Study of the solid-liquid extraction from two plants, *Salvia coccinea* (Lamiaceae) and *Syzygium sakalavarum* (Myrtaceae): modeling of isotherms

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ABSTRACT

The aim of this work is to thermodynamically model the solid-liquid extraction of solutes from two plants Salvia coccinea and Syzygium sakalavarum in order to be able to determinate the most adequate extraction method. Two types of solvent were used, water and ethanol 20%. For this, the solid-liquid extraction is assimilated to desorption in the liquid phase. Several tests, with different masses of these plants powder, were carried out in order to obtain experimental points. These were introduced into software in order to obtain the corresponding isotherm for each test. The isotherms obtained experimentally are classified according to IUPAC classification. 4 of these isotherms are associated with the type VI isotherm. However, no model exists in the literature to present this type of isotherm. So a new model has to be created. The type VI isotherm is made up of a set of simple isotherms that make up a step. The modeling of each step has shown that these simple isotherms are type V which demonstrates the mesoporous nature of plant materials and therefore shows the usefulness of extracting solutes at high temperature.

Keywords: - *extraction, Salvia coccinea, Syzygium sakalavarum, modelization, isotherm, desorption*

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1. Introduction

Solid-liquid extraction is a very old operation, encountered especially in the agro-food, pharmaceutical and perfume industries [1-3]. Most of the solids used in these industries are almost vegetable matter [1, 4- 6]. To ensure the quality of the processes to be implemented, industries must do preliminary studies on plants, especially on the mechanisms for extracting the components of a material they want to exploit. These preliminary studies can be carried out based on existing models. Knowledge of the physical characteristics of plant material leads to the improvement of the extraction method which allows to increase the quantity extracted and to optimize the experimental conditions in a production industry.

The aim of this study is to determinate the characteristics of the solid-liquid extraction isotherms of two plants, *Salvia coccinea* and *Syzygium sakalavarum*, by the most common solvents, water (H_2O) and ethanol (EtOH) 20%.

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Solid-liquid extraction is a method of separating one or more constituents of a solid mixture from a liquid phase [2]. Desorption is also a method of separation by detaching the molecules adsorbed on the solid by a liquid phase or by a gas phase [7]. These two methods can be considered as equivalent. To model the solid-liquid extraction, it is thus assimilated to desorption. For desorption model, it is necessary to know the adsorption isotherm that corresponds to it [8]. According to IUPAC classification, there are six types of adsorption-desorption isotherms [8-10]. Models already exist for modeling these types except for type VI. Several isothermal models exist but for this study, the Langmuir and Mahle models are used.

The Langmuir model is the best known and the most used of models (equation 1) [8, 11-13].

$$q_e = q_m \frac{K_L.C_e}{1 + K_L.C_e} \qquad \text{(Equation 1)}$$

This model is determinated from the kinetic theory of gases on a flat surface. This model assumes that the surface of the adsorbent is uniform; the interaction between the adsorbed molecules is negligible and the heat of adsorption is independent of the rate of recovery of the solid surface. It is used for monomolecular adsorption to a surface of the adsorbent at equilibrium. This model corresponds to the type I isotherms of IUPAC classification; therefore it is applicable for monolayer adsorbates [14, 15].

Mahle's model (Equation 2) is created to simplify the relationship between capillary condensation and the Kelvin equation.

$$q_e = \frac{q_m}{\tan^{-1}\left(\frac{1-A}{B}\right) - \tan^{-1}\left(\frac{-A}{B}\right)}\left(\tan^{-1}\left(\frac{C_e - A}{B}\right) - \tan^{-1}\left(\frac{-A}{B}\right)\right) \qquad \text{(Equation 2)}$$

This model is applicable for the obtained curve of adsorption on porous solids and corresponds to the type V isotherm of IUPAC classification. This equation has properties such as the finite saturation limit, the ease of calculating Henry's constant and the heat of absorption. It is therefore expressed by the partial pressure or the quantity of the adsorbed phase and the parameter values attributable to the distribution function [16]. But in this study, experiment is carried out in the liquid phase, then, is considered the quantity of the adsorbed phase.

Nomenclature

q_e : adsorbable quantity or extract quantity (= desorbed) (g)

q_m : maximal adsorbable quantity (g)

K_L : Langmuir constant (L.mg^{-1})

C_e : concentration in liquide phase at equilibrium (mg.L^{-1})

A, B : parameters of model characterising distribution

m_0 : solide masse for the 1st experiments (g)

Δm : difference between two successive experiments (g)

m_f : solid mass of the last experiment (g)

V : volume of solution (mL)

1. Materials and methods

After harvesting, plant material, consisting of aerial part of *Salvia coccinea* and leaves of *Syzygium sakalavarum*, are dried away from heat and solar light. In the case of *Salvia* coccinea and *Syzygium sakalavarum*, the drying took respectively three and four weeks. Then, they are crushed with a mortar and pestle to obtain powders. During extraction, experiments were carried out in glass vials containing 50 mL of extraction solvent. In first experiments, distilled water was used as extraction solvent and in second, it was ethanol 20%. After pouring in extraction solvent, vegetable powders are introduced into vials. Solutions are stirred at room temperature for three hours, using a multiple shaker (fig 1).

Fig -1: Multiple shaker

Masses introduced for each series of experiments are given in Table 1.

Table -1: Operational conditions for the four series of experiments

Solid / Mass solid/solvent	*Salvia coccinea*		*Syzygium sakalavarum*	
	H_2O	EtOH 20%	H_2O	EtOH 20%
m_0 (g)	0.25	0.25	0.25	0.25
Δm (g)	0.25	0.25	0.25	0.25
m_f (g)	5	5	9.25	7
Number of experiments	20	20	37	28

Solution is then filtered with cotton wool over a funnel. filtrate is collected in a graduated cylinder to measure its volume and weighed using a Sartorius balance to measure its mass. Once filtration has been carried out, washing is carried out in order to recover quantities of solute remaining on the surface of plant material. Then, filtrate is poured into a 100 mL flask. Drying is carried out by evaporating extraction solvent from filtrate, using a rotary evaporator, until a concentrated solution is obtained. This last is poured into crystallizer and placed in an oven. Extract is considered completely dry when its mass no longer varies. When extracts are dry, they are weighed using a Sartorius balance, this mass is the desorbed quantity q_e.

For tracing isotherms, extracted quantities are entered into the Excel software before calculating the liquid phase concentrations at equilibrium C_e (equation 3).

$$C_e = \frac{q_e}{V} \qquad \text{(Equation 3)}$$

Quantities extracted versus concentration in the liquid phase at equilibrium gives extraction isotherm considered as equivalent to desorption isotherm. After processing the data in Excel, isothermal curves are obtained. Their appearance compared with the types of isotherm in IUPAC classification allows identifying the type of model to be studied. Several models are tested and the one that best, which correlates with the experimental data, is retained while taking into account the assumptions made by authors of model.

2.1 Resolution of Langmuir equation

Solving Langmuir equation is performed by linearizing Equation 1 which leads to Equation 4.

$$\frac{C_e}{q_e} = \frac{1}{q_m.K_L} + \frac{C_e}{q_m} \qquad \text{(Equation 4)}$$

After determining these two parameters, they are treating by Kaleidagraph to obtain their adjusted values.

2.2 Resolution of Mahle equation

Solving Mahle equation for each step is done on Kaleidagraph software. For the first step, Mahle's model (equation 2) is applied and remains unchanged. The first step to be modeled is presented in chart 1. Only the first 6 points are thus used for the modelization.
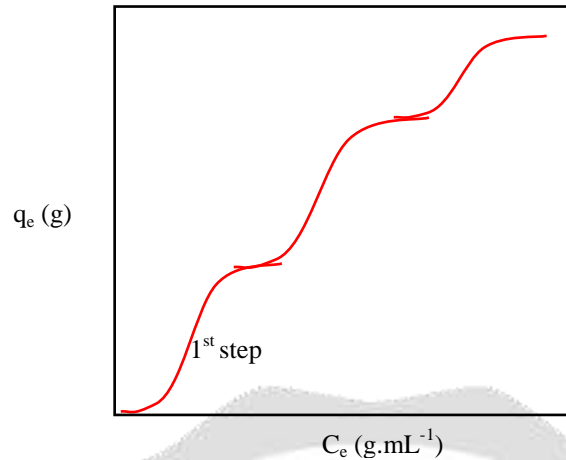
Chart -1: Curve of the first step

For the second step, points of first step are no longer used. 6th to 11th points are used. A change of variable $q_e \rightarrow q_e - q_{en}$ and $C_e \rightarrow C_e - C_{en}$ is applied.

$$\begin{cases} q_e \rightarrow q_e - q_{e_n} \\ C_e \rightarrow C_e - C_{e_n} \end{cases}$$

Two new parameters C_{en} and q_{en} are determinate graphically or by considering coordinates of the last point of previous step as starting point of new step (chart 2).
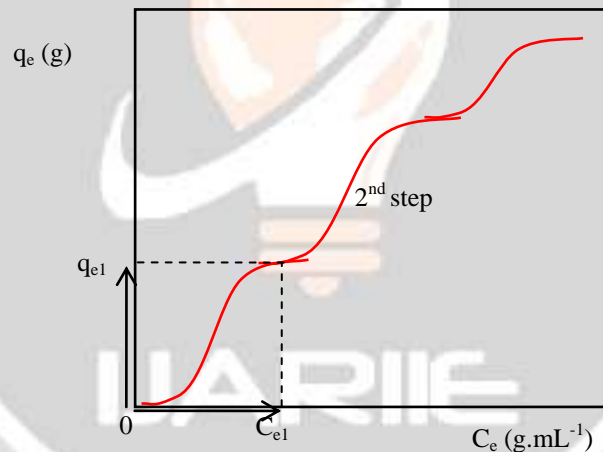

Chart -2: Curve of the second step with parameters C_{e1} et q_{e1}

Thus, Mahle's equation is slightly modified for the second step (equation 5).

$$q_e - q_{e1} = \frac{q_m}{\tan^{-1}\left(\frac{1-A}{B}\right) - \tan^{-1}\left(-\frac{A}{B}\right)}\left(\tan^{-1}\left(\frac{(C_e - C_{e1})-A}{B}\right) - \tan^{-1}\left(-\frac{A}{B}\right)\right) \quad \text{(Equation 5)}$$

Relation q_e versus C_e becomes (equation 6)

$$q_e = \frac{q_m}{\tan^{-1}\left(\frac{1-A}{B}\right) - \tan^{-1}\left(\frac{-A}{B}\right)}\left(\tan^{-1}\left(\frac{(C_e - C_{e1})-A}{B}\right) - \tan^{-1}\left(\frac{-A}{B}\right)\right) + q_{e1} \quad \text{(Equation 6)}$$

Equation 7 is used in Kaleidagraph to model the 2nd step.
By applying the same method for the 3rd step, an identical equation is obtained (equation 7) but parameters C_e, q_e take new values as before.

$$q_e = \frac{q_m}{\tan^{-1}\left(\frac{1-A}{B}\right) - \tan^{-1}\left(-\frac{A}{B}\right)} \left(\tan^{-1}\left(\frac{(C_e - C_{e2}) - A}{B}\right) - \tan^{-1}\left(-\frac{A}{B}\right) \right) + q_{e2} \quad \text{(Equation 7)}$$

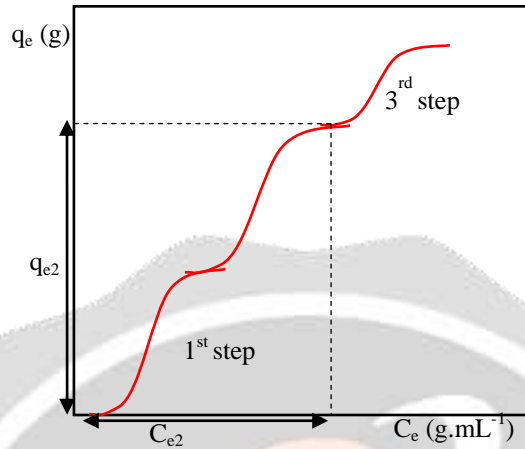Method for graphically determining C_e and q_e parameters is shown in Chart 3.

Chart -3: Curve of the third step with parameters C_{e2} et q_{e2}

2.2 Determination of model parameters

In this work the maximum quantity q_m has not yet been determinated so it is considered as one of the parameters to search in the model. Experimental data corresponding to a step are introduced into the Kaleidagraph software then they are presented in the form of point clouds. Then software automatically gives values of these parameters as well as error and regression. Same method is applied to the other two steps. Therefore, three Mahle's models are obtained. These three models are then transported to Maple software to combine them into a single curve.

3. Results and discussions

From the experimental results, the isotherms of each series of tests presented by the desorbed quantity q_e versus equilibrium concentration C can be plotted and modeled.

3.1 *Salvia coccinea*-water

Concerning the first series of extraction with *Salvia coccinea*, chart 4 corresponding to q_e = f(C_e) gives the extraction isotherm of the solutes in aqueous phase.
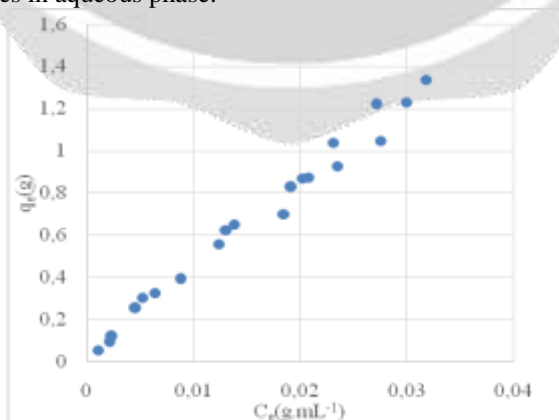

Chart -4: Extraction isotherm of hydrosoluble solutes in *Salvia coccinea*

After visual analysis of the experimental points, the isotherm contains steps. Five steps exist, the first step of which is made up of the first 6 trials, the 2nd step from 6th to 12th trials, so it contains 6 points, the 3rd step from 12th to 17th trials, then it contains 5 points, the 4th step contains only 3 points, the same for the 5th step. Data processing was carried out by Kaleidagraph software, parameters with their error and statistical coefficient R^2 corresponding to each step are therefore determinate as shown in Table 2.

Table -2: Model type V parameters of *Salvia coccinea*-water extraction

Parameters	1st step	2nd step	3rd step
q_m (g)	0.42 ± 0.03	0.367 ± 0.009	$0.20 \pm 0.15 \ 10^{-3}$
A	$(3.02 \pm 0.18) \ 10^{-3}$	$(5.67 \pm 0.04) \ 10^{-3}$	$(2.918 \pm 0.001) \ 10^{-3}$
B	$(2.17 \pm 0.04) \ 10^{-3}$	$(6.36 \pm 1.17) \ 10^{-4}$	$(-1.92 \pm 0.01) \ 10^{-4}$
C_{en} (g.mL^{-1})	-	0.006	0.01
q_{en} (g)	-	0.32	0.68
R^2	0.99	0.99	1

After data processing, only three steps were modeled because number of points is insufficient for 4th and 5th steps. These last two steps are influenced by the solubilization of solutes in extraction solvent. After changing these parameters by these values, Equations 6, 9 and 10 are simplified. They are inserted into Maple software so that they can be combined into an equation. Then Maple produces model as Equation 10;

$$q_e = \begin{cases} 0.17 \quad \tan^{-1}(459.72C_e - 1.39) + 0.16 & for \quad C_e \le 0.006 \\ 0.12 \quad \tan^{-1}(1725.86C_e - 20.87) + 0.50 & for \quad C_e \le 0.016 \\ 0.07 \quad \tan^{-1}(1829.29C_e - 34.43) + 0.77 & for \quad C_e \le 0.023 \end{cases}$$ (Equation 10)
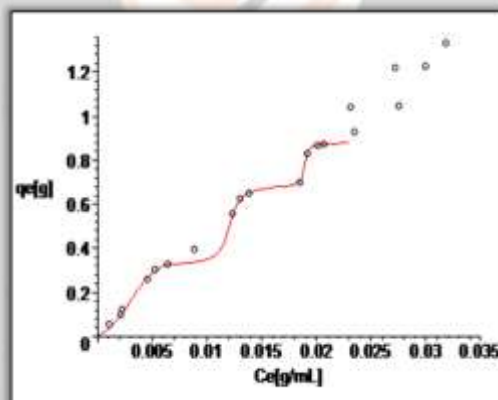
This gives curve shown in chart 5.

Chart -5: Type IV model of extraction isotherm solutes of *Salvia coccinea* in aqueous medium

Mahle's modeling is acceptable for each type VI step because points are correlated with the proposed model. Therefore, type VI isotherm can be a sequence of type V isotherms. However, type V isotherm and type VI isotherm correspond to multilayer desorption, therefore each step of type VI isotherm are multi-layered. Type V isotherm corresponds to sorption of solute from a solid phase containing a micro or mesopore [17]. This isothermal model show that height of steps decreases from 1st to 3rd step. Then, distribution of solutes for each layer in solid is non-uniform. Solutes are concentrated inside external pores.

3.2 *Salvia coccinea*-ethanol 20%

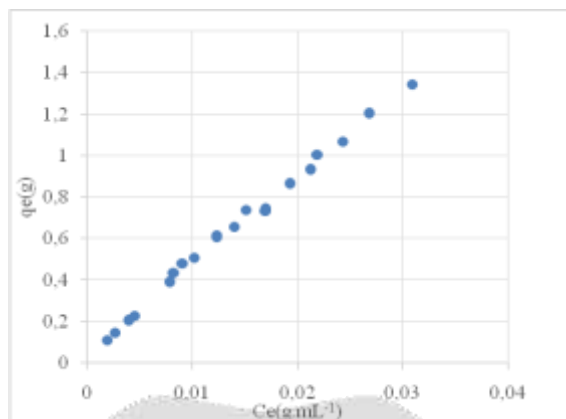Results of extractions for the second series give an extraction isotherm with 6 steps (Chart 6).

Chart -6: Extraction isotherm of *Salvia coccinea* solutes with ethanol 20%

First step is presented by the 4 first trials, second step from 4th to 8th trials, so it contains 5 points, 3rd step from 8th to 12th trial (4 points), 4th step from 13th to 15th trial (3 points), 5th step from 16th to 18th trial (3 points) and the 6th step is indistinguishable. It is difficult to attribute the rest of experiments, which is the last two points to steps due to deficiency of points. After processing data, only first the three steps were modeled because of insufficient points at the 4th and 5th steps. Statistical coefficient at each step and model parameters values are grouped in Table 3.

Table -3: Model type V parameters of *Salvia coccinea*-EtOH 20% extraction

Parameters	1st step	2nd step	3rd step
q_m (g)	0.33 ± 0.05	$0.33 \pm 5.14 \ 10^{-9}$	0.25 ± 0.01
A	$(1.44 \pm 0.47) \ 10^{-3}$	$0.003 \pm 1.81 \ 10^{-11}$	$(3.53 \pm 0.11) \ 10^{-3}$
B	$(2.58 \pm 1.06) \ 10^{-3}$	$0.85 \ 10^{-3} \pm 8.17 \ 10^{-11}$	$(0.65 \pm 0.28) \ 10^{-3}$
C_{en} (g.mL^{-1})	-	0.004	0.01
q_{en} (g)	-	0.22	0.51
R^2	0.99	1	0.99

These three models are assembled in Maple to obtain a continuous curve (equation 11).

$$q_e = \begin{cases} 0.1607 \quad \tan^{-1} \quad (386.6079 \quad C_e - 0.5568) + 0.8165 \quad for \ C_e \leq 0.0045 \\ 0.1141 \quad \tan^{-1} \quad (1167.3787 \quad C_e - 8.9689) + 0.3682 \quad for \ C_e \leq 0.0102 \\ 0.0839 \quad \tan^{-1} \quad (1524.8581 \quad C_e - 20.9411) + 0.6265 \quad for \ C_e \leq 0.0230 \end{cases} \quad \text{(Equation 11)}$$

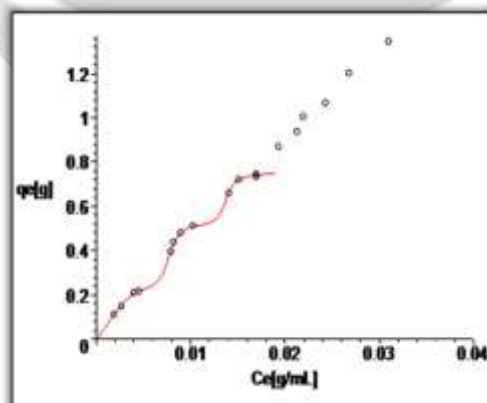The comparison of the model with the experimental data is presented in Chart 7.

Chart -7: Type VI model of extraction isotherm solutes of *Salvia coccinea* in alcoholic 20% medium

These three steps can be modeled because the diffusion of solutes in the extraction solvent is sufficient. The lack of points is due to the lower solubilization rate of solutes in extraction solvent. The preceding interpretations are also valid for the model of this series of experiments.

Comparing the two isotherms of *Salvia coccinea* obtained by two different solvents, adsorption is faster using 20% ethanol. This is due to nature of solvent because ethanol can solubilize more solutes. Therefore, nature of desorbed solutes can also be different. In addition, 20% ethanol is less viscous than water, which promotes internal and external diffusion.

3.3 *Syzygium sakalavarum*-water

Concerning extraction with water solutes of *Syzygium sakalavarum*, plotting amount desorbed versus equilibrium concentration obtained experimentally gives extraction isotherm in Chart 8.
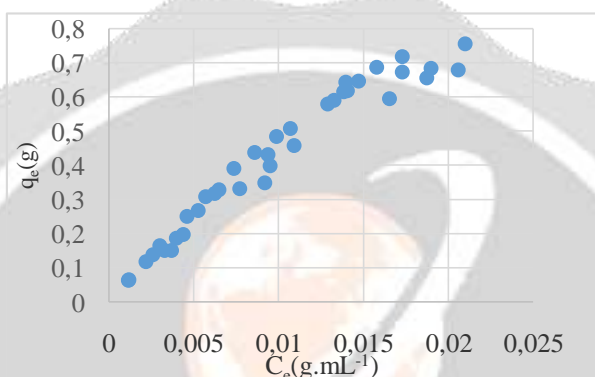

Chart -8: Extraction isotherm of hydrosoluble solutes of *Syzygium sakalavarum*

Extraction results for this 3rd serie are divided into two groups of points; the first group is formed by 14 points and the second by 23 points. Curve of the first group is associated with type I isotherm and the second corresponds to type VI isotherm containing landings, but only three steps are clear. So, extraction isotherm of *Syzygium sakalavarum* is formed by two isotherms. These groups of experimental points are then treated successively by Kaleidagraph software to determinate model parameters, the first by Langmuir type I model and the second by Mahle type V model.

3.3.1 Modeling of the type I isotherm

Parameters of Langmuir model with the respective errors are shown in Table 4. The statistical coefficient corresponding to these values is 0.991.

Table -4: Langmuir model parameters of *Syzygium sakalavarum*-water extraction

Parameters	Values
q_m (g)	2.14 ± 0.63
K_L	28.58 ± 3.67

The Langmuir model is introduced into Maple software with values of these parameters and gives the curve shown in chart 9.
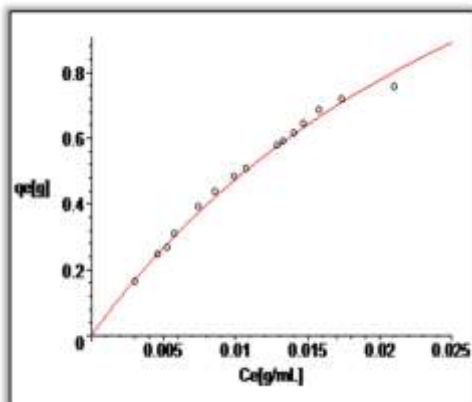
Chart -9: Langmuir model (type I) of extraction isotherm solutes of *Syzygium sakalavarum* in aqueous medium

3.3.2 Modeling of the type VI isotherm

There are 6 points in the first step of isotherm, the 2nd step has 5 points and the 3rd step contains 4 points. Model parameters with errors and statistical coefficient are shown in Table 5.

Table -5: Isotherm type V parameters of *Syzygium sakalavarum*-water extraction

	1st step	2nd step	3rd step
q_m (g)	0.30 ± 0.08	0.15 ± 0.006	$0.13 \pm 1.02.10^{-5}$
A	$(1.02 \pm 0.81).10^{-3}$	$(1.21 \pm 0.16).10^{-3}$	$2.56.10^{-3} \pm 4.86.10^{-7}$
B	$(2.78 \pm 1.49).10^{-3}$	$(4.48 \pm 1.22).10^{-4}$	$-2.02.10^{-4} \pm 3.32.10^{-7}$
C_{en} (g.mL^{-1})	-	0.004	0.006
q_{en} (g)	-	0.18	0.33
R^2	0.98	0.99	1

By rewriting this model with the value of these parameters, equation 12 has been transcribed by Maple.

$$q_e = \begin{cases} 0.16 \quad \tan^{-1} \quad (359.59 \quad C_e - 0.37) + 0.05 \quad for \ C_e \leq 0.0044 \\ 0.05 \quad \tan^{-1} \quad (2188.61 \quad C_e - 11.134) + 0.26 \quad for \ C_e \leq 0.0077 \ \text{(Equation 12)} \\ 0.04 \quad \tan^{-1} \quad (4959.08 \quad C_e - 44.95) + 0.39 \quad for \ C_e \leq 0.013 \end{cases}$$

Plot of model with experimental points are shown in chart 10.
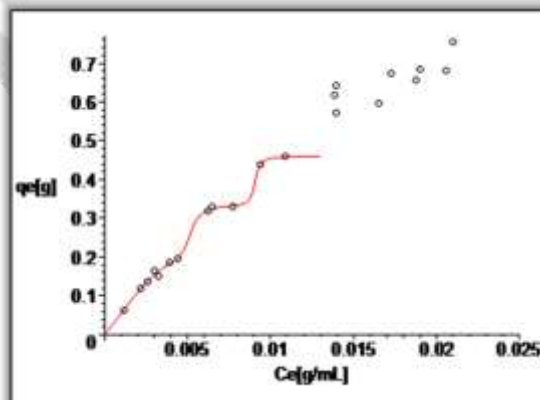

Chart -10: Mahle model (type VI) of extraction isotherm solutes of *Syzygium sakalavarum* in aqueous medium

The three visible steps can be modeled. The rest of the experimental points on type VI isotherm may be the 4th step but due to insufficient points or experimental errors steps are discontinuous. The increase in number of points from

the first to the last desorbed layer can be caused by solutes solubilization in solvent. On the last step or the first desorbed layer, hydrosoluble solutes are in small amounts causing this lack of point. More solvent penetrates into pore of solid, more quantity of desorbed solutes increases or, perhaps, diffusion of solutes towards water is the cause of this increase.

Type I model obtained previously can be the result of washing after filtration. Experimental points adapt well to Langmuir's type I model. Then, solutes remaining on plant material surface after filtration form a monolayer. Type I isotherm is therefore desorption of a monolayer solutes which corresponds to a non-porous surface of the solid. For type VI isotherm, desorption takes place layer by layer, starting in the reverse direction of adsorption. Each layer is considered as layers which include sub-layers because it is modeled by type V model corresponding to a multilayer desorption on a mesoporous surface. Then, isotherm of *Syzygium sakalavarum* with water shows that solutes are located in a non-porous part and a porous part of solid surface. In pores, solutes are arranged in multilayers, and on the smooth surfaces they are in a monomolecular layer. By combining these two isothermal curves on Maple, curve shown in Chart 11 is obtained.
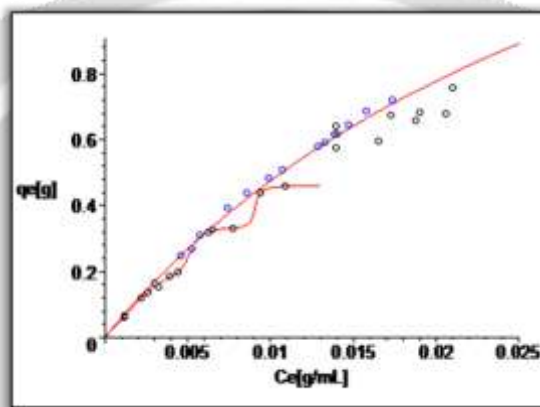

Chart -11: Superposition of the two extraction isotherm models of *Syzygium sakalavarum* in aqueous medium

3.3 *Syzygium sakalavarum*-ethanol 20%

Chart 12 shows the extraction isotherm of *Syzygium sakalavarum* with 20% ethanol. This isotherm is obtained by plotting curve q_e versus concentration C_e.
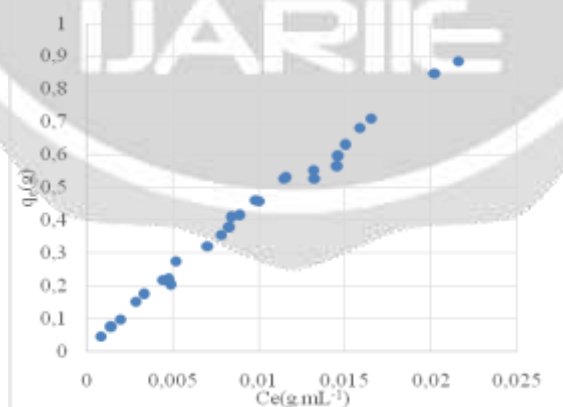

Chart -12: Extraction isotherm of *Syzygium sakalavarum* solutes with ethanol 20%

After analyzing the experimental data, this isotherm contains approximately 6 steps but only the first 4 steps are distinct. The 1st step is made up of the 1st 6 trials, the 2nd step contains 5 points, the 3rd step has 5 points, the 4th step contains 5 points. Experimental data are processed by Kaleidagraph software and parameters with errors and statistical coefficients of each step are obtained. These values are shown in Table 6.

Table -6: Isotherm type V parameters of *Syzygium sakalavarum*-EtOH 20% extraction

	1st step	2nd step	3rd step	4th step
q_m (g)	0.26 ± 0.04	0.15 ± 0.02	0.159 ± 0.004	0.082 ± 0.009
A	0.001 ± 0.0001	0.002 ± 0.0001	0.00136 ± 0.00002	0.0023 ± 0.0005
B	0.002 ± 0.0005	0.0004 ± 0.0002	0.00058 ± 0.00008	-0.00009 ± 0.00033
C_{en} (g.mL^{-1})	-	0.003	0.007	0.009
q_{en} (g)	-	0.17	0.32	0.46
R^2	0.99	0.92	0.99	0.96

Indeed, its equation becomes (equation 13):

$$q_e = \begin{cases} 0.1157 \quad \tan^{-1} \quad (577.1339 \quad C_e - 0.8028) + 0.0783 & for \ C_e \leq 0.0034 \\ 0.0543 \quad \tan^{-1} \quad (2506.7054 \quad C_e - 12.8010) + 0.2502 & for \ C_e \leq 0.0070 \\ 0.0582 \quad \tan^{-1} \quad (1706.2226 \quad C_e - 14.2677) + 0.3898 & for \ C_e \leq 0.0100 \\ 0.0263 \quad \tan^{-1} \quad (10944.8706 \quad C_e - 123.8105) + 0.5003 & for \ C_e \leq 0.0140 \end{cases} \quad \text{(Equation 13)}$$

Representative curve of this model is shown in chart 13.
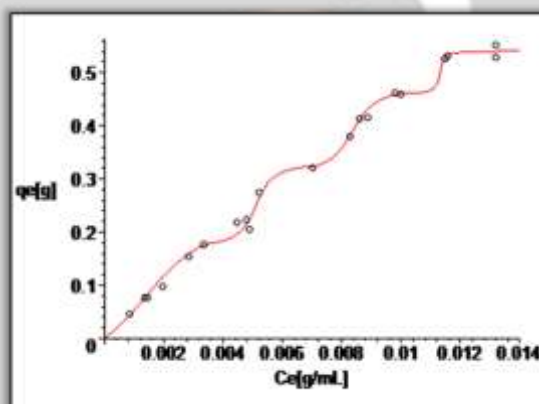
Chart -13: Type VI model of extraction isotherm solutes of *Syzygium sakalavarum* in alcoholic 20% medium

After processing data, these four steps are all modelizable. Plant isotherm of this plant in alcohol 20% is different of in water. It has now only one type VI isotherm, an association of Mahle type V isotherm model. Each model correlates well with the experimental results so each layer has sub-layers. Desorption of these sub-layers seems to take place on mesoporous surface. Since solutes placed on the mesoporous surface have sub-layers, each layer of the type VI isotherm is desorbed onto a mesoporous surface. Therefore, type VI isotherm is considered to result from desorption of layers placed in pores.

Adsorption kinetics are faster than that of desorption. So solid-solvent extraction velocity is slow. For *Salvia coccinea*, isotherm obtained with the two extraction solvents are of the same type, but for *Syzygium sakalavarum*, they are differentiated by the existence of a type I isotherm with water.

4. CONCLUSIONS

The objective is to determinate extraction isothermal curves of plants, *Salvia coccinea* and *Syzygium sakalavarum*, to know their physical characteristics. It is indeed predictable that a plant is porous, however, diffusion can be different from one plant to another. Extraction isotherms results confirm that plant materials have a porous structure, more precisely a mesoporous structure. There are extraction methods suitable for this structure favoring optimization of production yield, for example, when using water as an extraction solvent, it is preferable to wash after filtration, on the other hand when of the use of EtOH, washing is not very important. Experimental points are almost correlated with curve obtained from model; modeling of isotherm is therefore validated. Extraction isotherm type depends on plant material and extraction solvent used. Extract ion of solutes from the same solid with two different extraction solvents can give two different types of isotherm. Type V adsorption isotherm is normally obtained at low

temperature so desorption isotherm must be carried out at a high temperature. To maximize yield, it is necessary that solutes extraction from both plants takes place at high temperature. Improvement in efficiency leads to a reduction in errors on extraction isotherm, which corresponds to already existing model optimization. In the case of this study, extraction method used is maceration which gives only a low yield. So most suitable method of extraction will be decoction or percolation. Regarding limiting step, material transfer depends on plant structure, uniformity of particle sizes, method of grinding; therefore, to properly study and complete this study, the effects of grinding and sieving mode will be considered as perspectives.

6. REFERENCES

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