

## Original Research Article

### OPTIMIZATION OF EXTRACTION CONDITIONS OF POLYPHENOLS AND FLAVONOIDS FROM *OLAX SUBSCORPIOIDEA* LEAVES, A MEDICINAL PLANT TRADITIONALLY USED IN KORHOGO (COTE D'IVOIRE).

#### ABSTRACT

##### Objective:

Secondary metabolites including polyphenols and flavonoids play a key role in the expression of the active ingredients of several medicinal plants in Côte d'Ivoire including *Olax subscorpioidea* Oliv. This study was initiated to contribute to improving the conditions for extracting polyphenols and flavonoids from the leaves of *Olax subscorpioidea* Oliv harvested in Korhogo.

##### Material and Methods

The effect of four independent variables on polyphenol and flavonoid content was evaluated using experimental designs; these are extraction time (12h-24h), evaporation temperature (45°C – 55°C), plant matter-solvent ratio (1/20 g/mL – 1/5 g/mL) and ethanol proportion (20% - 100%). The Central Composite design (CCD) allowed the optimization of responses after screening by Plackett and Burman.

##### Results

The optimal extraction conditions were obtained with an ethanolic proportion of 88.29% and 17g of *Olax subscorpioidea* Oliv leaves dissolved in 100 mL of ethanol. The experimental values (96.31 ± 5.29 mg Eq AG/g for polyphenols and 62.44 ± 4.58 mg Eq Q/g) close to those expected (94.51 mg Eq AG/g for polyphenols and 58.22 mg Eq Q/g for flavonoids) confirmed the relevance of the mathematical model used and the success of the response surface methodology in optimizing the extraction conditions of the system studied.

##### Conclusion

These results show that by taking into account the extraction conditions during this preliminary study, we manage to optimize the quantities of secondary metabolites of therapeutic interest.

**Keywords:** Polyphenols, flavonoids, *Olax subscorpioidea*, CCD.

## 1. INTRODUCTION

Worldwide, interest in bioactive compounds is becoming increasingly important in several different fields such as pharmaceuticals and cosmetics [1]. According to [2], more than 80% of populations in the African continent use plants for health care. WHO indicates that in Côte d'Ivoire, traditional medicine is

experiencing unprecedented growth and constitutes the pillar of primary health care for the majority of the population thanks to its geographical, economic and cultural accessibility. The therapeutic effect of a plant is related to the presence of secondary metabolites that provide plants with remarkable active ingredients[3]. These secondary metabolites are widely used as valuable compounds in pharmaceuticals, cosmetics, fine chemicals[4]. Among these secondary metabolites, polyphenols and flavonoids found in the leaves of *Oxalysubscorpioidea* Oliv. are a good source of remarkable biological activities[5]. However, extraction parameters such as solvent, extraction time, temperature, liquid-solid ratio, and particle size could affect the content of flavonoids and polyphenols extracted in *Oxalysubscorpioidea* Oliv leaves. It is therefore essential to improve and develop experimental extraction using the response surface methodology (RSM). This methodology is an effective statistical and mathematical technique, used to study the impact of many factors and their interactions at the same time[6]. Thus, the aim of this work is to optimize the conditions for extracting polyphenols and flavonoids from the leaves of *Oxalysubscorpioidea*, a medicinal plant traditionally used in Korhogo.

## **2. MATERIAL AND METHODS**

### **2.1. Material**

#### **2.1.1. Plant material**

The plant material, which consists of the leaves of *Oxalysubscorpioidea* Oliv, was collected in Korhogo. These fresh leaves were authenticated by the National Floristic Center of Félix HOUPOUET- BOIGNY University (Abidjan, Côte d'Ivoire).

#### **2.1.2. Chemicals material and Reagents**

The different concentrations of hydroethanolic extracts of *Oxalysubscorpioidea* Oliv were prepared using distilled water (w/v). Concerning the determination of the amount of total polyphenols, folin-ciocalteu reagent diluted 1/10 calcium carbonate solution ( $75 \text{ g}\cdot\text{L}^{-1}$ ),  $\text{FeCl}_3$  solution were used. Regarding the determination of the amount of flavonoids, the following reagents were used:  $\text{NaNO}_2$  (5%, w/v),  $\text{AlCl}_3$  (10 %, w/v),  $\text{NaOH}$  (1 M). All other chemicals and reagents used were of analytical grade and obtained from standard sources.

### **2.2. Methods**

#### **2.2.1. Extraction by the Plackett and Burman plan according to Hadamard notation .**

Maceration extraction was performed according to the method defined by [7] modified. The modification concerned the factors studied. 10 g of plant material were placed in a glass bottle containing 100 mL of

extraction solvents under continuous stirring. The extraction duration (12–24 h), the plant material–solvent ratio ( 1/20 g/mL – 1/5 g/mL ) and the proportion of solvent (distilled water and ethanol: 20% – 100 %) were predetermined using the experimental design of Plackett and Burman summarized in **Table 1**. Then, the mixture was filtered twice with cotton and twice with filter paper. An oven was used (45°C – 55°C) to remove the extraction solvent. The crude extracts were preserved in bottles and stored at 4°C until further use. Eight (8) experiments were carried out.

**Table 1:** Compiled factor matrix with actual values in the table of experimentation in Plackett and Burman Plan case

Factors Tests	X <sub>1</sub> (h)	X <sub>2</sub> (%)	X <sub>3</sub> (°C)	X <sub>4</sub> (g/m L)
1	+(24)	+(100)	+(55)	-(1/20)
2	-(12)	+(100)	+(55)	+(1/5)
3	-(12)	-(20)	+(55)	+(1/5)
4	+(24)	-(20)	-(45)	+(1/5)
5	-(12)	+(100)	-(45)	-(1/20)
6	+(24)	-(20)	+(55)	-(1/20)
7	+(24)	+(100)	-(45)	+(1/5)
8	-(12)	-(20)	-(45)	-(1/20)

### 2.2.2. Extraction by the Central Composite Plane

The optimization of the extraction of polyphenols and flavonoids was carried out using the Central Composite design. The previous screening allowed us to find the influential factors X<sub>2</sub> ( proportion of solvent) and X<sub>4</sub> (plant matter-solvent ratio); they were selected for the experiment in the central composite design. For this design, each factor has 5 levels (-α, -1, 0, +1 and +α). Referring to [8], the combination of the levels of the 2 factors led to 13 tests comprising 4 factorial tests, 4 star tests and 5 tests in the center of the domain. The experimental table of this design is shown in **Table 2**. The quadratic mathematical model expected for the factors X<sub>2</sub> and X<sub>4</sub> which are taken into account for the study is as follows:  $Y = a_0 + a_2X_2 + a_4X_4 + a_{24}X_2X_4 + a_{22}X_2^2 + a_{44}X_4^2$

**Table 2: Experimental table in the Central Composite Plan**

Factors			
Tests		X <sub>2</sub> (%)	X <sub>4</sub> (g/mL)
1		31.71	7/100
2		88.29	7/100
3		31.71	17/100
4		88.29	17/100
5		20	3/25
6		100	3/25
7		60	1/20
8		60	1/5
9		60	3/25
10		60	3/25
11		60	3/25
12		60	3/25
13		60	3/25

**a<sub>0</sub>**= Theoretical effect or general average of all observed responses

**a<sub>1</sub>** = effect of extraction time (X<sub>1</sub>) on Y<sub>1</sub> and Y<sub>2</sub>

**a<sub>2</sub>** = effect of the proportion of ethanol (X<sub>2</sub>) on Y<sub>1</sub> and Y<sub>2</sub>

**a<sub>3</sub>** = effect of the evaporation temperature (X<sub>3</sub>) on Y<sub>1</sub> and Y<sub>2</sub>

**a<sub>4</sub>** = effect of the plant material-solvent ratio (X<sub>4</sub>) on Y<sub>1</sub> and Y<sub>2</sub>

### 2.2.3. Analysis of response variations

#### 2.2.3.1. Quantification of total polyphenol content

The method of [9] was used to determine total polyphenols concentration. A volume of 2.5 mL of diluted Folin-Ciocalteu reagent (1/10) was added to 30 µL of extract. The mixture was kept for 2 minutes in the dark at room temperature (30 ± 2 °C), then 2 mL of calcium carbonate solution (75 g.L<sup>-1</sup>) was added. Then, the mixture was placed for 15 minutes in a water bath at 50 °C, then cooled rapidly. The absorbance was read by ultraviolet spectrophotometer at the wavelength λ = 760 nm. A calibration line was made with gallic acid at different concentrations. Analyses were performed in triplicate and polyphenol content was expressed as milligram gallic acid equivalent ( mgEq AG /g ) per gram of *Ola x subscorpiodea* Oliv extract.

### 2.2.3.2. Quantification of flavonoid content

The method of [10] was used to determine total flavonoids content [10]. In a 25 mL flask, 0.75 mL of 5% (m/v) sodium nitrite ( $\text{NaNO}_2$ ) was added to 2.5 mL of *Olaxsubscorpioidea* Oliv extract. The mixture was supplemented with 0.75 mL of 10% (m/v) aluminum chloride ( $\text{AlCl}_3$ ) and incubated for 6 minutes in the dark at room temperature ( $30 \pm 2$  °C). After incubation, 5 mL of sodium hydroxide ( $\text{NaOH}$ , 1N) was added and the volume was made up to 25 mL. The mixture was shaken vigorously before being assayed using a UV-visible spectrophotometer at  $\lambda = 510$  nm. A calibration curve was obtained under the same conditions as those of the extract using a concentration range from 0 to 100  $\mu\text{g/mL}$  of quercetin solution. The flavonoid content was expressed as milligram equivalent of quercetin (mg Eq Q/g) per gram of *Olaxsubscorpioidea* Oliv extract from the calibration curve using the following equation:  $Y' = 0.0255X$ ,  $R^2 = 0.9812$  where  $Y'$  is the absorbance and  $X'$  is the quercetin equivalent (mg/g).

### 2.2.4. Statistical analysis

All results were expressed as means  $\pm$  standard deviations and all experiments were performed in triplicate. Experimental data were analyzed by Microsoft Office Excel 2016 software. Design Expert version 13 software was used to perform analysis of variance (ANOVA) and the degree of significance of the data was taken at probabilities 0.05; 0.01 and 0.001.

## 3. RESULTS AND DISCUSSION

The experimental responses obtained in the case of screening the factors influencing the quantity of polyphenols and flavonoids are reported in **Table 3**. The quantity of polyphenols varies between  $100 \pm 10.10$  mg Eq AG/g and  $236.66 \pm 5.77$  mg Eq AG/g. As for the quantity of flavonoids, it varies between  $66.66 \pm 5.77$  mg Eq Q/g and  $123.33 \pm 4.72$  mg Eq Q/g. Statistical analysis allowed the screening of factors influencing the amount of total polyphenols and total flavonoids contained in the leaves of *Olaxsubscorpioidea* Oliv. by calculating the coefficients by multiple linear regression.

The coefficients of the four factors studied (extraction time ( $X_1$ ), ethanol proportion ( $X_2$ ); evaporation temperature ( $X_3$ ) and plant material-solvent ratio ( $X_4$ )) are shown in **Table 4**.

**Table 3:** Experimental matrix and experimental results in the Plackett and Burman design

Tests	X <sub>1</sub> (h)	X <sub>2</sub> (%)	X <sub>3</sub> (°C)	X <sub>4</sub> (g/mL)	Y <sub>1</sub> (mg Eq AG/g)	Y <sub>2</sub> (mg Eq O/g)
1	24	100	55	1/20	120 ± 10.70	93 ± 2.64
2	12	100	55	1/5	100 ± 10.10	80 ± 4.00
3	12	20	55	1/5	136.66 ± 9.0	93.33 ± 6.50
4	24	20	45	1/5	173.33 ± 8.70	110 ± 10.00
5	12	100	45	1/20	110 ± 9.80	76.66 ± 5.77
6	24	20	55	1/20	196.66 ± 11.5	133.33 ± 9.07
7	24	100	45	1/5	106.66 ± 4.93	66.66 ± 5.77
8	12	20	45	1/20	236.66 ± 5.77	123.33 ± 4.72

X<sub>1</sub> = extraction time; X<sub>2</sub> = proportion of ethanol; X<sub>3</sub> = evaporation temperature and X<sub>4</sub> = plant material – solvent ratio.

Y<sub>1</sub> corresponds to the quantity of total polyphenols and Y<sub>2</sub> is the quantity of flavonoids.

**Table 4:** Statistical estimation of coefficients (effect of factors)

	Polyphenols (Y <sub>1</sub> )			Flavonoids (Y <sub>2</sub> )		
	Coefficients	Error Type	2 x Standard error	Coefficients	Error Type	2 x Standard error
a <sub>0</sub>	147.49 <sup>ns</sup>	9.35	18.7	97.04 <sup>ns</sup>	3.82	7.64
a <sub>1</sub>	1.67 <sup>ns</sup>	9.35	18.7	3.71 <sup>ns</sup>	3.82	7.64
a <sub>2</sub>	<b>-38, 33 *</b>	9.35	<b>18.7</b>	<b>-1796 *</b>	3.82	<b>7.64</b>
a <sub>3</sub>	-9.17 <sup>ns</sup>	9.35	18.7	2.88 <sup>ns</sup>	3.82	7.64
a <sub>4</sub>	<b>-18, 33 *</b>	9.35	<b>18.7</b>	<b>-9.54 *</b>	3.82	<b>7.64</b>

<sup>ns</sup>: not significant \* : significant

a<sub>0</sub> = Theoretical effect or general average of all observed responses

a<sub>1</sub> = effect of extraction time (X<sub>1</sub>) on Y<sub>1</sub> and Y<sub>2</sub>

a<sub>2</sub> = effect of the proportion of ethanol (X<sub>2</sub>) on Y<sub>1</sub> and Y<sub>2</sub>

a<sub>3</sub> = effect of the evaporation temperature (X<sub>3</sub>) on Y<sub>1</sub> and Y<sub>2</sub>

a<sub>4</sub> = effect of the plant material-solvent ratio (X<sub>4</sub>) on Y<sub>1</sub> and Y<sub>2</sub>

A coefficient is said to be statistically significant if its absolute value is strictly greater than twice the experimental standard deviation, i.e.  $|coef| > 2 \delta$  [11].

These results show that only the effects of factors X<sub>2</sub> and X<sub>4</sub> significantly influence (p < 0.05) the responses Y<sub>1</sub> and Y<sub>2</sub>. We obtain the following mathematical models:

$$Y_1 = 147.49 - 38.33X_2 - 18.33X_4$$

$$Y_2 = 97.04 - 17.96X_2 - 9.54X_4$$

By solving the above two equations, the conditions for which  $X_1$  and  $X_4$  are at their minimum value are determined. When the extraction of polyphenols and flavonoids is done in 20% ethanol proportion and with a material ratio plant-solvent of 1/20 g/mL, we obtain the maximum quantity of secondary metabolites; that is  $Y_{1max} = 236.3$  mg Eq AG /g for polyphenols and  $Y_{2max} = 133.33$  mg Eq quercetin / g for flavonoids . The extraction of polyphenols and flavonoids is therefore favored by a small proportion of ethanol and also with a small plant material-solvent ratio.

Regarding the optimization of the extraction conditions of total polyphenols and total flavonoids, 13 tests were carried out according to the recommendations of the Central Composite Plan by keeping the extraction time and the evaporation temperature constant. The results obtained are shown in Table 5. With an extraction time of 24 h and an evaporation temperature of 55°C, the maximum quantity of polyphenols obtained was  $106 \pm 5.44$  mg Eq AG/g. Under these same conditions, the total flavonoid content of the extract obtained was  $62 \pm 4.58$  mg Eq Q/g. These results are significantly higher than those obtained by Konan (2005)with  $35.49 \pm 0.29$  mg Eq AG/g of ethanolic extract for polyphenols and  $22.69 \pm 0.69$  mg Eq Q/g of extract for flavonoids[12].

Table 6 shows the estimation of the coefficients of the studied factors ( $X_2$  and  $X_4$ ), their interaction and their quadratic effects. The analysis of this table indicates that the quadratic effects of the factors  $X_2$ (proportion, %) and  $X_4$ (ratio, g/mL) positively influence the extraction of total polyphenols from *Olaxsubscorpioidea*Olivleaves ( $p < 0.05$ ). The analysis of the same Table 6 also indicates that the proportion (%) and the ratio (g/mL) as well as the quadratic effect of the proportion of ethanol are statistically significant ( $p < 0.05$ ) on the extraction of total flavonoids from *Olaxsubscorpioidea*Olivleaves. The interaction effect between the proportion of ethanol (%) and the ratio (g/mL) as well as the quadratic effect of the plant material - solvent ratio do not positively influence ( $p > 0.05$ ) the extraction of total flavonoids from the leaves of *Olaxsubscorpioidea*Oliv. The relationships between factors and responses result in the following polynomial forms:

$$Y_1 = 39.24 + 0.01X_2^2 - 7375X_4^2$$

$$Y_2 = 24.67 + 0.86X_2 + 8.91X_4 + 0.01X_2^2$$

**Table 5** :Experimental matrix and experimental results in the Central Composite Plane

Tests	Factors		Answers	
	$X_2$ (%)	$X_4$ (g/mL)	$Y_1$ (mg Eq AG/g)	$Y_2$ (mg Eq Q/g)
1	31.71	7/100	$89 \pm 2.64$	$46 \pm 2.00$
2	88.29	7/100	$81 \pm 7.21$	$50 \pm 4.10$
3	31.71	17/100	$75 \pm 6.31$	$50 \pm 4.00$
4	88.29	17/100	$96 \pm 5.29$	$62 \pm 4.58$

5	20	3/25	99 ± 7.00	41 ± 3.60
6	100	3/25	106 ± 5.44	46 ± 2.00
7	60	1/20	46 ± 5.56	53 ± 4.41
8	60	1/5	61 ± 6.44	57 ± 5.57
9	60	3/25	87 ± 8.52	58 ± 1.00
10	60	3/25	94 ± 6.00	61 ± 7.00
11	60	3/25	89 ± 10.03	55 ± 1.67
12	60	3/25	100 ± 6.55	56 ± 3.61
13	60	3/25	100 ± 4.00	56 ± 2.64

$X_2$  = proportion of ethanol;  $X_4$  = ratio of plant material to solvent.

$Y_1$  corresponds to the amount of total polyphenols and  $Y_2$  is the amount of flavonoids.

**Table 6 :** Estimation of coefficients of factors, interactions and quadratic effect factors

Coefficients	Polyphenols ( $Y_1$ )	Flavonoids ( $Y_2$ )
	Values of coefficients	
$a_0$	39.24 ***	24.67 *
Linear		
$a_2$	-1.43 ns	0.86 *
$a_4$	1518.00 ns	8.91 *
Interaction		
$a_{24}$	5.13 ns	1.41 ns
Quadratics		
$a_{22}$	0.01 *	0.01 ***
$a_{44}$	-7375.00 ***	-165.00 ns
$R^2$	0.91	0.87
$p$ -default adjustment	0.36	0.22

ns: not significant; \*: significant at 5%; \*\*: significant at 1%; \*\*\*: significant at 0.1%

\* = significant coefficients

$a_0$  = the value of the response at the central point of the experimental domain; the average of all theoretical responses.

$a_2$  = effect of the proportion of ethanol ( $X_2$ ) on  $Y_1$  and  $Y_2$

$a_4$  = effect of the plant material-solvent ratio ( $X_4$ ) on  $Y_1$  and  $Y_2$

$a_{24}$  = effect of the interaction between  $X_2$  and  $X_4$  on  $Y_1$  and  $Y_2$

$a_{22}$  = quadratic effect of the proportion of ethanol ( $X_2^2$ ) on  $Y_1$  and  $Y_2$

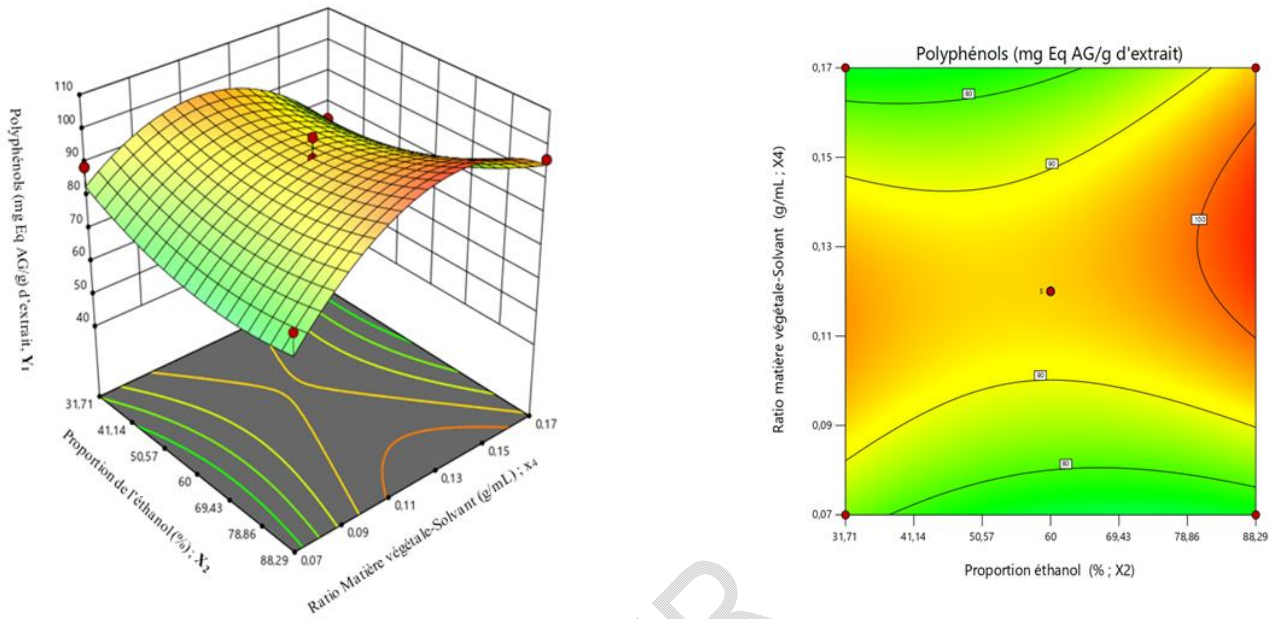
$a_{44}$  = quadratic effect of the plant material - solvent ratio ( $X_4^2$ ) on  $Y_1$  and  $Y_2$

The effects of ethanol proportion ( $X_2$ ) and plant matter-solvent ratio ( $X_4$ ) on the amounts of flavonoids and polyphenols were plotted in response surface mode (3D and 2D) as shown in **Fig 1 and Fig2**. The 3D response surface is a graphical representation of the regression equation. It allows visualizing the relationship between the responses and the experimental levels of each factor and the type of interactions between the test variables [13].

**Fig 1** shows that the amount of polyphenols increases with the simultaneous increase in the proportion of ethanol and the plant matter-solvent ratio until reaching a threshold. This threshold is linked to the limiting factor (plant matter-solvent ratio), an increase beyond the threshold of which leads to a decrease in the polyphenol content. The negative quadratic effect of the observed ratio can be explained by the saturation of the extraction medium [14]. As for the flavonoid content (**Fig 2**), the surface mode representation of the effect of the proportion of ethanol and the plant matter-solvent ratio on the extraction shows a significant influence ( $p < 0.05$ ) of the technological parameters. By increasing the proportion of ethanol and the plant matter-solvent ratio, the amount of flavonoids also increases up to a threshold. The effect of these two factors is significant but beyond the threshold value reached by the limiting factor (ethanol), the flavonoid content decreases. Indeed, the increase in the proportion of alcohol (ethanol) promotes the degradation of cell membranes which improve the permeability of the solvent in the solid matrix [15]. However, beyond the threshold value, that is to say at high concentrations of alcohol (ethanol), the resulting polarity is inappropriate for the extraction of flavonoids which are antioxidants; this explains the decrease in flavonoid content beyond the threshold value [16].

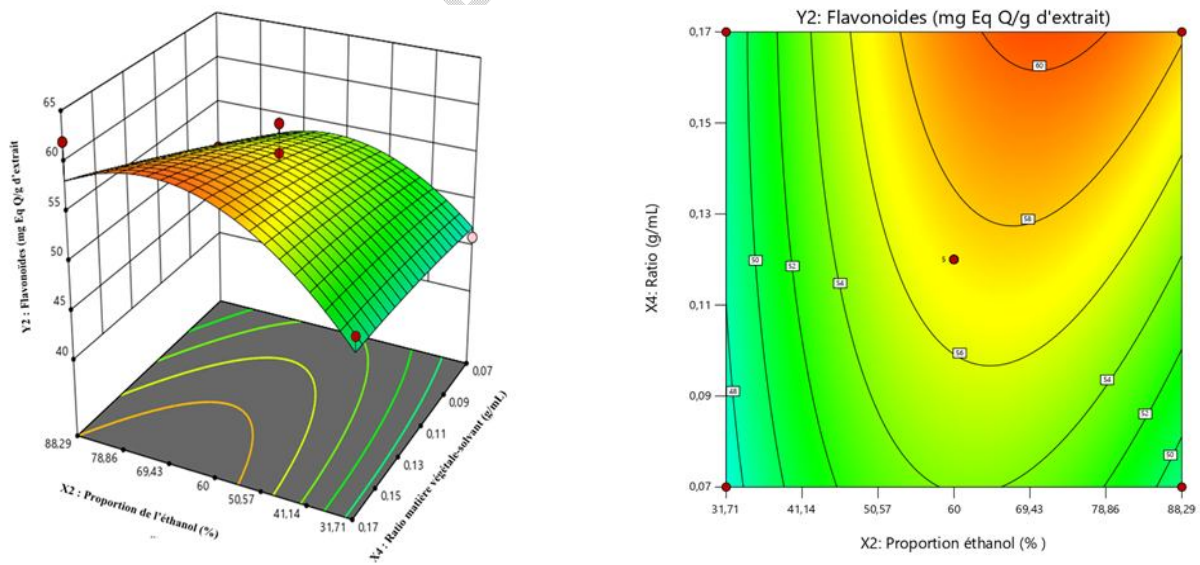
Table 7 presents the common optimal conditions for the extraction of polyphenols and total flavonoids. To simultaneously optimize the total polyphenol and flavonoid content of *Ola xsubscorpiodea* Oliveleaves, their extraction must be carried out in a solvent consisting of 88.29% ethanol with a plant material-solvent ratio of 0.17 g/mL. Whether in the amount of polyphenols (94.51 mg Eq AG/g versus 96.31 mg Eq AG/g) or flavonoids (58.22 mg Eq Q/g versus 62.44 mg Eq Q/g), there is no significant difference ( $p > 0.05$ ) between the expected values and those obtained by experiment; this result is in agreement with those obtained by Koffi *et al.* (2005) [17]; this author confirmed that PCC is

a method successfully applied in the framework of the optimization of the extraction of total flavonoids and total antioxidants from the leaves of *A. cordifolia*.



### 3D 2D

**Figure 1:** Influence of different parameters ( $X_2$ ,  $X_4$ ) on the extraction of total polyphenols (3D and 2D)



### 3D 2D

**Figure 2:** Influence of different parameters ( $X_2$ ,  $X_4$ ) on the extraction of flavonoids (3D and 2D)

**Table 7** : Experimental validation of the PCC

Answers	Expected values	Experimental values
Polyphenols (mg Eq AG/g)	94.51 <sup>a</sup>	96.31 ± 5.29 <sup>a</sup>
Flavonoids (mg Eq Q/g)	58.22 <sup>b</sup>	62.44 ± 4.58 <sup>b</sup>

Identical letters in the same row indicate that there is no statistical difference.

### Conclusion

By taking into account the extraction environment and the interactions between the factors influencing the extraction, we were able to have a better quantity of flavonoids and polyphenols from the leaves of *Olaxsubscorpioidea* Oliv. Among the technological factors studied through the Plackett and Burman Plan, only the plant matter-solvent ratio and the proportion of ethanol were identified as those that significantly influence the extraction of polyphenols and flavonoids. The composite central plan, through the second-degree polynomial model, was satisfactory in describing and predicting the optimal conditions for the simultaneous extraction of these two biomolecules; it is therefore necessary to carry out the extraction in an ethanolic proportion of 88.29% and with a ratio of 17g of *Olaxsubscorpioidea* Oliv leaves dissolved in 100 mL of ethanol. Applying these optimum conditions, the predicted values of polyphenols and flavonoids were successively 94.51 mg Eq AG/g and 58.22 mg Eq Q/g of *Olaxsubscorpioidea* Oliv leaf extract. Our results prove that the leaves of this plant are rich in natural phenolic compounds that can substitute synthetic compounds and be used as a source of natural product.

### CONSENT

It is not applicable

### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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