Journal of Advances in Medical and Pharmaceutical Sciences

23(8): 1-11, 2021; Article no.JAMPS.75869
ISSN: 2394-1111

Optimization of the Extraction of Total Flavonoids and Antioxidant Activity from *Lippia multiflora* Moldenke (Verbenaceae) Leaves using Experimental Design

Emmanuel N'Dri Koffi[1*], Yeboue Koffi François Kouakou[2], Bleouh Yves Nyamien[3], Roland Kouadoueu Deli[4] and Louise Atchibri Anin[4]

[1]Department of Sceinces and Technologies, Advanced Teachers Training College of Abidjan (ENS), 08 BP 10 Abidjan 08, Côte d'Ivoire.
[2]Department of Biochemistry - Genetic, Training and Research Unit of Biological Sciences, Peleforo Gon Coulibaly Korhogo University, BP 1328 Korhogo, Côte d'Ivoire.
[3]Institute of Agropastoral Management, University Peleforo Gon Coulibaly Korhogo, BP 1328 Korhogo, Côte d'Ivoire.
[4]Laboratory of Nutrition and Food Safety (LNSA), Research Training Unit of Sciences and Food Technologies, Nangui Abrogoua University, 02 BP 801 Abidjan 02, Côte d'Ivoire.

Authors' contributions

This work was carried out in collaboration among all authors. Author ENDK designed the study, performed the statistic alanalysis, wrote the protocol and wrote the first draft of the manuscript. Authors YKFK and BYN managed the analyses of the study. Author RKD did the literature searches. Author LAA read and approved the final manuscript..

Article Information

DOI: 10.9734/JAMPS/2021/v23i730250
Editor(s):
(1) Dr. Bogdan Socea, "Carol Davila" University of Medicine and Pharmacy Bucharest, Romania.
Reviewers:
(1) Miricescu Daniela, University of Medicine and Pharmacy, Romania.
(2) Sharad Vats, Banasthali Vidyapith, India.
(3) R. Anitha, Bharathi women's college, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/75869

Original Research Article

Received 20 August 2021
Accepted 30 October 2021
Published 08 November2021

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

ABSTRACT

The leaves of *Lippia multiflora* contain secondary metabolites including flavonoids which have an important antioxidant activity. This study aims to optimize the extraction conditions of total flavonoids and the antioxidant activity of these leaves. To achieve this, the Plackett-Burman design was used for the screening of the factors influencing the extraction, then the central composite design was implemented for the optimization itself. The effects of five factors, such as the plant-to-solvent ratio, the nature of the extraction solvent, the extraction time, the extraction method and the size of the *L. multiflora* leaves, on the extraction of total flavonoids and the antioxidant activity were studied. Results of Plackett-Burman design indicated that factors influencing both flavonoids extraction and antioxidant activity were the solid-liquid ratio and extraction time. The predicted optimal conditions for the highest flavonoids content from *L. multiflora* leaf with better antioxidant activity were found with aqueous decoction for 30 min with 3.5 g of cut leaves in 100 mL of distilled water. Using the predicted conditions, experimental responses were 87.18 ± 1.03 mg/g QE and 372.34 ± 4.04 µmol/g TE for total flavonoids and antioxidant capacity, respectively. Under the above-mentioned conditions, the experimental results are very close to predicted one. Thus, *L. multiflora* leaf can be considered as a natural source of flavonoids content with good antioxidant activity.

Keywords: Lippia multiflora leaves; antioxydant activity; flavonoids; Plackett-Burman design; central composite design.

1. INTRODUCTION

In Africa, plants used in traditional medicine have an important place in the treatment of various ailments as well as the food survival of populations. According to the WHO [1], 80% of African populations use traditional medicine for their care and health maintenance. Plant, as a source of medicine, is gaining international popularity because of its natural benefits availability in local communities, cheaper to purchase and ease of administration. Herbal medicine may be also useful for alternative treatment due to side effects and drug resistance [2, 3, 4]. Among these plants, *L. multiflora* (Verbenaceae) commonly known as "savannah tea" is an aromatic plant that grows spontaneously in sub-saharan Africa [5]. Its leaves are used as a remedy for various diseases, namely hyperthermia, high blood pressure, coronary heart disease and atherosclerosis [6]. It should therefore be noted that all the benefits conferred to this plant are linked to the presence of secondary metabolites such as flavonoids [7]. Indeed, flavonoids are endowed with antioxidant, anti-inflammatory, antimutagenic, anti-carcinogenic properties [8,9]. The importance of flavonoids is increasing due to their benefit effects on human health [8,10,11]. In addition, these compounds interest the food and cosmetic industries [12]. The classic methods of extracting these molecules are maceration, infusion and decoction [13]. During the extraction procedure, parameters such as extraction temperature, duration of extraction, solid-liquid ratio, the size of plant material and extraction method should be taken into account [14]. However, the non-control of the extraction conditions of these molecules could influence the chemical composition of the extracts and therefore have an impact on the expected therapeutic effect. Thus, experimental design is used for optimization of the extraction condition of bioactive compounds [15, 16, 17]. The aim of this study is to optimize the extraction condition of flavonoids and the antioxidant activity from *L. multiflora* leaves in order to participate in the valorization of this product to households and the industrial world.

2. MATERIALS AND METHODS

2.1 Plant Material

The fresh leaves of *L. multiflora* were collected on the northern site of Felix Houphouët Boigny National Polytechnic Institute of Yamoussoukro during 2019-2020 period. The study area is bounded by the coordinates 6°51'36 N and 6°51'72 N ; and 5°14'50 W and 5°14'72 W. The morphotype was identified according to leaf morphology as described by Watson and Dallwitz [18] and Aké [19]. Once in the laboratory, one part of the collected leaves were directly dried away from light until a constant weight was obtained, while the other was cut using a chisel before drying.

2.2 Chemical Reagents

The chemical reagents used for this study were of analytical grade. Among these chemical reagents, sodium nitrite, aluminum chloride, sodium hydroxide and methanol were purchased from Carlo Erba (Spain). The 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox), quercetin, and potassium peroxodisulfate were provided by Sigma-Aldrich (Germany). The 2, 2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) was purchased from Biochem (France).

2.3 Methods

2.3.1 Extraction by infusion or decoction

An aliquot of 1 to 7 g of *L. multiflora* leaves (powder or cut leaves), was infused in 100 mL of solvent (distilled water or water acidified with citric acid) or heated refluxed for 5 to 30 min. The leaf extracts were obtained after the filtration of the mixtures using hydrophilic cotton and Whatman Paper N°1.

2.3.2 Plackett-Burman design

This experimental design was carried out to screening the main extraction factors such as solid-liquid ratio ($X_{1)}$, extraction time (X_2), solvent used (X_3) and size of plant material (X_4). These factors that may influence the extraction of biomolecules from *L. multiflora* leaves have led to 8 trials [20]. For each factor, a higher level (+1) and lower level (-1) were determined (Table 1).

The expected responses for each trial were the flavonoids content and antioxidant activity of the extract obtained. The relationship between the factors studied and the expected responses was established using a 1st order mathematical model:

$$Y_n = b_0 + \sum b_i X_i$$

With $\mathbf{Y_n}$: the answer sought, b_0 : the constant of the model and b_i : the linear coefficient of the variable X_i.

2.3.3 Central composite design

A central composite design was put in place as part of the optimization of the flavonoids content and antioxidant activity of *L. multiflora* extracts, following screening factor test result. This experimental design was made from the combination of 2 factors (X_1 and X_3) in 5 coded levels (-1.414, -1, 0, +1, and + 1.414) (Table 2).

The execution plan of the central composite design includes 13 tests including 4 factorial trials, 4 axial trials and 5 center point trials. The expected responses for each trial were the flavonoids content and antioxidant activity of the extract obtained.

2.3.4 Determination of total flavonoids content

Total flavonoids were analyzed according to the method described by Marinova *et al*. **[21]**.

To a 0.75 mL of $NaNO_2$ distilled water solution (5%, w/v) contained in a 25 mL volumetric flask, was added to a 2.5 mL aliquot ~~portion~~ of the sample. The color reaction was kept for better developing during 5 min without light and at room temperature. Then, to 0.75 mL of $AlCl_3$ solution (10%, w/v) was added and covered for 6 minutes. Subsequently, 5 mL of sodium hydroxide (NaOH, 1N) was added to the previous volume to reach 25 mL. The solution obtained is well stirred before analyzing with a UV-vis spectrophotometer. The reading of absorbance was taken at 510 nm with distilled water as a reference. The triplicate trials were performed and the flavonoids content was given in milligrams quercetin equivalent per gram of dry matter (mg/g QE).

Table 1. Coded and extended levels of independent variables

Independent variables	Symbol	Coded/extended levels of independent variables	
		Inferior (-1)	Superior (+1)
Ratio (g/mL)	X_1	1/100	7/100
Extraction solvent	X_2	Water	Water/Citric acid (0.01N)
Extraction time	X_3	5	30
Extraction mode	X_4	Infusion	Decoction
Leaf size	X_5	Cut	Whole

Table 2. Coded and extended levels of independent variables

Independent variables	Coded/extended levels of independent variables				
	-1.414	-1	0	+1	+1.414
Ratio (X_1 ; g/mL)	1	1.88	4	6.12	7
Time (X_3 ; min)	5	8.66	17.5	26.34	30

2.3.5 Evaluation of antioxidant activity

The TEAC assay was carried out using the method described by Teow [22] with some modifications. The $ABTS^{\circ+}$radical cation was produced by mixing 8 mmole.L^{-1} of ABTS salt solution and 3 mmole.L^{-1} of potassium persulphate solution. The solution obtained was then incubated, at room temperature (30 ±2 °C) during 16 hours without light. The analytical solution was prepared extemporaneously before each dosing series by diluting the ABTS stock solution with methanol until an absorbance of 0.7±0.02 at the wavelength of 734 nm could be obtained. A volume of 3.9 mL of $ABTS^{\circ+}$ mixture diluted in methanol was added to a weak volume (0.1 mL) of sample (standard or extract). After vigorous stirring, the mixture was incubated during 6 min in the dark at room temperature. The absorbance of the mixture was read using a UV spectrophotometer visible at 734 nm. The results were given in micromole of Trolox equivalent per gram of extract (µmol/g TE) by comparing the percentage of degradation of $ABTS^{\circ+}$ by Trolox to that of the sample. The percentage of degradation of $ABTS^{\circ+}$ (A) was expressed as follows:

$$A\ (\%) = (A_{blank} - A_{extrait}) / (A_{blank} \times 100)$$

With, $\mathbf{A_{734blanc}}$= absorbance of the blank; $\mathbf{A_{734extract}}$ = absorbance of the extract after incubation.

2.3.6 Statistical Analysis

Results were given as means ± standard deviations of triplicate measurements. One-way analysis of variance (ANOVA) with Tukey's test was used to determine the significant differences (p = .05). The Statistica 8.0 software was employed to design and analyze the experimental data of experimental designs.

Experimental responses data were fitted to the second order polynomial model and regression coefficients were obtained. The form of this second-order polynomial model proposed for the response surface analysis was given as follows:

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ij} X_i X_j + \sum \beta_{ii} X^2$$

With β_0, β_i, β_{ii}, β_{ij} are regression coefficients for intercept, linear, quadratic and interaction terms, respectively. X_i and X_j are coded value of the independent variables while k equals to the number of the tested factors (k=2). The ANOVA tables were generated; the effect and regression coefficients of individual linear, quadratic and interaction terms were determined. The significances of all terms in the polynomial were given by computing the F-value at p = .001, p = .01 or p = .05.

3. RESULTS

3.1 Screening of the Effect of Parameters on Flavonoids Extraction and Antioxidant Activity

The plackett-Burman design was used for screening the effect of five variables wich include solid-liquid ratio (X_1 ; g/mL), solvent used (X_2), extraction time (X_3 ; min), extraction mode (X_4) and leaf size (X_5) on flavonoids extraction and antioxidant activity from *L. multiflora* leaves. Table 3 presents the total flavonoids content and antioxidant activity for the eight trials. The flavonoids content range from 1.71 to 78.75 mg/g QE. In addition, the antioxidant activity of the different extracts is varying between 8.06 to 320.54 µmol/g TE.

The relationship between the independent variables and the experimental responses is established by multiple linear regression. Table 4 presents the values of the coefficients which influence the independent variables for each experimental response (total flavonoids content and antioxidant activity).

Table 4 indicates that only the extraction time (X_3) and the extraction mode (X_4) influence significatively the total flavonoids extraction (p = .05). The mathematical model obtained was:

$$Y_1 = 30.09 + 26.99X_3 + 28.59X_4 \quad (1)$$

Moreover, the antioxidant activity of the extract is strongly impacted by the solid-liquid ratio and the

extraction mode (p = .05). The mathematical model is as follows:

$$Y_2 = 108.23 + 124.74\ X_1 + 95.36X_4 \quad (2)$$

Ultimately, the factors that influenced both the extraction of total flavonoids and antioxidant activity were the solid-liquid ratio ($X_{1)}$, the extraction time (X_3) and the extraction mode (X_4).

3.2 Optimization of Extraction Conditions Using Central Composite Design

In the case of simultaneous optimization of total flavonoids extraction conditions and antioxidant activity, 13 trials were performed according to the recommendations of the central composite design ; the solid-liquid ratio and extraction time were used as variables and the other factors (X_2 = water, X_4 = decoction and X_5 = cut leaves) were kept constant. The results are mentionned in Table 5. The total flavonoids content of the different trials vary from 22.92 to 85.00 mg/g QE, while those of antioxidant activity range from 42.24 to 339.44 µmol/g TE. The high amount of flavonoids and antioxidants were recorded from the axial points.

Fig. 1 shows a good correlation between the flavonoids content and the antioxidant activity of the different extracts (R^2 = 0.73).

The data in Table 5 made it possible to establish the relationships between the factors and the responses studied. Table 6 presents the values of the coefficients affecting each factor and their significance.

Table 3. Flavonoids content and antioxidant activity of extracts according to the Plackett-Burman design

Independent variables					Experimental responses	
X_1	X_2	X_3	X_4	X_5	Y_1	Y_2
1/100	Acidified water	30	Infusion	Whole leaves	19.33	13.40
7/100	Water	5	Infusion	Whole leaves	1.71	47.12
1/100	Acidified water	5	Infusion	Cut leaves	7.67	8.06
7/100	Water	30	Infusion	Cut leaves	34.48	173.60
1/100	Acidified water	30	Decoction	Cut leaves	78.75	143.92
1/100	Water	5	Decoction	Whole leaves	31.67	18.04
7/100	Acidified water	5	Decoction	Whole leaves	10.00	141.14
7/100	Water	30	Decoction	Cut leaves	57.13	320.54

Y_1 : Total flavonoids content expressed in milligrams of quercetin equivalent per gram of dry matter (mg/g QE); Y_2 : antioxidant activity expressed in micromole of Trolox equivalent per gram of dry matter (µmol / g TE).

Table 4. Statistical coefficients of each independent variable for each response

Coefficients	Values of the coefficients of the variables for each equation					
	Total Flavonoids (mg/g QE)			Antioxidant activity (µmol/g TE)		
	Valeurs	2σ	*p-value*	Valeurs	2σ	*p-value*
b_0	30.09**	4.14	0.005	108.23**	15.36	0.005
b_1	-8.52^{ns}	8.28	0.176	124.74*	30.73	0.014
b_2	-13.12^{ns}	8.28	0.086	25.11^{ns}	30.73	0.244
b_3	26.99*	9.56	0.029	74.63	35.48	0.052
b_4	28.59*	8.28	0.02	95.36*	30.73	0.024
b_5	-15.33^{ns}	9.56	0.085	-69.29^{ns}	35.48	0.059
R^2	0.99			0.99		
R^2 ajusted	0.95			0.96		

** : p=.05 ; ** : p=.01 ;*** : p=.001 et ns : not significant*

Table 5. Experimental matrix and results of the central composite design

Trials	Variable levels		Experimental responses	
	X_1 (Ratio, g/mL)	X_3 (Time, min)	Y_1 (mg/g QE)	Y_2 (µmol/g TE)
Factorial trials	-1	+1	61.99	303.06
	-1	-1	29.41	52.3
	+1	-1	57.04	101.1
	+1	+1	74.38	330.98
Axial trials	0	-1.414	46.67	42.24
	-1.414	0	22.92	80.86
	0	+1.414	85.00	339.44
	+1.414	0	70.00	255.38
Center point trials	0	0	56.67	196.76
	0	0	63.33	210.44
	0	0	65.00	232.86
	0	0	63.33	204.18
	0	0	51.67	188.86

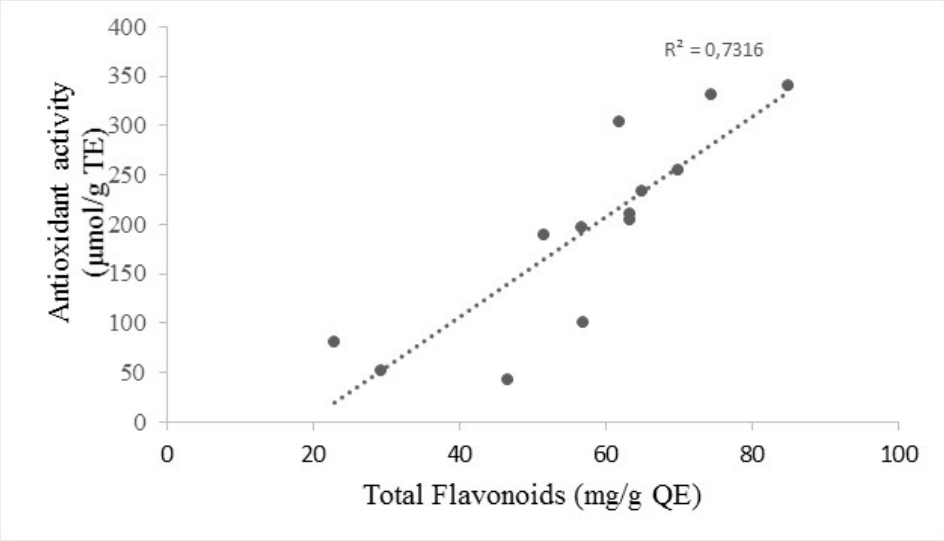

Fig. 1. Correlation between antioxydant activity and flavonoids content

Table 6. Statistical estimates of the coefficients for each response studied

	Total flavonoids			Antioxidant activity		
Coefficients		**2σ**	***p-value***	**Coefficients**	**2σ**	***p-value***
Constant	**60.00**	5.05	< 0.001	**206.58**	14.98	< 0.001
Linear						
b_1	**26.68**	8.00	< 0.01	**80.94**	23.72	< 0.01
b_3	**26.07**	8.00	< 0.01	**225.59**	23.72	< 0.001
Quadratic						
b_{11}	**-13.83**	8.60	< 0.05	-29.86^{ns}	25.50	> 0.05
b_{33}	5.66^{ns}	8.60	> 0.05	-7.01^{ns}	25.50	> 0.05
Interaction						
b_{13}	-7.62^{ns}	11.30	> 0.05	-10.44^{ns}	33.42	> 0,05
R^2		0.94			0.95	

ns : not significant

Table 6 shows that the solid-liquid ratio and the extraction time influence positively the extraction of total flavonoids from *L. multiflora* leaves (p = .01). In addition, there are a significant quadratic effect of the solid-liquid ratio on the extraction of total flavonoids of *L. multiflora* (p = .05). The mathematical model resulting is as follows:

$$Y_1' = 60 + 26.68\ X_1 + 26.07X_3 - 13.83\ X_1^2 \quad (3)$$

The effect of the solid-liquid ratio and the extraction time on the flavonoids extraction is highlighted in Fig. 2. By increasing the solid-liquid ratio and the extraction time, flavonoids content increases again until a threshold. The threshold reached would be due to the quadratic effect of the solid-liquid ratio which is significant at 5%.

Likewise, the ratio and the extraction time affect signicantly the extraction of total antioxidants from the leaves of *L. multiflora* (p =.01). However, the quadratic effects of these parameters and their interaction are not statistically significant at the 5% threshold. All of this translates into the following equation:

$$Y_2' = 206.58 + 80.94\ X_1 + 225.59X_3 \quad (4)$$

Fig. 3 shows the effect of the solid-liquid ratio and extraction time on the antioxidant activity of *L. multiflora* leaf extracts. As in the case of total flavonoids, it is noted that by increasing the solid-liquid ratio and prolonged the extraction time to a certain threshold, the antioxidant activity of *L. multiflora* leaf extracts also increases. The quadratic effect is however less pronounced, therefore insignificant (p =.05).

3.3 Experimental Validation of Optimal Extraction Conditions

The optimal conditions for the common extraction of flavonoids and antioxidants from *L. multiflora* leaves were found with extraction by aqueous decoction for 30 min with 3.5 g of cut leaves in 100 mL of water (Table 7). These optimal extraction conditions are predicted using desirability function (Statistica 8.0). Under the above-mentioned conditions, the experimental results are very close to predicted one. Furthermore, this analysis demonstrates that there were no significant differences between the predicted and experimental values (p = .05).

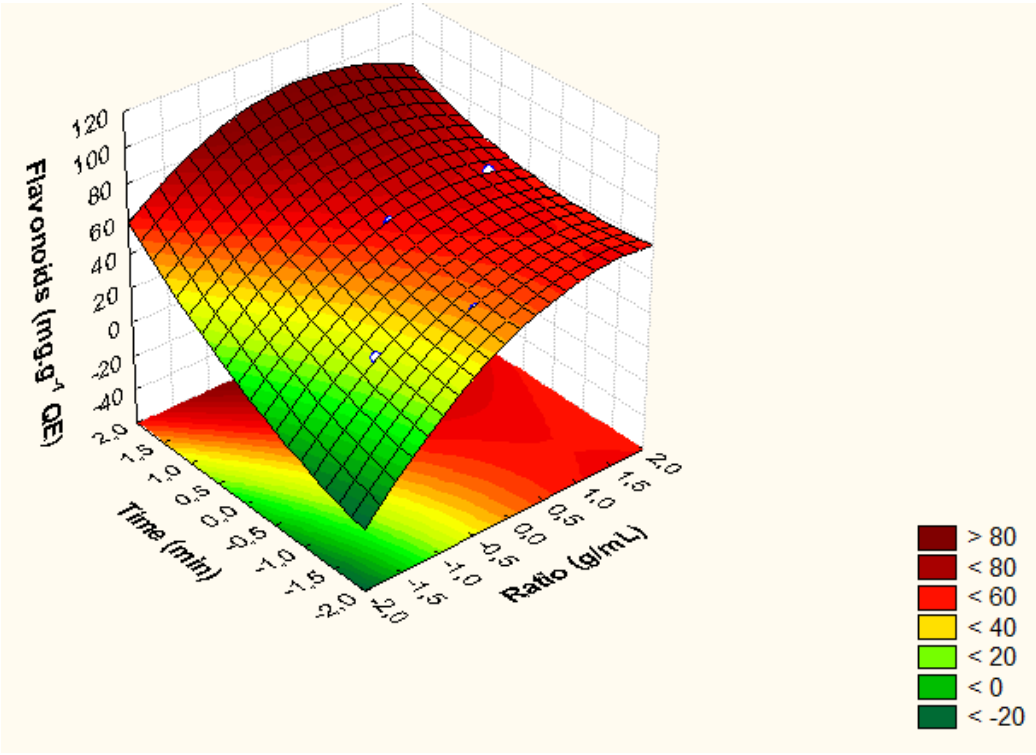

Fig. 2. Effect of ratio and extraction time on flavonoid extraction from *L. multiflora* leaves

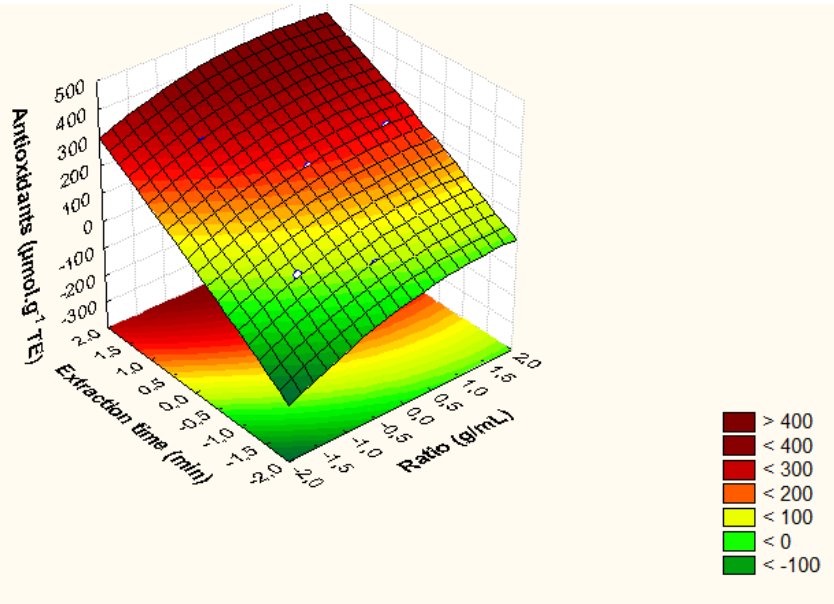

Fig. 3. Effect of the solid-liquid ratio and extraction time on the antioxidant activity of *L. multiflora* leaves.

Table 7. Predicted and experimental values of responses under optimal conditions

Optimal conditions	flavonoid content (mg/g QE)		Antioxidant activity (µmol/g TE)	
	Predicted value	Experimental value	Predicted value	Experimental value
X_1 = 3.5 g/100 mL X_2 = water X_3 = 30 min X_4 = Decoction X_5 = Cut leaves	86.18[a]	87.18 ± 1.03[a]	374.58	372.34 ± 4.04

The values followed by the same lowercase letter are not statistically different at the threshold of 5%.

4. DISCUSSION

Most studies carried out on *L. multiflora* have related to the characterization of essential oils in flowers and leaves [23, 24, 25]. In addition, some authors have highlighted the presence of secondary metabolites including flavonoid compounds in extracts of this plant [26]. Flavonoids, by their properties as free radical scavengers, are involved in the prevention of oxidative damage caused by oxygen species activated on cellular molecules [27].

The infusion and decoction applied during this study indicate a thermostability of these molecules. Thus, increasing the temperature would promote the diffusion of biomolecules from the plant matrix to the extraction solution [28]. Similar results were obtained by Nyamien *et al.* [29] during the extraction of extractables from the cola nut. Also, Sathishkumar *et al.* [30].showed linearity between the diffusion coefficient of phenolic compounds and the increase of temperature.

The best solid-liquid ratio to extract flavonoids and antioxidants is 3.5/100 (w/v); above this ratio, flavonoids content found are low due to the saturation phenomenon [29]. The use of a suitable plant-solvent ratio basically makes it possible to obtain an optimum extraction of the compounds from a plant matrix.

Comparing the effect of different solvents on flavonoid extraction and antioxidant activity, the aqueous solution was the best solvent for analysis. This result is in agreement of the work carried out by Koffi [14]. According to this author,

the best solvent for better extraction of total flavonoids from the dry leaves of *Justicia secunda* was water. The choice of solvent depends on the type of plant, part of plant to be extracted, nature of the bioactive compounds, and the availability of solvent [31]. In general, polar solvents such as water, methanol and ethanol are used in extraction of polar compound [32, 33]. In fact, water dissolves a wide range of substances, nontoxic, non-inflammable and highly polar [32, 34].

The study of the size of the plant material showed that the cut leaves promoted better extraction of bioactive compounds from the leaves of *L. multiflora.* Thus, the reduction in the size of *L. multiflora* leaves would have allowed the increase of the contact surface between the plant material and the solvent ; this therefore promotes a better extraction of the bioactive compounds [29,30].

The optimal extraction conditions are found with aqueous decoction for 30 min with 3.5 g of leaves cut in 100 mL of water. The total flavonoids content of the optimized extracts (87.17 mg/g EQ) was higher than those recorded by Soro *et al.* [35] (14.57mg/g QE, 11.67 and 10.82 mg/g QE). Under the above-mentioned conditions, the experimental results are very close to predicted one. As a result, the central composite design used has been successfully applied for optimizing the extraction of total flavonoids and antioxidants of *L. multiflora* [36]. The relationship between flavonoids content and antioxidant activity showed that flavonoids from *L. multiflora* extracts could be responsible for 73% of antioxidant activity. Indeed, according to several authors, antioxidants such as flavonoids are the group of phenolic compounds with the greatest antioxidant activity [9, 31]. Therefore, *L. multiflora* extract could be advised as an alicament to prevent various chronic diseases such as cancers and cardiovascular diseases [37].

5. CONCLUSION

This study permitted to optimize the extraction conditions of flavonoids and antioxidant activity of *L. multiflora* leaves using the Plackett & Burman and the composite central designs. To obtain an extract of *L. Mutiflora* leaves containing the maximum of flavonoids having better antioxidant activity, the extraction should be carried out by aqueous decoction for 30 min with 3.5 g of leaves cut in 100 mL of water. Under these conditions, the amount of flavonoids and antioxidant activity obtained experimentally were similar to those predicted. In view of these results, the use of central composite design would be an efficient way to optimize the antioxidant activity and flavonoids content of *L. multiflora* leaves.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Who (2002). Stratégie de l'OMS pour la médecine traditionnelle pour 2002- 2005, Genève, 20.
2. Pandey AM, Tripathi SH. Concept of standardization, extraction, and pre-phytochemical screening strategies for herbal drug. Journal of Pharmacognosy and Phytochemistry. 2014;2(5):115-119.
3. Azwanida NN. A review on the extraction methods use in medicinal plants, principle, strength, and limitation. Medicinal & Aromatic Plants. 2015;4 (3):196.
4. Ingle KP, Deshmukh AG, Padole DA, Dudhare MS, Moharil MP,Khelurkar VC. Phytochemicals: Extraction methods, identification and detection of bioactive compounds from plant extracts. Journal of Pharmacognosy and Phytochemistry. 2017;6 (1):32-36.

5. Jim SI, Wudeneh L, Mariana S, Dan A. Agribusiness in sustainable natural African plant Product: Lippia tea. Jim Simon, PhD Center for New Use Agriculture and Natural Plant Products available. 2001;105.
6. Abena AA, Diatewa M., Gakosso G, Gbeassor M, Hondi-Assah T,Ouamba J. Analgesic, antipyretic and anti-inflammatory effects of essential oil of *Lippia multiflora*. Fitoterapia. 2003;74(3) : 231-236.
7. Juliani HR., Wang M, Moharram H, Asante-Dartey J, Acquaye D, Koroch AR, Simon JE. Intra specific variations in quality control parameters, polyphenol profile, and antioxidant activity in wild populations of *Lippia multiflora* from Ghana. Journal of the American Chemical Society. 2006 ; 925 : 126–142.
8. Panche AN, Diwan AD, Chantal SR, Chandra R.Flavonoids: An overview. Journal of Nutrition and Science. 2016 ; 5: 1-15.
9. Tzanova M, Vasil A, Zvezdelina Y, Donika I, Toncho D. Selectivity of Current Extraction Techniques for Flavonoids from Plant Materials. Process. 2020 ; 1222.
10. Doughari JH. Phytochemicals: Extraction Methods, Basic Structures and Mode of Action as Potential Chemotherapeutic Agents. In Phytochemical. A Global Perspective of Their Role in Nutrition and Health; Rao, V., Ed.; InTechOpen: Rijeka, Croatia, 2012; 1–33.
11. Kumar S, Pandey K.A. Chemistry and Biological Activities of Flavonoids: An Overview. The Scientific World Journal. 2013 ; (162750) : 16.
12. Kerry N, Abbey M. Red wine and fractionated phenolic compounds prepared from red wine inhibit low density lipoprotein oxidation in vitro. Atherosclerosis.1997; 135: 93–102.
13. Bohui GSP, Adima AA, Niamké FB, N'Guessan JD. Etude comparative de trois méthodes d'extraction des flavonoides totaux à partir des feuilles de plantes médicinales: *Azadirachta indica* et *Psidium guajava*.Journal de la Société Ouest-Africaine de Chimie. 2018; 046: 50 – 58.
14. Koffi E. Contribution à l'optimisation de l'extraction et à l'étude chimique des polyphénols de deux plantes de Côte d'Ivoire: *Tectona grandis* Linn (Verbenaceae) et *Justicia secunda* Vahl (Acanthaceae). Thèse de doctorat de Phytochimie et substance naturelle, faculté des Sciences Fondamentales et Appliquées (SFA). Abidjan : Université NanguiAbrogoua, 2014.
15. Koffi EN, Meudec, E, Adjé, FA, Lozano PR , Lozano YF, Bekro YA. Effect of reverse osmosis concentration coupled with drying processes on polyphenols and antioxidant activity obtained from *Tectona grandis* leaf aqueous extracts. Journal of Applied Research on Medicinal and Aromatic Plants. 2015 ; 2 (2) : 54–59.
16. Nyamien Y, Adjé F, Niamké F, Koffi E, Chatigre O, Adima A,Biego HG. Effect of Solvents and Solid-Liquid Ratio on Caffeine Extraction from Côte d'Ivoire Kola Nuts (*Cola nitida*).International Journal of Science and Research. 2015; 4 (1): 218-222.
17. Yao KJ, Actchibri AO, Koffi NE, N'da KP,Adima AA. Optimisation of total flavonoids and total antioxidants extraction from Dacryodesedulis leaves. International Journal of Current Research. 2016; 8(11): 42130-42135.
18. Watson L, Dallwitz MJ. Les feuilles des usines fleurissantes : Descriptions, illustrations, identification et recherche documentaire, 14 ème Version. 1992 ; 127-133.
19. Aké AL. Flore de la Côte d'Ivoire: Catalogue systématique, biogéographique et écologie, 2. Boissiera 58, Mémoires de Botanique Systématique, Abidjan, Université Félix Houphouët Boigny. 2002 ; 171
20. Plackett RL, Burman JP. The design of optimum multi factirial experiments. Biometrica. 1946 ; 33 (4) : 305-325.
21. Marinova D, Ribavora F, Antanassova M. Total phenolics in bulgarian fruits and vegetables. J of the Univer of Chem Tech and Metal. 2005; 40 (3):255-260.
22. Teow CC, Truong VD, Mc Feeters RF, Thompson RL, Kenneth VP, Yencho GC. Antioxidant activities, phenolic and b-carotene contents of sweet potato genotypes with varying flesh colours. Food Chemistry. 2007; 103: 829 – 838.
23. Kanko C, Koukoua G, N'Guessan YT, Fournier J, Pradère JP, Toupet L. Contribution à l'étude phytochimique de *Lippia multiflora* (Verbenaceae). Comptes rendus de Chimie. 2004 ;7 (10-11) : 1029–1032.
24. Oussou KR, Jean BB, Coffi K, Affouet MK. Etude Chimique et Activité Anti diarrhéique

des Huiles Essentielles de deux plantes aromatiques de la pharmacopée ivoirienne. European Journal of Scientific Research. 2008 ; 24 (1) : 94-103.
25. Owolabi MO, Akintayo O, Labunmi L, Matthew OO, William NS, Maria CP. Chemical Composition and Antibacterial Activity of the Essential Oil of Lippia multiflora Moldenke from Nigeria. Records of Natural Products. 2009 ; 3 : 4 170-177.
26. KankoC. Contribution à l'étude phytochimique de Lippiamultiflora (Verbenaceae). Abidjan : Université de Cocody–Abidjan, 1995.
27. Yun-Ching C, Kai-Xun H, An-Chung H, Yung-Chyuan H,Chau-Jong W."Hibiscus anthocyanins-rich extract inhibited LDL oxidation and oxLDL-mediated macrophages apoptosis". Food and Chemical Toxicology. 2006; 44 (7): 1015-1023.
28. Shi J, JianmeiYu, Joseph Pohorly, Christopher JY, Mike B, Ying W. Optimization of the extraction of polyphenols from grape seed meal by aqueous ethanol solution. Food, Agriculture & Environment. 2003 ; 1(2) : 42-47.
29. Nyamien Y, Adjé F, Niamké F, Koffi E, Chatigre O, Adima A, Biego HG. "Effect of Solvents and Solid-Liquid Ratio on Caffeine Extraction from Côte d'Ivoire Kola Nuts (Cola nitida)."International Journal of Science and Research. 2015;4(1):218-222.
30. Sathishkumar T, Shanmgam S, Rajasaekaram P, Sadavivam S,Manikandan V. Optimization of flavonoïds extraction from the leaves of *Tabernaemontana hyena* wall. Using L16 orthogonal design. Nature and science. 2008; 6(3):10 – 21.
31. Abdullahi R, Mainul H. Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. Journal of Pharmacology and Bioallied Science. 2020; 12 (1):1-10.
32. Das K, Tiwari K, Shirvastava K. Technique for evaluation of medicinal plant product as antimicrobial agents: Curent methods and future trends. Journal of Medicinal Plants and Research. 2010;4(2): 104-111.
33. Altemimi A, Lakhssassi N, Azam B, Dennis GW, David A. Phytochemicals : Extraction, isolation, and identification of bioactive coupounds from plant extracts. plants. 2017; 6: 42.
34. Tiwari P, Kumar B, Kaur G, Kaur H. Phytochemical screening and extraction: A review. International Pharmacology Science. 2011; 1 (1): 98-106.
35. Soro C. Caractérisation biochimique et activités biologiques des feuilles de *Lippia multiflora* (Verbenaceae) «thé de savane» cultivé en milieux forestier et savanicole de Côte d'Ivoire, faculté des sciences et technologies des aliments. Abidjan, Université Nangui Abrogoua, 2016.
36. Chan SW, Lee CY, Yap CF, Wan Aida WM, Ho CW. Optimisation of extraction conditions for phenolic compounds from limaupurut (*Citrus hystrix*) peels. International Food Research Journal. 2009; 16: 203-213.
37. Obouayeba AP, Souleymane M, Lydie B, Dodehe Y, Tanoh HK, Jean David N."Cardioprotective and anti-inflammatory activities of a polyphenols enriched extract of *Hibiscus sabdariffa* petal extracts in wistar rats." Journal of Pharmacognosy and Phytochemistry. 2015;4 (1):57–63.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/75869