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Research Article

Face-Centered Central Composite Design for the Optimization of the Extraction of Phenolic Compounds from Kernels and Shells of *Raphia farinifera* and Evaluation of the Antioxidant, Antimicrobial, and Anti-Inflammatory Activities

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This study reports the extraction of phenolic compound from shells and kernels of the *Raphia farinifera* fruit and the biological activities of the extract. A face-centered composite design was established to optimize the extraction conditions: ethanol/water ratio (0-100%), solvent/powder ratio (10-30 mL/g), and extraction time (90-180 min). Subsequently, the extracts obtained under the optimal conditions were used for the evaluation of the radical scavenging capacity, the capacity to chelate ferric ions, and the antimicrobial and anti-inflammatory activities. The optimal extraction conditions for the shells are an extraction time of 134.24 min, 65% ethanol in water, and a solvent/substrate ratio of 21.16 mL/g, and for the kernel, an extraction time of 180 min, 94.3% ethanol in water, and a solvent/substrate ratio of 18.6 mL/g. In these conditions, the phenolic compounds were 95.36 mg EAG/L for the shell extract and 139.72 mg EAG/L for the kernel extract. The antioxidant activity revealed that the half-maximal inhibitory concentrations (IC_{50}) of the kernel extracts are 22.32 μg/mL and 55.73 μg/mL for the shells. A reducing activity of Fe^{3+} ions with an activity of 308.39 μg EAA/mg for the kernel extracts and 293 μg EAA/mg for the shells was observed. *B. cereus* was the most sensitive microorganism with a minimum inhibitory concentration (MIC) equal to the minimum bactericidal concentration (MBC) with a value of 156.25 ppm for the kernel extract while the shell extract showed MIC of 625 ppm and MBC of 2500 ppm. The IC_{50} values for the denaturation of proteins by extracts of shells and kernels are 0.76 μg/mL and 0.56 μg/mL, respectively. Membrane stabilization revealed IC_{50} values of 1054.54 μg/mL and 1339 μg/mL for the shell and kernel extracts, respectively. This work has shown the potential of *Raphia farinifera* extracts for the food industry and cosmetics.

1. Introduction

The plant kingdom represents an inexhaustible source of active compounds synthesized metabolically to meet some of its needs. Thanks to their biological activities (antibacterial, anticancer, antifungal, antioxidant, etc.), these molecules are a concrete response to several diseases affecting humanity [1]. They also make a major contribution, through their application, to progress in several sectors such as cosmetics, agrifood, and health [2]. These active compounds include phenolic compounds, compounds with several unique aromatic rings coupled to one or more hydroxyl groups. These compounds are found in fruit, legumes, vegetables, tea, wine, coffee, and many other plant products [3]. Phenolic compounds, which are secondary metabolites, are responsible for numerous organoleptic characteristics of plant foods and have many therapeutic virtues [4, 5]. They play a vital role, mainly in the fight against cancers, cardiovascular diseases, and lipid peroxidation, thus explaining their great use in the manufacture of drugs. They are also involved in protecting plants against various microbial attacks.

Raphia, which belongs to the Palmaceae or Arecaceae family, is a plant found in swampy and semiswampy areas of equatorial forests or derived savannas [6]. *Raphia farinifera* is one of the *Raphia* species found in Cameroon. Although this plant is widely exploited by craftsmen, very little research has been done on it to date [7, 8]. Therapeutic values have been reported for the *Raphia* fruit, but little research has been carried out on its use as a food. Nevertheless, a few studies have been carried out. Yapna et al. [9] carried out a phytochemical study of *Raphia farinifera* kernel extracts and identified eight compounds, namely, diosgenin, diosgenin-3-O-α-L-rhamnopyranosyl-(1→2)-D-glucopyranoside, catechin, epiafzelechin, ellagic acid, aurantiamide acetate, crotonic acid, and sitosterol. The work of Tapondjou et al. [10] isolated eight steroidal saponins from the mesocarp of *Raphia farinifera* fruits, by combined column chromatography and RP-HPLC methods. Cytotoxicity testing of all saponins against bladder carcinoma cells (ECV-304) revealed that, for high cytotoxicity, a sugar chain of at least three sugar moieties attached to the C-3 of the steroidal saponin is required. Oluwaniyi et al. [11] carried out a comparative phytochemical study of the pulp, shell, and kernel of *Raphia hookeri* and *Raphia farinifera* and showed that extracts from different parts of the two fruits had phytochemical compounds such as terpenes, cardiac glycosides, and saponins whose concentration in the extracts depended on the nature of the solvent used. To our knowledge, no studies have been carried out to optimize the extraction of phenolic compounds from different parts of the fruit and to demonstrate their biological properties.

The extraction of phenolic compounds from plant matrices is subject to several factors which must be mastered if the operation is to be carried out properly. Furthermore, in the context of sustainable development and the development of green chemistry, these operations to isolate bioactive compounds must be safe and have a low impact on the environment. The efficiency of the extraction of these phenolic compounds depends largely on the extraction conditions and mainly on the solvent [12–14]. To the best of our knowledge, there are no studies on the effect of extraction parameters on the recovery of phenolic compounds from the different parts of the *Raphia farinifera* fruit. Several types of polar organic solvents such as ethyl acetate, acetone, methanol, or acetone and their combination with water are often reported as the best for the extraction of phenolic compounds from various plant matrices [5]. The water/ethanol combination is often considered the most appropriate of the combinations due to a positive Environment, Health, and Safety (EHS) for ethanol and the fact that it is relatively safe for human consumption [5]. However, the proportion of solvents remains to be determined, as it depends on the nature of the matrix [12, 15] and may also be influenced by other extraction conditions such as time and the solid/liquid ratio, which cannot be elucidated using the usual one-factor-at-a-time methods. Design of experiment (DOE) methodology is known as a method of planning experiments to minimize the cost of experimentation while obtaining the maximum amount of information about the phenomenon, such as the possible interactions between the factors likely to influence extraction. The use of this approach, which is essential for good control of extraction conditions, also makes it possible to find the optimum extraction conditions.

The phenolic compounds extracted may have numerous biological properties, such as antioxidant, antimicrobial, and anti-inflammatory properties. To date, no activity has been reported for extracts from the various parts of the *Raphia farinifera* fruit.

The aim of the present work is to determine the conditions for extracting phenolic compounds from the shells and kernels of the *Raphia farinifera* fruit using a face-centered composite design in which the factors are the percentage of ethanol in the aqueous solution used as the extraction solvent, the liquid/solid ratio, and the extraction time, with the concentration of phenolic compounds as a response. The optimal conditions will be used to produce extracts that will be used to assess antioxidant activity by two methods (DPPH and FRAP), antimicrobial activity on five microorganisms of medical interest (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 11966, *Salmonella enteritidis* 155A, and *Candida albicans* spp.), and anti-inflammatory activity (protein denaturation and membrane stabilization).

2. Materials and Methods

2.1. Preparation of Plant Material. The fruits of *Raphia farinifera* were harvested in the West region of Cameroon at Bafoussam Ier, Batoukop village, in March 2022 in a marshy vegetative environment with geographical coordinates between 5 degrees 25 north latitude and 5 degrees 30 east longitude. The botanical identification of this fruit was carried out and authenticated by the National Herbarium of Cameroon (Yaoundé) in comparison with the herbarium sample No. 40 964/HNC of the collector Fotius G., No. 3088. The fruit comes from a plant belonging to the *Arecaceae* family, with the scientific name *Raphia farinifera*(Gaertn Hyander).



(a) (b)

Figure 1: Palm trees with the fruits of *Raphia farinifera* (a); the fruits of *Raphia farinifera* (b).

The fruit (Figure 1) was taken to the laboratory, where it was sorted, cleaned, and peeled to obtain the shell, pulp, and kernel. The various components were dried at 50°C in a Memmert® oven, then crushed and sieved using a 450 μm mesh sieve.

2.2. Proximate Composition of Kernels and Shells of Raphia farinifera. The moisture, ash, fat, and protein contents of the kernel and shell were determined by the AOAC method [16]. The total carbohydrate content was determined by difference via the equation below (eq. (1) according to Sompong et al. [17].

$$\%\text{total carbohydrate} = 100 - (\%\text{moisture} + \%\text{fat} + \%\text{ash} + \%\text{protein}). \quad (1)$$

2.3. Plant Material Extraction Process. Five grams of powder was mixed with different solvent ratios (10 to 30 mL/g) of a distilled water/ethanol mixture in which the proportion of ethanol varied from 0 to 100%. The mixture was subjected to magnetic stirring at a speed of 700 rpm at room temperature for a predetermined time (90 to 180 min). After extraction, the extracts were recovered by filtering the mixture using a vacuum device and Whatman No. 4 filter paper and stored in amber glass bottles at 4°C until analysis.

2.4. Experimental Design and Model Validation. A face-centered composite design was developed to model and optimize the extraction of phenolic compounds from *Raphia farinifera* shells and kernels. It comprised nineteen (19) trials, including 8 trials for the factorial design, 6 for the trials of the axes, and 5 for the trials at the center of the study area. The factors studied were the percentage of ethanol in a water/ethanol mixture (0 to 100%), the solvent/powder ratio (10 to 30 mL/g), and the extraction time (90 to 180 min). The various tests are presented in Table 1. Trials were carried out in triplicates, and the mean total phenolic compound concentration of these trials was used as a response. A second-degree polynomial model including linear, quadratic, and interaction terms was postulated and used to fit the experimental data.

$$y = \alpha_0 + \sum_i \alpha_i x_i + \sum_{ii} \alpha_{ii} x_i^2 + \sum_{ij} \alpha_{ij} x_i x_j + \varepsilon, \quad (2)$$

where y is the concentration of total phenolic compounds, x_i and x_j are the independent variables, α_0 is the equation constant, α_i is the linear coefficient, α_{ii} is the quadratic coefficient, α_{ij} is the interaction coefficient, and ε is the error.

The equation used to convert the coded variables into the real variables used in the tests is as follows:

$$x_i = \frac{X_i - \bar{X}_{oi}}{\Delta X_i}, \quad (3)$$

where x_i is the coded variable, X_i is the value of the corresponding real variable, $\bar{X}_{oi}$ is the central value in current units, and ΔX_i is the step size of the variable in question.

Various criteria were used to check the adequacy of the postulated second-degree polynomial model, namely, the coefficients of determination R^2 and adjusted R^2, the bias factor (B_f), the accuracy factor (A_f), and the absolute analysis of deviation from the mean (AADM). The formulae used for B_f, A_f, and AADM are presented in eqs. (4), (5), and (6), respectively.

Table 1: Face-centered composite design with real and coded variables and experimental and predicted responses from the second-degree polynomial model.

Essay	Coded variables			Real variables			Experimental values		Predicted values	
No.	Time (min) x_1	Percentage ethanol (%) x_2	Solvent/powder ratio (mL/g) x_3	Time (min) X_1	Percentage ethanol (%) X_2	Solvent/powder ratio (mL/g) X_3	TPC (mg GAE/L) shell extract Y_1	TPC (mg GAE/L) kernel extract Y_2	TPC (mg GAE/L) shell extract Y_1	TPC (mg GAE/L) kernel extract Y_2
1	-1	-1	-1	90	0	10	22.72 ± 1.19	35.47 ± 2.79	21.93	33.10
2	1	-1	-1	180	0	10	29.61 ± 1.19	40.9 ± 1.54	24.05	35.85
3	-1	1	-1	90	100	10	55.04 ± 1.78	126.61 ± 1.12	53.68	128.05
4	1	1	-1	180	100	10	60.21 ± 2.54	130.69 ± 0.17	63.54	129.38
5	-1	-1	1	90	0	30	50.96 ± 1.73	50.84 ± 1.18	47.51	49.65
6	1	-1	1	180	0	30	33.55 ± 1.41	55.37 ± 0.75	34.78	51.43
7	-1	1	1	90	100	30	52.84 ± 2.12	113.78 ± 1.50	58.27	116.34
8	1	1	1	180	100	30	52.61 ± 0.24	116.84 ± 0.72	53.28	116.71
9	-1	0	0	90	50	20	74.96 ± 1.64	123.27 ± 3.41	75.13	122.84
10	1	0	0	180	50	20	73.35 ± 2.16	113.64 ± 2.84	73.69	124.39
11	0	-1	0	135	0	20	50.25 ± 0.67	43.78 ± 0.83	58.83	56.338
12	0	1	0	135	100	20	92.02 ± 2.27	139 ± 5.74	83.95	136.44
13	0	0	-1	135	50	10	73.08 ± 2.48	93.552 ± 2.75	77.47	100.84
14	0	0	1	135	50	30	89.02 ± 1.53	100.08 ± 2.07	85.13	102.78
15	0	0	0	135	50	20	97.31 ± 1.58	127.25 ± 2.41	91.24	119.62
16	0	0	0	135	50	20	89.82 ± 1.60	122.65 ± 3.58	91.24	119.62
17	0	0	0	135	50	20	90.57 ± 1.99	117.23 ± 4.08	91.24	119.62
18	0	0	0	135	50	20	88.14 ± 1.71	120.25 ± 0.42	91.24	119.62
19	0	0	0	135	50	20	91.35 ± 1.30	130.72 ± 0.25	91.24	119.62

TPC: total phenolic compound; GAE: gallic acid equivalent.

$$B_f = 10^B \text{ with } B = \frac{1}{n}\sum_{i=1}^{n} \log\left(\frac{Y_{i,\text{theo}}}{Y_{i,\text{exp}}}\right), \quad (4)$$

$$A_f = 10^A \text{ with } A = \frac{1}{n}\sum_{i=1}^{n} \left|\log\left(\frac{Y_{i,\text{theo}}}{Y_{i,\text{exp}}}\right)\right|, \quad (5)$$

$$\text{AADM} = \left(\frac{1}{n}\right)\sum_{i=1}^{n}\left(\frac{\left|Y_{i,\text{exp}} - Y_{i,\text{theo}}\right|}{Y_{i,\text{exp}}}\right), \quad (6)$$

where Yi, exp and $Yi, theo$ represent the experimental and theoretical responses from the model for the experiment i, respectively. n is the number of experiments.

2.5. Assessment of the Content of Total Phenolic Compound. The total polyphenol content of *Raphia farinifera* fruit shell and kernel extracts was estimated using Folin-Ciocalteu according to the method described by Kemegne et al. [18] with a few modifications. Briefly, 100 μL of each extract was mixed with 500 μL of the one-tenth-diluted Folin-Ciocalteu reagent. After 4 min, 400 μL of an aqueous sodium carbonate solution (Na_2CO_3, 7.5%) was added. The mixture was stirred and left for 2 h at room temperature. Absorbance was measured at 765 nm using a UV/visible spectrophotometer (Jenway 6405®) against a blank represented by 100 μL of distilled water to which 500 μL of Folin-Ciocalteu and 400 μL of 7.5% sodium bicarbonate had been added. Gallic acid was used as the standard, and the polyphenol content for each extract was expressed as μg gallic acid equivalent per milligram extract (μg GAE/mg extract).

2.6. Measurement of Anti-Free Radical Activity Using the DPPH Method. The ability of the different extracts to trap the DPPH radical was assessed using the method described by Sánchez-Moreno et al. [19] with a few modifications. 50 μL of the extract at different concentrations (10–100 μg/mL) was added to 2 mL of an ethanolic solution of DPPH (0.1 mM). The mixture was homogenized and incubated in the dark at room temperature for 30 min. The control, without extract, was represented by the solution of DPPH with ethanol. Ascorbic acid at different concentrations (10–100 μg/mL) was used as the standard, and absorbance was read at 515 nm using a UV/visible spectrophotometer (Jenway 6405®). The results for each extract are expressed as a percentage of scavenging of DPPH, calculated basing of the equation below:

$$\%\text{scavenging of DPPH} = \frac{\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{Control}}} \times 100. \quad (7)$$

2.7. Evaluation of Antioxidant Activity by the FRAP Method. The reducing power of iron (Fe^{3+}) in the extracts was determined using the method described by Oyaizu [20]. 250 μL was mixed with 625 μL of 0.2 M phosphate buffer (pH 6.6) and 625 μL of 1% potassium ferricyanide solution ($K_3Fe[CN]_6$). The mixture was then incubated in a water bath at 50°C for 20 min; then, 625 μL of 10% trichloroacetic acid was added to stop the reaction, followed by the addition of 625 μL of distilled water and 125 μL of an aqueous solution of iron III chloride (0.1% $FeCl_3$). The absorbance of the reaction mixture was read at 700 nm against a blank prepared similarly, replacing the extract with distilled water. The standard antioxidant used was ascorbic acid, whose absorbance was measured under the same conditions as the sample. The results for each extract are expressed as μg ascorbic acid equivalent per gram of dry extract (μg EAA/g dry extract).

2.8. Evaluation of Antimicrobial Activity by the Microdilution Method. The antimicrobial activity of the extracts obtained was individually tested against two Gram-negative bacteria (*Escherichia coli* ATCC 25922 and *Salmonella enteritidis* 155A) and two Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923 and *Bacillus cereus* ATCC 11966). *Candida albicans* was a yeast clinical isolate obtained from the Centre Hospitalier Universitaire de Yaoundé (Cameroon); the bacteria strains were kindly donated by the Laboratory of Food Microbiology of the University of Bologna (Italy). Microorganisms stored at -80°C were cultured at 37°C for 24 h twice in Mueller Hinton liquid culture medium before use.

The microdilution method was carried out following the recommendations of CLSI [21]. A stock solution was first prepared by diluting the extract in 10% DMSO. Simultaneously, 10^6 cells/mL of bacterial inoculum were prepared in Mueller Hinton broth from an overnight broth culture. Next, 2 μL of the stock solution was added to 200 μL of bacterial inoculum to reach 2500 ppm as the first test concentration. From this concentration, a double dilution was performed using the bacterial inoculum to obtain concentrations ranging from 2500 ppm to 2.44 ppm, followed by incubation at 37°C for 24 h (after mixing with a vortex). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were defined as in CLSI [21]. The presence of viable bacteria after incubation was assessed by adding 200 μL of a 10 ppm triphenyltetrazolium chloride solution; the color changes towards red indicate the presence of viable cells. The antibacterial effect was bactericidal or bacteriostatic depending on the MBC/MIC ratio. If MBC/MIC = 1 – 2, the effect is bactericidal, and if MBC/MIC = 4 – 16, the effect is bacteriostatic [22].

2.9. Evaluation of Anti-Inflammatory Activity by Inhibition of Protein Denaturation. Inhibition of protein denaturation was assessed using the method described by Ranaweera et al. [23] with some modifications. To each tube containing 100 μL of the test sample (extract or diclofenac) at concentrations ranging from 0.6 to 0.1 mg/mL, 0.5 mL of 5% Bovine Serum Albumin (BSA) prepared in 1 mL of phosphate-buffered saline (PBS) at pH 7.4 was added. The control consisted of a mixture of 0.5 mL BSA and 1 mL PBS. The tubes were shaken and incubated at 37°C for 15 minutes. After incubation, the tubes were heated in a water bath at 70°C for 10 minutes. Following heating, 1 mL of distilled water was added to each tube, and the optical density was read at 660 nm.

$$\%\text{inhibition} = \frac{\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{test}}}{\text{Absorbance}_{\text{Control}}} \times 100. \quad (8)$$

Table 2: Proximate composition of *Raphia farinifera* shell and kernel powders.

	Ash ($g \cdot 100\,g^{-1}$ dry matter)	Protein ($g \cdot 100\,g^{-1}$ dry matter)	Total carbohydrate ($g \cdot 100\,g^{-1}$ dry matter)	Lipid ($g \cdot 100\,g^{-1}$ dry matter)	Moisture (g/100 g wet basis, wb)
Shell	6.05 ± 0.07^a	3.89 ± 0.05^a	83.5 ± 0.28^a	6.62 ± 0.01^a	15.10 ± 0.14^a
Kernel	1.40 ± 0.02^b	4.78 ± 0.09^b	86.26 ± 0.48^b	7.56 ± 0.00^b	10.00 ± 0.05^b

All means in the same column showing different superscripts differ significantly ($p < 0.05$).

2.10. Assessment of Anti-Inflammatory Activity by Haemolysis. To assess the membrane stabilization of erythrocytes, a suspension of red blood cells from fresh beef whole blood collected in heparin tubes (10 mL) at the Yaoundé abattoir (SODEPA) was prepared. The tubes were then centrifuged at 3000 revolutions per minute (rpm) for 10 minutes, then washed three times with an equal volume of normal saline (NaCl, 0.9%) at 3000 rpm for 5 minutes. Blood volume was measured and reconstituted as a 10% (*v*/*v*) suspension with normal saline. The reaction mixture consisted of 1 mL of extract at different concentrations and 1 mL of red blood cell suspension (10%). Normal saline was used as the control and sodium diclofenac as the reference. The tubes were incubated at 37°C for 10 minutes, then in a water bath at 60°C for 20 minutes. After cooling, the reaction mixture was centrifuged at 3000 rpm for 5 minutes, and the absorbance of the supernatant was determined at 560 nm using a UV/visible spectrophotometer (Jenway 6405®).

$$\%\text{inhibition} = \frac{\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{test}}}{\text{Absorbance}_{\text{Control}}} \times 100. \tag{9}$$

2.11. Data Analysis. All experiments were performed in triplicate, and results were expressed as mean ± standard deviation. Differences were considered significant at the 5% probability level ($p < 0.05$). Calculation of the various terms in the regression equation and statistical analysis of the design were estimated using STATGRAPHICS Centurion 18 version 18.1.12 (64-bit) software. Sigmaplot version 14 software was used to produce the various plots. The effective concentration (EC50) was determined using GraphPad Prism V. 5.00 software by plotting the percentage inhibition curve as a function of concentration.

3. Results and Discussion

3.1. Proximate Composition of Kernels and Shells of Raphia farinifera. Table 2 presents ash, protein, carbohydrate, lipid, and moisture content of the *Raphia farinifera* shell and kernel powders. The recommended moisture content of powders for good long-term storage is 10%. The water content of the shell is higher than this value, compared with the moisture content of the kernel. The mineral fraction is higher for the shell (6.05%) than for the kernel. This mineral fraction is low compared with that of palm kernels reported by Ikubanni et al. [24] and kernels of certain species of Cucurbitaceae grown in Nigeria [25]. On the other hand, the kernel has high protein, carbohydrate, and lipid content, significantly different ($p < 0.05$) from the shell. León-López et al. [26] also showed a significant difference in composition between the shell and kernel of *Moringa oleifera* seeds.

3.2. Modeling and Optimization of the Extraction of Phenolic Compounds from the Shells and Kernels of Raphia farinifera. In this study, the influences of time, the solvent/solid ratio, and the percentage of ethanol in the water/ethanol mixture on the polyphenol concentration of *Raphia farinifera* shell and kernel extracts were evaluated. For this purpose, a face-centered composite design was produced, and the results of the 19 trials are presented in Table 1.

From the different conditions investigated, it appears that the extracts obtained from the kernels lead to the highest concentrations of polyphenols. For extracts obtained from kernels, the lowest total phenol content (TPT) was 35.47 mg/L, with a maximum value of 139 mg/L, while for shells these values were 22.72 mg/L and 97.31 mg/L, respectively. These results illustrate the influence of the nature of the plant matrix on the polyphenol concentration of the plant extracts studied. Based on these data, models in coded and real variables were developed, and the expressions are given in the equations below.

Coded variables:

$$\begin{aligned}\text{TPC}_{\text{coded kernel}} &= 119.60 + 0.7470x_1 + 40.06x_2 + 0.9688x_3 \\ &\quad + 3.89x_1^2 - 0.3525x_1x_2 - 0.2400x_1x_3 \\ &\quad - 23.18x_2^2 - 7.06x_2x_3 - 17.75x_3^2, \\ \text{TPC}_{\text{coded shell}} &= 91.24 - 0.7190x_1 + 12.56x_2 + 3.83x_3 \\ &\quad - 16.83x_1^2 + 1.93x_1x_2 - 3.71x_1x_3 - 19.85x_2^2 \\ &\quad - 5.25x_2x_3 - 9.93x_3^2.\end{aligned} \tag{10}$$

Real variables:

$$\begin{aligned}\text{TPC}_{\text{Real kernel}} &= -0.461224 - 0.483235X_1 + 2.03197X_2 \\ &\quad + 7.97597X_3 + +0.001920X_1^2 - 0.000157X_1X_2 \\ &\quad - 0.000533X_1X_3 - 0.009271X_2^2 \\ &\quad - 0.014130X_2X_3 - 0.177515X_3^2, \\ \text{TPC}_{\text{Real shell}} &= -164.82601 + 2.34969X_1 + 1.13909X_2 \\ &\quad + 5.99452X_3 - 0.008310X_1^2 + 0.000859X_1X_2 \\ &\quad - 0.008250X_1X_3 - 0.007939X_2^2 \\ &\quad - 0.010495X_2X_3 - 0.099321X_3^2.\end{aligned} \tag{11}$$

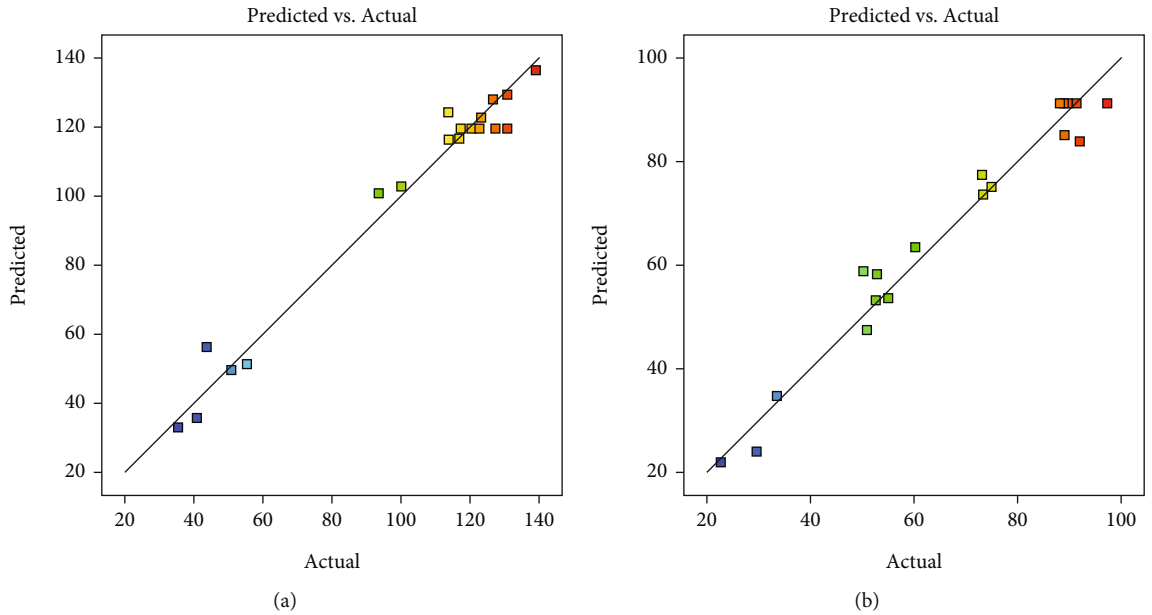


Figure 2: Diagnosis plot for the model adequacy of total polyphenol from the kernel (a) and shell (b) of *Raphia farinifera*.

To assess the relevance of these models, the coefficient of determination was evaluated, giving values of 96.87 and 97.39 for the shell and kernel models, respectively. These values mean that the models obtained explain 97.39% and 96.86%, respectively, of the variability in polyphenol concentration in the kernel and shell extracts due to the different factors investigated. The adjusted coefficient of determination ranged from 94.78 (kernels) to 93.76% (shells), which is higher than the 80% value recommended by Joglekar and May [27]. Figure 2 also shows that the observed and predicted values do not deviate too far from the first bisector, illustrating good agreement between the values predicted by the model and the experimental values. Other studies, such as those by Ross [28], Baranyi et al. [29], and Muala et al. [30], have recommended adding other indicators to the adjusted coefficient of determination to judge the relevance of the model, such as the bias factor (B_f), the accuracy factor (A_f), and the absolute mean deviation analysis. The recommended values are close to 1 for the bias and accuracy factors and close to 0 for the absolute mean deviation analysis. Regardless of the model obtained, the values obtained for these different indicators are in line with the recommendations (Table 3). The lack-of-fit test was not significant, as the p values obtained were greater than 0.05 (Table 3). All the indicators used allow us to conclude that the quadratic models obtained provide a very good description of the phenolic compound extraction process from the two matrices used.

The various models obtained include linear, quadratic, and interaction terms. To judge the relevance of these different terms, an analysis of variance was carried out, and the results are presented in Table 4. The results show that four terms are significant for the kernel model (linear effect of ethanol percentage, interaction of ethanol percentage and solvent/powder ratio, quadratic effect of ethanol percentage, and quadratic effect of solvent/powder ratio) and five terms for the shell model (linear effect of ethanol percentage, interaction of ethanol percentage and solvent/powder ratio, quadratic effect of time, quadratic effect of ethanol percentage, and quadratic effect of solvent/powder ratio) (Table 4).

Table 3: Validation indicators of the different models.

	Shell	Kernel
R^2 (%)	96.87	97.39
R^2_{adjust} (%)	93.74	94.78
AADM	0.0004	-0.0004
B_f	0.9966	1.0008
A_f	1.0583	1.0577
Lack of fit (p value)	0.0917	0.1365

The linear effect of ethanol percentage was the most significant term ($p < 0.0001$) regardless of the model obtained. These results agree with the fact that the selection of the solvent is a critical parameter for the extraction of polyphenols from different matrices [31]. Abd-El-Aziz et al. [4] also showed that the percentage of ethanol in a water/ethanol mixture was the most significant factor during the extraction of polyphenols from the aerial parts of *Leontodon hispidulus*. Whatever the model, the linear term for the percentage of ethanol had a positive sign, and the quadratic effect had a negative sign. These results illustrate that ethanol, at low proportions in the mixture, favors the extraction of polyphenols, but at high percentages, ethanol leads to a decrease in the polyphenol concentration of the different extracts (Figures 3 and 4). The results obtained are in line with previous studies suggesting that binary solvent systems were more efficient for the extraction of polyphenols from plant materials compared to monosolvent systems (water or pure

Table 4: Analysis of variance for the models of total polyphenol content extract from shell and kernel of *Raphia farinifera*.

Factors		Kernel extract p value	Shell extract p value
Linear term	X_1: time	0.7677	0.7072
	X_2: percentage of ethanol	**<0.0001**	**<0.0001**
	X_3: solvent/power ratio	0.7131	0.0689
Interaction term	X_1X_2	0.9045	0.3756
	X_1X_3	0.9349	0.1071
	X_2X_3	**0.0010**	**0.0323**
Quadratic term	X_1X_1	0.4359	**0.0011**
	X_2X_2	**0.0010**	**0.0003**
	X_3X_3	**0.0054**	**0.0208**

P values less than 0.05 indicate that the effects of factors are significantly different from zero at the 95.0%.

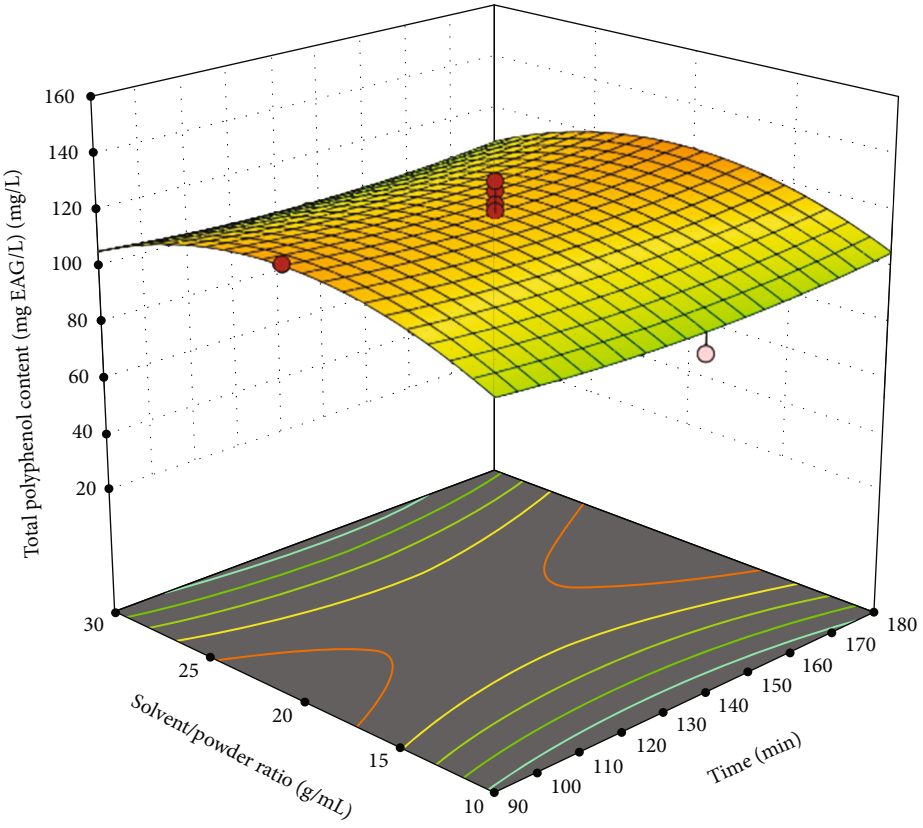
ethanol) [32, 33]. It should be noted that increasing the percentage of ethanol leads to a change in the polarity of the solvent and thus modifies the affinity of the solvent towards the different polyphenols. Özbek et al. [32] observed that for more than 50% ethanol in a water-ethanol mixture, the concentration of polyphenols in *Pistacia vera* L. shell extracts decreased. Hikmawanti et al. [12] reported that extracting dried leaves of *Sauropus androgynus* with 50% ethanol in a water/ethanol mixture led to better polyphenol extraction yields than at 70 and 96%. Lohvina et al. [5] evaluated the effect of ethanol concentration (30, 50, 70, and 96% [*v*/*v*] ethanol in water) as a solvent for extracting polyphenols from *Trigonella foenum-graecum* L. seeds. They showed that the optimum concentration for extracting polyphenols is 70%.

The solvent/powder ratio, through its interaction with the percentage of ethanol and its quadratic effect, was also found to be significant. The significance of the interaction of the solvent/powder ratio with the percentage of ethanol would mean that the influences of the percentage of ethanol in the medium observed are conditioned by the values taken by the solvent/powder ratio whatever the matrix used (Figures 3 and 4). Although the linear effects of this ratio are positive, they do not significantly influence the concentration of polyphenols in the different extracts. It is observed that the quadratic effects are significant and negatively influence the extraction of polyphenols within the different matrices (Figures 3 and 4). These observations can be explained by the fact that the more solvent can penetrate the cells, the more phenolic compounds can be extracted in the solvent [34]. Above a certain threshold, the strong presence of the solvent favors the extraction of nonphenolic compounds from the medium, which is likely to lead to polymerization reactions with the polyphenols, resulting in the formation of complexes and a reduction in the polyphenols in the medium.

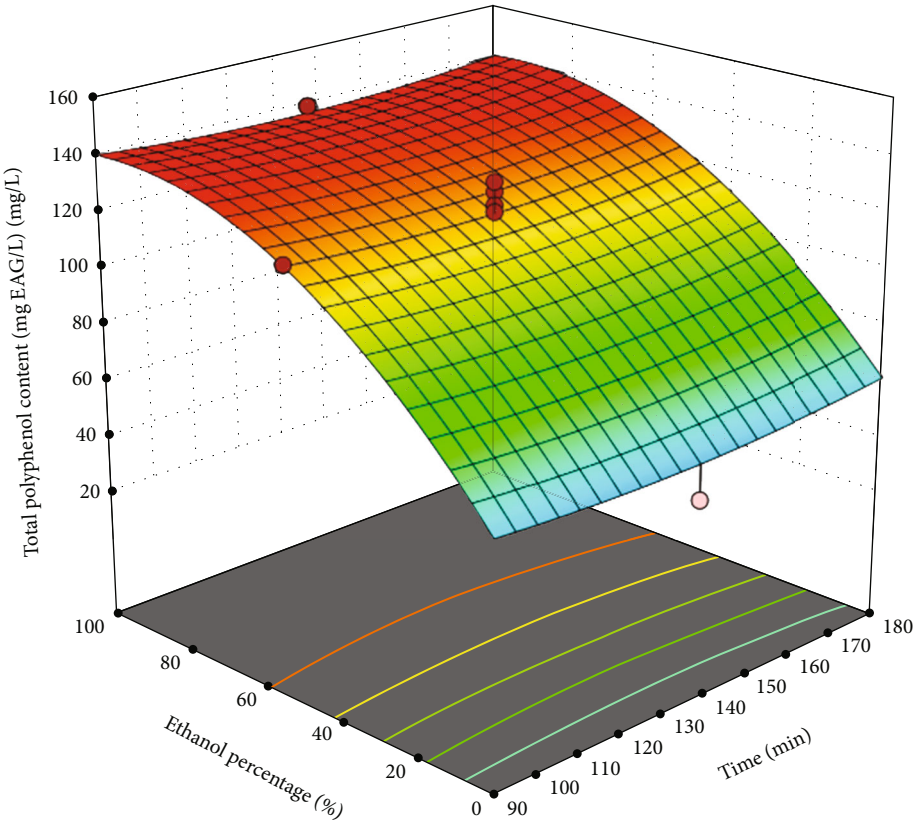
Reaction time is a crucial parameter for minimizing the cost of the phenolic compound extraction process [34]. Whatever the model obtained, the linear effects and interactions with the percentage of ethanol and the solvent/powder ratio are not significant. Only its quadratic effect in the shell model is significant and negatively influences the extraction of phenolic compounds within this matrix, thus illustrating the fact that excessive reaction time induces a decrease in the concentration of phenolic compounds in the extracts obtained from shells (Figures 3 and 4). Several studies have reported that a prolonged extraction time could degrade phenolic compounds due to their oxidation following prolonged exposure to light or oxygen [35]. Ciric et al. [36] during the extraction of polyphenols from *Allium sativum* L. found a quadratic effect of time negatively influencing the extraction of phenolic compounds, and a decrease was observed for extraction times greater than 13.50 min.

The aim of the experiments was to determine the influence of the various factors on the concentration of polyphenols in *Raphia farinifera* shell and kernel extracts and to determine the optimum conditions for extracting the polyphenols. Numerical optimization of the various models, based on the experimental data, enabled us to determine these optimum conditions, which are a reaction time of 134.24 min, an ethanol percentage of 65%, and a solvent/liquid ratio of 21.16 for a polyphenol concentration of 93.35 mg/L for the shell extracts. The kernels had a polyphenol concentration of 141.76 mg/L under conditions where the reaction time was 180 min, the ethanol percentage was 94.3%, and the solvent/liquid ratio was 18.61. Tests were carried out under these conditions, and the values obtained were 95.36 mg/L and 139.72 mg/L for shell and kernel extracts, respectively. These values are not significantly different from those predicted by the model.

3.3. Antioxidant Activity of Ethanolic Extracts of Raphia farinifera Shells and Kernels. The optimum conditions for extracting phenolic compounds from the shells and kernels of *Raphia farinifera* were used to produce extracts to be used for assessing antioxidant activity by two methods (FRAP and DPPH). Figure 5 shows the free radical scavenging activity of the extracts and a reference antioxidant, ascorbic acid, against the DPPH radical. The inhibition power is a function of the concentration of the antioxidant and the extracts. At 10 μg/mL, a percentage of inhibition of 27.91, 33.96, and



(a)



(b)

Figure 3: Continued.

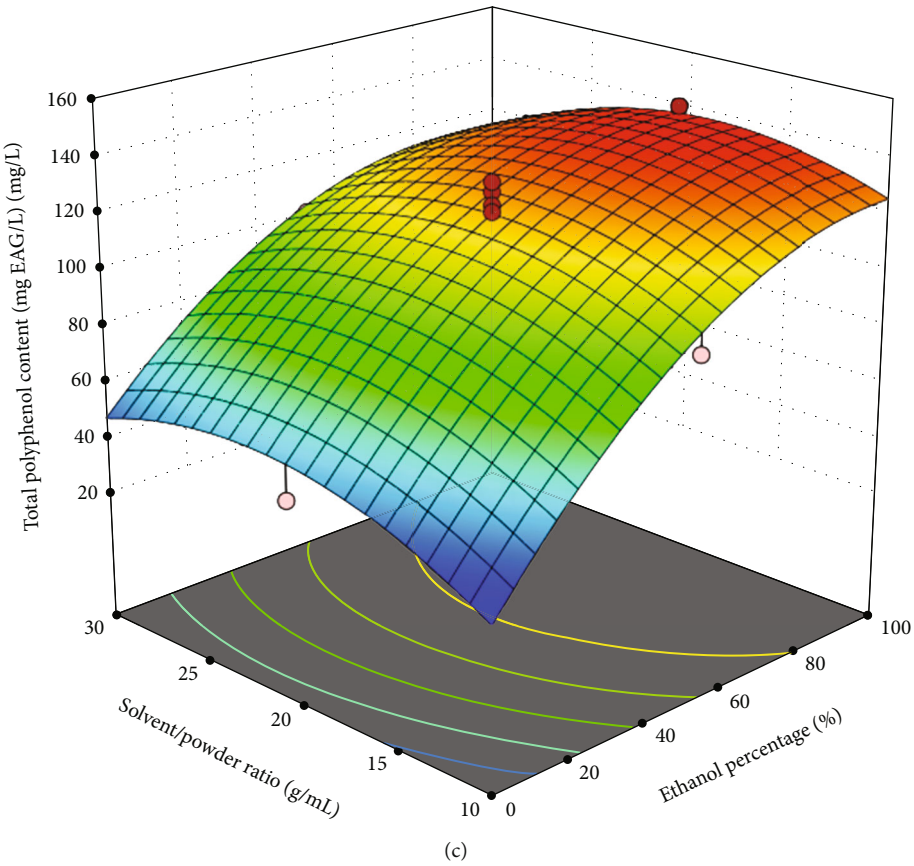


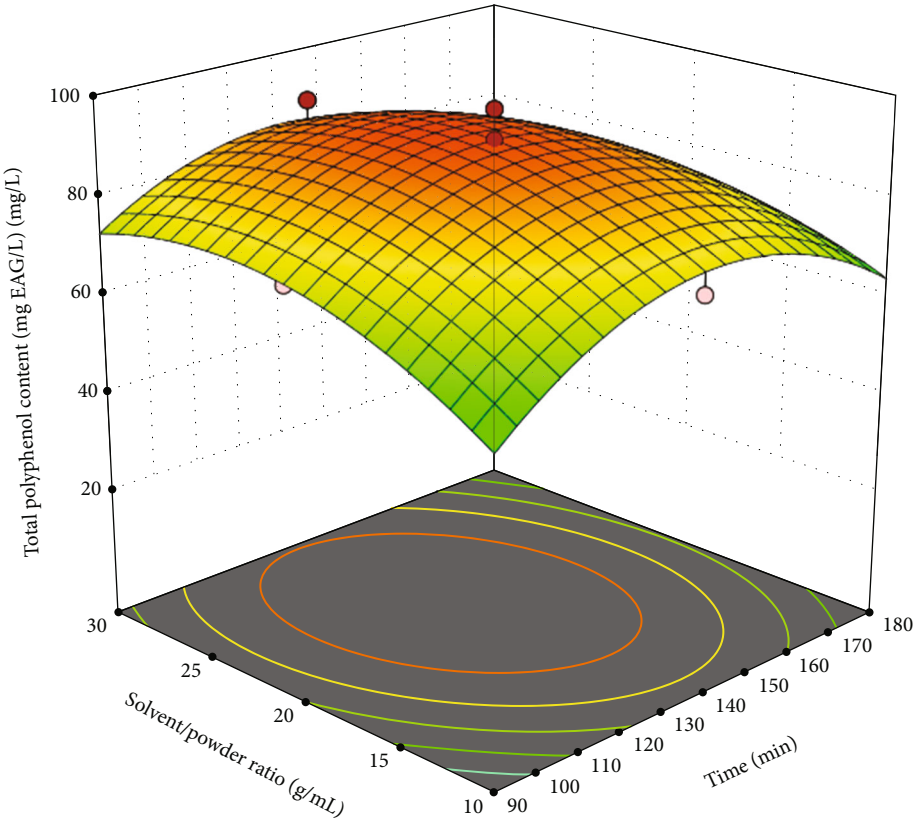
Figure 3: 3D response surface plots for the total polyphenol content of the extract from the kernel of *Raphia farinifera* (mg EAG/L). (a) Effect of solvent/powder ratio, time (min), and their interaction on the total polyphenol content. (b) Effect of ethanol percentage, time (min), and their interaction on the total polyphenol content. (c) Effect of solvent/powder ratio (g/mL), ethanol percentage (%), and their interaction on the total polyphenol content.

41.35% was obtained with extracts of shells, kernels, and ascorbic acid, respectively. This percentage increased significantly when the concentration of the kernel and ascorbic acid extracts varied from 10 μg/mL to 60 μg/mL, reaching percentage inhibition values of over 80%. Above 60 μg/mL, the inhibition potency of kernel extracts and ascorbic acid is not significantly different, and an increase in concentration does not result in a significant increase in inhibition potency. Whatever the concentration, ascorbic acid had the greatest inhibitory power, followed by kernel extract and shell extract. These results are in line with several studies reporting antiradical activity as a function of the different parts of the same plant [37, 38]. To better compare the antiradical activities, Table 5 presents the antiradical activity expressed as the quantity of antioxidants required to reduce the initial concentration (IC_{50}) of the DPPH radical by 50%. The smallest quantity was obtained with ascorbic acid (14.27 μg/mL), followed by stone extract (22.32 μg/mL), which represents less than half the concentration of the shell extract (55.73 μg/mL) required for 50% inhibition of the DPPH radical. These results show that kernel extracts have greater anti-free radical properties than shell extracts. The differences observed can be explained by the different nature of the phenolic compounds present in the different extracts.

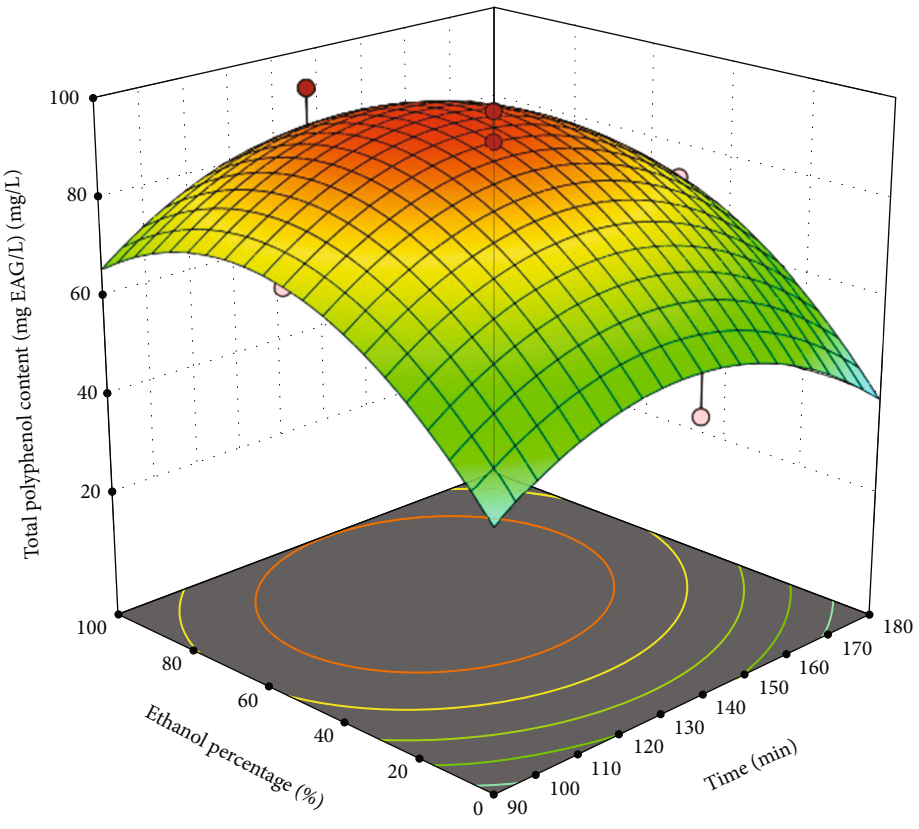
In addition to the free radical scavenging activity, the reducing power of the different extracts, an essential mechanism of antioxidant activity, was evaluated by the reduction of the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}). The results obtained showed that the activities were significantly different, at 308.39 ± 1.70 μgEAA/mg and 293637gEAA/mg, respectively, for kernel and shell extracts. These results show that ethanolic extracts of the *Raphia farinifera* kernel have a high reducing power compared with those of shells. Furthermore, whatever the antioxidant activity assessment method used, the ethanolic extract of the *Raphia farinifera* kernel has a high activity. The reducing capacity of a sample identifies a considerable potential antioxidant activity. Hence, the FRAP assay is used to examine the reduction of ferric iron (Fe^{3+}) into ferrous iron (Fe^{2+}).

3.4. Antimicrobial Activity of Ethanolic Extracts of Shells and Kernels of Raphia farinifera. The antimicrobial activities of *Raphia farinifera* shell and kernel extracts obtained under optimal conditions were also assessed using the *in vitro* microdilution method. The corresponding antimicrobial activities are presented in Table 6.

Of the two types of extract evaluated, the most active was that obtained from the nucleus (kernel) of *Raphia farinifera*.



(a)



(b)

Figure 4: Continued.

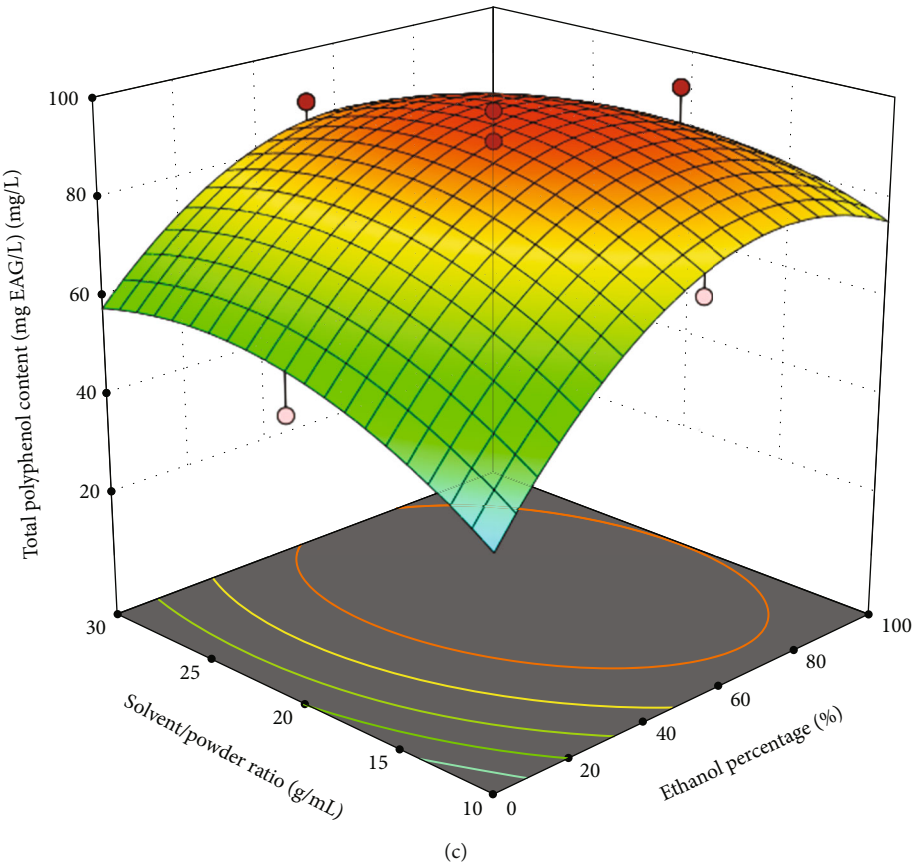


Figure 4: 3D response surface plots for the total polyphenol content of the extract from the shell of *Raphia farinifera* (mg EAG/L). (a) Effect of solvent/powder ratio, time (min), and their interaction on the total polyphenol content. (b) Effect of ethanol percentage, time (min), and their interaction on the total polyphenol content. (c) Effect of solvent/powder ratio (g/mL), ethanol percentage (%), and their interaction on the total polyphenol content.

A significant inhibitory effect was shown by the kernel extract against *B. cereus* (MIC = 156.25 ppm) and *S. aureus* (MIC = 312.5 ppm). A moderate inhibitory effect was observed for the shell extract against *B. cereus* (MIC = 625 ppm) and *S. aureus* (MIC = 1200 ppm) as well as for the stone extract against *S. enteritidis* (MIC = 625 ppm). *E. coli* showed low sensitivity (MIC = 2500 ppm) to both extracts. Both shell and kernel extracts were ineffective against the growth of *C. albicans* (MIC > 2500). A bactericidal effect (MBC/MIC = 1) of the kernel extract and a bacteriostatic effect of the shell extract could be observed with *B. cereus*. These results could be largely attributed to the high content of phenolic compounds contained in the ethanolic extracts of *Raphia farinifera* shells and kernels. Indeed, the extraction of phenolic compounds for which antimicrobial activity has already been demonstrated [39, 40] was optimized in this study.

In 2011, Fratianni et al. [41] determined the phenolic composition and antimicrobial activity by disc diffusion of the ethanolic extract of Annurca (*Malus domestica* var. Annurca). The authors showed that the extract contained abundant phenolic compounds (rutin, epicatechin, dicaffeoylquinic acid, and caffeic acid) and demonstrated antimicrobial activity against three species of *Bacillus cereus* (inhibition diameters of between 11 and 14 mm) and two species of *Escherichia coli* (inhibition diameter of 10 mm).

The antimicrobial activities of phenolic compounds produced by enzymatic extraction from oven-dried and freeze-dried residues of black grape (*Vitis vinifera* × (*Vitis labrusca* × *Vitis riparia*)), apple (*Malus domestica* cv Jonagold), and yellow pitahaya (*Hylocereus megalanthus*) were investigated by disk diffusion and minimum inhibitory concentration assays [42]. The results of this study demonstrated enhanced antimicrobial capacity for many extracts; the lowest MIC (12.5 mg/mL) was found for black grape samples against *Bacillus cereus* and *Bacillus subtilis* and for apple and pitahaya samples against *Pseudomonas putida*.

3.5. *In Vitro* Anti-Inflammatory Activity of *Raphia farinifera* Extracts

3.5.1. Inhibition of Protein Denaturation. One of the major, well-documented causes of inflammation is protein denaturation. As extracts of *Raphia farinifera* shells and kernels are rich in phenolic compounds, they are likely to contain anti-inflammatory activity, the capacity of which would be to inhibit protein denaturation following stress. An *in vitro* assessment of this activity in relation to the denaturation

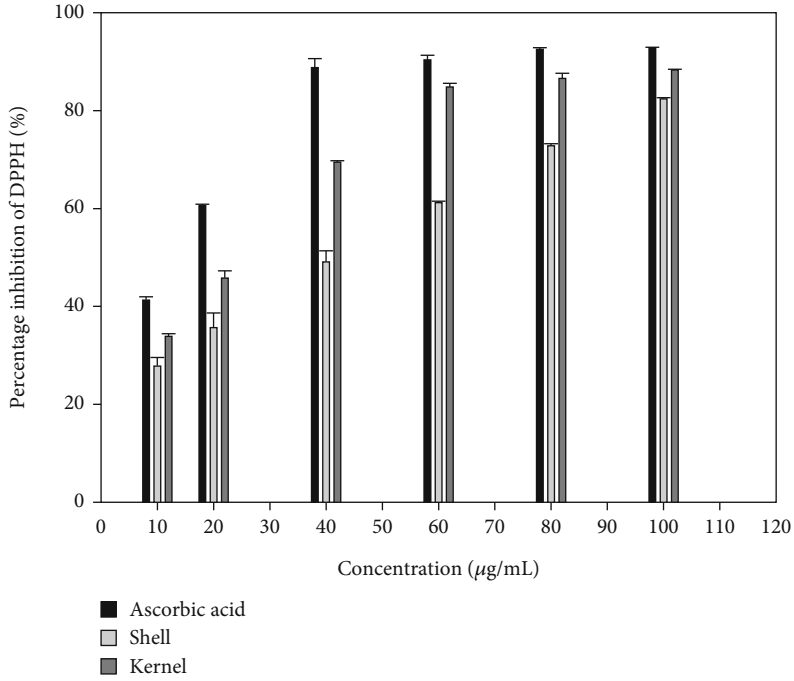


Figure 5: Percentage of inhibition of DPPH radicals according to the concentrations of ascorbic acid and extracts from the shells and kernel of *Raphia farinifera*.

Table 5: IC_{50} of free radical DPPH of ascorbic acid and extracts of shells and kernels of *Raphia farinifera*.

	Shell extract	Kernel extract	Ascorbic acid
IC_{50} (μg/mL)	55.73	22.32	14.27

of bovine serum albumin (BSA) was therefore carried out. Based on the results shown in Figure 6, the inhibition power ranged from 19.79 to 53.4% for the kernel extract and from 10.77 to 36.49% for the shell extract. Regardless of the concentration of the extracts studied, the ethanolic extract of the kernels produced higher inhibition potencies than that of the shell. The activity of *Raphia farinifera* shell and kernel extracts depends on their concentration. These results are in line with several studies [43–45] which also found that the protective activity of plant extracts against protein denaturation was dependent on extract concentration. The disruption of electrostatic and hydrogen bonds and disulphide bridges, which enable the secondary and tertiary structures of proteins to be maintained, associated with the production of self-antigens, is often attributed to protein denaturation in certain arthritic diseases [43, 46]. Potential antiarthritic activity is often attributed to compounds that prevent these changes. Extracts of *Raphia farinifera* shells and kernels could therefore be endowed with this activity and help to reduce the production of auto-antiantigens. Although these extracts may have this activity, it remains low compared with the activity of the standard used, sodium diclofenac. The IC_{50} of the standard is 170 μg/mL, while those of the shell and kernel extracts are 760 and 560 μg/mL, respectively (Table 7).

3.5.2. Membrane Stabilization. The potential of *Raphia farinifera* shell and kernel extracts to inhibit erythrocyte membrane lysis was assessed. Figure 7 shows the influence of the concentration of the extracts and the standard (Diclofenac Sodium) on the percentage of inhibition of erythrocyte cell lysis. The inhibition percentages of the extracts varied from 17.55 to 40.78% for the shells and 5.47 and 28.84% for the kernel. This shows that the inhibition power depends on the concentration of the extract used. These results agree with those of Aidoo et al. [43] and Kherbache et al. [45]. These authors respectively showed that extracts of *Helichrysum stoechas* and bergaptene, a furocoumarin present in many medicinal plants, have a concentration-dependent erythrocyte membrane stabilization activity. Despite the extract concentrations used, shell extracts led to higher inhibition percentages than kernel extracts. At concentrations of 600 and 800 μg/mL, shell extracts gave inhibition percentages similar to those of diclofenac, the standard used. A study of the haemolysis inhibitory power of the two extracts shows that they both have haemolysis inhibitory activity. The shell extracts had a lower IC_{50} than those of the kernel, at 1054.54 μg/mL and 1339.78 μg/mL, respectively, confirming the fact that *Raphia farinifera* shell extracts have a better capacity to prevent lysis of red blood cells than *Raphia farinifera* kernel extracts. In addition, the IC_{50} values of the various extracts were higher than those of the standard, illustrating the fact that these extracts are significantly less active than diclofenac. The results showed that the kernel extracts had a significantly higher polyphenol content than those of the shells. However, the shell extracts had a lower IC_{50} than those of the kernels, illustrating a better ability to inhibit protein denaturation. These results could be

Table 6: Antimicrobial activities of extracts of shells and kernels of *Raphia farinifera* against some microbial species.

Microbial species	*E. coli*			*S. enteritidis*			*B. cereus*			*S. aureus*			*C. albicans*		
Concentration (ppm)	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC
Extract of solid kernel	2500	>2500	ND	625	>2500	ND	156.25	156.25	1	312.5	>2500	ND	>2500	>2500	ND
Extract of solid shell	2500	>2500	ND	>2500	>2500	ND	625	2500	4	1200	>2500	ND	>2500	>2500	ND

E. coli: *Escherichia coli*; *S. enteritidis*: *Salmonella enteritidis*; *B. cereus*: *Bacillus cereus*; *S. aureus*: *Staphylococcus aureus*; >2500 ppm: no inhibition observed at 2500 ppm; MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration; ND: not determined.

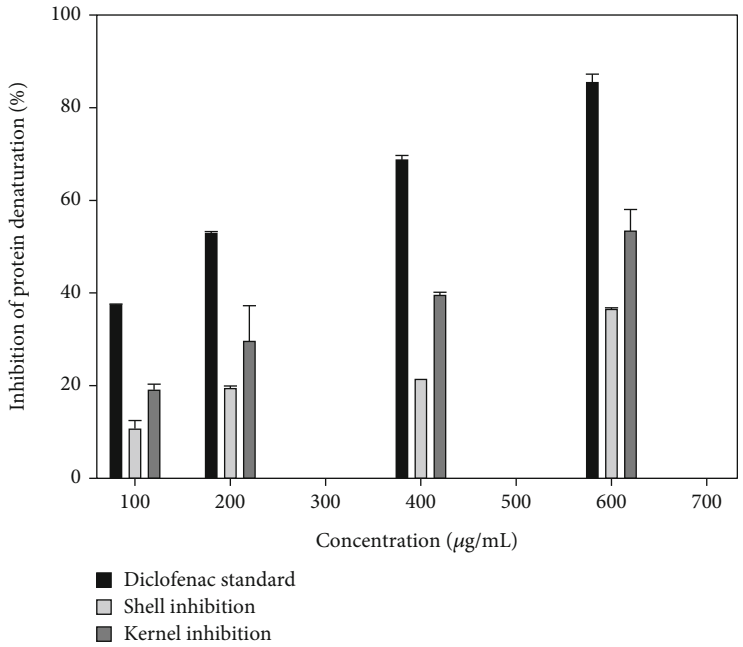


Figure 6: Effects of the concentration of *Raphia farinifera* shell and kernel extracts and sodium diclofenac on inhibition of protein denaturation.

Table 7: IC_{50} for the in vitro anti-inflammatory activities of *Raphia farinifera* shell and kernel extracts and the standard.

	Shell extract	Kernel extract	Diclofenac sodium
		Membrane stabilization	
IC_{50} (μg/mL)	1054.54	1339.78	1045.15
		Protein denaturation	
IC_{50} (μg/mL)	760	560	170

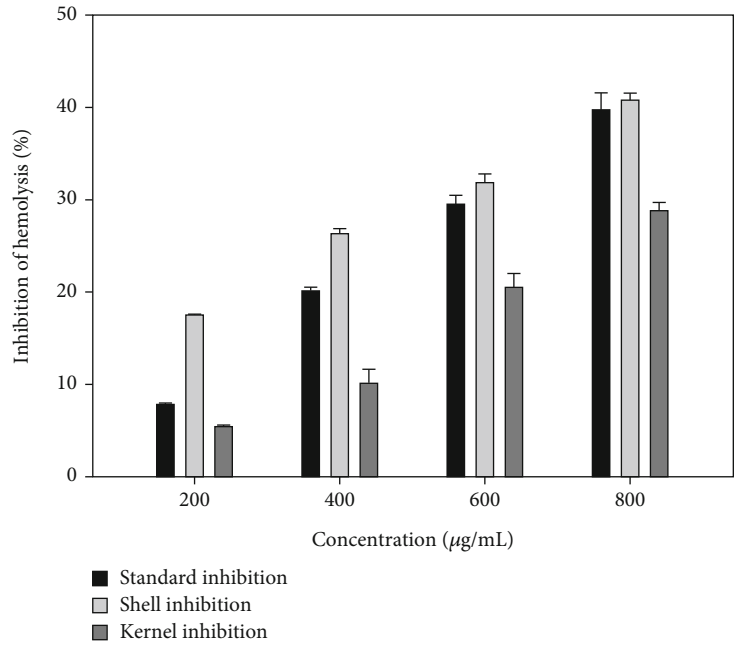


Figure 7: Percentage of inhibition of erythrocyte lysis as a function of the concentrations of diclofenac sodium and extracts from the shells and kernel of *Raphia farinifera*.

explained by the fact that shell extracts are richer in certain molecules specific to the inhibition of haemolysis than kernel extracts. In [11], Oluwaniyi et al. in a comparative study of the compounds present in the pulp, kernel, and shell of *Raphia farinifera* showed that the pulp and kernel lacked flavonoids, whereas the shell had some. One of the activities of flavonoids, well known from several studies, is to stabilize the membrane of erythrocytes when they are subjected to stress [47, 48].

4. Conclusion

In this study, the optimization of extraction conditions for phenolic compounds from *Raphia farinifera* shells and kernels using a face-centered composite plane was successfully carried out to study the influences of extraction time, solvent/powder ratio, percentage of ethanol in the water, and their interaction on the concentration of phenolic compounds in the extracts. The developed mathematical models have a high level of significance with a R^2_{adjusted} of 94.78% and 93.76% for the kernels and shells, respectively. The quadratic models were validated, reflecting good agreement between the experimental data and those predicted by the two models. The percentage of ethanol in the water was found to be the most significant factor. Evaluation of the antioxidant, antimicrobial, and anti-inflammatory activities showed that shell and kernel extracts are endowed with these activities, the extent of which depends on the nature of the substrate (shell or kernel). The positive correlation obtained between phenolic content and the various biological activities shows that polyphenols are mainly responsible for antioxidant, anti-inflammatory, and antimicrobial properties. Based on this work, hydroethanol extracts of *Raphia farinifera* shells and kernels could be considered to improve the antioxidant properties and stability of certain products, subject to the identification of the phenolic compounds present in the extracts, which could be the subject of future work.

Data Availability

The data collected during the different experiments for this study are available from the corresponding author upon request.

Conflicts of Interest

The authors of this work claim that they have no conflicts of interest.

Authors' Contributions

A.K.M. and D.V.T.T. conducted the different experiments and prepared the first version of the paper. A.G.K. coordinated the microbiological analyses and participated in the proofreading of the paper. M.M.P supervised experiments on antioxidant activity and participated in the proofreading of the document. A.S.Y.P coordinated experiments on anti-inflammatory activity and antioxidant activity. P.A.N.K participated in the writing and proofreading of the document and conducted data analysis from the experiments and the experimental design for the modelling. G.A.A with M.R.M designed and supervised the experiments and revised the paper.

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