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

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Authors’ contributions

This work was carried out in collaboration among all authors. Author ENDK designed the study, performed the statistical analysis, 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.

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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; antioxidant 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 Biotech (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 (X₁), extraction time (X₂), solvent used (X₃) and size of plant material (X₄). 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 \( Y_n \) : 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 (X₁ and X₃) 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 NaNO₂ 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 AlCl₃ 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>
<thead>
<tr>
<th>Independent variables</th>
<th>Symbol</th>
<th>Coded/extended levels of independent variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio (g/mL)</td>
<td>X₁</td>
<td>1/100</td>
</tr>
<tr>
<td>Extraction solvent</td>
<td>X₂</td>
<td>Water</td>
</tr>
<tr>
<td>Extraction time</td>
<td>X₃</td>
<td>5</td>
</tr>
<tr>
<td>Extraction mode</td>
<td>X₄</td>
<td>Infusion</td>
</tr>
<tr>
<td>Leaf size</td>
<td>X₅</td>
<td>Cut</td>
</tr>
</tbody>
</table>

Table 1. Coded and extended levels of independent variables

<table>
<thead>
<tr>
<th>Ratio (g/mL)</th>
<th>X₁</th>
<th>1/100</th>
<th>Superior (+1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extraction solvent</td>
<td>X₂</td>
<td>Water</td>
<td>Water/Citric acid (0.01N)</td>
</tr>
<tr>
<td>Extraction time</td>
<td>X₃</td>
<td>5</td>
<td>30</td>
</tr>
<tr>
<td>Extraction mode</td>
<td>X₄</td>
<td>Infusion</td>
<td>Decoction</td>
</tr>
<tr>
<td>Leaf size</td>
<td>X₅</td>
<td>Cut</td>
<td>Whole</td>
</tr>
</tbody>
</table>

Table 2. Coded and extended levels of independent variables
response surface analys.

The form of this second order polynomial model and regression coefficients were obtained. The experimental data were fitted to the 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:

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

With \( \beta_0, \beta_i, \beta_{ij}, \beta_{ii} \) 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 which 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 significantly the total flavonoids extraction (\( p = .05 \)). The mathematical model obtained was:

\[
Y = 30.09 + 26.99X_3 + 28.59X_4
\]

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:

\[
Y_2 = 108.23 + 124.74 X_1 + 95.36 X_4
\]

(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 \((X_1)\), the extraction time \((X_3)\) and the extraction mode \((X_4)\) 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 mentioned 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

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Experimental responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>(X_1)</td>
<td>(X_2)</td>
</tr>
<tr>
<td>1/100</td>
<td>Acidified water</td>
</tr>
<tr>
<td>7/100</td>
<td>Water</td>
</tr>
<tr>
<td>1/100</td>
<td>Acidified water</td>
</tr>
<tr>
<td>7/100</td>
<td>Water</td>
</tr>
<tr>
<td>1/100</td>
<td>Acidified water</td>
</tr>
<tr>
<td>1/100</td>
<td>Water</td>
</tr>
<tr>
<td>7/100</td>
<td>Acidified water</td>
</tr>
<tr>
<td>7/100</td>
<td>Water</td>
</tr>
</tbody>
</table>

\(Y_1\): 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

<table>
<thead>
<tr>
<th>Coefficients</th>
<th>Values of the coefficients of the variables for each equation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Flavonoids (mg/g QE)</td>
</tr>
<tr>
<td></td>
<td>Valeurs</td>
</tr>
<tr>
<td>(b_0)</td>
<td>30.09**</td>
</tr>
<tr>
<td>(b_1)</td>
<td>-8.52**</td>
</tr>
<tr>
<td>(b_2)</td>
<td>-13.12**</td>
</tr>
<tr>
<td>(b_3)</td>
<td>26.99*</td>
</tr>
<tr>
<td>(b_4)</td>
<td>28.59*</td>
</tr>
<tr>
<td>(b_5)</td>
<td>-15.33**</td>
</tr>
<tr>
<td>(R^2)</td>
<td>0.99</td>
</tr>
<tr>
<td>(R^2) adjusted</td>
<td>0.95</td>
</tr>
</tbody>
</table>

*: \(p=.05\); **: \(p=.01\); ***: \(p=.001\) et ns: not significant
Trials | Variable levels | Experimental responses
---|---|---
| $X_1$ (Ratio, g/mL) | $X_2$ (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 | 0 | 65.00 | 232.86
-1.414 | 0 | 90.00 | 210.44
0 | 1.414 | 70.00 | 255.38
Center point trials
0 | 0 | 51.67 | 188.86

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

<table>
<thead>
<tr>
<th>Coefficients</th>
<th>Total flavonoids 2σ</th>
<th>p-value</th>
<th>Antioxidant activity 2σ</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>60.00</td>
<td>5.05</td>
<td>&lt; 0.001</td>
<td>206.58</td>
</tr>
<tr>
<td>Linear $b_1$</td>
<td>26.68</td>
<td>8.00</td>
<td>&lt; 0.01</td>
<td>80.94</td>
</tr>
<tr>
<td>$b_2$</td>
<td>26.07</td>
<td>8.00</td>
<td>&lt; 0.01</td>
<td>225.59</td>
</tr>
<tr>
<td>Quadratic $b_{11}$</td>
<td>-13.83</td>
<td>8.60</td>
<td>&lt; 0.05</td>
<td>-29.86$^\text{ns}$</td>
</tr>
<tr>
<td>$b_{22}$</td>
<td>5.66$^\text{ns}$</td>
<td>8.60</td>
<td>&gt; 0.05</td>
<td>-7.01$^\text{ns}$</td>
</tr>
<tr>
<td>Interaction $b_{12}$</td>
<td>-7.62$^\text{ns}$</td>
<td>11.30</td>
<td>&gt; 0.05</td>
<td>-10.44$^\text{ns}$</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.94</td>
<td>0.95</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$ns$: not significant

Fig. 1. Correlation between antioxidant activity and flavonoids content
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:

\[
Y_1 = 60 + 26.68X_1 + 26.07X_3 - 13.83X_1^2
\] (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 significantly 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:

\[
Y_2 = 206.58 + 80.94X_1 + 225.59X_3
\] (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. 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

<table>
<thead>
<tr>
<th>Optimal conditions</th>
<th>flavonoid content (mg/g QE)</th>
<th>Antioxidant activity (µmol/g TE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Predicted value</td>
<td>Experimental value</td>
</tr>
<tr>
<td>$X_1$ = 3.5 g/100 mL</td>
<td>86.18$^a$</td>
<td>87.18 ± 1.03$^a$</td>
</tr>
<tr>
<td>$X_2$ = water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$X_3$ = 30 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$X_4$ = Decoction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$X_5$ = Cut leaves</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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,
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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] (14.57 mg/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. Multiflora* 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


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


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.


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