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RESEARCH ARTICLE

OPTIMISATION OF TOTAL FLAVONOIDS AND TOTAL ANTIOXIDANTS EXTRACTION FROM *DACRYODES EDULIS* LEAVES

[1]Jean-Baptiste Kouame YAO, [1,*]Louise Ocho-Anin Atchibri, [2]Emmanuel N'Dri KOFFI, [3]Philippe Kessé N'DA and [3]Augustin Amissa ADIMA

[1]Laboratory of Nutrition and Food safety (LNSA), Nangui-Abrogoua University, Abidjan, Côte d'Ivoire
[2]Advanced Teachers Training College of Abidjan (ENS), Department of Sciences and Technologies, 08 BP 10 Abidjan 08, Cote d'Ivoire
[3]Laboratory of Water Chemistry and Natural Substance, Training and Research Department of GCAA, Felix HOUPHOUËT-BOIGNY National Polytechnic Institute, BP 1093 Yamoussoukro, Côte d'Ivoire

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**Corresponding author:*
Louise Ocho-Anin Atchibri,
Laboratory of Nutrition and Food safety (LNSA), Nangui-Abrogoua University, Abidjan, Côte d'Ivoire.

ABSTRACT

Aim: The aim of this study is to apply experimental design to optimize extraction of total flavonoid and total antioxidant from *Dacryodes edulis* leaves.
Study Design: D. *edulis* leaves were collected from March to April 2016 around Agboville in the southern part of Cote d'Ivoire. Afterwards, the leaves were transferred to the laboratory for drying.
Place and Duration of Study: This study was carried out from June to July 2016 in the Chemistry Laboratory of Water and Natural Substances, at Felix Houphouet Boigny National Polytechnic Institute of Yamoussoukro, Cote d'Ivoire.
Methodology: The harvested leaves were subdivided in two groups (Group 1 and Group 2). The leaves of Group 1 were dried in an oven at 40 ° C for 5 days and those of Group 2, dried in the shade for 2 weeks. Then the effect of 5 parameters (solid-liquid ratio, drying method, extraction solution, extraction time and the extraction mode) was observed on the extraction of total flavonoids and total antioxidants. For this purpose, we used two experimental designs: Plackett-Burman design used to identify the most important variables which influence the extraction process and the full factorial design (2k, k = 3), used to optimize the extraction conditions.
Results: Results showed that the ratio (w/v), the drying mode and the extraction mode had significant effects on the extraction of total flavonoids and total antioxidants from D. *edulis* leaves. The optimal experimental conditions for the highest amount of total flavonoids and total antioxidants are obtained through aqueous maceration from dried leaves of D. *edulis* in oven for 60 minutes with a solid-liquid ratio of 1/100 (w/v). The optimized sample presented a total flavonoid content of 100.33± 1.53 mg/g EQ for antioxidant activity of 314.81 ± 11.33mg/g TE. These experimental results were very close to the predicted values.It was observed that total flavonoids showed a good correlation with total antioxidants of the sample.
Conclusion: The extraction of total flavonoids and total antioxidants from D. *edulis* leaves are influenced by the ratio (w/v), the drying method and the extraction mode. Their optimal extraction condition is the maceration in water of D. *edulis* leaves that have been dried in oven for 60 min with a solid-liquid ratio of 1/100 (w/v). We came across a strong correlation between total flavonoids and total antioxidants. The good antioxidant capacity of the aqueous extract of D. *edulis* leaves could play an important role for the population in the fight against diseases related to oxidative stress.

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INTRODUCTION

The plant under investigation is *Dacryodes edulis*, which belongs to the family of Burseraceae (Chevalier, 1916). *D. edulis* is commonly called safou "African plum", "African pear" or "native pear (Awono *et al.,* 2002). This plant has long been used in the traditional medicine of some African countries to treat various ailments such as wound, dysentery and fever diseases, such as anemia, malaria, headache, fever and skin diseases (Dike *et al.,* 2012). Some studies conducted on antioxidant activities of *D. edulis* leaves extracts have concluded that their extracts possess a good antioxidant activity (Agbor *et al.,* 2007; Nguefack, 2009) which can be useful in treating oxidation-associated diseases,such as cancer and diabetes (Moise *et al.,* 2012). Dai and Mumper (Dai and Mumper, 2010) were reported that the antioxidant activities of plant extracts are due to their bioactive constituents such as flavonoids. (Heim *et al.,* 2007) confirmed theantioxidant and radical scavenging activities of flavonoids. The presence of these compounds was noticed in *D. edulis*leaves extract (Ajibesin, 2011). Flavonoids present on *D. edulis* leaves extract couldplay an important role in the defenseof

humanbody against cardiovascular, aging and cancer diseases (Oroian and Escriche, 2015; Wang *et al.,* 2009; Bobe *et al.,* 2009). The extraction procedure of these phenolic compounds is influenced by many parameters such as their chemical nature, the extraction method used, the sample particle dimension and the extraction time (Dai and Mumper, 2010). Thus, one can make use of an experiment design (statistics and mathematics method) for the optimization. The optimization of the extraction of phenolic compounds from plants using experimental design has been used successfully by some authors (Koffi *et al.,* 2015; Nyamien *et al.,* 2015; Koffi, 2014). This study aims to apply the experiment design to optimize the extractions of flavonoid and the total antioxidants of *D. edulis* leaves with non-toxic solvent by using experiment designs. For so doing, five parameters (solid-liquid ratio, drying method, extraction solution, extraction time and the extraction mode) have been chosen. ThePlackett and Burman design has been used to determine factors or parameters that could influence the total flavonoids and total antioxidants extractions. After the screening, the optimization has been performed by the full factorial design, taking into account factors that influence the extraction with the interaction effects between these factors.

MATERIALS AND METHODS

Plant material

The *D. edulis* leaves were harvested in the city of Agboville, southern part of Cote d'Ivoire. Afterwards the harvested leaves were subdivided in two groups (Group 1 and Group 2). The leaves of Group 1 were dried in an oven at 40 ° C for 5 days and those of Group 2, dried in the shade for 2 weeks.

Chemicals

All chemical reagents used were of analytical grade. They are: Sodium nitrite, aluminum chloride, sodium hydroxide, ethanol and methanol which were purchased from Carlo Erba (Spain). Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid), quercetin, and potassium peroxodisulfate were purchased from Sigma-Aldrich (Germany). ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6- sulphonic acid) diammonium salt) was purchased from Biochem (France).

Plackett and Burman Design

The Plackett-Burman experimental design was used for screening the effect of five independent variables (factors). These factors include vegetal to liquid ratio (w/v) (X_1), drying method (X_2), extraction solution (X_3), extraction time (X_4), and extraction mode (X_5) on total flavonoids extraction and antioxidant activity from *D. edulis* leaves. For each independent variable, the low (-1) and high (+1) levels were tested (Table 1). The independent variables were screened in eight combinations according to Plackett-Burman design matrix (Rajendran *et al.,* 2007). Two dependent variables (experimental responses) were studied: total flavonoids (Y_1) and total antioxidants (Y_2). The relationship between factors and experimental responses is shown by the following equation:

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

Where Y_n is a predicted response, b_o is a model constant and b_i is a variable linear coefficient.

Full Factorial Design

A 2^3 full factorial design was employed in the present study to identify the relationship existing between the responses functions and process variables (Aboua *et al.,* 2010). In this design three factors were evaluated, each at two levels and experimental trials were performed for 8 possible combinations. The solid-liquid ratio (X_1), the drying method (X_2), and the extraction mode (X_5) were used as factors (Table 2), while total flavonoids content (Y_1) and antioxidant activity (Y_2) were taken as experimental responses. In the full factorial design, the main as well as the interaction effects of various factors are determined by fitting the data into 1st order polynomial equation:

$$Y_n = b_0 + b_1X_1 + b_2X_2 + \ldots. + b_kX_k + \ldots + b_{12}X_1X_2 + \ldots + b_{k\text{-}1k}X_{k\text{-}1}X_k + \ldots + b_{1\ldots k}X_1X_2\ldots X_k$$

Where Y_n was the measured response, b_i is the main effect of the factors X_i, b_{ij} is the interaction effect between the factors i and j and b_o is a constant term.

Analytical Methods

Determination of total flavonoids content

Total flavonoids were determined by the aluminum chloride colorimetric method described by Marinova *et al.* 2005. In a 25 mL volumetric flask, 0.75 mL of sodium nitrite ($NaNO_2$) distilled water solution (5%, w/v) was added to 2.5 mL aliquot of the sample. The color reaction was left to develop for 5 min in the dark and at room temperature. Then, 0.75 mL of aluminum chloride ($AlCl_3$) distilled water solution (10 %, w/v) was added and incubated for 6 minutes. After incubation, 5 mL of sodium hydroxide (NaOH 1M) were added and the volume made up to 25 mL. After gentle mixing, the solution absorbance was measured at λmax = 510 nm. A standard calibration curve of quercetin (0.1 to 1.0 $mg.mL^{-1}$) was plotted to calculate the results. The total flavonoids content was expressed as milligram per gram quercetin equivalents (mg/g QE). Samples were analyzed in triplicate

Trolox equivalent antioxidant capacity (TEAC) using $ABTS^{\bullet+}$ radical-scavenging assay

Antioxidant capacity was determined using the procedure reported by Koffi *et al.* 2013. The $ABTS^{\bullet+}$ solution was prepared by mixing equal volumes of $ABTS^{\bullet+}$ salt solution (87.7 mg salt in 20 mL distilled water, 8 $mmol.L^{-1}$) and potassium persulphate ($K_2S_2O_8$) solution (0.0162 g salt in 20 mL distilled water, 3 $mmol.L^{-1}$). The final 40 mL stock solution obtained was kept at room temperature in the dark for 16 hours before use. The volume of stock solution needed for TEAC assay was first diluted with methanol in order to obtain an absorbance of 0.7 ± 0.02 at λ_{max}= 734 nm. A sample volume of 0.1 mL was mixed with 3.9 mL of diluted $ABTS^{\bullet+}$ stock solution and the mixture was incubated for 6 min exactly, in the dark at 30 °C. Absorbance was measured at λmax= 734 nm, and had to be higher than 20% of the absorbance of the diluted $ABTS^{\bullet+}$ stock solution itself, otherwise the sample solution had to be diluted accordingly. Pure Methanol was used as a blank solution. Trolox solutions with concentrations ranging from 0 to 500 $\mu mol.L^{-1}$ were used as the standard. TEAC was expressed as milligram per gram trolox equivalent (mg/g TE). Samples were analyzed in triplicate.

Statistical Analysis

Results were expressed as mean ± standard deviation of three duplicate. Data were evaluated by one-way analysis of variance (ANOVA) using *Statistica* 8.0 (Statsoft, Inc., USA) solfware. Newman-keuls test was performed to determine significant difference at *P*=.05. Coefficient and experimental standard deviations were determined by the method of linear regression.

RESULTS AND DISCUSSION

Screening of variable effects on total flavonoid and total Antioxidant extraction

The effects of five factors (solid-liquid ratio (w/v) (X_1), drying method (X_2), extraction solution (X_3), extraction time (X_4), and extraction mode (X_5)) on extraction of total flavonoids and antioxidant activity from *D. edulis* leaves were studies. According to Plackett-Burman design, eight experiments were carried out. Table 3 shows experiment conditions and results obtained. The minimum amount of total flavonoids (42.67± 1.5 mg/g EQ) is obtained through extraction by infusion of *D. edulis* leaves dried in the shadow with a ratio of 1/1000 (w/v) in a mixture of ethanol/water (70/30) (Experiment 3). On the other hand, the maximum amount of total flavonoids (115.33 mg EQ /g) was obtained in experiment 7, when experiment was carried out during 60 min by aqueous maceration of oven dried leaves of *D. edulis* with liquid-solid ratio at 1/100 (w/v). On the other hand, the highest amount of total flavonoids (115.33 mg/g EQ), is obtained through a 60 min extraction by aqueous maceration of *D. edulis* leaves dried in oven with a solid-liquid ratio (w/v) of 1/100 (Experiment 7). The antioxidant capacity of the different samples obtained by applying the Plackett-Burman design, varies between 91.45 and 441.07mg/g TE.

The weakest antioxidant capacity is obtained through an extraction by infusion of *D. edulis* leaves during 60 min, dried in the shadow with a ratio of 1/1000 (w/v) in a mixture ofethanol/water (70/30, v/v). On the other hand, the maximum amount of total flavonoid 441.07 mg/g TE is obtained through extraction, within 60 min, by aqueous maceration of *D. edulis* leaves dried in oven with a ratio of 1/100 (w/v). Table 4 illustrates estimation and statistics of linear regression coefficient. A coefficient is reported to be statistically significant if its absolute value is strictly superior to the double of the experimental standard deviation, that is $|coef|>2\sigma$ (Assidjo *et al.,* 2005). The analysis of table 4 shows that for total flavonoid (Y_1), the coefficients b_o, b_1, b_2 and b_5 are significant. The extraction of total flavonoids is thus influenced by the solid-liquidratio (X_1) (w/v), the drying method (X_2), and the extraction mode (X_5). Yet, the influence of extraction mode is higher than those of solid-liquid ratio and the drying method. The response (Y_2) which corresponds to total antioxidants is influenced by the following technological parameters: the solid-liquidratio (X_1) (w/v), the drying method (X_2) and the extraction mode (X_5) for, the coefficients associated to the different parameters are significant. In short, the dominating technological parameters for the extraction of flavonoids and total antioxidants of *D. edulis* are: the solid-liquidratio (X_1) (w/v), the drying method (X_2) and the extraction mode (X_5).

Optimization of extraction conditions using 2^3 full factorial designs

The minimum amount of total flavonoids obtained from the matrix of the full factorial design is 47.66 mg/g EQ (table 5). In contrast, the maximum amount of flavonoids (100.66 mg/g EQ) is obtained by aqueous maceration with a solid-liquid ratio of 1/100 (w/v) of dried leaves in oven for 60 min. The heat supply during the extraction (infusion) could damage the extraction of total flavonoids. Indeed, a highest temperature could oxidize the flavonoids and it could therefore reduce their extraction (Yaqin *et al.,* 2005) also a high temperature could decrease the fluid density and may reduce the extraction efficiency (Sathishkumar *et al.,* 2008). The amount of total flavonoids in *D. edulis* leaves is higher than those of *D. edulis* leaves methanolic extract (78.8mg EQ/g) (Omoregie and Okugbo, 2014), *D. edulis*seed boiled extract (50.02mg EQ/g) obtained by Ogunmoyole *et al.* 2012. In comparison with other leaves, the quantity of total flavonoids extract from *D. edulis*leaves is superior to those of *Moringa* (27 mg/g EQ) (Sreelatha and Padma, 2009) and*Aloysia triphylla* (6.81 mg/g EQ) (Cheurfa and Allem, 2015). The antioxidant capacity of the samples obtained through tests of full factorial design varies from 128.20 to 324.34 mg/g TE. The highest value of total antioxidants (324.34 mg/g TE) is obtained from aqueous maceration of *D. edulis* leaves dried in oven with a ratio of 1/100 (w/v). When the extraction is performed through aqueous infusion of *D. edulis* leaves dried in the shade with a ratio of 1/1000 (w/v), we obtain a weaker value (128.20 mg/g TE). This result is similar to the one obtained during the extraction of total flavonoid from *D. edulis* leaves. The different values of the coefficients assigned to each parameter for the Y_1 and Y_2 responses are shown in, The statistical analysis of data shows that for total flavonoids (Y_1) only the solid-liquid ratio (X_1) and the drying mode (X_2) are significant. Yet no interaction was observed between the different factors. The resulting mathematic model is:

$$Y_1 = 70.87 + 35.07\ X_1 + 13.07\ X_2 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (1)$$

As for the total antioxidants, we notice significant effects of the solid-liquid ratio (X_1), the drying mode (X_2) and the extraction mode (X_5). The ratio (X_1) is the most influent parameter(p =.01). No interaction was observed between the different factors. The resulting mathematic model is:

$$Y_2 = 221.58 + 136.91\ X_1 + 35.14X_2 - 24.71X_5 \quad \ldots\ldots\ldots\ldots\ (2)$$

At the end of these different analyses, the optimal condition extraction of flavonoids and total antioxidant determined thanks to the full factorial design is: the ratio (X_1) = +1 (1/100), the drying mode (X_2) = +1 (oven drying) and the extraction solution (X_3) = -1 (water). Thus the solid-liquid ratio of 1/100 (w/v) seems much more preferable to that of 1/1000 (w/v). This result is in accordance with the mass transfer principle, where transmission force during this transfer is the gradient of the concentration between the solid and the liquid. This force becomes important when a higher solvent to solid-liquid ratio is used (Al-farsi and Lee, 2008; Gaboriaud, 1996). Similar results on the solid-liquid ratio upon the extraction of polyphenols have been reported for date stones by Al-farsi and Lee 2008 and for the leaves of *Inga edulis* by Silva *et al.* 2007. The oven drying of the *D. edulis* leaves could prevent the biotransformation of flavonoids in other secondary metabolites thanks to the enzyme activity that sometimes remains during the air drying of plants (ABDALI Youness, 2015).

Table 1. Factors levels used in Plackett-Burman design

Factors	Technological parameters	Coded levels / True value or state	
		Low (-1)	High (+1)
X_1	Vegetal-liquid ratio	1/1000	1/100
X_2	Drying method	Shade drying	Oven drying
X_3	Extraction solution	Water	Ethanol/Water (70/30)
X_4	Extraction time	15	60
X_5	Extraction Mode	Maceration	Infusion

Table 2. Factors levels used in 2^3 factorial designs

Factors	Technologicalparameters	Coded levels	
		Low (-1)	High (+1)
X_1	Vegetal-liquid ratio	1/1000	1/100
X_2	Drying method	Shade drying	Oven drying
X_5	Extraction mode	Maceration	Infusion

Table 3:Sample and results according to the Plackett-Burman design

Test set	Technological parameter levels					Experimental responses	
	X_1	X_2	X_3	X_4	X_5	Y_1[a]	Y_2[b]
1	1	1	1	-1	1	85.33 ± 0.6	185.44 ± 5.41
2	-1	1	1	1	-1	79.67 ± 0.6	309.49 ± 3,00
3	-1	-1	1	1	1	42.67 ± 1.5	91.45 ± 0.68
4	1	-1	-1	1	1	74.67 ± 0.6	198.82 ± 4.72
5	-1	1	-1	-1	1	55 ± 1.7	147.29 ± 10.84
6	1	-1	1	-1	-1	95 ± 1.7	314.59 ±10,74
7	1	1	-1	1	-1	115.33 ± 0.6	441.07 ±8,83
8	-1	-1	-1	-1	-1	60.67 ±2.1	249.94 ±7,74

Y_1: total flavonoids; Y_2 total antioxidants; a: mg/g EQ; b: mg/g TE

Table 4 Statistical estimates of coefficient responses of Plackett-Burman design

Coefficient	Coefficient and standard deviations for each equation					
	Total flavonoids			Antioxidant activity		
	Values	2σ	*P-value*	Values	2σ	*P-value*
bo	242.26^{***}	8.97	0.000	76.04^{***}	1.36	0.000
b_1	85.44^{*}	17.95	0.011	33.08^{**}	2.71	0.002
b_2	57.12^{*}	17.95	0.023	15.58^{**}	2.71	0.007
b_3	-34.04^{ns}	17.95	0.063	-0.75^{ns}	2.71	0.636
b_4	35.89^{ns}	17.95	0.057	4.08^{ns}	2.71	0.095
b_5	-173.02^{**}	17.95	0.003	-23.25^{**}	2.71	0.003
R^2	0.99			0.99		

ns not significant; *: Significant data at p = .05; **: Significant data at p = .01; *** Significant data at p = .001; R^2 regression coefficient

Table 5. Experimental design (2^k, k=3) and corresponding responses

Test set	Level of technological parameters			Experimental responses	
	X_1	X_2	X_5	Y_1[a]	Y_2[b]
1	1/1000	S	M	47.66 ±1.54	143.53 ±15.06
2	1/100	S	M	81.00±1.00	283.57 ±0.84
3	1/1000	O	M	57.66 ±0,58	184.30 ±6.58
4	1/100	O	M	100.66 ±1.53	324.34 ±11.66
5	1/1000	S	I	50.00±1.00	128.20 ±9,50
6	1/100	S	I	78.66 ±1.15	260.73 ±5.57
7	1/1000	O	I	58.00±0.00	156.46 ±7.93
8	1/100	O	I	93.30±2,89	291.49 ±8.80

S: shade drying; O: oven drying; M: maceration; I: infusion; Y_1: total flavonoids; Y_2: antioxidant activities; a: mg / g EQ; b: mg TE / g.

Table 6. Statistic estimate of full factorial design coefficient reponses

Coefficient	Coefficient and standard deviations for each equation					
	Total flavonoids			Antioxidant activity		
	Values	2σ	*P-value*	Values	2σ	*P-value*
bo	70.87**	0.75	0.003	221.58***	0.62	0.000
b_1	35.07*	1.51	0.013	136.91**	1.25	0.003
b_2	13.07*	1.51	0.036	35.14*	1.25	0.011
b_5	-1.75^{ns}	1.51	0.259	-24.71*	1.25	0.016
b_{12}	4.07^{ns}	1.51	0.117	0.62	1.25	0.500
b_{15}	-3.09^{ns}	1.51	0.152	-3.13^{ns}	1.25	0.125
b_{25}	-1.75^{ns}	1.51	0.259	-5.64^{ns}	1.25	0.070
R^2	0.99			1.00		

ns not significant; *: Significant data at p = .05; **: Significant data at p = .01; *** Significant data at p = .001; R^2 regression coefficient

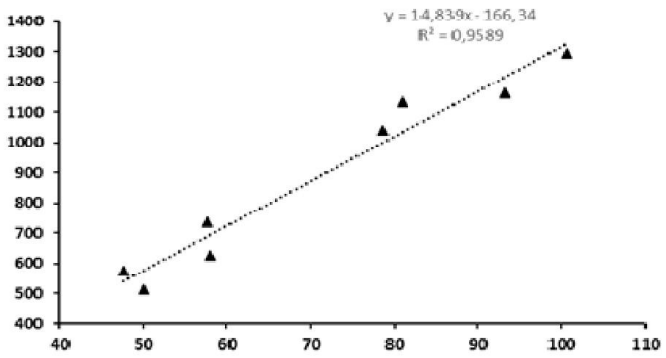

Figure 1. Correlation between the antioxidant capacity and the amount of flavonoids of the aqueous extract of *D. edulis* leaves

Correlation between flavonoids and antioxidant activity

In this study, good correlation (R^2= 0.96) are observed between total flavonoid and total antioxidant from aqueous extract of *D. edulis* leaves (Figure 1). This result indicates that antioxidant activity of aqueous leaf extract of *D. edulis* is strongly related to total flavonoid. This result is consistent with that of Ajibesin (Ajibesin, 2011) which stated that the antioxidant activity leaves *D. edulis* was attributed to the presence of flavonoids. Similar correlation between flavonoids with antioxidant activity was observed byLizcano *et al.* 2010 and Cortés-Rojas *et al.* 2011. Indeed, according to Ghasemzadeh and Ghasemzadeh (Ghasemzadeh and Ghasemzadeh, 2011) flavonoids is a powerful antioxidant which could contribute to the reinforcement of the antioxidant activities of *D. edulis* leaves.

Experimental validation of predicted optimal condition

The results of the full factorial design have permitted to determine the extraction optimal conditions of total flavonoids and the total antioxidants of *D. edulis* leaves. To better extract these compounds, the extraction should be through aqueous maceration of the leaves dried in oven with a solid-liquid ratio of 1/100 (w/v), for 60 min. The assays performed in such conditions have permitted to experimentally validate the predictions of the full factorial design (Table 7). The amount of total flavonoid extract (100.33 mg/g QE) is substantially equal to that predicted by the mathematic model (100.28 mg/g QE). The statistical analyses show that there is no significant difference between the two values at $p = .05$. Thus the experimental results of the total flavonoids are similar to those predicted by the mathematic model. As well, at the level of total antioxidants, the statistical analysis shows that there is no significant difference on the threshold of 5% between the value obtained and the one predicted. It comes out from these analyses that the experimental results performed are similar to those predicted by the different mathematic models. The full factorial design used for the optimization of the extraction of the flavonoids and the total antioxidant of *D. edulis* leaves is applied successfully in the experimental field chosen (Rao and Padmanabhan, 2013).

Conclusion

This work has permitted to find optimal conditions for extraction of total flavonoids and total antioxidants from *D.* This work has permitted to find optimal conditions for extraction of total flavonoids and total antioxidants from *D. edulis* leaves dried in the shade and in oven.For so doing, the Plackett-Burman design has been used to obtain the parameters that influence the extraction of the flavonoids and total antioxidants that are the solid-liquid ratio, the drying mode and the extraction mode. The application of these parameters in a full factorial design permitted us to optimize the extraction of flavonoid and total antioxidants. The maximum amount of total flavonoids and total antioxidantsare obtained with leaves dried in oven with a ratio of 1/100 (w/v) by aqueous maceration, for 60 min. We observed a strong correlation between total flavonoid and total antioxidants. The strong value of the total antioxidants of aqueous extracts of *D. edulis* leaves could play a crucial role for the population in the fight against ailments related to oxidative stress. A more precised study of these factors as well as the study and the identification of other phenolic compounds families should be undertaken.

Authors' Contributions

This work was carried out in collaboration between all authors. Author J-BKY designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors'LO-AA, ENK, PKN and AAA managed the study and the literature searches. All authors read and approved the final manuscript.

REFERENCES

ABDALI Youness, 2015. Effet des modes de séchage sur la teneur en flavonoïdes et l'activité antioxydante de Matricaria chamomilla. Mémoires Licence Sciences & Techniques «Biotechnologie et Valorisation des PhytoRessources». Université sidi mohamed ben abdellah faculte des sciences et techniques de fes, 27p.

Aboua, N., Yao, B., Gueu, S., Trokourey, A. 2010. Optimization by experimental design of activated carbons preparation and their use for lead and chromium ions sorption. *Res. J. Agric. Biol. Sci.,* 6(6):665-670.

Agbor, G., Kuate, D., and Oben, J. 2007. Medicinalplants can be good source of antioxidants: Casestudy in Cameroon. *Pak. J. Biol. Sci.,* 10(4):537-544.

Ajibesin K. 2011. *Dacryodes edulis* (G.Don) H.J.Lam: A review on its medicinal, phytochemical and economical properties. *Res. J. Med. Plant*., 5(1):32-41.

Al-farsi M. and Lee C. Optimization of phenolics and dietary fiber extraction from date seeds; J. Food Chem. 2008. 108: 977-985.

Assidjo, E., Yao, B., Akou, E., Ado, G. 2005. Optimisation of the treatment conditions of cocoa butter in order to reduce non-quality. *J. Chemom.,* 19:543-548.

Awono, A., Ndoye, O., Schreckenberg, K., Tabuna, H., Issen, F. and Temple, L. 2002. Production and marketing of safou (*Dacryodes edulis*) in Cameroon and internationally: Market development issues. Forests, *Treesand Livelihoods*, 12(1-2):125-147.

Bobe, G., Peterson, J.J., Gridley, G., Hyer, M., Dwyer, J.T., Brown, L.M. 2009. Flavonoid consumption and esophageal cancer among black and white men in the United States. *Int. J. Cancer*, 125,1147-1154.

Cheurfa Mand Allem, R. 2015. Évaluation de l'activité antioxydante de différents extraits des feuilles d'Aloysia triphylla (L'Hérit.) d'Algérie in vitro Phytothérapie, 14(3):181-187.

Chevalier, A. 1916. Les végétaux utiles de l'Afrique tropicale française. Laforêt et les bois du Gabon. Paris : Challamel, Fench.

Cortés-Rojas, D., Souza, C., Oliveira, W. 2011. Optimisation of the extraction of phenolic compounds and antioxidant activity from aerial parts of Bidens pilosa L. using response surface methodology. *Int J Food Sci Technol.,* 46(11): 2420-2427.

Dai, J. and Mumper, R.J. 2010. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules*, 15(10):7313-7352.

Dike, I., Obembe, O. and Adebiyi, F. 2012. EthnobotanicaL survey for potential anti-malarial plants in south-western Nigeria. *J.Ethnopharmacol*, 144(3):618-626.

Gaboriaud, R. 1996. Physico-chimie des solutions ; Edition MASSON, Paris., 208-228.

Ghasemzadeh, A. and Ghasemzadeh, N. 2011. Flavonoids and phenolic acids: role and biochemical activity in plants and human. *J. Med. Plants Res.,* 5(31): 6697-6703.

Heim, K. E., Taqliaferro, A.R., Bobilya, D.J. 2002. Flavonoids antioxidants: Chemistry, metabolism and structure-activity relationship. *J. Nutr. Biochem*., 13(10), 572-584.

Koffi, E. 2014. Contribution à l'optimisation de l'extraction et a l'etude chimique des polyphenols de deux plantes de Côte-d'Ivoire : *Tectona grandis* Linn (Verbenaceae) et *Justicia secunda* Vahl (Acanthaceae) Thèse de Doctorat de l'Université Nangui Abrogoua, Côte-d'Ivoire, 169 p.

Koffi, E., Le Guernevé, C., Lozano, P,, Meudec, E., Adjé, F., Bekro, Y. et al. 2013. Polyphenol extraction and characterization of *Justicia secunda* Vahl leaves for traditional medicinal uses. *Ind Crops Prod.,* 49:682-689.

Koffi, E.N., Cissé, I., Kassi, A.B.B., Lozano, P.R., Adima, A.A., Assidjo, E.N., Bekro, Y.A. 2015. Optimization of ultrasound-assisted extraction of phenolic antioxidants from Tectona grandisleaves, using experimental design. *European Journal of Medicinal Plants*, 10(3):1-10.

Lizcano, L., Bakkali, F., Ruiz-Larrea, M. and Ruiz-Sanz, J. 2010. Antioxidant activity and polyphenol content of aqueous extracts from Colombian Amazonian plants with medicinal use. *Food Chem.,* 119, 1566–1570.

Marinova, D., Ribavora, F. et al., 2005. Antanassova M. Total phenolics in bulgarian fruits and vegetables. *J. Univ. Chemi. Technol.* M. et al., 40(3): 255-260.

Moise, M., Benjamin, L., Etienne M., Thierry, G., Ndembe Dalida, K., Doris, T., et al. 2012. Intake of Gnetum Africanum and *Dacryodes Edulis*, Imbalance of Oxidant/Antioxidant Status and Prevalence of Diabetic Retinopathy in Central Africans. PLoS ONE, 7(12).

Nguefack, E. 2009. Hypoglycemic, Hypolipidemic and Antioxidant Activity of Some Cameroonian Medicinal Plants. Lyon, France, p. 49.

Nyamien, Y., Chatigre, O., Koffi, E., Adima, A. and Biego, H. 2015. Optimization of Polyphenols Extraction Method from Kola Nuts (*Cola nitida* Vent. Schott & Endl.) Using Experimental Design. *British Biotechnology Journal,* 7(1): 40-50.

Ogunmoyole, T., Kade, I., Johnson, O. and Makun, O. 2012. Effect of boiling on the phytochemical constituents and antioxidant properties of African pear Dacryodes edulis seeds in vitro. *Afri. J. of Biochem. Res.,* 6(8):105-114.

Omoregie, E. and. Okugbo, O. 2014. In vitro antioxidant activity and phytochemical screening of methanol extracts of Ficus capensis and Dacryodes edulis leaves. *J. Pharm. Bioresources.,* 11(2), 66-75.

Oroian, M., Escriche, I. 2015. Antioxidants: Characterization, natural sources, extraction and analysis. *Food Res Int.,* 74:10-36

Rajendran, A., Thirugnanam, M., Thangavelu, V. 2007. Statistical evaluation of medium components by Plackett-Burmann experimental design and kinetic modeling of lipase production by Pseudomonas fluorescens. *Indian J. Biotechnol.,* 6:469-478.

Rao, S. and Padmanabhan, G. Linear modeling of the electrochemical machining process using full factorial design of experiments. *J. Adv. Mech. Eng.,* 2013; 1: 13-23.

Sathishkumar, T., Shanmugam, S., Rajasaekaran, P., Sadavivam, S., Manikandan, V. 2008. Optimization of flavonoids extraction from the leaves of Tabernaemontana heynena Wall. using L_{16} orthogonal design. *Nat. Sci.,* 6(3):10-21.

Silva, E. M., Rogez, H. and Larondelle, Y. 2007. Optimisation of extraction of phenolics from *Inga edulis* leaves using response surface methodology; *Journal of Separation and PurificationTechnology*., 55: 381-387.

Sreelatha, S. and Padma, P. 2009. Antioxidant Activity and Total Phenolic Content of Moringa oleifera Leaves in Two Stages of Maturity. *Plant Foods for Human Nutrition*., 64:303-311.

Wang, L., Lee, I.M., Zhang, SM, Blumberg, JB, Buring, J.E., Sesso, H.D. 2009. Dietaryintake of selected flavonols, flavones, and flavonoid-rich foods and risk of cancer in middle-aged and older women. *Am.J.Clin.Nutr.,* 89, 905-912.

Yaqin, X., Rui, Z., Hong, F. 2005. Studies on the optimal process to extract flavonoids from red-raspberry fruits. *Nat. Sci.,* 3(2): 43-46.

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