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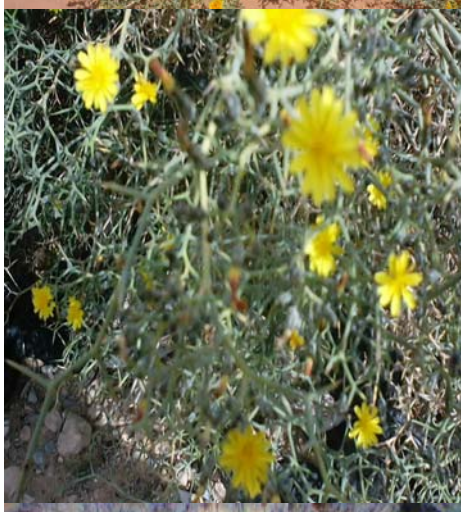

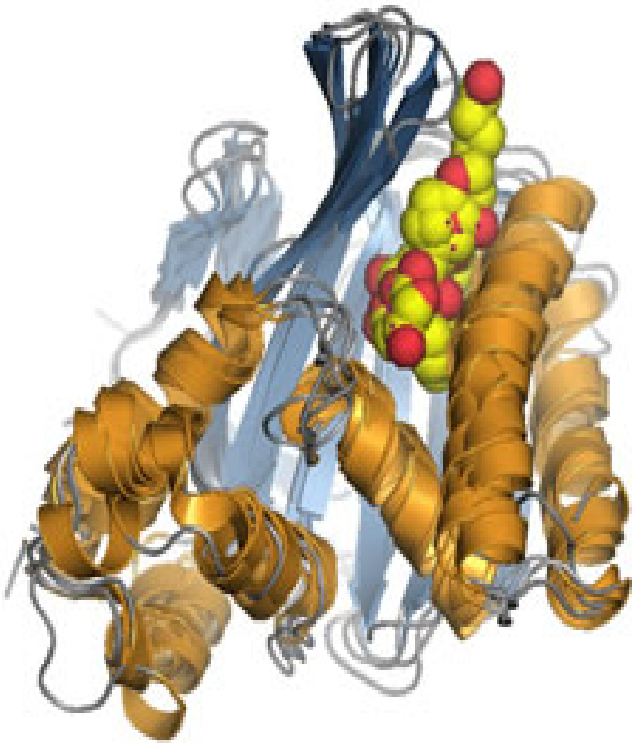

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Contribution to the study of antioxidant activity for flavonoids compounds. Application for QSAR modeling.

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Abstract. *During the past few years, marqued with great development in biotechnologies, the exploitation of natural substances from all sources (used as products as themselves or sources of new molecules) has attracted increasing interest from manufacturers and society. The phenomenon finds many applications in food, cosmetics and fragrance industries. From this background, and following the work done in the Phytochemistry and Organic Synthesis Laboratory (Bechar University) for the identification and characterization of natural products, Qantitative Structure Activity Relationships had been carried out to represent the antioxidant activity (log CI50) of flavonoids series and its derivatives. Quantum chemistry calculations at DFT/B3LYP scale, had been used with the calculation basis 6-31G **. The antioxidant activity values (IC50) have been reported from the literature. The correlation between the biological activity and structural properties have been obtained using the multilinear regression method. To obtain the QSAR model with high predictive capacity, the original database has been filled randomly in a training set and test set. The ranges of biological activity provided by developed QSAR model are in good accordance with the experimental data. We show in this manuscript that antioxidant activity of flavonoids and its derivatives can be linked to Fukui's indices on some key molecules.*

Key Words: flavonoids, DFT, QSAR, Antioxidant activity, Fukui's indice.

I. Introduction

The flavonoids represent a group of natural compounds which has been the subject of sustained activity in scientific research for many decades. They are important constituents in our nutrition. They are present in many foods, dark chocolate, green tea and citrus, they are consumed accordingly as a balanced diet. Their extraction, concentration, or the selection of varieties with high content of flavonoids allows manufacturers to offer functional products for health benefits **[1,2].**

In 2009, nearly 4000 flavonoids have been identified, and classified into several classes **[3]**. Indeed, flavonoids appear to have different effects on health that researchers rely heavily. Numerous studies have shown the benefits of antioxidants on health, and it is now recognized that flavonoids would be part of the molecules having such effects. Other benefits have since been described: anti-viral, anti-allergic, antiplatelet, anti-inflammatory, anti-tumor…….
In the continuous of our work to the characterization of natural products **[4-7]**, we were interested in this study to the antioxidant activity of flavonoids. We turned to another type of theoretical approach: the structure / activity relationships using QSAR (Quantitive Structure Activity relationship) study. Researchers interested in this type of study rely on empirical rules and theoretical simulation tools that allow them to accurately calculate the properties of molecules based on their structure.
Our goal therefore is to develop effective new QSAR model for the prediction of the antioxidant activity of flavonoids using Fukui indices as descriptors. It is based on experimental data of antioxidant activity derived from the literature **[8].**

II. Computational method

All molecular structures used in the study to develop QSAR models have been calculated with Gaussian03 software **[9]**. We have used the Density Functional Theory (DFT) **[10]** through B3LYP hybrid functional **[11]** and 6-31G (d,p) basis. Calculation of vibrational frequencies has been performed after geometry optimization on a same theory level to be sure that no optimized structure could display any imaginary frequency. Radical structures have been calculated through Kohn-Sham formalism with DFT/UB3LYP unrestricted spin **[12].** All structures have then been used to calculate molecular descriptors, which are Fukui indices.

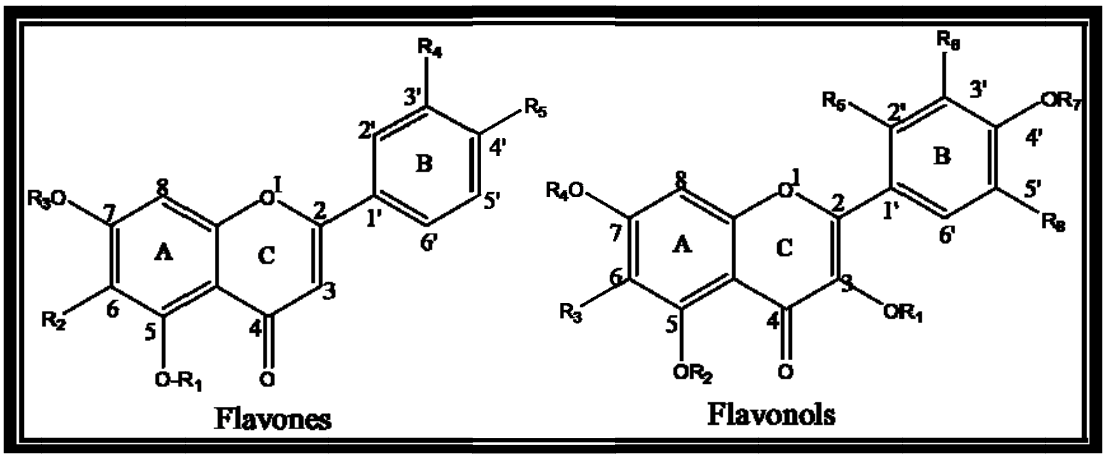

Fig1. flavonoids types structure

III. Results and discussion

1. Quantitative chemical study of the structures of flavonoids:

Today, more than 6000 flavonoids have been identified. The basic structure of the flavonoid and systematic numbering are summarized in Figure 2

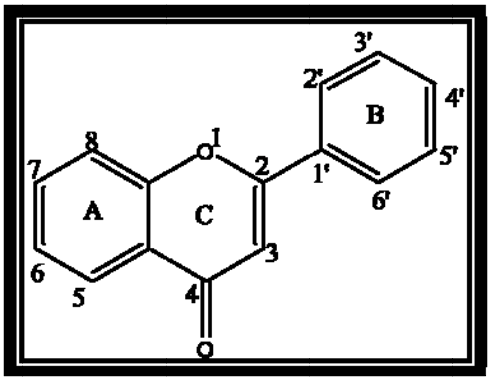

Fig 2. Basic Structure of a flavonoid

All the basic structures can be substituted into the following preferred positions: 3, 5, 7, 3 ', 4' and 5 ' by OH groups, or by other groups (CH3).

Table1: stabilization energy of flavonoids and their derivatives calculated by B3LYP/6-31G **

flavones	**Nomenclature**	**E(a.u)**
1	6-Hydroxyluteolin(R_2, R_4 ,R_5-*OH)*	-1104.18156317
2	Luteolin-7-glucoside *(*R_3- Glu, R_4 ,R_5-*OH)*	-1600.40550631
3	Baicalein (R_2-*OH)*	-953.73984487
4	Scutellarein *(*R_2, R_5-*OH)*	-1028.96120694
5	Baicalin R_2-*OH*, R_3- Glu*)*	-1638.53006531
6	Plantaginin *(*R_2,R_5-*OH*, R_3- Glu)	-1600.41000611
7	Luteolin *(*R_2,R_5-*OH)*	-1028.96182298
8	Sorbarin*(*R_2,R_5-*OH*, R_3-***rha****)*	-1564.50485791
9	Cosmosiin (R_3- Glu ,R_5-*OH)*	-2021.40889739
10	Acacetin R_5-*OMe*	-1525.18517083
Flavonols		
1	Quercimeritrin (R_4- Glu)	-1600.41251106
2	Rhamnetin(R_4-*Me*, R_7-*OH)*	-1143.49423737
3	Quercetin (R_6-*OH)*	-1104.18935288
4	Rutin *(*R_1- Glu, R_6-*OH)*	-2250.48561244
5	Chrysosplenol D *(*R_1, R_4-*Me*, R_3-*OMe*, R_6-*OH)*	-1297.30153768
7	Quercitrin(R_1-*Rha*, R_6-*OH)*	-1639.71458374
8	Chrysosplenol C (R_1, R_4-*Me*, R_3-*OH*, R_6-*OMe)*	-1297.30175868
9	Isoquercitrin (R_1- Glu, R_6-*OH)*	-1675.61738021
10	Isorhamnetin (R_6-*OMe)*	-1143.48648530
11	Oxyayanin A(R_1, R_4 R_7-*Me*, R_5,R_8-*OH)*	-1297.29250009
12	Kaempferol	-1028.96912058
13	Tetramethylquercetin (R_2, R_4 R_7-*Me*, R_6-*OMe)*	-1261.40092471

The geometric parameters of the compounds are summarized in Tables (2 ,3 and 4)

Table 2: bond lengths **(Å)** calculated with the B3LYP/6-31G** basis set.

flavones	**O1C2**	**C7C8**	**C7C6**	**C6C5**	**C2C3**	**C2C1'**	**C1'C6'**	**C6'C5'**	**C5'C4'**	**C4'C3'**
1	1.366	1.390	1.401	1.394	1.353	1.473	1.403	1.393	1.391	1.406
2	1.362	1.390	1.404	1.384	1.359	1.475	1.405	1.391	1.396	1.395
3	1.366	1.390	1.401	1.394	1.475	1.404	1.403	1.395	1.396	1.391
4	1.367	1.390	1.401	1.395	1.354	1.471	1.407	1.389	1.400	1.399
5	1.364	1.393	1.399	1.407	1.353	1.475	1.405	1.391	1.396	1.395
6	1.364	1.393	1.407	1.408	1.355	1.471	1.404	1.389	1.400	1.399

7	1.367	1.390	1.401	1.395	1.353	1.474	1.403	1.392	1.3917	1.405
8	1.364	1.393	1.398	1.407	1.355	1.471	1.404	1.389	1.400	1.399
9	1.367	1.391	1.400	1.397	1.353	1.471	1.404	1.389	1.400	1.399
10	1.367	1.390	1.401	1.396	1.353	1.471	1.404	1.389	1.400	1.399
Flavonols										
1	1.378	1.389	1.405	1.392	1.364	1.464	1.408	1.390	1.398	1.399
2	1.378	1.393	1.406	1.393	1.363	1.465	1.407	1.393	1.390	1.405
3	1.379	1.389	1.405	1.391	1.363	1.465	1.407	1.393	1.390	1.405
4	1.371	1.390	1.403	1.393	1.367	1.473	1.405	1.393	1.390	1.406
5	1.370	1.389	1.404	1.401	1.367	1.473	1.405	1.394	1.390	1.405
7	1.371	1.389	1.402	1.394	1.362	1.475	1.402	1.397	1.392	1.407
8	1.370	1.390	1.402	1.393	1.367	1.473	1.405	1.391	1.396	1.409
9	1.369	1.389	1.403	1.393	1.365	1.472	1.404	1.393	1.390	1.406
10	1.379	1.389	1.405	1.391	1.363	1.464	1.407	1.391	1.396	1.409
11	1.367	1.392	1.404	1.396	1.363	1.472	1.403	1.390	1.411	1.399
12	1.379	1.389	1.405	1.391	1.363	1.464	1.408	1.390	**1.398**	**1.399**
13	1.378	1.390	1.408	1.394	1.363	1.464	1.404	1.394	1.397	1.413

Table 3: bond angles **(°)**calculated with the B3LYP/6-31G** basis set.

flavones	**O1C2C1'**	**C2'C3'C4'**	**C1'C6'C5'**	**C2C3C4**	**C5C6C7**
1	112.160	120.606	120.381	123.056	121.710
2	111.760	120.391	120.526	122.579	120.429
3	112.113	120.468	120.526	123.003	121.701
4	112.059	121.220	121.075	123.100	121.703
5	112.193	120.480	120.534	123.171	119.846
6	112.099	121.247	121.084	123.273	119.664
7	112.041	119.670	120.477	123.261	120.375
8	112.121	121.237	121.083	123.250	119.805
9	112.093	121.243	121.089	123.197	119.901
10	112.084	121.226	121.075	123.194	119.858
Flavonols					
1	112.505	121.400	120.797	122.838	119.702
2	112.492	120.818	120.179	122.766	119.988
3	112.485	120.839	120.156	122.742	120.224
4	110.865	119.026	120.122	121.958	120.435
5	111.216	120.892	120.206	122.283	120.822
7	110.694	120.615	120.039	121.210	120.467
8	111.264	121.882	120.331	122.351	121.086
9	111.243	120.787	120.188	122.368	120.465
10	112.501	121.771	120.253	122.775	120.226
11	112.532	119.758	123.345	122.125	120.201
12	112.486	121.387	120.806	122.811	120.218
13	112.528	121.610	120.731	122.839	119.775

Table 4: torsion angles [º] calculated with the B3LYP/6-31G** basis set

flavones	**O1C2C1'C2'**	**O1C2C1'C6'**	**C3C2C1'C6'**	**C3C2C1C2'**
1	18.569	-161.619	18.996	-160.814
2	11.711	-168.242	12.340	-167.705
3	18.810	-161.312	19.428	-160.448
4	16.767	-163.318	17.212	-162.701
5	18.572	-161.467	19.352	-160.607
6	15.996	-163.999	16.633	-163.369

7	0.029	-179.970	0.03074	-179.969
8	16.673	-163.256	17.353	-162.716
9	15.596	-164.646	16.029	-163.727
10	16.098	-163.947	16.684	-163.269
Flavonols				
1	1.921	-178.053	2.067	-177.957
2	0.016	-179.984	0.015	-179.984
3	0.015	-179.984	0.018	-179.982
4	19.580	-158.725	21.510	-160.184
5	20.585	-158.425	21.554	-159.434
7	-36.203	139.781	-39.585	144.429
8	-19.396	159.542	-20.462	160.598
9	-23.246	155.444	-25.100	156.208
10	-1.374	178.863	-1.133	178.628
11	43.302	-136.082	41.491	-139.123
12	0.011	-179.988	0.010	-179.988
13	1.397	-178.803	1.251	-178.547

This series of compounds is limited to two types of the most representative of flavonoid family molecules : flavones and flavonols . These molecules are found to be the subject of most research.

The most stable conformation were obtained for Cosmosiine and Rutin which it characterized by the substitution of a glucoside group in position R_3 and R_1 respectively. The presence of sugar increases their stability by the link that forms between the aglycone and the ose especially those in the 3-position (for flavonols) or in 7 (for flavones), and sometimes in 6 or 8 if these positions are hydroxylated (figure **3)**.

Concerning torsion angles that connect the ring B and the rest of each molecule are respectively 15.596° and 19.580° for Cosmosiine and Rutin. This angle informs us about the planarity of the molecule; it is also influenced by the nature of the flavonoid and the presence of substituent. Generally , flavonols are planar because of electron delocalization throughout the molecule.

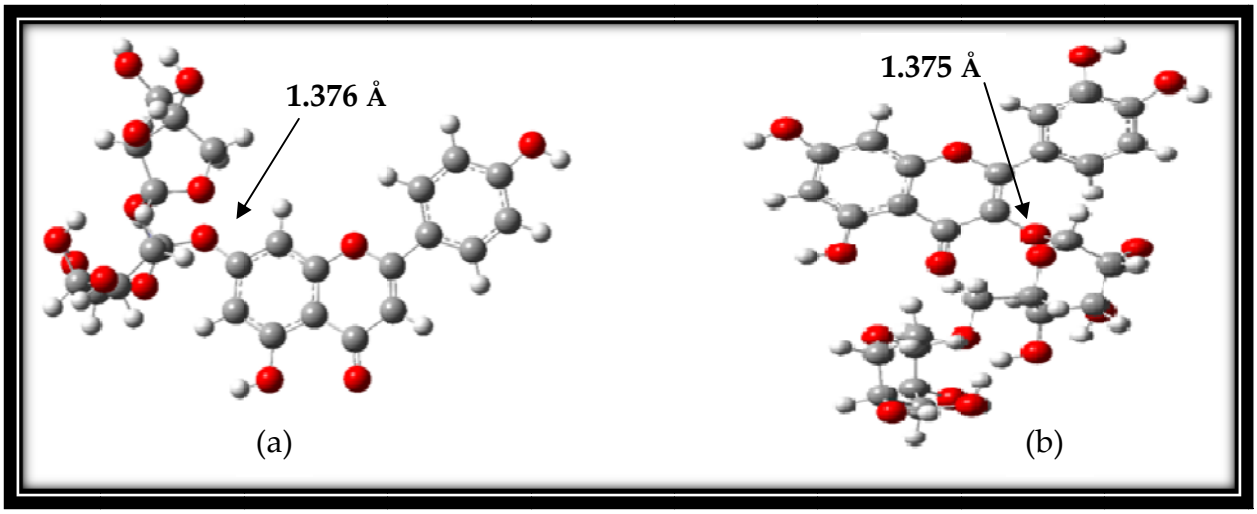

Fig 3 : optimized structure by (B3LYP/9-31G**) of the Cosmosiin (a), and Rutine (b)

2. Indices de Fukui

The frontier orbital (FO) theory proposed by Fukui **[13]** provides a general qualitative approach to understand and interpret chemical reactions. In this theory, the distribution of electron densities in the FOs (HOMO and LUMO) is recognized as the principal factor governing the stereoselective behavior of a molecule with respect to an approaching reagent. Parr and Yang **[14]** used a finite difference approximation (FDA) to evaluate the derivative Fukui function, *f*(**r**) and identified three types of Fukui functions, *f*+(**r**), *f*-(**r**), and *f*0(**r**), which correspond to electrophilic, nucleophilic, and free-radical attack, respectively. The explicit expressions of *f*R(**r**) (R) +, -, and 0) are given by

$f_k^+ = [q_k(N+1) - q_k(N)]$ for a nucleophilic attack

$f_k^- = [q_k(N) - q_k(N-1)]$ for an electrophilic attack

$f_k^0 = [q_k(N+1) - q_k(N-1)]/2$ for a radical attack

Charge analysis to calculate Fukui indices has been conducted using NPA approach **[12].** Fukui indices calculation results using NPA's population analysis are listed in (**Table5**)

Table 5. Fukui indices (B3LYP/6-31G**).

Nom des composés	f_K^+		f_K^-		f_K^0	
	(O)	(C)	(O)	(C)	(O)	(C)
Cirsiliol	0.114	0.018	0.076	0.153	0.095	0.0855
6-Hydroxyluteolin	0.104	0.046	0.08	0.133	0.092	0.0895
Luteolin-7-glucoside	0.105	0.037	0.068	0.13	0.0865	0.0835
Baicalein	0.103	0.052	0.098	0.149	0.1005	0.1005
Scutellarein	0.104	0.046	0.086	0.155	0.095	0.1005
Baicalin	0.097	0.033	0.078	0.112	0.0875	0.0725
Plantaginin	0.106	0.028	0.079	0.128	0.0925	0.0780
Luteolin	0.103	0.052	0.067	0.137	0.085	0.0945
Sorbarin	0.107	0.027	0.078	0.127	0.0925	0.0770
Apiin	0.104	0.038	0.069	0.145	0.0865	0.0915
Cosmosiin	0.105	0.036	0.073	0.152	0.089	0.0940
Quercimeritrin	0.105	-0.007	0.065	0.103	0.085	0.0480
Rhamnetin	0.105	-0.001	0.059	0.097	0.082	0.0480
Quercetin	0.106	0.001	0.063	0.098	0.0845	0.0495
Rutin	0.113	0.033	0.065	0.122	0.089	0.0775
Chrysosplenol D	0.109	0.022	0.073	0.113	0.091	0.0675
Hyperin	0.105	0.049	0.061	0.11	0.083	0.0795
Quercitrin	0.103	0.051	0.063	0.113	0.083	0.082
Chrysosplenol C	0.107	0.029	0.08	0.110	0.0935	0.0695
Isoquercitrin	0.111	0.029	0.066	0.129	0.0885	0.0790
Isorhamnetin	0.105	0.001	0.063	0.101	0.084	0.0510
Oxyayanin A	0.105	0.025	0.06	0.100	0.0825	0.0625
Kaempferol	0.107	0.001	0.065	0.104	0.086	0.0525
Tetramethylquercetin	0.101	-0.002	0.056	0.096	0.0785	0.047

3. QSAR models of flavonoids antioxidant activity

In the current study, a QSAR model is presented for logIC50 of 24 flavonoides involving theoretical descriptors, which have been calculated from molecular structure.

The structures, the distribution of experimental data, the logarithm of inhibitory activity (IC50) for the Full Set of 24 flavonoids and derivatives is shown in table 7.
In a QSAR study, generally, the quality of a model is expressed by its fitting ability and prediction ability, and of these the prediction ability is the more important. In order to build and test the model, a data set of 23 compounds was separated into a training set of 18 compounds, which were used to build the model and a test set of 5 compounds, which were applied to test the built model. With the selected descriptors, we have built a linear model using the training set data, and the following equation was obtained:

$$n = 12,\ R^2 = 0.7015,\ F = 6.2658,\ S^2 = 0.0968$$
$$\log IC_{50} = -2.4152 - 20.991 D_1 + 49.023\ D_2 + 24.400\ D_3$$

Table 6 . Three-parameter model including $\log IC_{50}$ as external

No	X	±ΔX	t -test	Descripteurs
0	-2.4152	1.0417	-2.3186	**Intersection**
1	20.991	196.93	-0.1066	D_1 - (f^+_k)
2	49.023	208.46	0.2352	D_2 - (f^-_k)
3	24.400	406.42	-0.06	D_3 - (f^0_k)

In this equation, N is the number of compounds, R^2 is the squared correlation coefficient S^2 is the squared cross-validation coefficient, and F is the Fisher F statistic
The built model was used to predict the test set data and prediction results are given in Table 7 and fig4

Table 7 : structures of flavones, flavonols, experimental and calculated value of antioxidant activity (LogIC50)

flavones	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	$LogIC_{50}$ exp.	$LogIC_{50}$ calculé.
1	H	OH	H	OH	OH	-	-	-	0.9604	0.9677
2	H	H	GLU	OH	OH	-	-	-	0.9956	1.1560
3	H	OH	H	H	H	-	-	-	1.0338	1.3577
4	H	OH	Glu	H	H	-	-	-	1.0976	0.6259
5	H	OH	Glu	O	OH	-	-	-	1.1401	1.3688
6	H	OH	rha	H	OH	-	-	-	1.2688	1.3652
7	H	H	Glu^2-api	H	OH	-	-	-	1.8112	1.6751
8	H	H	glu	H	OH	-	-	-	2.3664	1.9871
Flavonols										
1	Glu-rha	H	H	H	H	OH	H	OH	0.9576	0.9941
2	Me	H	OMe	Me	H	OH	H	H	0.9836	1.0278
3	H	H	H	H	H	OMe	H	H	1.2166	1.2708
4	H	H	H	H	H	H	H	H	1.3581	1.3934

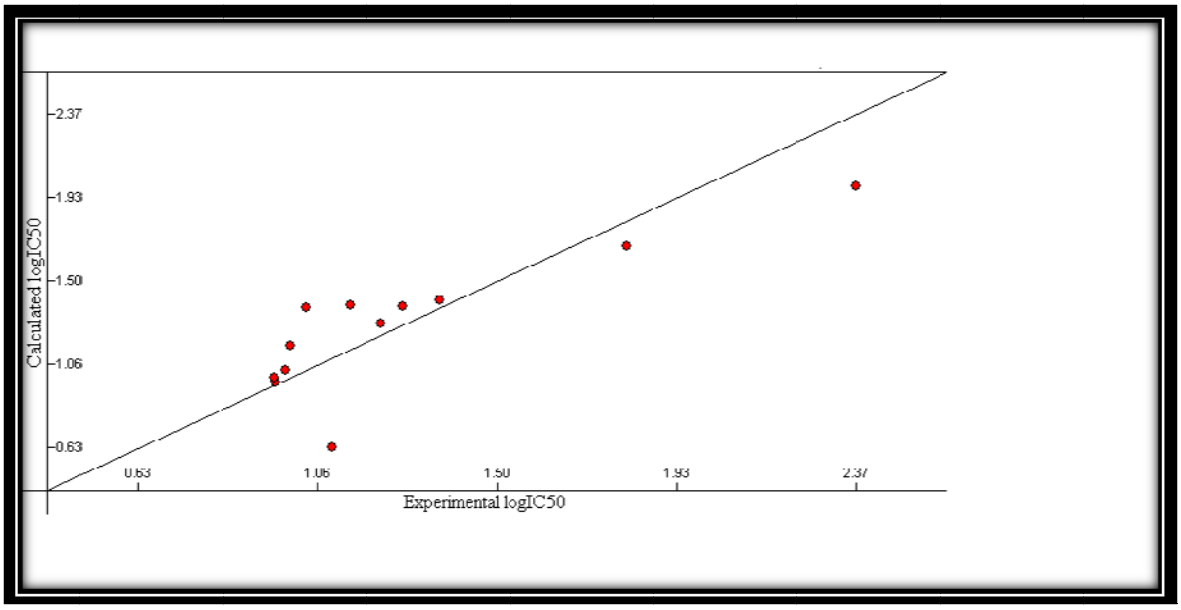

Fig 4. Linear regression line between experimental and calculated log IC_{50} with MLR. (Three parameters model).

As can be seen from Table 7, the calculated values for the $\log IC_{50}$ are in good agreement with those of the experimental values. The predicted values for $\log IC_{50}$ for the compounds in the training and test sets using equation $\log IC_{50}$ were plotted against the experimental $\log IC_{50}$ values in Figure 4.

We then used selected model to classify 5 new molecules, being a test basis, and decide if they belong to learning set group or not. Error rate comes up to 10 %. Results can be thus compared to learning results we got with the model.

Model predictions based on test prove to be satisfactory and most of recorded errors can be explained.

Correlation coefficient with learning set is excellent (**$R^2 = 0.7015$**) and is close to **≈ 1.**

Correlation graph points (**Fig 4**) are all close to ideal line with slope = 1 and Y-axis origin = 0. The model thus explains every molecule activity from learning set with minor error.

IV. Conclusion

In this article, a QSAR study of 24 flavonoides and derivatives. was performed based on the theoretical molecular descriptors calculated by the GAUSSIAN software and selected. The built model was assessed comprehensively (internal and external validation) and all the validations indicated that the QSAR model built was robust and satisfactory, and that the selected descriptors could account for the structural features responsible for the flavonoids derivatives properties of the compounds. The QSAR model developed in this study can provide a useful tool to predict the $\log IC_{50}$ of new compounds and also to design new compounds with high $\log IC_{50}$

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