular charge-transfer complexes are formed when electron donors and electron acceptors were interact [1]. The bond between the components of the complex was postulated to arise from the partial transfer of an electron from the base to the empty orbital of the acceptor. The Charge transfer complexes [2] have unique absorption bands in the ultraviolet-visible region. Some of the charge transfer complexes containing chloranil as an acceptor have been reported e.g. Tetra ThioFulvalene-Chloranil[3], Tetra ThioFulvalene-imidazole-Chloranil[4], p-phenylenediamine-Chloranil[5], Tetra Methyl-p-PhenyleneDiamine-Chloranil[6], Aniline-Chloranil[7], aromatic amines and nitrogen heterocycles-Chloranil[8], N-Aryldithiocarbmates-Chloranil[9], phosphine oxide and tri-n-butylphosphate-Choranil[10] etc.

π -Acceptors such as 7,7,8,8-tetracyano quinodimethane (TCNQ), tetracyanoethylene (TCNE), 2,3-dichloro-5,6-dicyano-1,4-benzo-quinone(DDQ), 2,3,5,6-tetrabromo-1,4-benzoquinone(bromanil) and 2,3,5,6-tetrachloro-1,4-benzoquinone(choranil) are known to yield charge-transfer complexes and radical anions with a variety of electron donors[11-12]. Formation of charge transfer complex between chloranil and dimethyl amine followed by formation of radical ions [13] are shown in Scheme.1.



Scheme 1

Free radical species are known to play important roles in biological systems such as mitochondria, signal transductions, and the immune system. In the human body the free radicals are continuously produced due to the oxygen utilization by the cells of the body. This generates a series of Reactive Oxygen Species (ROS) like super oxide anion (O_2^-) and hydroxyl (HO^\cdot) radicals and non-free radical species such as H_2O_2 singlet oxygen (O_2) and nitric oxide (NO).

The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids and can initiate degenerative disease. Antioxidant compounds like Phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide hydro peroxide or lipid peroxy and thus inhibit the oxidative mechanisms that lead to degenerative diseases. The spectroscopic method is conceived because of rapid formation of the complexes. ROS are highly reactive and can easily react with almost all the biological molecules including DNA, proteins, lipids and lipoproteins [14]. Antioxidants are of great interest because of their involvement in important biological and industrial processes. In general, compounds with antioxidant activity have been found to possess anticancer, anti-cardiovascular, anti-inflammation and many other activities [15-17]. The antioxidants are molecules that are mainly decelerate or prevent the oxidation reaction in vitro by terminating the oxidation of chain reaction [18]. The application of antioxidants in pharmacology is valuable to improve current treatments for diseases.

Phenolic compounds are chemical agents that exert their principle pharmacological and therapeutic effects by acting at peripheral sites to either enhance or reduce the activity of components of the sympathetic division on autonomic nervous system. These derivatives are acts as antioxidants[19]. The radical produced by decomposition of charge transfer complex can easily be trapped by Phenolic compounds. The optical density of charge transfer complex can be followed by spectroscopic method. The literature survey reveals that the any research group has not attempted to develop a spectroscopic method for in vitro bioassay of antioxidant nature of Phenolic compounds by charge transfer complex. Therefore this prompted us to develop a direct, sensitive and accurate for antioxidant bioassay of in vitro type and develop the high efficacy antioxidants by molecular modeling studies.

MATERIALS AND METHODS

General Analytical Procedure

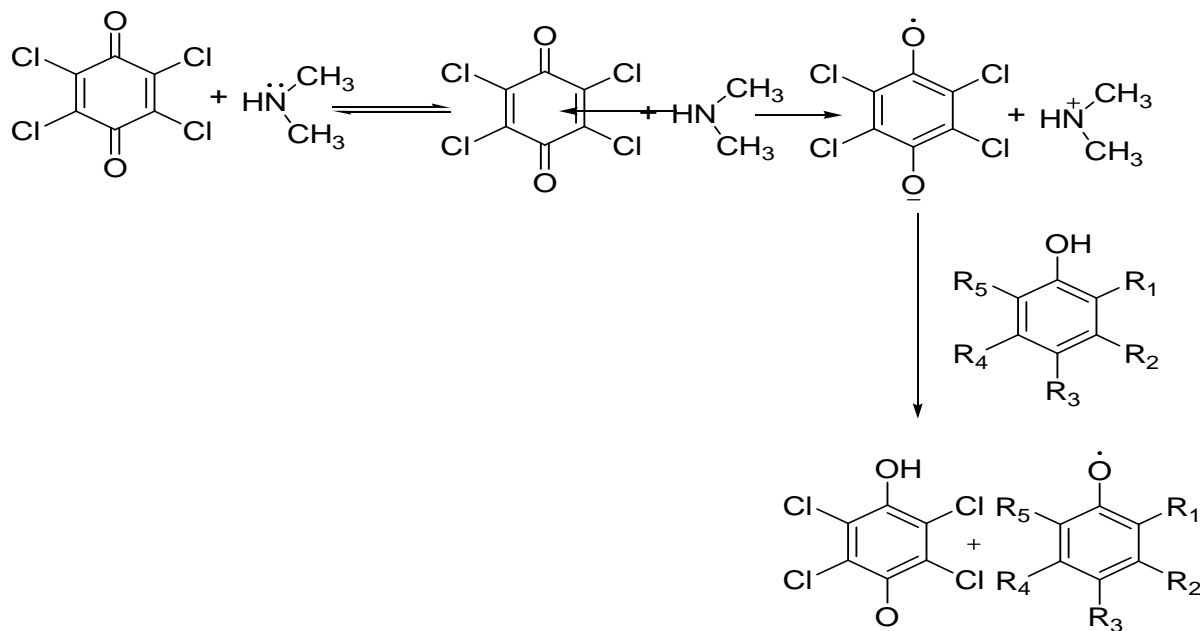
2,3,5,6-tetrachloro-1,4-benzoquinone(chloranil) was purchased from the AVRA SYNTHESIS. PVT.LTD. A/28/1/19, Road No.15, IDA, NACHARAM, HYD-501507. L-ASCORBIC ACID, 1,4-dioxane and Acetone solvents were purchased from the SD FINE- CHEM PVT.LTD.315-317, T.V.INDUSTRIAL ESTATE, 248,WORLI ROAD, MUMBAI-30. Systronics Version1.1PC based Double Beam Spectrophotometer 2202 with matched, 1cm Quartz cuvettes, Digital weighing balance, calibrated flasks, beakers, were used. Into a 100ml Calibrated flask, 25mg of 2,3,5,6-tetrachloro-1,4-benzoquinone(chloranil) was weighed accurately and dissolved in 2ml of 1,4-dioxane, and the volume made up to the mark with the same solvent then add 1ml of the dimethyl amine solution within 5 minutes complex was formed. It was then diluted quantitatively to obtain the suitable concentration. Into a 10ml Calibrated flask, 10mM concentration of Ascorbic acid was prepared by weighing 17.6mg of ascorbic acid accurately and dissolving in 2ml of distilled water. The volume was made up to the mark with the same solvent. It was used as standard and by maintaining the standard concentration each time 10ml of phenolic analogs solution was prepared.

In 10 ml Calibrated flasks, 4ml of the complex solution was placed and then 1ml of the sample solution was added. The absorbance of the solution was measured at the wavelength of maximum charge transfer bands i.e. at 412nm after the appropriate time interval at room temperature against blank. Absorbance was recorded and percentage of Radical Scavenging Activity [20] was calculated using the following formula.

$$\% \text{Radical Scavenging Activity} = \frac{A_b - A_a}{A_b} \times 100$$

Where A_b is the absorption of complex solution A_a is the absorption of the test sample

Sample concentration providing the 50% Radical Scavenging activity is taken as IC_{50} value.



Scheme 2

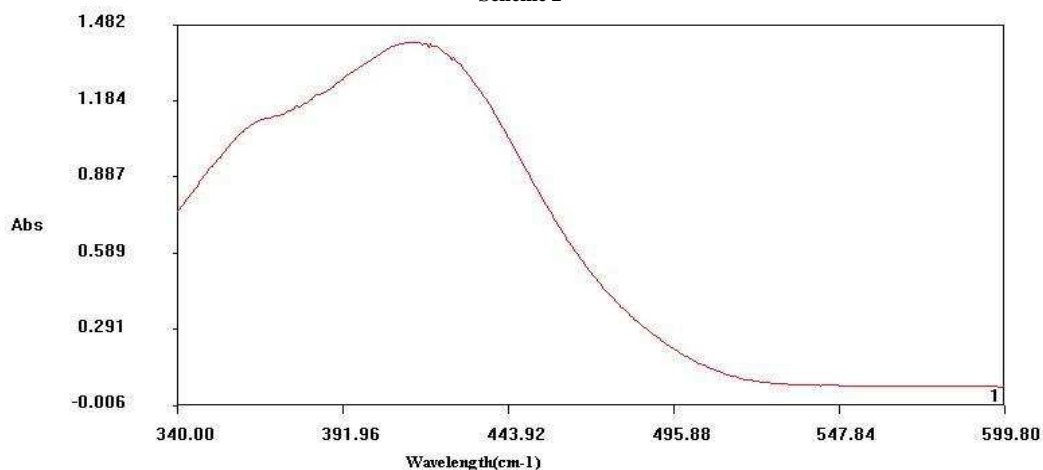


Figure. 1 Absorption Spectrum of Charge Transfer complex with Chloranil in 1,4-dioxane. Blank: 1,4-dioxane

On studying the charge transfer complex, the maximum peak was exhibited at 412 nm. Scheme.2 indicates the formation of charge transfer complex followed by chloranil radical. A simple method that has been developed to determine the antioxidant activity of Phenolic compounds utilizes the stable chloranil radical. When the odd electron in the chloranil free radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced chloranil. The resulting decolorization is stoichiometric with respect to number of electrons captured. In the present study Systronics Version1.1 PC based Double Beam Spectrophotometer 2202 in which Absorbance of

Phenolic compounds are noted with time and the standard was assessed on the basis of the radical scavenging effect of the stable 2,3,5,6-tetrachloro-1,4-benzoquinone(chloranil)free radical activity by novel method and expressed as IC₅₀value.

Table 1

| Compd | % of Radical Scavenging | IC ₅₀ value (uM) | Activity |
|-----------------|-------------------------|-----------------------------|----------|
| V ₁ | 29.46 | 16.97 | 7.77 |
| V ₂ | 20.40 | 24.51 | 7.61 |
| V ₃ | 18.07 | 27.67 | 7.56 |
| V ₄ | 32.79 | 15.25 | 7.82 |
| V ₅ | 52.34 | 9.55 | 8.02 |
| V ₆ | 6.81 | 73.42 | 7.13 |
| V ₇ | 37.53 | 13.32 | 7.87 |
| V ₈ | 46.60 | 10.73 | 7.97 |
| V ₉ | 50.14 | 9.97 | 8.00 |
| V ₁₀ | 51.42 | 9.72 | 8.01 |
| V ₁₁ | 35.91 | 13.92 | 7.86 |
| V ₁₂ | 43.06 | 11.61 | 7.93 |
| V ₁₃ | 11.26 | 44.40 | 7.35 |
| V ₁₄ | 49.57 | 10.09 | 8.00 |
| V ₁₅ | 47.86 | 10.45 | 7.98 |
| V ₁₆ | 63.63 | 7.86 | 8.10 |
| Ascorbic acid | 93.34 | 5.3568 | 8.27 |

Ascorbic acid is used as standard

BASIC STRUCTURE

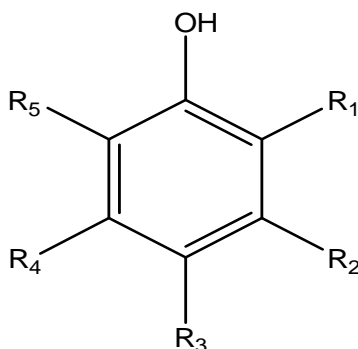


Table 2

| Compd | R ₁ | R ₂ | R ₃ | R ₄ | R ₅ |
|-----------------|-----------------|-----------------|--|-------------------------------|-----------------|
| V ₁ | H | OH | H | H | H |
| V ₂ | H | H | OH | H | H |
| V ₃ | H | H | H | H | H |
| V ₄ | OH | H | H | H | H |
| V ₅ | COOH | H | H | H | H |
| V ₆ | NO ₂ | H | NO ₂ | H | NO ₂ |
| V ₇ | CH ₃ | H | H | H | H |
| V ₈ | H | H | CH ₃ | H | H |
| V ₉ | NO ₂ | H | H | H | H |
| V ₁₀ | H | H | NO ₂ | H | H |
| V ₁₁ | NH ₂ | H | H | H | H |
| V ₁₂ | H | NH ₂ | H | H | H |
| V ₁₃ | H | H | NH ₂ | H | H |
| V ₁₄ | H | H | Cl | H | H |
| V ₁₅ | H | H | CH ₂ CH(NH ₂)COOH | H | H |
| V ₁₆ | H | H | H | C ₆ H ₅ | H |

COMPUTATIONAL CALCULATIONS

A series of compounds tested for Antioxidant activity was selected for the present study and the program of window Hyperchem software in [21] (<http://www.warezdestiny.com/free-hyp>) was used in modeling studies. The molecules were generated and the energy was minimized using molecular modeling pro. The physicochemical parameters, such as vertical ionization potentials (IP_v's) electron affinity (EA), electro negativity (χ), hardness (η), softness (S), electrophilic index (ω), partition coefficient (LogP), charges, hydration energy (HE) and polarisability (Pol) were obtained for Phenolic derivatives. The appropriate descriptors or parameters for the compounds were used as independent variables for deciding in Antioxidant activity.

Table 3 Values obtained for the AM₁ computational method

| Compd | IP _v | IP | EA | EN | η | S | ω | Logp | HE | Pol(A ^{o3}) |
|-----------------|-----------------|-------|--------|-------|--------|------|----------|-------|--------|-----------------------|
| V ₁ | -1.05 | 9.35 | -10.02 | 0.33 | 9.68 | 0.05 | 0.01 | 2.56 | -7.70 | 27.40 |
| V ₂ | -1.06 | 9.32 | -9.99 | 0.33 | 9.66 | 0.05 | 0.01 | 3.18 | -7.07 | 29.24 |
| V ₃ | -1.30 | 9.65 | -10.09 | 0.22 | 9.87 | 0.05 | 0.00 | 3.67 | -8.33 | 27.13 |
| V ₄ | -0.66 | 9.61 | -10.08 | 0.23 | 9.85 | 0.05 | 0.00 | 2.36 | -9.86 | 34.52 |
| V ₅ | -2.18 | 9.46 | -11.03 | 0.78 | 10.25 | 0.05 | 0.03 | -0.04 | -13.08 | 13.63 |
| V ₆ | -1.75 | 1.41 | -10.80 | -0.31 | 11.11 | 0.05 | 0.00 | -3.47 | -18.83 | 16.59 |
| V ₇ | -2.30 | 9.00 | -11.94 | 1.47 | 10.47 | 0.05 | 0.10 | 0.73 | -7.38 | 12.91 |
| V ₈ | -2.17 | 8.88 | -11.95 | 1.53 | 10.41 | 0.05 | 0.11 | 0.73 | -7.66 | 12.91 |
| V ₉ | -2.15 | 9.91 | -10.88 | 0.48 | 10.40 | 0.05 | 0.01 | -4.11 | -12.62 | 12.91 |
| V ₁₀ | -2.17 | 10.07 | -11.16 | 0.54 | 10.62 | 0.05 | 0.01 | -4.11 | -13.80 | 12.91 |
| V ₁₁ | -2.31 | 8.20 | -11.08 | 1.44 | 9.64 | 0.05 | 0.11 | -1.15 | -13.55 | 12.42 |
| V ₁₂ | -2.39 | 8.28 | -11.26 | 1.49 | 9.77 | 0.05 | 0.11 | -1.15 | -14.07 | 12.42 |
| V ₁₃ | -2.17 | 7.96 | -11.16 | 1.60 | 9.56 | 0.05 | 0.13 | -1.15 | -14.30 | 12.42 |
| V ₁₄ | -2.14 | 9.12 | -11.84 | 1.36 | 10.48 | 0.05 | 0.09 | 0.35 | -8.58 | 13.00 |
| V ₁₅ | -1.93 | 8.99 | -11.32 | 1.17 | 10.16 | 0.05 | 0.07 | -0.64 | -18.32 | 18.65 |
| V ₁₆ | -1.88 | 8.49 | -10.18 | 0.85 | 9.34 | 0.05 | 0.04 | 0.65 | -8.44 | 17.25 |

Table 4 values obtained for the PM3 computational method

| Compd | IP _v | IP | EA | EN | η | S | ω | LogP | HE | Pol(A ^{o3}) |
|-----------------|-----------------|-------|--------|-------|--------|------|----------|--------|--------|-----------------------|
| V ₁ | -1.08 | 9.37 | -9.92 | 0.27 | 9.64 | 0.05 | 0.00 | 2.56 | -7.52 | 27.40 |
| V ₂ | -1.08 | 9.35 | -9.89 | 0.27 | 9.62 | 0.05 | 0.00 | 3.18 | -7.02 | 29.24 |
| V ₃ | -1.32 | 9.67 | -10.01 | 0.17 | 9.84 | 0.05 | 0.00 | 3.67 | -8.09 | 27.13 |
| V ₄ | -0.85 | 9.47 | -9.83 | 0.18 | 9.65 | 0.05 | 0.00 | 2.36 | -9.94 | 34.52 |
| V ₅ | -2.23 | 9.40 | -10.92 | 0.76 | 10.16 | 0.05 | 0.03 | -0.04 | -13.20 | 13.63 |
| V ₆ | -1.73 | 11.46 | -10.85 | -0.31 | 11.15 | 0.04 | 0.00 | -13.47 | -19.20 | 16.59 |
| V ₇ | -2.38 | 9.06 | -12.05 | 1.49 | 10.55 | 0.05 | 0.11 | 0.73 | -7.35 | 12.91 |
| V ₈ | -2.26 | 8.95 | -12.06 | 1.55 | 10.50 | 0.05 | 0.11 | 0.73 | -7.62 | 12.91 |
| V ₉ | -2.18 | 9.90 | -10.83 | 0.46 | 10.37 | 0.05 | 0.01 | -4.11 | -12.59 | 12.91 |
| V ₁₀ | -2.21 | 10.17 | -11.25 | 0.54 | 10.71 | 0.05 | 0.01 | -4.11 | -13.81 | 12.91 |
| V ₁₁ | -2.42 | 8.09 | -11.08 | 1.49 | 9.58 | 0.05 | 0.12 | -1.15 | -13.60 | 12.42 |
| V ₁₂ | -2.52 | 8.12 | -11.13 | 1.50 | 9.63 | 0.05 | 0.12 | -1.15 | -14.08 | 12.42 |
| V ₁₃ | -2.26 | 7.84 | -11.02 | 1.59 | 9.43 | 0.05 | 0.13 | -1.15 | -14.31 | 12.42 |
| V ₁₄ | -1.84 | 9.01 | -11.73 | 1.36 | 10.37 | 0.05 | 0.09 | 0.35 | -8.54 | 13.00 |
| V ₁₅ | -1.97 | 9.08 | -11.47 | 1.20 | 10.28 | 0.05 | 0.07 | -0.64 | -18.38 | 18.65 |
| V ₁₆ | -1.94 | 8.59 | -10.26 | 0.83 | 9.43 | 0.05 | 0.04 | 0.65 | -8.41 | 17.25 |

Quantum chemical calculations at the AM1 [22] and PM3 [23] semi empirical theory levels, are employed for full optimization of the selected neutral compounds. The geometrical structures of the radicals studied are optimized independently from the neutral molecules prior to the calculation of energies, treated as open shell systems. Using the program of window hyper chem software Inc performs all calculations. The calculated vertical ionization potential (IP_v's) and electron affinity (EA) are corrected for zero-point energy, assuming a negligible error and thus saving computer-time. The IP_v are calculated as the energy differences between a radical cation and the respective neutral molecule; IP_v ($E_{\text{cation}} - E_{\text{neutral}}$) DFT and Koopmans's theorem ($IP = -\epsilon$ HOMO). The EA are computed as the energy differences between a neutral form and the anion molecule; $EA = E_{\text{neutral}} - E_{\text{anion}}$. The AM1 and PM3-based reactivity descriptors are obtained from Eqs. (1) – (2) [24-27].

The window version software SPSS10 [28] (SPSS Software. Consult <http://www.spss.com>) was used in the regression analysis. A relation between biological activity, expressed as Log₁/IC₅₀, and the physicochemical parameters and QSAR was analyzed statistically by fitting the data to correlation equations consisting of various

combinations of these parameters. The statistical optimization was used to propose the best correlation model. The matrix correlation uses the Pearson product moment correlation to measure the degree of linear relationship between two variables. The coefficient assumes a value between -1 and +1. If one variable tends to increase the other decreases, the correlation coefficient is negative. Conversely, if the two variables tend to increase together the correlation coefficient is positive. We obtained the correlation matrix between Antioxidant activity and respective calculated properties for Phenolic derivatives. The more relevant regression models were selected following criteria: The correlation coefficient I, the Fisher ratio values (F) and the standard deviations(s), standard error estimate (SEE), percentage of effective variable(%EV) and R^2 adjusted(R^2 adj).

The best equation was also tested for their predictive power using a cross-validation procedure. The cross-validation is a practical and reliable method for testing this significance. In principle, the so-called "leave-one-out" approach consist in developing a number of models with one sample omitted at the time. After developing each model, the omitted data is predicted and the differences between actual and predicted reduction potential (y) values are calculated. The sum of squares of these differences is computed and finally the performance of the model is given by PRESS (Predictive Sum of Squares) and SPRESS (Standard deviation of cross validation)[29]. The predictive ability of the model was also quantified in terms of the Q^2_{cv} [30].

The biological activity data and the physicochemical properties, IP, EA, EI, EN, Hard, Soft, LogP, HE and Polarisability of the Phenolic derivatives are given in Tables 3 and 4. The data from these tables were subjected to regression analysis. The Correlation matrices were generated with 16 analogs. The term close to 1 indicates high co-linearity, while the value below 0.5 indicates that no co-linearity exist between more than the two parameters. The perusal of correlation matrix indicates that IP, EN, Soft and EI are the predicted parameters from AM1 method. From regression methods backward, forward, removed and stepwise. IP, EN, Soft and EI were found to be explainable variable. The regression technique was applied through the origin using these explainable parameters.

$$\text{ACTIVITY} = 0.414(0.034)\text{IP} + 1.771(0.184)\text{EN} + 65.871(7.207)\text{Soft} - 14.863(2.079)\text{EI} \text{-----}(1)$$

N=16; R=1.00; $R^2=1.00$; R^2 adjusted=1.00; SEE=0.1218; F=16483; Sum of Squares=978; df=4; Mean Square=245; Q=8.21; %EV=100

Eq.1 explains the biological activity of 16 analogs. In this way, the predictive molecular descriptors IP, EN, Soft and EI were considered as variables. From the correlation matrix table, it reveals IP, EN, Soft and EI were found to be explainable variables. A parametric QSAR equation with IP, EN, Soft and EI was generated in PM3 method also.

$$\text{ACTIVITY} = 0.395(0.032)\text{IP} + 1.704(0.178)\text{EN} + 70.607(6.586)\text{Soft} - 14.438(2.073)\text{EI} \text{-----}(2)$$

N=16; R=1.00; $R^2=1.0$; R^2 adjusted=1.0; SEE=0.1254; F=15540; Sum of Squares=978; df=4; Mean Square=244; Q=7.97; %EV=100

In an attempt to investigate the predictive potential of proposed models, the cross-validation parameters (Q^2_{cv} and PRESS) were calculated and used. The predictive power of the equations was confirmed by leave-one-out (LOO) cross-validation method [31] where, compounds are deleted one after another and prediction of the activity of the deleted compound is made based on QSAR model. The cross-validation evaluates the validity of a model by how well it predicts the data rather than how well it fits the data. The cross-validation parameter, Q^2_{cv} , is mentioned in the respective equations.

$$Q^2_{cv} = (\text{SD} - \text{PRESS}) / \text{SD}$$

Where the PRESS (predictive residual sum of squares) and SD (standard deviation) values are obtained as

$$\text{PRESS} = \sum (\text{property observed} - \text{property predicted})^2$$

$$\text{SD} = \sum (\text{property observed} - \text{property mean})^2$$

Eq.1 and 2 of AM1 and PM3 methods respectively give good Q^2_{cv} values, which should be always smaller than %EV. A model is considered to be significant when $Q^2_{cv} > 0.48$

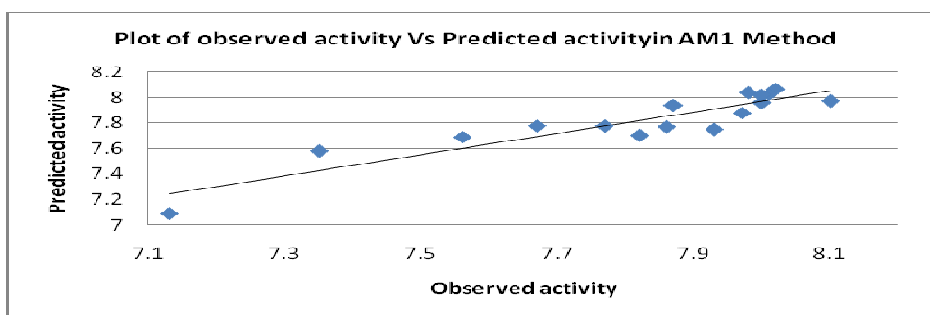


Figure.2

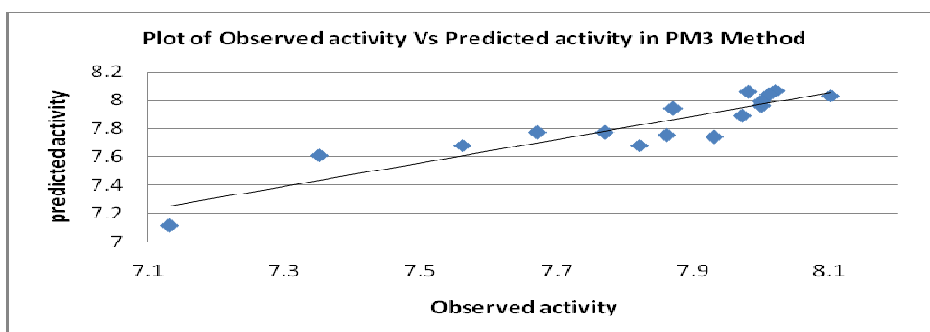


Figure.3

Another cross-validation parameter, PRESS is the sum of the squared differences between the actual and that predicted. The quality factor Q , is defined as the ratio of regression constants I to the standard error estimation (SEE), that is, $Q = R/SEE$. This indicates that the higher the value of R , and the lower the value of SEE, the higher is the magnitude of Q and the better will be the correlation. The plots confirmed the predictive ability of model as well as good correlation between the predictive activity and observed activity.

Table 5

| Compd | Observed Activity | Predicted Activity from AM1 method | Residual | Observed Activity | Predicted Activity from PM3 Method | Residual |
|-----------------|-------------------|------------------------------------|----------|-------------------|------------------------------------|----------|
| V ₁ | 7.77 | 7.78 | -.01 | 7.77 | 7.77 | .00 |
| V ₂ | 7.67 | 7.78 | -.11 | 7.67 | 7.77 | -.10 |
| V ₃ | 7.56 | 7.69 | -.13 | 7.56 | 7.68 | -.12 |
| V ₄ | 7.82 | 7.7 | .12 | 7.82 | 7.68 | .14 |
| V ₅ | 8.02 | 8.07 | -.05 | 8.02 | 8.07 | -.05 |
| V ₆ | 7.13 | 7.09 | .04 | 7.13 | 7.11 | .02 |
| V ₇ | 7.87 | 7.94 | -.07 | 7.87 | 7.94 | -.07 |
| V ₈ | 7.97 | 7.88 | .09 | 7.97 | 7.89 | .08 |
| V ₉ | 8 | 7.96 | .04 | 8 | 7.96 | .04 |
| V ₁₀ | 8.01 | 8.03 | -.02 | 8.01 | 8.04 | -.03 |
| V ₁₁ | 7.86 | 7.77 | .09 | 7.86 | 7.75 | .11 |
| V ₁₂ | 7.93 | 7.75 | .18 | 7.93 | 7.74 | .19 |
| V ₁₃ | 7.35 | 7.58 | -.23 | 7.35 | 7.61 | -.26 |
| V ₁₄ | 8 | 8.02 | -.02 | 8 | 7.99 | .01 |
| V ₁₅ | 7.98 | 8.04 | -.06 | 7.98 | 8.06 | -.08 |
| V ₁₆ | 8.1 | 7.97 | .13 | 8.1 | 8.03 | .07 |

DOCKING STUDIES:

Gold 2.0 version (Genetic optimization for Ligand Docking) docking program was evaluated in the present study. The Gold program uses Genetic Algorithm (GA) to explore the full range of ligand conformational flexibility and the rotational flexibility of selected receptor hydrogen's [32-34]. The mechanism for ligand placement is based on the fitting points. The program adds the fitting points to hydrogen-bonding groups on the protein and ligand and maps acceptor points in the ligand on donor points in the protein and vice versa. The docking poses are ranked based on

molecular mechanics like scoring functions. There are two different built-in scoring functions in the gold program that are gold fitness and chemscore.

Table 6 Gold Fitness by default settings

| File name | Fitness | S(hb_ext) | S(vdw_ext) | S(hb_int) | S(vdw_int) |
|--------------|---------|-----------|------------|-----------|------------|
| V1_ml_3.mol | 27.36 | 4.57 | 19.47 | 0.00 | -3.98 |
| V2_ml_1.mol | 28.59 | 4.62 | 20.75 | 0.00 | -4.57 |
| V3_ml_2.mol | 27.07 | 3.86 | 18.41 | 0.00 | -2.10 |
| V4_ml_3.mol | 27.62 | 5.01 | 19.89 | 0.00 | -4.73 |
| V5_ml_2.mol | 31.20 | 5.50 | 23.31 | 0.00 | -6.34 |
| V6_ml_2.mol | 39.77 | 5.98 | 28.16 | 0.00 | -4.93 |
| V7_ml_7.mol | 30.14 | 2.65 | 22.09 | 0.00 | -2.89 |
| V8_ml_1.mol | 30.78 | 3.07 | 22.11 | 0.00 | -2.68 |
| V9_ml_2.mol | 32.26 | 5.04 | 21.75 | 0.00 | -2.69 |
| V10_ml_3.mol | 33.12 | 4.29 | 22.87 | 0.00 | -2.61 |
| V11_ml_6.mol | 29.94 | 3.79 | 20.58 | 0.00 | -2.15 |
| V12_ml_1.mol | 31.03 | 4.15 | 20.96 | 0.00 | -1.95 |
| V13_ml_4.mol | 30.65 | 4.19 | 20.82 | 0.00 | -2.16 |
| V14_ml_3.mol | 30.61 | 3.50 | 21.19 | 0.00 | -2.03 |
| V15_ml_3.mol | 43.26 | 11.12 | 27.32 | 0.00 | -5.44 |
| V16_ml_1.mol | 38.36 | 3.48 | 27.88 | 0.00 | -3.47 |

Table 7 Chemscore by Default Settings

| Compd | Score | DG | S(hbond) | S(metal) | S(lipo) | DE(clash) | DE(int) |
|-------|-------|--------|----------|----------|---------|-----------|---------|
| V1 | 22.65 | -22.66 | 2.52 | 0.00 | 109.50 | 0.00 | 0.00 |
| V2 | 21.97 | -21.97 | 1.96 | 0.00 | 119.49 | -0.00 | 0.00 |
| V3 | 21.13 | -21.16 | 1.28 | 0.00 | 119.27 | 0.03 | 0.00 |
| V4 | 21.66 | -22.11 | 2.33 | 0.00 | 110.38 | 0.45 | 0.00 |
| V5 | 25.26 | -25.27 | 2.95 | 0.00 | 120.82 | 0.00 | 0.00 |
| V6 | 19.30 | -25.56 | 2.93 | 0.00 | 109.79 | 2.03 | 4.23 |
| V7 | 22.73 | -22.74 | 1.15 | 0.00 | 136.53 | 0.01 | 0.00 |
| V8 | 23.60 | -23.78 | 1.36 | 0.00 | 139.50 | 0.18 | 0.00 |
| V9 | 23.96 | -25.96 | 2.94 | 0.00 | 112.93 | 0.34 | 1.66 |
| V10 | 26.66 | -27.51 | 3.46 | 0.00 | 111.40 | 0.73 | 0.12 |
| V11 | 23.87 | -24.04 | 2.40 | 0.00 | 111.97 | 0.17 | 0.00 |
| V12 | 22.83 | -22.89 | 1.65 | 0.00 | 123.51 | 0.07 | 0.00 |
| V13 | 22.81 | -22.83 | 1.88 | 0.00 | 116.52 | 0.02 | 0.00 |
| V14 | 22.83 | -22.85 | 0.99 | 0.00 | 142.14 | 0.03 | 0.00 |
| V15 | 24.21 | -25.00 | 3.74 | 0.00 | 130.54 | 0.44 | 0.34 |
| V16 | 30.31 | -30.40 | 1.53 | 0.00 | 191.27 | 0.09 | 0.00 |

Table 8. Argus lab binding energy values

| Compound | Argus Dock | | GA Dock | |
|-----------------|--------------|--------------------|--------------|--------------------|
| | BE(Kcal/mol) | Elapsed time (sec) | BE(Kcal/mol) | Elapsed time (sec) |
| V ₁ | -8.01 | 2 | -7.98 | 1 |
| V ₂ | -7.85 | 1 | -7.71 | 1 |
| V ₃ | -8.02 | 2 | -8.00 | 1 |
| V ₄ | -8.61 | 2 | -8.60 | 1 |
| V ₅ | -8.49 | 2 | -8.49 | 2 |
| V ₆ | -8.55 | 2 | -7.28 | 2 |
| V ₇ | -8.31 | 2 | -8.76 | 1 |
| V ₈ | -8.84 | 2 | -8.84 | 4 |
| V ₉ | -8.64 | 2 | -8.96 | 1 |
| V ₁₀ | -8.30 | 1 | -8.43 | 1 |
| V ₁₁ | -7.53 | 2 | -7.62 | 1 |
| V ₁₂ | -7.44 | 2 | -7.45 | 1 |
| V ₁₃ | -7.59 | 2 | -7.59 | 1 |
| V ₁₄ | -8.23 | 2 | -8.71 | 2 |
| V ₁₅ | -9.84 | 2 | -8.98 | 7 |
| V ₁₆ | -10.45 | 2 | -10.38 | 2 |

Argus lab 4.0.1 is molecular modeling and docking software [35-36]. It is very flexible and can reproduce crystallographic binding orientation. Argus lab provides a user-friendly graphical interface and uses shape dock algorithm, to carry out docking studies. There are two different functions in Argus lab that are Argus Dock and GA dock to evaluate the binding energies of the ligands.

Auto dock used a simulated annealing (SA) technique for configurationally exploration with a rapid energy evaluation using grid-based molecular affinity potentials [37-40]. It is the combined advantages of exploring a large search space and a robust energy evaluation. This has proven to be a powerful approach to the problem of docking a flexible substrate into the binding site of a static protein. Input to the procedure is minimal. The researcher specifies a rectangular volume around the protein, the rotatable bonds for the substrate, and an arbitrary or random starting configuration, and the procedure produces a relatively unbiased docking.

The molecular docking program Auto dock 4.2 was used to determine the potential binding mode between the most active compounds and selected enzyme candidate target. The Lamarckian Genetic Algorithm (LGA) implemented in the Auto dock program was applied following a protocol of 10 independent runs with a population size of 150 individuals, a mutation rate of 0.02, and a crossover rate of 0.80 and an elitism value of 1. These parameters were sufficient for the objective of a first approach for target search.

Table 9 Auto dock results

| Compd | Binding Energy (Kcal/Mol) | RMSD (Å ⁰) | Estimated inhibition constant ki (µM) |
|-----------------|---------------------------|------------------------|---------------------------------------|
| V ₁ | -3.74 | 22.00 | 1820 |
| V ₂ | -3.36 | 19.44 | 3420 |
| V ₃ | -3.32 | 19.84 | 3710 |
| V ₄ | -3.56 | 20.38 | 2450 |
| V ₅ | -5.56 | 22.99 | 84.68 |
| V ₆ | -7.81 | 19.09 | 1.88 |
| V ₇ | -3.51 | 19.58 | 2690 |
| V ₈ | -3.40 | 19.49 | 3200 |
| V ₉ | -6.24 | 18.63 | 26.70 |
| V ₁₀ | -6.23 | 18.59 | 27.00 |
| V ₁₁ | -3.54 | 18.82 | 2540 |
| V ₁₂ | -3.19 | 17.23 | 4610 |
| V ₁₃ | -3.35 | 23.02 | 3530 |
| V ₁₄ | -3.48 | 18.515 | 2820 |
| V ₁₅ | -5.46 | 19.02 | 99.28 |
| V ₁₆ | -4.45 | 17.35 | 550.99 |

RESULTS AND DISCUSSION

Active site analysis:

Two different sites were identified by Spdbv software [41]. The most probable binding site was analyzed and the residues in the active site are GLY1602 and ser1641. The hydrogen bond interactions of heteroatom LDR with 3SE2 are shown in figure.4.

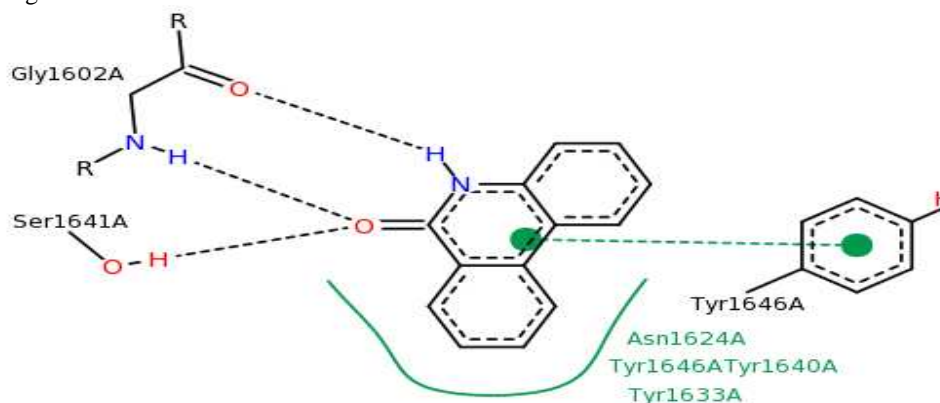


Figure. 4 Interactions of 3SE2:LDR:A:1

Experimental Results

The Free radical generation of Phenolic analogs was confirmed with the photo polymerization of acrylonitrile using charge transfer complex as initiator. Phenolic analogs were tested for antioxidant activity in the present experimental studies. As compared with the standard Ascorbic acid, the title compounds exhibited good antioxidant activity. The proposed procedure has the advantage in that most of the assays were performed in the visible region. The rapid development of colors at room temperature with non-corrosive reagents, the intensity, sensitivity and the stability of colors suggest obvious use of this method for the detection of the Antioxidant studies of the title compounds, so the title compounds exhibit good antioxidant activity.

Among the Twelve analogs studied, V₁₆ exhibited high antioxidant activity, very low IC₅₀ value and high percentage of radical scavenging, because bulky electron-donating groups increase the activity of the compounds which was followed by the analogs V₅, V₁₀, V₉ and V₁₄. The activity was least in V₆ because electron-withdrawing groups are decreasing the activity of the compounds, followed by V₁₃, both of which had high IC₅₀ values of 73.42uM and 44.40uM respectively.

Computational Results

In the present study, Quantitative Structure-activity relationship modeling was conducted on a series of Phenolic derivatives. In both semi empirical methods correlation coefficient R² is 1 and standard error estimation values AM1 and PM3 are 0.1218 and 0.1254 respectively. The statistical parameters from the models indicate that the data well fitted and have high predictive ability. The external predictive capability of the established model was evaluated by test set of 16 compounds. In both the semi-empirical methods AM1 and PM3, IP, EN, Soft and EI are the variables to explain the antioxidant activity are observed from the regression analysis. IP and Softness of the compounds are more activity is more observed. EN and EI are less then the activity is more observed. The Cross-validation correlation coefficients for AM1 and PM3 are 0.48 and 0.54 respectively. It is recommended that the bulky electron-donation groups at the R¹ and R³ position can increase the biological activities of the inhibitors. It is expected that the developed model could provide some useful information for the future synthesis of highly potent Transferase inhibitor. In present study, a test set of existing 16 compounds was tested for antioxidant activity. All ligands exhibited well gold score, Chemscore and good binding energy values. These results are analyzed both in terms of energy values (Auto dock binding energy ranged from -3.19 Kcal/mol to -7.81 Kcal/mol, Argus dock binding energy ranged from -7.44 to -10.45Kcal/mol and GA dock binding energy ranged from -7.28 to -10.38 Kcal/mol) and fitness scores (Gold score ranging from 27.07 to 43.26, Chem score ranging from 19.30 to 30.31).

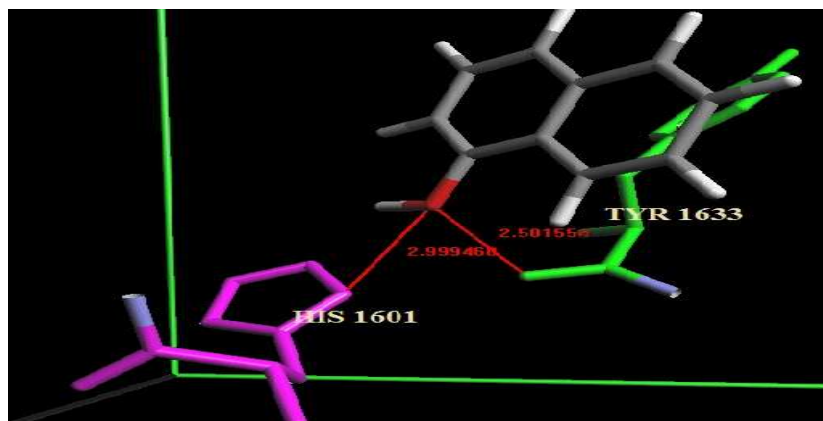


Figure.5 Argusdock best pose of V₁₆ molecule

Figure.6 Argus dock best pose of V₁₀ moleculeFigure.7 Auto dock best pose of V₁₆ moleculeFigure.8 Auto dock best pose of V₁₀ molecule

Docking results revealed that the substituted Phenolic compounds were found to be remarkable inhibitory activity on Transferase inhibitor. Among them V₅, V₁₀, V₉, V₁₄ and V₁₆ exhibited best Gold scores and Chem Scores and maximum binding energy values. The docking scores by using default settings were evaluated and given in Table[6-7]. The predicted scoring functions of these compounds have shown good correlation with binding energies. The Auto dock Binding energy, Root Mean Square Distance (RMSD) between protein-ligand, and enzyme inhibition

constant (K_i) are shown in Table-9. The Argus Lab binding energies and elapsed time were shown in table-8. It was found that all the ligands involve in hydrogen bonding with the binding site residue of Transferase inhibitor.

CONCLUSION

A novel spectrophotometric method for the determination of antioxidant activity of the Phenolic compounds using chloranil radical ion was studied in the present investigations. The present study, therefore confirms the suitability of chloranil for spectrophotometric analysis of title compound in the micro range. This method could also be applied to the quality control analysis of the investigated compounds.

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