International Research Journal of Pure & Applied Chemistry

22(7): 19-34, 2021; Article no.IRJPAC.72000
ISSN: 2231-3443, NLM ID: 101647669

QSPR/QSAR Modelling of the Antioxidant Properties of Some Flavonoids

R. Chabi Doco[1*], M. T. A. Kpota Houngue[1], Urbain A. Kuevi[1] and Y. G. S. Atohoun[1]

[1]Laboratory of Theoretical Chemistry and Molecular Spectroscopy (LACTHESMO), University of Abomey-Calavi, 03 BP 3409 Cotonou, Benin.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IRJPAC/2021/v22i730418
Editor(s):
(1) Dr. Farzaneh Mohamadpour, University of Sistan and Baluchestan, Iran.
Reviewers:
(1) Valerio Benedetti, University of Turin, Italy.
(2) Jigisha Anand, Deemed To Be University, India.
Complete Peer review History: https://www.sdiarticle4.com/review-history/72000

Original Research Article

Received 10 June 2021
Accepted 15 August 2021
Published 25 August 2021

ABSTRACT

Several methods exist when seeking to experimentally evaluate the antioxidant properties of a natural bioactive substance. In the case of flavonoids, the methods used are mainly based on the experimental determination of the percentage of inhibition (IC50) or the redox potential (E).
In the present work, a prediction study of the redox potential *E* and the inhibitory concentration *LogIC50* was carried out, using the AM1 and HF/6-311G(d,p) method.
At the end of this study, three (03) QSPR models were validated and retained, one (01) for the prediction of the redox potential and four (02) for the prediction of the inhibitory concentration :

- **The Redox Prediction Model**, developed at the AM1 approximation level, for which 96.43 of the experimental variance is explained by the descriptors :

$E = -0,29 + 0,22E_{Homo} + 0,11E_{Lumo} - 0,05\bar{\omega}^{-}$

- **The Inhibitory Concentration Prediction Models**, developed at the AM1 level, for which 96.35P of the experimental variance is explained by the descriptors :

$LogIC_{50} = -4,92 + 11,37E_{Homo} + 34,36E_{Lumo} + 0,67\bar{\omega}^{-}$

- **The Inhibitory Concentration Prediction Model**, developed at the HF/6-311G level (d, p), for which 99.96P of the experimental variance is explained by the descriptors. $LogIC_{50} = 62,40 + 80,25\ E_{Homo} - 28,44E_{lumo} + 52,01S - 71,26\ \eta - 6,11\mu$

**Corresponding author: E-mail: titiwan1980@gmail.com;*

The development of these QSPR models represents a significant advance in predicting the antioxidant properties of bioactive molecules such as flavonoids based on descriptors calculated by quantum chemical methods.

Keywords: Antioxidant; properties; QSAR/QSPR; quantum descriptors.

1. INTRODUCTION

The field of investigation is vast when it comes to experimentally evaluating the antioxidant properties of flavonoids. The methods used are mainly based on the experimental determination of the percentage inhibition (IC50) or the redox potential (E).

Indeed, several results published in the literature have shown that experimental parameters such as redox potential and inhibitory concentration allow to evaluate the antioxidant powers of bioactive molecules [1,2,3,4,5] (Jorgensen et *al.* 1999; Volikakis et *al.* 2000; Yamamura, 2003 and and Dragan AMI et *al.* 2017).

In the present work, QSAR/QSPR models for predicting antioxidant properties were developed. The aim is to find a linear relationship that will predict the experimental parameters E or IC50 as a function of quantum descriptors such as : electronic affinity (EA), ionisation energy (EI), hardness (η), softness (S), electronegativity (χ), electrophilic index (ω), energy (HOMO), energy (LOMO), energy gap (HOMO-LUMO), electrophilic index (ω), dipole moment (μ***,*** electron donor ($\overline{\omega}^-$) and electron acceptor ($\overline{\omega}^+$), used in our previous studies.

From the results obtained, the redox potential (E) and the inhibitory concentration (IC50) of the flavonoids will be predicted and consequently their antioxidant power.

2. MATERIALS AND METHODOLOGY

2.1 Materials

In our work, 29 flavonoids with known experimental redox potential (E) and inhibitory concentration (IC50) values were selected as the structural basis for study. These flavonoids are classified into two groups or series: the learning series with 19 molecules (≈ *2⁄3* of the base molecules) and the test series with 10 molecules (≈ *1⁄3* of the base molecules). The choice of molecules for the constitution of the groups is arbitrary. These molecules are coded M_i in order to simplify their notations.

The molecules of the learning set will be used to develop predictive models of redox potential and inhibitory concentration from quantum descriptors, and those of the test set for validation of the developed models. The experimental values of the redox potential and the inhibitory concentration of the molecules are taken from the literature [6].

2.2 Methodology

For the determination of the quantum descriptors on which the prediction models of the redox potential E and the inhibitory concentration IC50 of the studied molecules were developed, different levels of calculations were used. These are : AM1 and HF/6-311G (d, p). These levels of theory were chosen in view of the size of the molecules and the different quantum parameters to be evaluated.

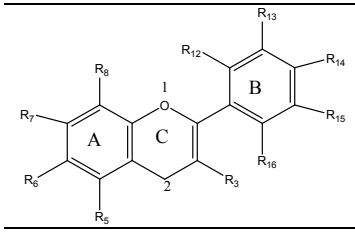
2.2.1 Statistical analysis

All molecules were optimised using the GAUSSIAN 09 program**.** Two software packages were used, according to their specificities, to perform the statistical analysis of the results and to plot the graph, i.e. XLSTAT and MATLAB**.** The choice of the quantum descriptors is based on two fundamental criteria.

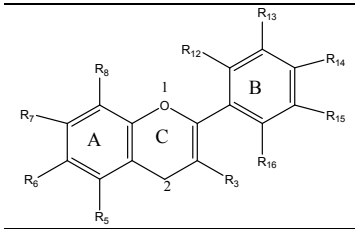
Criterion 1: By nature, the dependence of *Y* on X_i is assumed to be linear. Therefore the absolute value of the linear correlation coefficient between the property *Y* and the variables X_i must be greater than 0.50: **|R| ≥0.50**

Criterion 2: The different samples Y_i are assumed to be independent of each other. For two descriptors to be independent, the partial correlation coefficient (a_{ij}) between these two descriptors i and j must be strictly less than 0.70 : $a_{ij} < 0.70$

Table 1. Structure of the flavonoids studied



Basic structure of flavonoids											
Molecules	**C2**	**R3**	**R5**	**R6**	**R7**	**R8**	**R12**	**R13**	**R14**	**R15**	**Names**
M1	C	H	OH	H	OH	H	H	H	OH	OH	Epigallo catechin
M2	C	H	OH	H	OH	H	H	OH	OH	OH	Epigallocatechingallate
M3	C	Gallate	OH	H	OH	H	H	OH	OH	H	Epicatechin gallate
M4	C	Gallate	OH	H	OH	H	H	OH	OH	OH	Gallocatechin gallate
M5	C	Gallate	OH	H	OH	H	H	H	OH	OH	Catechin gallate
M6	C	OH	OH	H	OH	H	H	OH	OH	H	- Catechin
M7	C	OH	OH	H	OH	H	H	H	OH	OH	- Epicatechin
M8	C	OH	OH	H	OH	H	H	H	OH	OH	+ Epicatechin
M9	C	OH	OH	H	OH	H	H	OH	OH	H	+ Catechin
M10	C=O	OH	OH	H	OH	H	H	H	4-(2, 3, 4-trihydroxybutyl)-2-methoxyphenol		Silibinin
M11	C=O	H	H	OH	H	OH	H	OH	H	H	Luteolin
M12	C=O	H	H	OH	H	OH	H	H	H	H	Wogonin
M13	C=O	H	H	OH	H	OH	H	H	H	H	Apigenin
M14	C=O	OH	OH	H	H	OH	H	OH	H	H	Fustin
M15	C=O	OH	OH	OH	H	OH	H	H	H	H	Naringenin
M16	C=O	H	H	H	H	OH	H	H	H	H	Daidzein
M17	C=O	H	H	H	Glucose	H	H	H	OH	H	Daidzin
M18	C=O	H	H	H	OH	Glucose	H	H	OH	H	Puerarin
M19	C	OH	OH	H	OH	H	H	OH	OH	OH	Gallocatechin
M20	C=O	OH	OH	H	OH	H	H	OH	OH	OH	Myricetin
M21	C=O	OH	OH	H	OH	H	H	OH	H	H	Quercetin
M22	C=O	OH	H	H	OH	H	H	OH	H	H	Fisetin
M23	C=O	OH	OH	H	OH	H	H	H	H	H	Kaempferol



Basic structure of flavonoids

Molecules	C2	R3	R5	R6	R7	R8	R12	R13	R14	R15	Names
M24	C=O	OH	OH	H	OH	H	OH	H	H	H	Morin
M25	C=O	OH	OH	H	OH	H	H	H	H	H	Galangin
M26	C=O	Rutinose	H	H	H	H	H	OH	OH	H	Rutin
M27	C=O	Glucose	OH	H	OH	H	H	OH	H	H	Hyperoside
M28	C=O	OH	OH	OH	H	H	H	H	H	H	Baïcalein
M29	C=O	OH	OH	Glucose	H	H	H	H	H	H	Baïcalin

Table 2. Molecules in the learning and test series

Molecules in the learning series		Molecules in the test series	
N°	**Name of the molecules**	**N°**	**Name of the molecules**
M1	-(2R, 3R) Epigallocatechin	M20	Myricetin
M2	-(2R, 3R)-Epigallocatechin gallate	M21	Quercetin
M3	-(2S, 3R) -Gallocatechin gallate	M22	Fisetin
M4	(-) -(2S, 3R) -catechin gallate	M23	Kampferol
M5	-(2R, 3R)-Epicatechin gallate	M24	Morin
M6	(+) -(2S, 3S)-Epicatechin	M25	Galangin
M7	(-) -(2R, 3R) -Epicatechin	M26	Rutin
M8	(-) -(2S, 3R)-Catechin	M27	Hyperoside
M9	(+) -(2S, 3R)-Catechin	M28	Baicalein
M10	Silibinin	M29	Baicalin
M11	Luteolin		
M12	Wogonin		
M13	Apigenin		
M14	Fustin		
M15	Naringenin		
M16	Daidzein		
M17	Daidzin		
M18	Puerarin		
M19	-(2S, 3R) -Gallocatechin		

Chart 1. Quantum descriptors used. Debye (D)*; Electron-volt (eV)*

Quantum descriptors	Rating	Expression	Unit
Dipole moment	**μ**	-	(D)
Energy of the HOMO	E_{Homo}	-	*(eV)*
Energy from the LUMO	E_{Lumo}	-	*(eV)*
Electronic affinity	AE	$AE = -E_{Lumo}$ [12]	*(eV)*
Ionisation energy	IE	$IE = -E_{Homo}$ ([13]	*(eV)*
GAP (HOMO-LUMO)	Gap	Gap = $E_{Lumo} - E_{Homo}$	*(eV)*
Electronegativity	χ	$\chi = \frac{(IP + EA)}{2}$ [14]	*(eV)*
Hardness.	η	$\eta = \frac{(IP - EA)}{2}$ [15]	*(eV)*
Softness	S	$S = \frac{1}{2\eta}$ [16]	$(eV)^{-1}$
Electrophilic Index	ω	$\omega = \frac{\chi^2}{2\eta}$ [17]	*(eV)*
Donor electron power	$\bar{\omega}^-$	$\bar{\omega}^- = \frac{(3.EI+AE)^2}{16(EI-AE)}$ [18]	*(eV)*
Electron acceptor power	$\bar{\omega}^+$	$\bar{\omega}^+ = \frac{(EI+3.AE)^2}{16(EI-AE)}$ [19]	*(eV)*

The predictive power of a model is also based on the Tropsha criteria. If the three fifths (3/5) of the criteria are verified then the model has a good predictive power. Normality tests were also carried out to verify the quality of the confidence interval obtained. These are the Shapiro-Wilk and Durbin-Watson tests.

2.2.2 Theoretical descriptors

These are quantum descriptors calculated by quantum chemical methods. Chart 1 shows the quantum descriptors used in this study [7,8,9,10,11] [20-24].

2.2.3 Contribution of an explanatory variable to the prediction of a property

The contribution of an explanatory variable X_i noted C_{X_i} to the prediction of a property of *Y* is based on the statistical parameter t_test which indicates the significance of an explanatory variable in a model [25].

$$C_{X_i} = \frac{|t\_test(X_i)|}{\Sigma|(t\_test(X_i)|} \times 100$$

The contribution is expressed as a percentage (%), where $|t\_test(X_i)|$, the absolute value of the t_test of the variable X_i ; $\Sigma|(t\_test(X_i)|$ the sum of the absolute values of the t_test of all the variables X_i of the model. The higher C_{X_i} is, the greater the contribution of the explanatory variable X_i in the model developed [26].

3. RESULTS AND DISCUSSION

In Tables 3 to 6, the values of the calculated quantum descriptors and the values of the redox potential E and the inhibitory concentration IC50 of the molecules of the training and test series are recorded.

3.1 Selection of Quantum Descriptors for the Prediction of the *LogIC50* Inhibitory Concentration

The results for the final selection of the predictive quantum descriptors for the *LogIC50* inhibitory concentration are reported in Tables 8 and 9.

The results in Table 8 allow us to consider two groups of predictive quantum descriptors of *LogIC50* for the AM1 level:

- **Group 3**: LUMO Energy ($_{ELumo}$), HOMO Energy (*EHomo*) and Electron Donor Power ($\bar{\omega}^-$) ;
- **Group 4:** Energy of the HOMO (*EHomo*), Electron Acceptance Power ($\bar{\omega}^+$) and Electron Donor Power ($\bar{\omega}^-$).

The analysis of Table 9 reveals that the quantum descriptors selected for the prediction of *LogIC50 at* the HF/6-311G level (d, p) are: EHomo, $_{ELumo}$, S, η, χ and μ. These quantum descriptors allow us to consider two groups:

- **Group 5**: HOMO energy (*EHomo*), LUMO energy ($_{ELumo}$), Softness (*S*), Hardness (η) and Density (μ);
- **Group 6:** LUMO energy ($_{ELumo}$), Softness (S), Hardness (η), Electronegativity (χ), and Dipole moment (μ).

Table 3. Values of the quantum descriptors calculated at the AM1 level and the experimental values of the redox potential E and the inhibitory concentration IC50 of the training series

Code	Quantum descriptors										Experimental descriptors	
	EHomo	$_{ELumo}$	*Gap*	*χ*	*η*	*S*	*ω*	*μ*	$\bar{\omega}^+$	$\bar{\omega}^-$	*E*	*LogIC50*
M1	-0 .33	-0 .00	0.33	0.17	0.17	2.94	0.09	3.34	0.02	0 .02	-0.030	-4.98
M2	-0 .33	-0 .02	0.31	0.18	0.16	3.13	0.10	2.61	0.03	0 .21	0.020	-5.07
M3	-0 .33	-0 .00	0.33	0.17	0.17	2.94	0.09	3.12	0.02	0 .02	0.030	-4.68
M4	-0 .33	-0 .02	0.31	0.18	0.16	3.13	0.10	4.11	0.03	0 .21	0.080	-4.72
M5	-0 .33	-0 .01	0.32	0.17	0.16	3.13	0.09	0.57	0.03	0 .20	0.105	-4.64
M6	-0 .33	-0 .01	0.32	0.17	0.16	3.13	0.09	1.21	0.03	0 .20	0.280	-4.86
M7	-0 .32	-0 .01	0.31	0.17	0.16	3.13	0.09	2.78	0.02	0 .19	0.180	-4.03
M8	-0 .33	-0 .00	0.33	0.17	0.17	2.94	0.09	3.30	0.02	0 .02	0.185	-4.23
M9	-0 .33	-0 .00	0.33	0.17	0.17	2.94	0.09	1.56	0.02	0 .02	-0.060	-5.20
M10	-0 .33	-0 .01	0.32	0.17	0.16	3.13	0.09	4.13	0.02	0 .20	0.080	-4.70
M11	-0 .33	-0 .04	0.29	0.19	0.15	3.33	0.12	3.03	0.04	0 .23	0.180	-4.58
M12	-0 .33	-0 .03	0.30	0.18	0.15	3.33	0.11	2.87	0.04	0 .22	0.360	-
M13	-0 .34	-0 .03	0.31	0.19	0.16	3.13	0.11	2.35	0.04	0 .22	0.500	-
M14	-0 .33	-0 .02	0.31	0.18	0.16	3.13	0.10	2.43	0.03	0 .21	0.132	4.18
M15	-0 .34	-0 .02	0.32	0.18	0.16	3.13	0.10	2.64	0.03	0 .21	0.590	-
M16	-0 .32	-0 .02	0.30	0.17	0.15	3.33	0.10	1.98	0.03	0 .20	0.500	-
M17	-0 .32	-0 .02	0.30	0.17	0.15	3.33	0.10	3.03	0.03	0 .20	0.538	-
M18	-0 .33	-0 .03	0.31	0.18	0.16	3.13	0.10	1.40	0.04	0 .21	0.540	-
M19	-0 .33	-0 .00	0.33	0.17	0.17	2.94	0.09	1.06	0.02	0 .02	-0.030	-4.53

Table 4. Values of the quantum descriptors calculated at AM1 and the experimental values of the redox potential E and the inhibitory concentration IC50 of the test series

Code	Quantum descriptors										Experimental descriptors	
	E_{Homo}	E_{Lumo}	Gap	χ	η	S	ω	μ	$\bar{\omega}^{+}$	$\bar{\omega}^{-}$	E	$LogIC_{50}$
M20	-0 .32	-0 .04	0.28	0.18	0.14	3.57	0.12	1.06	0 .04	0 .22	-0.035	-4.80
M21	-0 .32	-0 .04	0.28	0.18	0.14	3.57	0.12	2.14	0 .04	0 .22	-0.020	-4.96
M22	-0 .32	-0 .04	0.28	0.18	0.14	3.57	0.12	2.39	0 .04	0 .22	-0.010	-4.89
M23	-0 .32	-0 .04	0.28	0.18	0.14	3.57	0.12	0.49	0 .04	0 .22	0.040	-4.89
M24	-0 .31	-0 .03	0.28	0.18	0.14	3.57	0.12	1.91	0 .04	0 .21	0.080	-5.00
M25	-0 .32	-0 .04	0.28	0.18	0.14	3.57	0.12	2.20	0 .04	0 .22	0.082	-4.60
M26	-0 .31	-0 .03	0.28	0.18	0.14	3.57	0.12	2.95	0 .04	0 .21	0.082	-4.52
M27	-0 .32	-0 .03	0.29	0.18	0.15	3.33	0.11	1.62	0 .04	0 .34	0.092	-4.42
M28	-0 .35	-0 .03	0.32	0.19	0.16	3.13	0.11	2.95	0 .04	0 .23	0.102	-4.29
M29	-0 .32	-0 .03	0.29	0.18	0.15	3.33	0.11	4.62	0 .04	0 .21	0.450	-4.01

Table 5. Values of the quantum descriptors calculated at the HF/6-311G level (d, p) and the experimental values of the redox potential E and the inhibitory concentration IC50 of the training set

Code	Quantum descriptors										Experimental descriptors	
	E_{Homo}	E_{Lumo}	Gap	χ	η	S	ω	μ	$\bar{\omega}^{+}$	$\bar{\omega}^{-}$	E	$LogIC_{50}$
M1	-0.29	-0.06	0.23	0.18	0.12	4.17	0.14	2.06	0.06	0.24	-0.030	-4.98
M2	-0.29	-0.06	0.23	0.18	0.12	4.17	0.14	2.61	0.06	0.24	0.020	-5.07
M3	-0.29	-0.06	0.23	0.18	0.12	4.17	0.14	2.45	0.06	0.24	0.030	-4.68
M4	-0.29	-0.06	0.23	0.18	0.12	4.17	0.14	1.02	0.06	0.24	0.080	-4.72
M5	-0.30	-0.08	0.22	0.19	0.11	4.55	0.16	2.73	0.08	0.27	0.105	-4.64
M6	-0.33	-0.08	0.25	0.21	0.13	3.85	0.17	8.10	0.08	0.27	0.280	-4.86
M7	-0.29	-0.09	0.20	0.19	0.10	5.00	0.18	4.21	0.10	0.29	0.180	-4.03
M8	-0.30	-0.08	0.22	0.19	0.11	4.55	0.16	3.68	0.08	0.27	0.185	-4.23
M9	-0.32	-0.08	0.24	0.20	0.12	4.17	0.17	3.70	0.08	0.28	-0.060	-5.20
M10	-0.30	-0.08	0.22	0.19	0.11	4.55	0.16	7.85	0.06	0.27	0.080	-4.70
M11	-0.31	-0.07	0.24	0.19	0.12	4.17	0.15	4.39	0.07	0.26	0.180	-4.58
M12	-0.31	-0.06	0.25	0.19	0.13	3.85	0.14	3.77	0.06	0.25	0.360	-
M13	-0.31	-0.07	0.24	0.19	0.12	4.17	0.15	3.35	0.07	0.26	0.500	-
M14	-0.31	-0.09	0.22	0.20	0.11	4.55	0.18	3.02	0.10	0.30	0.132	4.18

Code	Quantum descriptors										Experimental descriptors	
	E_{Homo}	E_{Lumo}	Gap	χ	η	S	ω	μ	$\bar{\omega}^{+}$	$\bar{\omega}^{-}$	E	$LogIC_{50}$
M15	-0.32	-0.09	0.23	0.22	0.12	4.17	0.20	3.91	0.09	0.30	0.590	-
M16	-0.30	-0.09	0.21	0.20	0.11	4.55	0.18	2.35	0.10	0.29	0.500	-
M17	-0.29	-0.08	0.21	0.19	0.11	4.55	0.16	4.30	0.08	0.27	0.538	-
M18	-0.30	-0.09	0.21	0.20	0.11	4.55	0.14	2.83	0.10	0.24	0.540	-
M19	-0.34	-0.08	0.26	0.21	0.13	3.85	0.17	10.45	0.08	0.29	-0.030	-4.53

Table 6. Values of the quantum descriptors calculated at the HF/6-311G level (d, p) and the experimental values of the redox potential E and the inhibitory concentration IC50 of the test series

Code	Quantum descriptors										Experimental descriptors	
	E_{Homo}	E_{Lumo}	Gap	χ	η	S	ω	μ	$\bar{\omega}^{+}$	$\bar{\omega}^{-}$	E	$LogIC_{50}$
M20	-0.30	-0.13	0.17	0.22	0.09	5.55	0.27	4.12	0.11	0.32	-0.035	-4.80
M21	-0.31	-0.10	0.21	0.21	0.11	4.55	0.20	3.25	0.14	0.36	-0.020	-4.96
M22	-0.31	-0.12	0.19	0.22	0.11	4.55	0.22	4.76	0.08	0.27	-0.010	-4.89
M23	-0.30	-0.08	0.22	0.19	0.11	4.55	0.16	4.54	0.14	0.36	0.040	-4.89
M24	-0.31	-0.12	0.21	0.22	0.11	4.55	0.22	1.55	0.18	0.39	0.080	-5.00
M25	-0.30	-0.13	0.17	0.22	0.09	5.55	0.27	1.97	0.18	0.39	0.082	-4.60
M26	-0.30	-0.13	0.17	0.22	0.09	5.55	0.27	1.82	0.18	0.36	0.082	-4.52
M27	-0.31	-0.13	0.18	0.22	0.09	5.55	0.27	3.91	0.14	0.36	0.092	-4.42
M28	-0.31	-0.12	0.19	0.22	0.11	4.55	0.20	1.81	0.13	0.34	0.102	-4.29
M29	-0.31	-0.11	0.20	0.21	0.10	5.00	0.22	5.27	0.11	0.32	0.450	-4.01

Table 7. Selection of descriptors at the HF/6-311G (d,p) level of approximation

Redox potential E			**Inhibitory concentration IC50**		
Equations	Linear correlation coefficient **IRI**	Descriptor rejected if **IRI < 0.50**	Equations	Linear correlation coefficient **IRI**	Descriptor rejected if **IRI < 0.50**
E and EHomo	0.03	Rejected	*LogIC50 and EHomo*	0.64	Withheld
E and $_{ELumo}$	0.43	Rejected	*LogIC50 and* $_{ELumo}$	0.56	Withheld
E and Gap	0.45	Rejected	*LogIC50 and Gap*	0.53	Withheld
E and χ	0.44	Rejected	*LogIC50 and* χ	0.66	Withheld
E and η	0.44	Rejected	*LogIC50 and* η	0.63	Withheld
E and S	0.41	Rejected	*LogIC50 and S*	0.64	Withheld
E and ω	0.44	Rejected	*LogIC50 and* ω	0.68	Withheld
E and μ	0.32	Rejected	*LogIC50 and* μ	0.52	Withheld
E and $\bar{\omega}^+$	0.42	Rejected	*LogIC50 and* $\bar{\omega}^+$	0.67	Withheld
E and $\bar{\omega}^-$	0.45	**Rejected**	*LogIC50 and* $\bar{\omega}^-$	0.68	**Withheld**

Table 8. Selection of descriptors at the AM1 level of approximation

Redox potential E			**Inhibitory concentration IC50**		
Equations	Linear correlation coefficient **IRI**	Descriptor rejected if **IRI < 0.50**	Equations	Linear correlation coefficient **IRI**	Descriptor rejected if **IRI < 0.50**
E and EHomo	0.61	Withheld	*LogIC50 and EHomo*	0.66	Withheld
E and $_{ELumo}$	0.81	Withheld	*LogIC50 and* $_{ELumo}$	0.66	Withheld
E and Gap	0.30	**Rejected**	*LogIC50 and Gap*	0.49	**Rejected**
E and χ	0.17	**Rejected**	*LogCI50 and* χ	0.18	**Rejected**
E and η	0.26	**Rejected**	*LogIC50 and* η	0.49	**Rejected**
E and S	0.29	**Rejected**	*LogIC50 and S*	0.31	**Rejected**
E and ω	0.42	**Rejected**	*LogIC50 and* ω	0.22	**Rejected**
E and μ	0.12	**Rejected**	*LogIC50 and* μ	0.10	**Rejected**
E and $\bar{\omega}^+$	0.61	Withheld	*LogIC50 and* $\bar{\omega}^+$	0.66	Withheld
E and $\bar{\omega}^-$	0.61	Withheld	*LogIC50 and* $\bar{\omega}^-$	0.66	Withheld

3.2 Selection of Quantum Descriptors for the Prediction of Redox Potential

The results for the final selection of the predictive quantum descriptors of the redox potential E are given in Tables 8 and 9.

The analysis of the results allows us to consider two groups of predictive quantum descriptors for the AM level1 :

- **Group 1**: LUMO energy ($_{ELumo}$), HOMO energy (*EHomo*) and electron donor power ($\bar{\omega}^-$**) ;**
- **Group 2:** Energy of the HOMO (*EHomo*), electron acceptor ($\bar{\omega}^+$) and electron donor power ($\bar{\omega}^-$**).**

For the HF/6-311G (d, p) method, no descriptor was retained, therefore there is no predictive model for redox potential at this level of calculation.

3.3 QSPR Model of the Predictive Quantum Descriptors of the Redox Potential *E* of the Inhibitory Concentration $LogIC_{50}$

Based on **the** learning **set** and the selected predictive descriptors, the aim were to :

- Establish one or more QSPR model(s) for predicting the redox potential *E* and the inhibitory concentration *LogIC50* per calculation level.
- to carry out an analysis of the statistical parameters of the QSPR models developed.

The results of this work allowed the validation of the best models for predicting the redox potential

E and the *LogIC50* inhibitory concentration of flavonoids.

3.3.1 QSPR model of predictive quantum descriptors of redox potential by the AM1 method

In order to select the group to be used for the regression equation of the QSPR model at the AM1 level of calculation, the Fisher coefficients of the two groups 1 and 2 compared and the most significant group in the Fisher sense selected.

Analysis of the results in Tables 10 and 11 shows that the Fisher coefficient ($_{F1}$) for Group 1 was higher than the Fisher coefficient (F2) for Group 2 : $\mathbf{F_2 < F_1}$ **;** this means that the regression equation for Group 1 will be more significant than that for Group 2. Therefore, the quantum descriptors of group 1 can be preferred to establish the QSPR model of the AM1 level of redox potential.

The ANOVA table for Model 1, which was used to perform the analysis of variance, is shown in Table 10. This ANOVA table indicates that the p-value (0.0005E-6) is less than α=0.05, showing that the regression equation of model 1 is significant in predicting redox potential.

The results of the multilinear regression obtained from the descriptors of group 1 are shown in Table 11.

The regression equation for model 1 is as follows :

$$E = -0.29 + 0.22E_{Homo} + 0.11E_{Lumo} - 0.05\bar{\omega}^{-}$$

3.3.2 Contribution of the AM1 level quantum descriptors in the prediction of the redox potential E

According to the absolute values of the t-test in Table 12, the importance of the quantum descriptors of the AM1 level in Model 1 is in the following order

$$\bar{\omega}^{-} < E_{Homo} < E_{Lumo}$$

Indeed, the contribution calculations show that the Lumo Energy ***(ELumo)*** makes a contribution of 48.35P in predicting the redox potential, the electron donor power ($\bar{\omega}^{-}$ **)** and the HOMO Energy (***EHomo***) make a contribution of 25.48P and 26.18P respectively. It is clear that the LUMO energy (***ELumo***) is the main predictive descriptor of the redox potential.

Table 9. ANOVA table of the quantum descriptors of group 1 of the AM1 level

	DS	SC	MSC	$_{F1}$	P-value
Regression	3	0.58	0.19	10.68	0.0005E-6
Residue	15	0.27	0.01		
Total	18	0.86			

Table 10. ANOVA table of the quantum descriptors of group 2 of the AM1 level

	DS	SC	MSC	F2	P-value
Regression	3	0.10	0.03	0.70	0.5634E-5
Residue	15	0.75	0.05		
Total	18	0.86			

Table 11. Values of the regression coefficients of group 1 for model 1

	Coefficients	Standard deviation	Test t	P-value
constants	-0.29	5.93	5.93	0.01
EHomo	0.22	0.02	0.02	0.20
ELumo	0.11	0.04	0.04	0.01
$\acute{\omega}^{-}$ ¿¿	-0.05	0.02	0.02	0.92

3.3.2 QSPR model of the predictive quantum descriptors of the *LogIC50* inhibitory concentration at the AM1 level: model 2

The ANOVA tables (Tables 12 and 13**)** show that the Fisher coefficient (F4) of group 3 is higher than the Fisher coefficient (F5) of group 4: $\mathbf{F_5 < F_4}$ **;** therefore, the quantum descriptors of group 4 can be preferred to establish the QSPR model of the AM1 level for the prediction of the inhibitory concentration.

The results of the multilinear regression obtained from the descriptors of group 4 are shown in Table 14.

The regression equation for model 2 is as follows:

$$LogIC_{50} = -4.92 + 11.37\ E_{Homo} + 34.36\ E_{Lumo} + 0.67\bar{\omega}^{-}$$

The ANOVA table for the model (Table 15) indicates that the p-value (0.00021E-7) is less than α = 0.05. Thus the regression equation of model 2 is significant in predicting the inhibitory concentration.

3.3.3 Contribution of AM1 quantum descriptors to the prediction of the inhibitory concentration

According to the absolute values of the t-test in Table 15, the importance of the quantum descriptors of the AM1 level in Model 2 was in the following order

$$\bar{\omega}^{-} < E_{Homo} < E_{Lumo}$$

The contribution calculations show that the Lumo Energy ($_{ELumo}$) makes a contribution of 60.66□ in predicting the redox potential, the electron donor power ($\bar{\omega}^{-}$) and the HOMO Energy (*EHomo*) make a contribution of 37.71□ and 2.25□ respectively. It is clear that the LUMO Energy ($_{ELumo}$) is the main descriptor predicting the inhibitory concentration.

3.3.4 QSPR model of the predictive quantum descriptors of the *LogIC50* inhibitory concentration at the HF/6-311G level (d, p): model 3

The Fisher coefficients for groups 5 and 6 are provided by the ANOVA tables in Tables 16 and 17. From the analysis of the results, the Fisher coefficient (F11) of group 5 is higher than the Fisher coefficients (F12) of group 6**;** its regression equation will be more significant than that of group 6.

The ANOVA table for Model 3 indicates that the p-value (p-value = 0.026E-8) is smaller than α=0.05. This shows that the regression equation of Model 3 is significant in predicting the inhibitory concentration of the molecules.

The results of the multilinear regression are shown in Table 17.

Table 12. ANOVA table of the quantum descriptors of group 3 of level AM1

	DS	SC	MSC	F4	P-value
Regression	3	2.84	3.96	4.33	0.00021E-7
Residue	15	3.87	3.59		
Total	18	6.71			

Table 13. ANOVA table of quantum descriptors for group 4 at AM1 level

	DS	SC	MSC	F5	P-value
Regression	3	3.65	7.82	1.16	0.00356E-4
Residue	15	4.06	8.20		
Total	18	5.71			

Table 14. Values of the regression coefficients of model 2 at AM1 level

	coefficients	Standard deviation	Test t	P-value
constants	-4.92	3.09	-3.36	0.00
EHomo	11.37	6.56	1.73	0.10
$\boldsymbol{E_{Lumo}}$	34.36	12.13	2.83	0.01
$\bar{\omega}^{-}$	0.67	6.38	0.10	0.91

The regression equation for model 6 is as follows:

$$LogIC_{50} = 62.40 + 80.25\ E_{Homo} - 28.44\ E_{lumo} + 52.01\ S - 71.26\ \eta - 6.11\mu$$

3.4 Statistical Parameters of Model 1 of Models 1, 2 and 3

The results of the statistical parameters are reported in Table 19.

The results show that :

- The redox potential is strongly correlated with the quantum descriptors of the AM1 level as R= 0.9820. In addition 96.43P of the experimental variance of the redox potential is explained by the descriptors of model 1. It can be said that model 1 is validated and can be retained as a model for predicting the redox potential of the studied molecules.
- The *LogIC50* inhibitory concentration is correlated with the quantum descriptors at the AM1 and HF/6-311G levels (d, p). Indeed, 96.35P and 99.96P of the experimental variance of the inhibitory concentration are explained by the descriptors of model 2 and model 3 respectively. It can be said that models 2 and 3 are validated and can be retained as a predictive model for the inhibitor concentration.

3.5 Internal LOO Validation of Models 1, 2 and 3

The results are reported in Table 20. They indicate that :

- Model 1 has a very high predictive capacity as 94.9P of the molecules in the training set have their redox potential predicted.
- Model 3 has a very high predictive ability, 98.9P of the molecules in the training set have their predicted inhibitory concentration.
- The model has a very high predictive capacity (Q^2_{Loo} = 0,941) because 94.1P of the molecules in the training set have their predicted inhibitory concentration

Table 15. ANOVA table of the quantum descriptors of group 5 of level HF/6-311G (d, p)

	DS	SC	MSC	F11	P-value
Regression	5	3.77	2.75	4.26	0.026E-8
Residue	13	3.16	2.55		
Total	18	6.93			

Table 16. ANOVA table of the quantum descriptors of group 6 of the HF/6-311G level (d, p)

	DS	SC	MSC	F12	P-value
Regression	5	6.65	1.32	0.83	0.546E-3
Residue	13	0.79	9.29		
Total	18	7.44			

Table 17. Values of the regression coefficients for group 5

	coefficients	Standard deviation	Test t	P-value
constants	62.40	63.74	0.05	0.95
EHomo	80.25	86.62	1.57	0.13
Elumo	-28.44	16.71	-2.35	0.03
S	52.01	40.83	0.36	0.72
η	-71.26	24.56	-0.13	0.89
μ	-6.11	7.58	-0.80	0.43

Table 18. Statistical parameters for the external validation of models 1, 2 and 3

Models	*n*	*R*	*R2*	*Rzaj*	*S*	*F*	*FIT*
Model1	19	0.9820	0.9643	0.9582	0.0755	4.3492	0.230
Model2		0.9816	0.9635	0.8575	0.0850	4.3397	0.023
Model 3		0,998	0,996	0,8936	0,2498	4,2614	0.080

3.6 External Validation of Models 1, 2 and 3

The results are reported in Table 20 and show that :

- Model 1, has high predictive power (Q2ext = 0.891) as 89, 1.P of the molecules in the test series have their redox potential predicted. In addition 93, 50% of the experimental variance in redox potential is explained by the quantum descriptors of model1 at the AM1 level.
- Model 2, has a high predictive power. Indeed, 97.80P of the molecules in the test series have their redox potential predicted. In addition 98.30% of the experimental variance of the inhibitory concentration is explained by the quantum descriptors of model 2 at the AM1 level.
- Model 3 has high predictive power as 96.10P of the molecules in the test series have their redox potential predicted. Also, 97.70P of the experimental variance in the percentage of inhibition is explained by the quantum descriptors of model 3 at the HF/6-311G level (d, p).

3.7 Verification of Tropsha Criteria for Models 1, 2 and 3

The analysis of the results is recorded in Table 21 and shows that :

- For model 1 only criteria 1, 2 and 4 are verified; i.e. the of Tropsha's criteria. The model is therefore efficient in predicting the redox potential
- For model 2, only criteria 1, 2 and 4 are verified while for model 3, all 5 criteria are verified. Models 2 and 3 therefore perform very well in predicting the inhibitory concentration.

3.8 Normality Tests of Models

3.8.1 Normality tests for model 1

- **Shapiro-Wilk test (*Epréd*)**

This test gives the following results: w = 0.390; p-value = 0.879; α =0.05

Table 19. Statistical parameters for internal and external validation of models 1, 2 and 3

Model 1	**Internal**	***n***	***PRESS***	Q^2_{Loo}	S_{Press}	
		19	0.197	0.949	0.170	
	Extern	***n***	R^2_{ext}	***PRESS***	***Q²ext***	S_{PRESS}
		10	0.935	0.149	0.891	0.044
Model 2	**Internal**	***n***	***PRESS***	Q^2_{Loo}	S_{Press}	
		19	0.221	0.989	0.134	
	Extern	***n***	R^2_{ext}	***PRESS***	***Q²ext***	S_{PRESS}
		10	0.983	0.149	0.978	0.116
Model 3	**Internal**	***n***	***PRESS***	Q^2_{Loo}	S_{Press}	
		19	0.256	0.941	0.322	
	Extern	***n***	R^2_{ext}	***PRESS***	***Q²ext***	S_{PRESS}
		10	0.977	0.198	0.961	0.143

Table 20. Tropsha criteria verification for the models developed [15] (T. M. Martin et al.; 2012)

Criteria	**Model 1**	**Model2**	**Model 3**
Critrion 1 = $R^2_{ext} > 0.70 > 0.70$	0.935	0.953	0.877
Critrion 2 = *Q²ext* > 0.60	0.891	0.918	0.853
Critrion 3 = $\frac{R^2_{ext}-R^2_0}{R^2_{ext}}$ **< 0.1 et 0.85 < *k* < 1.15**	0.010	0.058	0.139
Critrion 4 = $\frac{R^2_{ext}-R'^2_0}{R^2_{ext}}$ **< 0.1 et 0.85 < *k'* < 1.15**	0.011	0.041	1.528
Critrion 5= $\lvert R^2_{ext} - R^2_0 \rvert \leq 0.30$	0.03	0.023	0.121

Interpretation of the test: Since the calculated p-value is above the alpha threshold significance level (0.879 > 0.05), it is concluded that the predicted values of the redox potential by model 1 follow a normal distribution.

- **Durbin-Watson test (residuals) :**

This test gives the following results: U= 0.785; p-value = 0.4860; α = 0.05

Interpretation of the test: Since the calculated p-value is above the alpha significance level (0.4860 > 0.05), it is concluded that the residuals are not autocorrelated. Therefore, they do not contain any information that could influence the prediction of model 1.

3.8.2 Normality tests of model 2

- **Shapiro-Wilk test**

This test gives the following results: w = 0.239; p-value = 0.067; α =0.05

Interpretation of the test: Since the calculated p-value is above the alpha threshold significance level (0.067 > 0.05), it is concluded that the predicted values of the inhibitory concentration by model 2 follow a normal distribution.

- **Durbin-Watson test:**

This test gives the following results: U=0.463; p-value = 0.1137; α =0.05

Interpretation of the test: Since the calculated p-value is above the alpha significance level, the residuals are not autocorrelated. They do not contain any information that could influence the prediction of model 2.

3.8.3 Normality tests of model 3

The results are as follows:

- **Shapiro-Wilk test**

This test gives the following results: w=0.105; p-value = 0.077; α =0.05

Interpretation of the test: Since the calculated p-value is above the alpha threshold significance level (0.077 > 0.05), it is concluded that the predicted values of their inhibitory concentration by model 3 follow a normal distribution.

- **Durbin-Watson test:**

This test gives the following results: U=0.993; p-value = 0.1232; α =0.05

Interpretation of the test: Since the calculated p-value is above the alpha significance level, the residuals do not contain information that could influence the prediction of model 3.

3.8.4 Predicted model equations

From the various statistical tests in Table 18 we can deduce that the equations of the models are as follows:

Model 1: Prediction of the redox potential, which is summarized as follows:

$$E = -0.29 + 0.22E_{Homo} + 0.11ELumo - 0.05\bar{\omega}^{-}$$

***n*=**19; R**=**0.9820 ; $\mathbf{R^2}$ **=**0.643 ; $\boldsymbol{R^2_{aj}}$ **=** 0.9582 ; ***S* =** 0.0755 ; ***F*=**4.3492 ; ***FIT*=**0.230

Model 2: Prediction of the inhibitory concentration is summarised as follows at the AM1 level:

$$LogIC_{50} = -4.92 + 11.37E_{Homo} + 34.36\ E_{Lumo} + 0.67\bar{\omega}^{-}$$

***n*=**19; ***R* =** 0.9816*;* $\boldsymbol{R^2}$ **=** 0.9635; $\boldsymbol{R^2_{aj}}$ **=** 0.8575; ***S*=**0.0850; ***F*=**4.3492 ; ***FIT* =** 0.0230

Model 3: The prediction regression equation is summarised as follows:

$$LogIC_{50} = 62.40 + 80.25\ E_{Homo} - 28.44\ E_{lumo} + 52.01\ S - 712.6\ \eta - 6.11\mu$$

***n*=**19; ***R* =**0.998; $\mathbf{R^2}$ **=**0.998; $\boldsymbol{R^2_{aj}}$ **=** 0.996; ***S*=** 0.24936; ***F*=**1.4614; ***FIT*=**0.08

3.9 Predicted Values of Redox Potential and Inhibitory Concentration of 29 Flavonoids by Models 1, 2, 3

Table 21 shows the predicted values of redox potential and percentage inhibition of the 29 flavonoids by models 1, 2 and 3.

These results show that there is good agreement between the model values and the experimental values published in the literature.

Table 21. Experimental and predicted values of redox potential and inhibitory concentration of the 29 flavonoids by the models

Code	*E*		*LogIC50*		
	Exp	**Mod1**	**Exp**	**Mod 2**	**Mod3**
M1	-0.035	-0.038	-4.80	-4.84	-4.87
M2	-0.020	-0.021	-4.96	-4.95	-4.91
M3	-0.010	-0.008	-4.89	-4.91	-4.85
M4	0.040	0.038	-4.89	-4.91	-4.85
M5	0.080	0.076	-5.00	-4.97	-4.95
M6	0.082	0.086	-4.60	-4.66	-4.68
M7	0.082	0.086	-4.52	-4.53	-4.49
M8	0.092	0.097	-4.42	-4.39	-4.37
M9	0.102	0.107	-4.29	-4.27	-4.25
M10	0.450	0.453	-4.01	-3.98	-3.94
M11	0.180	0.177	-4.58	-4.57	-4.54
M12	0.360	0.356	-	-	-
M13	0.500	0.496	-	-	-
M14	0.132	0.134	4.18	4.15	4.13
M15	0.590	0.591	-	-	-
M16	0.500	0.503	-	-	-
M17	0.538	0.537	-	-	-
M18	0.540	0.542	-	-	-
M19	-0.030	-0.028	-4.53	-4.51	-4.49
M20	-0.030	-0.032	-4.98	-4.95	-4.94
M21	0.020	0.023	-5.07	-5.02	-5.03
M22	0.030	0.027	-4.68	-4.65	-4.62
M23	0.080	0.084	-4.72	-4.76	-4.73
M24	0.105	0.101	-4.64	-4.65	-4.62
M25	0.280	0.279	-4.86	-4.78	-4.74
F26	0.180	0.177	-4.03	-3.97	-3.95
M27	0.185	0.183	-4.23	-4.21	-4.18
M28	-0.060	-0.059	-5.20	-5.21	-5.25
M29	0.080	0.076	-4.70	-4.67	-4.65

4. CONCLUSION

A prediction study of the redox potential *E* and the inhibitory concentration *LogIC50* was performed, using the semi-empirical methods AM1 and HF/6-311G (d, p).

- ✓ The application of the descriptor selection criteria made it possible to determine and retain 6 groups of quantum descriptors, including 2 groups of descriptors for the prediction of the redox potential *E* and 4 groups of descriptors for the prediction of the inhibitory concentration *LogIC50*. The antioxidant properties of the molecules depend strongly on these groups of descriptors.
- ✓ From the multilinear regression analysis, several prediction models (one model for redox potential and two for inhibitory concentration) were established from the quantum descriptors. The established models are validated and perform well according to Tropsha criteria.

The development of these QSPR models represents a significant advance in the prediction of antioxidant properties of bioactive molecules such as flavonoids based on descriptors calculated by quantum chemical methods. This is a contribution to the database of the two main parameters (E and IC50) involved in the prediction of antioxidant properties of bioactive molecules.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Hendrickson HP, Kaufman AD, Lunte CE. J. Pharm. Biomed Anal. 1994;12:325.

2. Jovanovic SV, Steenken S, Simic MG, Hara Y. Antioxidant properties of flavonoids: Reduction potentials and electron, transfer reductions of flavonoid radicals. In: Rice-Evans, C. A.; Packers, L., eds. Flavonoids in health and disease. New York: Marcel Dekker, Inc. 1996;137-161.
3. Labuda J, Buková M, Heilerová L, Šilhár S, Štepánek I. Evaluation of the redox properties and anti/pro-oxidant effects of selected flavonoids by means of a DNA-based electrochemical biosensor. Anal Bioanal Chem. 2003;376:168-173.
4. José T, Alexandra G, Manuela E, Garrido JG, Fernanda B. Hydroxycinnamic acid antioxidants: An electrochemical overview, hindawi publishing corporation bio med research international. Article ID 251754. 2013;11.
5. Lien EJ, Ren S, Bui HH, Wang R. Free Radic. Biol. Med. 1999;26:285.
6. Bin Y, Akira K, Kensunke A, Fumiyo K. Estimation of the antioxidant activities of flavonoids from their oxidation potentials. Analytical sciences; the Japan society for analytical chemistry. 2001;1.
7. Pearson RG. Hard and soft acids and bases, J. Am. Chem. Soc. 85, 3533-3539, (1963),(2001).
8. R. G. Parr, R. A. Donnelly, M. Levy, W. E. Palke. J. Chem. Phys. 1978;68:3801-3807.
9. Geerlings P, De Proft F, Langenaeker W. Chem. Rev, 2003 ;103 :1793-1874.
10. Caro A, Zagal JH, Bedioui F, Adamo C, Cardenas-Jiron GI. J. Phys. Chem. A. 2004;108:6045-6051.
11. De Vleeschouwer F, Jaque P, Geerlings P, Toro-Labbe A, De Proft F. J. Org Chem. 2010;75:4964-4974.
12. Morrel H Cohen, Adam W. Journal of Statistical Physics, On Hardness and Electronegativity Equalization in Chemical Reactivity Theory; 2006.
13. Bonin KD. Kresin electric-dipole polarizabilities of atoms molecules and clusters. World Scientific, Singapore, VV; 1997.
14. Payán G, Sergio A, Norma Flores H, Antonino P, Manuel Piñón M, Daniel G. Computational molecular characterization of the flavonoid rutin. Chemistry Central Journal. 2010;4:12.
15. Laffly. Multiple regression: principles and application examples; 2006.
16. Golbraikh A. Tropsha, beware of q2 J. Mol. Graph. Model. 2002;20:269-276.
17. Dragan A, Duanka D, Drago B, Vesna R, Bono L, Nenad T. SAR and QSAR of the antioxidant activity of flavonoids. Current Medicinal Chemistry. 2007;14:827-845.
18. Durbin J, Watson GS. Testing for serial correlation in least squares regression II. Biometrika. 1951;38(1-2):159-179.
19. Tetko V, Sushko I, Pandey AK, Zhu H, Tropsha A, Papa E, Oberg T, Todeschini R, Fourches D, Varnek A. J. Chem. Inf. Model. 2008;48:1733.
20. Jovanovic SV, Steenken S, Hara Y, Simic MG. Reduction potentials of flavonoid and model phenoxyl radicals which ring in flavonoids is responsible for antioxidant New York: Marcel Dekker, Inc. 1998;137-161.
21. Jaworska J, Jeliazkova NN, Aldenberg T. Altern. Lab. Anim. 2005;33:445-459.
22. Jorgensen LV, Cornett C, Justesen U, Skibsted LH, Dragsted LO. Free Rad. Res. 1998;29:339.
23. Medjdoub GA. Contribution to the study of chemical reactivity using conceptual DFT, application to heterocycle chemistry; 2012.
24. Chattaraj PK, Perez P, Zevallos J, Toro-Labbe A. J. Phys. Chem. A. 2006 ;104 :4272-4283.
25. Rubalya V, S, Neelamegam P. Selective ABTS and DPPH- radical scavenging activity of peroxide from vegetable oils , International Food Research Journal. Journal homepage. 2015;22(1):289-294.
26. Shapiro SS, Wilk MB. An analysis of variance test for normality (Complete Samples). Biometrika. 1965;52(3 and 4):591-611.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/72000