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

Secondary metabolites, including polyphenols and flavonoids, play a key role in the expression of the active principles of several medicinal plants from Côte d'Ivoire, such as *Olax subscorpioidea* Oliv. This study was initiated to help improve conditions for extracting polyphenols and flavonoids from *Olax subscorpioidea* Oliv leaves harvested in Korhogo. Experimental design methodology was used. The effect of four independent variables on polyphenol and flavonoid content was evaluated: extraction time (12h-24h), evaporation temperature (45°C - 55°C), plant material-solvent ratio (1/20 g/mL - 1/5 g/mL) and ethanol proportion (20% - 100%). The Central Composite Design (CCD) was used to optimize responses after factor screening using the Plackett and Burman design. Optimum extraction conditions were obtained with an ethanolic proportion of 88.29% and 17g of *Olax subscorpioidea* Oliv leaves dissolved in 100 mL ethanol. The experimental values (96.31 ± 5.29 mg Eq AG/g for polyphenols and 62.44 ± 4.58 mg Eq Q/g) were close to those expected (94.51 mg Eq AG/g for polyphenols and 58.22 mg Eq Q/g for flavonoids), confirming the relevance of the mathematical model used and the success of the response surface methodology in optimizing the extraction conditions of the system studied. These results show that by taking extraction conditions into account in this preliminary study, we can optimize the quantities of secondary metabolites of therapeutic interest.

Key words: 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]. About 80% of the world population consult the traditional health practitioners for plant-based traditional medicines, either in their liquid and dry form [2]. 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 *Olax*

subscorpioidea 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 *Olax subscorpioidea* 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 *Olax subscorpioidea*, 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 *Olax subscorpioidea* Oliv, was collected in Korhogo. These fresh leaves were authenticated by the National Floristic Center of Félix HOUPHOUET- BOIGNY University (Abidjan, Côte d'Ivoire).

2.1.2. Chemicals material and Reagents

The different concentrations of hydroethanolic extracts of *Olax subscorpioidea 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 $g.L^{-1}$) were used. Regarding the determination of the amount of flavonoids, the following reagents were used: $NaNO_2$ (5%, w/v), $AlCl_3$ (10 %, w/v), 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 (PBP) according to Hadamard notation.

Maceration extraction was performed according to the method defined by Zirihi modified [7]. The modification concerned the factors studied. Olax subscorpioidea Oliv leaves were harvested in the morning, washed and dried in the shade at room temperature in the Laboratory for two weeks. The dry matter was ground to powder using a grinder (IKAMAG®, Electronic: 1500g Multi-Function DieInlogrator). To prepare the extracts, 10 g to 68 g (X_4) of powder, was dissolved in a solvent consisting of a mixture of distilled water and ethanol or ethanol alone (Solvent, X_2 : 200 mL to 400 mL). The macerate was stirred with continuous agitation using an automatic electric shaker, in the laboratory at

25 °C for a set time (Extraction time, X_1 : 12 h to 24 h). Next, the extract was filtered successively through cotton (2 times) and Wattman N°1 paper (1 time). The filtrate obtained was then Oven dried at various temperatures (Evaporation temperature of extraction solvent, X_3 : 45 °C to 55 °C) to remove the extraction solvent. The residue obtained, which constitutes the hydro-ethanolic or ethanolic extract, was used for the determination of polyphenols and flavonoids content. The modification of extraction parameters, carried out by PBP, enabled 8 experiments to be carried out, as shown in **Table 1**.

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

Factors / Tests	X_1 (h)	X_2 (%)	X_3 (°C)	X_4 (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 Disign

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_2 (proportion of solvent) and X_4 (plant matter-solvent ratio); These variables (**Table 2**) 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 Feinberg, 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 [8]. The experimental table of this design is shown in **Table 3.** The quadratic mathematical model expected for the factors X_2 and X_4 which are taken into account for the study is as follows: $\mathbf{Y = a_0 + a_2X_2 + a_4X_4 + a_{24}X_2X_4 + a_{22}X_2^2 + a_{44}X_4^2}$

$\mathbf{a_o}$ = Theoretical effect or general average of all observed responses

$\mathbf{a_1}$ = effect of extraction time (X_1) on Y_1 and Y_2

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

a_3 = effect of the evaporation temperature (X_3) on Y_1 and Y_2

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

Table 2 : Experimental range of selected factors in the case of the composite central design

Independent variables	Symbols	Coded levels / Actual values				
		1.414(α)	- 1	0	+1	+1.414(α)
Proportion of ethanol (%)	X_2	20	31.71	60	88.29	100
Ratio of plant matter to solvent (g/mL)	X_4	1/20	7/100	3/25	17/100	1/ 5

Table 3: Experimental table in the Central Composite Disign

		Coded and actual values of factors			
Test groups	Test N°	X_2(%)		X_4	
Factorial tests	1	-1	(31.71)	-1	(7/100)
	2	+1	(88.29)	-1	(7/100)
	3	-1	(31.71)	+1	(17/100)
	4	+1	(88.29)	+1	(17/100)
Star tests	5	-1.414	(20)	0	(3/25)
	6	+1.414	(100)	0	(3/25)
	7	0	(60)	-1.414	(1/20)
	8	0	(60)	+1.414	(1/5)
Center tests	9	0	(60)	0	(3/25)
	10	0	(60)	0	(3/25)
	11	0	(60)	0	(3/25)
	12	0	(60)	0	(3/25)
	13	0	(60)	0	(3/25)

Each line of the table corresponds to an experiment;

Assay N°1: X_4 corresponds to 7 g of plant extract (*Olax subscorpioide*a) dissolved in 100 mL of hydroethanol solvent containing 31.71 % of ethanol (X_2);

Assay N°6: X_4 corresponds to 3 g plant extract dissolved in 25 mL solvent containing 100% of ethanol (X_2).

2.2.3. Analysis of response variations

2.2.3.1. Quantification of total polyphenol content

The method of Wood was used to determine total polyphenols concentration. A volume of 2.5 mL of diluted Foling-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 $g.L^{-1}$) 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 at 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 (mg Eq AG /g) per gram of *Olax subscorpiodea* Oliv extract [9].

2.2.3.2. Quantification of flavonoid content

The method of Marinova was used to determine total flavonoids content [10]. In a 25 mL flask, 0.75 mL of 5% (m/v) sodium nitrite ($NaNO_2$) was added to 2.5 mL of *Olax subscorpioidea Oliv* extract. The mixture was supplemented with 0.75 mL of 10% (m/v) aluminum chloride ($AlCl_3$) and incubated for 6 minutes in the dark at room temperature (30 ± 2 °C). After incubation, 5 mL of sodium hydroxide (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 λ = 510 nm. A calibration curve was obtained under the same conditions as those of the extract using a concentration range from 0 to 100 µg/mL of quercetin solution. The flavonoid content was expressed as milligram equivalent of quercetin (mg Eq Q/g) per gram of *Olax subscorpioidea* 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 ± 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 4**. The quantity of polyphenols varies between 100 ± 10.10 mg Eq AG/g and 236.66 ± 5.77 mg Eq AG/g**.** As for the quantity of flavonoids, it varies between 66.66 ± 5.77 mg Eq Q/g and 123.33 ± 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 *Olax subscorpioidea* 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 5**.

Table 4: Experimental matrix and experimental results in the Plackett and Burman design

Tests	X_1 (h)	X_2 (%)	X_3 (°C)	X_4 (g/mL)	Y_1 (mg Eq AG/g)	Y_2 (mg Eq Q/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_1 = extraction time; **X_2** = proportion of ethanol; X_3 = evaporation temperature and **X_4** = plant material – solvent ratio.

Y_1 corresponds to the quantity of total polyphenols and **Y_2** is the quantity of flavonoids.

Table 5: Statistical estimation of coefficients (effect of factors)

	Polyphenols (Y_1)			Flavonoids (Y_2)		
	Coefficients	Error Type	2 x Standard error (δ)	Coefficients	Error Type	2 x Standard error (δ)
a_0	147.49 ns	9.35	18.7	97.04 ns	3.82	7.64
a_1	1.67 ns	9.35	18.7	3.71 ns	3.82	7.64
a_2	**-38.33 ***	9.35	**18.7**	**-17.96 ***	3.82	**7.64**
a_3	-9.17 ns	9.35	18.7	2.88 ns	3.82	7.64
a_4	**-18.33 ***	9.35	**18.7**	**-9.54 ***	3.82	**7.64**

ns: not significant *****: significant

a_0 = Theoretical effect or general average of all observed responses

a_1 = effect of extraction time (X_1) on Y_1 and Y_2

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

a_3 = effect of the evaporation temperature (X_3) on Y_1 and Y_2

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

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

These results show that only the effects of factors X_2 and X_4 significantly influence ($p < 0.05$) the responses Y_1 and Y_2. 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 6.** With an extraction time of 24 h and an evaporation temperature of 55°C, the maximum quantity of

polyphenols obtained was 106 ± 5.44 mg Eq AG/g. Under these same conditions, the total flavonoid content of the extract obtained was 62 ± 4.58 mg Eq Q/g. These results are significantly higher than those obtained by Konan (2005) with 35.49 ± 0.29 mg Eq AG/g of ethanolic extract for polyphenols and 22.69 ± 0.69 mg Eq Q/g of extract for flavonoids [12].

Table 7 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 *Olax subscorpioidea* Oliv leaves ($p < 0.05$). 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 *Olax subscorpioidea Oliv* leaves. 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 *Olax subscorpioidea* Oliv. The relationships between factors and responses result in the following polynomial forms:

$$\mathbf{Y_1 = 39.24 + 0.01X_2^2 - 7375X_4^2}$$

$$\mathbf{Y_2 = 24.67 + 0.86X_2 + 8.91X_4 + 0.01X_2^2}$$

Table 6 : Experimental matrix and experimental results in the Central Composite Plane

	Factors		Answers	
Tests	**X_2 (%)**	**X_4 (g/mL)**	**Y_1 (mg Eq AG/g)**	**Y_2 (mg Eq Q/g)**
1	31.71	7/100	89 ± 2.64	46 ± 2.00
2	88.29	7/100	81 ± 7.21	50 ± 4.10
3	31.71	17/100	75 ± 6.31	50 ± 4.00
4	88.29	17/100	96 ± 5.29	62 ± 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 7 : Estimation of coefficients of factors, interactions and quadratic effect factors

	Polyphenols (Y_1)	Flavonoids (Y_2)
Coefficients	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

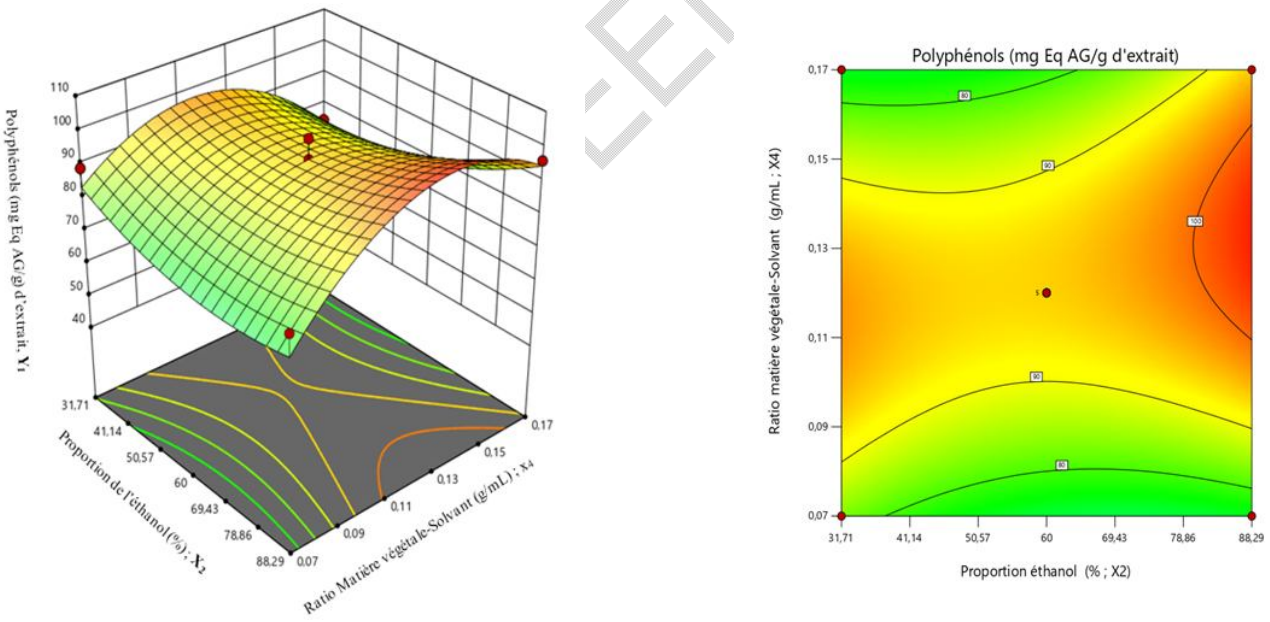
a_{44} = quadratic effect of the plant material - solvent ratio (X_4^2) on Y_1 and Y_2

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 Fig 2** . 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

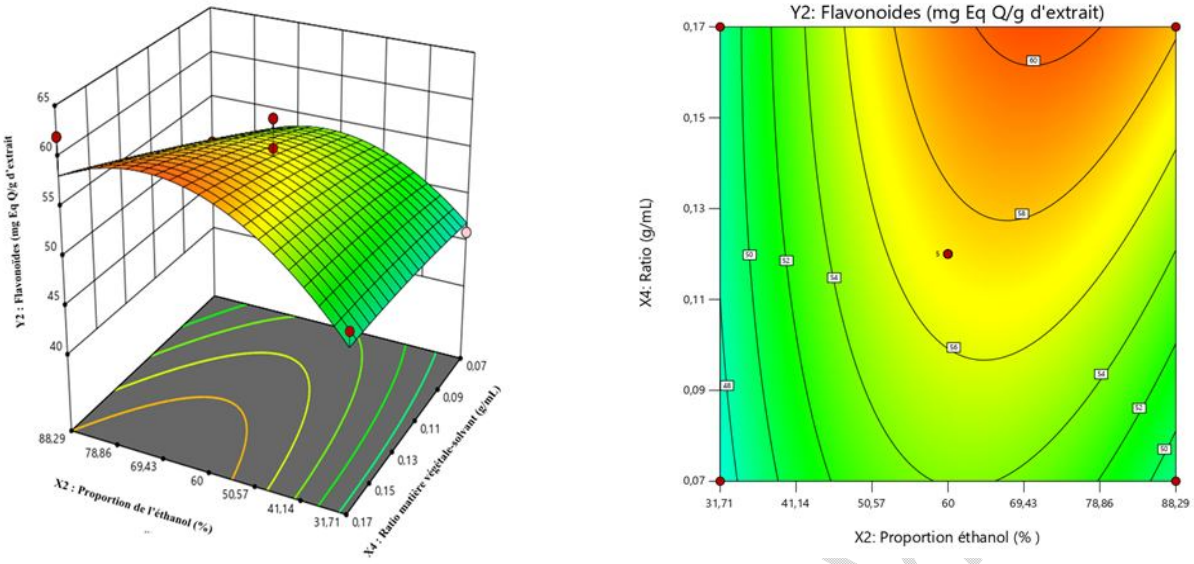
the limiting factor (ethanol) , the flavonoid content decreases. Indeed, the increase in the proportion of alcohol (ethanol) promotes the degradation of cell membranes which improve the permeability of the solvent in the solid matrix [15]. However, beyond the threshold value, that is to say at high concentrations 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 8 presents the common optimal conditions for the extraction of polyphenols and total flavonoids. To simultaneously optimize the total polyphenol and flavonoid content of *Olax subscorpiodea* Oliv leaves, 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* [17].



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 8: Experimental validation of the PCC

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

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 Olax subscorpioidea* 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 17 g of *Olax subscorpioidea* 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 *Olax subscorpioidea 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.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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